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Cover picture: FTS Tourism
Dear Colleagues,

It is my great pleasure and honour to hold the 59th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research on September 4–9, 2011 in Antalya, Turkey. This congress series has been organized annually since 1953 and has become the most important and popular congress in Europe in its respected field. It is the first time the congress is organized in Turkey. Turkey is a large peninsula bridging the east and the west at the junction of two continents and has been a passage way between Europe and Asia and even Africa. Due to its geographic location Turkey has been a melting pot of civilizations, cultures and nations, and is full of history and home to diverse traditions. It is a land of many firsts since history starts here. Thanks to its climatically and phytogeographically unique position and its transect ranging from sea level (0 m) to the peak of the Ararat mountain (5137 m) the flora of Turkey is rich and diverse with over 12,000 flowering plant taxa recorded of which 33% are endemic. Anatolia is the land of Galenus of Pergamon and Dioscorides of Anavarza. Pedanius Dioscorides, a physician in the Roman Army had written his famous Materia Medica in the 1st century AD. His birthplace Anavarza is in Kozan, Adana in Southern Turkey not too far from Antalya.

The 59th Congress has attracted global attention and there are participants from all parts of the world. Its scientific level is high thanks to the efforts of the Scientific Committee. High rate of rejects were due to the meticulous work of the reviewers who gave it time and effort to keep the scientific level as high as possible.

Main topics of the Congress are as follows:
- New Trends in Pharmacognosy
- Traditional and Natural Medicines
- Lead Finding from Nature
- Antimicrobials – What’s next?
- Endophytes – Importance in Pharmacognosy
- Natural Immune Enhancers
- Nutraceuticals, Cosmeceuticals, Functional Foods – Prevention of Metabolic Diseases
- Essential Oils – Analysis, Bioactivities, Uses, Therapeutical Potential
- Biotechnology and Nanobiotechnology
- Advances in the Analysis of Natural Products

Ten plenary and two keynote lectures will be presented by distinguished scientists. 73 short lectures will be presented in three parallel sessions. Numerous researchers will be able to report their research findings in 900 poster presentations. In addition, young researchers will be able to present their papers at two parallel Young Researchers Workshops. There will also be three more Permanent Committee Workshops of the GA on regulatory affairs, pharmacology, agriculture and quality of natural products. An additional workshop will be held on Traditional Chinese Medicine (TCM). 31 lectures will be presented in the workshops. All in all over 1100 scientific presentation will be made at the congress.

I would like to thank the Executive and the Advisory Board members of the GA for their help and encouragement during the preparatory stages of the Congress. I wish to extend my grateful thanks to Georg Thieme Verlag KG for processing such a huge number of abstracts in a short time. My special thanks go to the members of the Organizing Committee and to the Congress Organizing Company FTS who have done their utmost to offer you a successful, satisfying and enjoyable congress.

I wish you all a fruitful congress which I hope will strengthen old friendships and develop new ones in a friendly, scientific and cultural atmosphere. I hope everybody enjoys their stay in sunny Antalya, gets the opportunity to discover hidden beauties of the region and Turkey, and takes home new scientific knowledge and unforgettable memories.

Prof. Dr. K. Hüsnü Can Başer
President of the 59th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research
Abstracts | 59th International Congress of the GA | 4th–9th September 2011, Antalya, Turkey

1. Natural products in modern life science

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With a realistic threat against biodiversity in rain forests and in the sea, a sustainable use of natural products is becoming more and more important. Basic research directed against different organisms in Nature could reveal unexpected insights into fundamental biological mechanisms but also new pharmaceutical or biotechnological possibilities for more immediate use. Many different strategies have been used prospecting the biodiversity of Earth in the search for novel structure-activity relationships, which has resulted in important discoveries in drug development. However, we believe that the development of multidisciplinary incentives will be necessary for a future successful exploration of Nature. With this aim, one way would be a modernization and renewal of a venerable proven interdisciplinary science, Pharmacognosy, which represents an integrated way of studying biological systems. This has been demonstrated based on an explanatory model where the different parts of the model are explained by our ongoing research. Anti-inflammatory natural products have been discovered based on ethnopharmacological observations, marine sponges in cold water have resulted in substances with ecological impact, combinatorial strategy of ecology and chemistry has revealed new insights into the biodiversity of fungi, in depth studies of cyclic peptides (cyclotides) has created new possibilities for engineering of bioactive peptides, development of new strategies using phylogeny and chemography has resulted in new possibilities for navigating chemical and biological space, and using bioinformatic tools for understanding of lateral gene transfer could provide potential drug targets. A multidisciplinary subject like Pharmacognosy, one of several scientific disciplines bridging biology and chemistry with medicine, has a strategic position for studies of complex scientific questions based on observations in Nature. Furthermore, natural product research based on intriguing scientific questions in Nature can be of value to increase the attraction for young students in modern life science. References: Bohlin L, Gornysson U, and Backlund A (2007) Pure and Appl Chem 79: 763 – 774. Larsson S, Backlund A, and Bohlin L (2008) Phytochem Lett 1: 131 – 134. Bohlin L, Gornysson U, Alsmark C, Wedén C, and Backlund A (2010) Phytochem Rev 9: 279 – 301.

2. Combination of ethnopharmacological knowhow with modern in silico tools

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Computational methods are valuable tools in current drug discovery and development processes. They aim at analyzing, understanding, and predicting the bioactivity of a compound with respect to a specific biological target, and have been applied successfully in medicinal chemistry. Their application in natural product research is however affected with some challenges, such as limited availability of high quality natural product databases, often restricted or laborious access to individual compounds for testing, and lack of chemoinformatics experience with secondary metabolites. This asks for a sensible application of data mining tools in this prospering field of lead finding from nature. The hyphenation of in silico strategies with knowledge from ethnopharmacology offers a unique opportunity to benefit from a combined theoretical and empirical approach. Herbal remedies, which are used since centuries, represent a particularly promising resource for drug leads. These thus defined multicomponent mixtures play a dominant role in healthcare worldwide with increasing importance, and have been used as a traditional and biomedical uses and with links between multi-target molecular pharmacology and clinical medicine. This presentation describes strategies how to integrate computational strategies in pharmacognostic workflows to disclose promising bioactive compounds or hidden information about affected pharmacological targets. I will focus specifically on the expectations, possibilities and limits when using computational tools in phytochemistry, and present some recent examples of these highly complementary, but synergistic approaches, viz. in silico – in traditio. Keywords: computational methods, in silico, ethnopharmacology, drug discovery.

3. Biological Activities of Essential Oils

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Essential Oils (natural mixtures of single fragrance compounds) do possess biological properties. Besides psychological influences, these rather small and lipophilic molecules with distinct physiological activities upon inhalation and/or topical administration which can be shown in animal experiments as well as in human studies. After a short introduction about the correct definition of the term “Essential Oils” new research results on the application of fragrance compounds in therapy are presented. Examples of the biological properties of these natural compounds will be discussed and a great bow drawn from the effects on the human autonomic and central nervous system to “other effects”. These cover e.g. anti-inflammatory activities, anti-oxidative ones and penetration enhancing properties, anti-microbial and insect repellent activities and the possibility to use these small molecules in cancer prevention or therapy and against Alzheimer’s disease. Some studies on the biochemical mechanism of such effects are also presented as well as methods to investigate the above activities of essential oils.

4. Infectious diseases and natural products. What is next?

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Infectious diseases caused by bacteria, viruses, parasites and fungi are results of complex interactions between the pathogen, host and the environment. The early discovery of quinine, followed by the antibiotics and more recently artemisinin has brought a new, realistic hope in the control of infections that once ravaged the humankind. However, the widespread use of these drugs and globalization has led to the development of multidrug-resistant pathogens worldwide. On the other hand, the pharmaceutical industry that previously relied extensively on natural products as source of new molecules for anti-infective drug discovery and development has undergone a significant de-emphasis in NP research. The main research activity currently falls on to academia and requires novel approaches for tackling infectious diseases. This lecture will emphasize the opportunities and the challenges in this area and highlight new application forms and areas for research. Specific examples from our own research will be presented in order to point out the potential of vast variety of natural products derived from plants, marine organisms and other sources in both prophylaxis and the chemotherapy of infectious diseases caused by parasites (e. g. malaria, trypanosomiasis, leishmaniasis, schistosomiasis and river blindness) and (myco)bacteria (e. g. tuberculosis). Target determination, molecular properties (lipophilicity, permeability, drug-likeness) and pharmacokinetic properties of some natural and natural product-derived synthetic leads will also be included. Keywords: Infectious diseases, natural products, drug discovery, prophylaxis, chemotherapy.

5. Natural products derived from traditional chinese medicine as novel inhibitors of the epidermal growth factor receptor in cancer cells

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The success of established anticancer drugs is frequently hampered non-sufficient tumor specificity leading to side effects and drug resistance. Oncogene products and tumor suppressors are exquisite targets developing more specific drugs with improved features. The epidermal growth factor receptor (EGFR) became an important target for drug development. However, clinical application of EGFR tyrosine kinase inhibitors resulted in resistance to EGFR-targeting drugs due to the selection of EGFR-mutated variants. This phenomenon forced the search for novel inhibitors of EGFR and downstream signaling cascades. We investigated the therapeutic role of the EGFR in human tumor biopsies (lung cancer, glioblastoma, head and neck cancer) by comparative genomic hybridization and immunohistochemistry [1 – 4,6] and identified phytochemicals (dicentrine, camptothecin derivatives, artesunate etc.) affecting EGFR signaling [5,7 – 9]. Here, we report on recent achievements...

L6 The role of pharmacokinetics in natural products research

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In recent years the number of studies investigating the pharmacodynamic effects of botanicals has increased exponentially, often reporting pharmacological effects of botanical extracts with insignificant bioactivities obtained in irrelevant in vitro bioassays. The data interpretation from these in vitro assays for their efficacy in animals and humans is based on the assumption that a sufficient concentration of active constituents can reach the target sites of action in the body. This interpretation can be misleading since the pharmacokinetic properties of a compound are completely ignored. Although important, there is still limited information available regarding herbal pharmacokinetics. This might be due to the following reasons: (i) the active constituents are not known; (ii) the study of herbal pharmacokinetics is extraordinarily complex because extracts are multicomponent mixtures which contain several chemical constituents. Therefore, concentrations of single compounds in the final product are in the lower mg range per dose. (iii) The resulting plasma concentrations are often in the μg to pg per liter range. As a consequence analytical methods determining bioavailability and pharmacokinetics of natural compounds have to be sufficiently sensitive. Advanced techniques such as GC-MS/MS or HPLC-MS/MS can be used nowadays to accomplish these goals. A better understanding of the pharmacokinetics and bioavailability of natural compounds can help in designing rational dosage regimen; and it can further help to link data from pharmacological assays with clinical effects. In this presentation, pharmacokinetic studies will be discussed that have been conducted for some of the top-selling botanicals worldwide, including artichoke, echinacea, mangosteen and valerian.

L7 Overview of Dietary Supplements in USA

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Herbal product studies cannot be considered scientifically valid if the product tested was not authenticated and characterized in order to ensure reproducibility in the manufacturing of the product in question. In the case of botanicals, misidentification of the collected plant, adulteration with other species or contamination with extraneous ingredients are possibilities in which reproducibility may be affected unknown to the manufacturer. Many studies refer to the use of standardized material, but in reality they are referring to chemical standardization. While chemical standardization is important, its utility is limited when the starting material is not well characterized botanically. Although the resulting studies are sound with respect to the actual product tested, adequate authentication of the product cannot be compared to other products on the market. Also, a comparison of one study to another cannot be made due to inconsistencies in the identity of the botanical matrix. The tools needed for authentication of the field plant material also depend on the plant and process involved. This could be as straightforward as botanical/morphological identification or as elaborate as genetic or chemical profiling. These controls are also critical for the evaluation of pharmacological, toxicological and clinical studies of the botanical supplements. Keywords: Herbal products, botanical supplement, authentication

L8 Discovery and applications of naturally occurring cyclic peptides

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Over recent years more than 200 examples of ribosomally synthesized head-to-tail cyclised peptides have been discovered in bacteria, plants and animals [1]. The cyclotides [2] are the largest family of these circular proteins and have applications in drug design [3] and agriculture [4]. They occur in plants from the Violaceae (violet), Rubiaceae (coffee) and Cucurbitaceae (cucumber) families and have a diverse range of biological activities, including iewtonic, anti-HIV, and insecticidal activities the latter suggesting that their natural function is in plant defence. Individuals plants express suites of 10 – 100 cyclotides. Cyclotides typically comprise ~30 amino acids, and incorporate three disulfide bonds arranged in a cystine knot topology. The combination of this knotted structure with a circular backbone makes the cyclotides impervious to enzymatic breakdown and makes them exceptionally stable. This presentation will describe the discovery of cyclotides in plants, their structural characterization, evolutionary relationships and their applications in drug design. Their stability and compact structure makes them an attractive protein framework onto which bioactive epitope peptides can be grafted to stabilize them. Keywords: Cyclic peptides; cyclotides; drug design; NMR; protein structure References: [1] Crail D J (2006) Science 311: 1561 [2] Gruber C W et al (2008) The Plant Cell 20: 2471 – 2483. [3] Henrys S T, Crail D J (2010) Drug Discovery Today 15: 57 64. [4] Barbeta B L et al (2008) PNAS 105: 1221 – 1225

L9 Natural immunomodulators – A Drug Discovery Perspective

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The immune system is an adaptive complex system which poses a challenge to therapeutic intervention. Inflammation is known to be involved in numerous disease processes like autoimmune attack and carcinogenesis and can be targeted by immunopharmacological agents. However, it is far less clear how the immune system may be stimulated [1]. In this talk a new view on how the immune system recognizes and activates plant food shall be provided. During the evolution of the immune system different endogenous systems have evolved that modulate inflammatory processes, such as the pattern recognition receptors (e.g.,TLRs) and the arachidonic acid lipids. Studying the molecular interactions in these biochemical units by small organic molecules provides insight into how inflammation may be regulated and ultimately manipulated. The endocannabinoid system is a stress signal-integrating lipid signaling network and provides great opportunities to treat inflammatory diseases and bone degeneration with the potential to link nutrition and inflammation [2 – 4]. Keynote: Inflammation, immune system, immunomodulation, drug discovery, endocannabinoid system References: [1] Gertsch J, Viveros-Paredes JM, Taylor P (2010) Plant immunomodulants: Scientific paradigm or myth? J Ethnopharmacol PMID: 20620205 [2] Gertsch J, Leonti M, Raduner S, Raczi I, Chen JZ, Xie XQ, Altmann KH, Karsak M, Zimmer A (2008) Beta-carophyllene is a dietary cannabinoid. Proc Natl Acad Sci USA 105(26):9099 – 104 [3] Gertsch J (2008) Anti-inflammatory cannabinoids in diet: Towards a better understanding of CB(2) receptor action? Commun Integr Biol 1(1):26 – 8. [4] Schuly W., Viveros Paredes J M, Kleyer J, Hufner F, Anavi-Goffer S, Raduner S, Altmann KH, Gertsch J (2011) Mechanisms of Osteoarthritis Inhibition by a Novel Class of Biphenyl-type Cannabinoid CB2 Receptor Inverse Agonists. Chem Biol in press

L10 Pharmacognosy in Turkey

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This presentation will give an overview on the wide range of studies of Pharmacognosy in Turkey, since its establishment as a discipline during the Ottoman Empire, up until contemporary times. Studies of Pharmacognosy have begun in 1839 together with the official education of
Pharmacognosy, particularly using plant–originated natural product has played a central role in natural science. Even in these days, the importance of pharmacognosy is not reduced at all. However, due to innate bias and changing socio-economic aspects of our life [1], in parallel the last 10 years have recognized a revolution in biology basically as a result of three main developments: (i) shotgun and next-generation genome sequencing, (ii) genome-scale molecular analysis using omics-technologies, and (iii) computer-assisted analysis, modeling and interpretation of biological data [1, 2]. Metabolomics – the measurement of the complete small molecule fraction in a biological system – is a relatively young technology, however, has already reached an importance comparable with proteomics and transcriptomics platforms [3]. Metabolomics and proteomics emerged in parallel with the development of novel mass spectrometric techniques. Natural product research is intimately bound to these analytical procedures. Accordingly, metabolomic technology is especially suited for the analysis of a wide range of chemical diversity in biological systems. Many of these biological systems providing the widest range of chemical diversity of natural products comprise symbiotic life forms between plants, fungi and bacteria. Although known and investigated for hundreds of years, this interaction undergoes a revival of deep interest because novel tools as described above are available, especially whole-genome sequencing and genome-scale molecular profiling. Whole plant–endophyte–ecosystem interactions can be observed in nature giving ample opportunities for the search of new natural compounds. Examples for the investigation of plant–endophyte–interactions are given and a vision is presented how the combination of novel technologies such as genome sequencing, metabolomics, proteomics and transcriptomics will increase our understanding of the mechanism of plant–endophyte–interaction and at the same time will amplify our existing portfolio of chemical diversity of natural products.

References:
[1] Weckwerth W (2011) Green Systems Biology – from metabolomics and systems biology approaches for the investigation of endophytes – plant–interaction – a vision for their importance in biotechnology and natural product research. Weckwerth W Department of Molecular Systems Biology, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria

Plants have shaped human life forms since their rising. Plants have ever served as food and feed and have a role as one of the richest resources for natural products and lead structures in drug research. Nowadays personalized medicinal and health approaches try to adapt diets to life style. Thus, intimate knowledge of the composition of our plant–derived food is one of the next cornerstones in nutritional physiology. Together with emerging themes, such a renewable energy resources to cope with global climate changes and limited energy resources the relevance of plant biology and biotechnology becomes dramatically important in the next decades. Consequently, it can be anticipated that plant biology and applications will have even more indispensable future roles in all sociocultural aspects of our life [1]. In parallel the last 10 years have recognized a revolution in biology basically as a result of three main developments: (i) shotgun and next-generation genome sequencing, (ii) genome-scale molecular analysis using omics-technologies, and (iii) computer-assisted analysis, modeling and interpretation of biological data [1, 2]. Metabolomics – the measurement of the complete small molecule fraction in a biological system – is a relatively young technology, however, has already reached an importance comparable with proteomics and transcriptomics platforms [3]. Metabolomics and proteomics emerged in parallel with the development of novel mass spectrometric techniques. Natural product research is intimately bound to these analytical procedures. Accordingly, metabolomic technology is especially suited for the analysis of a wide range of chemical diversity in biological systems. Many of these biological systems providing the widest range of chemical diversity of natural products comprise symbiotic life forms between plants, fungi and bacteria. Although known and investigated for hundreds of years, this interaction undergoes a revival of deep interest because novel tools as described above are available, especially whole-genome sequencing and genome-scale molecular profiling. Whole plant–endophyte–ecosystem interactions can be observed in nature giving ample opportunities for the search of new natural compounds. Examples for the investigation of plant–endophyte–interactions are given and a vision is presented how the combination of novel technologies such as genome sequencing, metabolomics, proteomics and transcriptomics will increase our understanding of the mechanism of plant–endophyte–interaction and at the same time will amplify our existing portfolio of chemical diversity of natural products.

References:
tivariate data analysis, brought us closer to the final goal of metabolomics, comprehensive evaluation of all metabolites in living organisms including plants. Of many analytical platforms NMR has been thought as one of the most promising techniques to cover all the metabolites in a short time despite its inherent low sensitivity compared with MS-based technology. In addition to the unambiguous advantages of NMR such as broad coverage of metabolite detection, the easiness of data handling for further statistic treatment and signal robustness have been attracting many metabolite scientists. In this presentation we discuss diverse applications of NMR-based metabolomics for chemical characterization of plants, plant physiology, and screening method of bioactive metabolites will be shown as well as a possible protocol developed by our groups [4].

References:
1. Andreadou I, Iliodromitis E, Mikros E, Skaltsounis A 1Department of Pharmacognosy and Natural Products, University of Athens; 2Department of Systematic and Evolutionary Botany, Bouchout, BE-1860, Meise, Belgium; 3University of Vienna, Department of Systematic and Evolutionary Botany, Renwegg 14, A-1090 Vienna, Austria;

Keyword: Cyclotides, disulfide-rich plant peptides with unique structural features of a cyclic backbone and three conserved disulfide bonds in a knotted arrangement, known as cyclic cystine knot. So far their presence has only been reported for species of the families of Rubiaceae, Violaceae, Cucurbitaceae and recently Fabaceae [1], but it is very likely that cyclotides are more widely distributed since their predicted number in Viola-L. belonging to the Apocynaceae family, to be a ‘cycloid-plant’ and for the first time we report sequences in this family confirming previous investigations [2]. This underpins earlier suggestions that cyclotides are one of the largest peptide classes within plants, confirming previous investigations [2].

This strategy was applied to the fractionation and purification of bioactive natural products from plant extracts: the on-line coupling of CPC-UV to HPLC-UV-MS/MS. The coupling of CPC and HPLC system, via a six position switching valve, reduces the time of complete fractionation procedure by direct on-line analyses of collected fractions and permits a guided fractionation step [3]. HPLC columns suitable for fast analysis, monolith and fused core columns, were evaluated to allow rapid analysis of compounds separated by CPC. Furthermore, the use of MS in tandem mode allows to get a direct structural identification of separated molecules. This strategy was applied to the fractionation and purification of bioactive compounds from berries and roots of sea buckthorn (Hippophae rhamnoides L., Elaeagnaceae), a Eurasian medicinal thorny bush, known to have various pharmacological effects [4].


On-line coupling of Centrifugal Partition Chromatography (CPC) to High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS/MS)

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Phytochemical analyses of food and medicinal plant extracts require rapid screening and detection strategies to identify bioactive natural products. Centrifugal Partition Chromatography (CPC), a free solid support liquid-liquid chromatography, is a well established method for the isolation of natural products and fractionation of complex samples at the preparative scale [1,2]. Nevertheless, even if the separation is monitored by detectors the composition of the different collected fractions must be evaluated by further High Performance Liquid Chromatography (HPLC) which is time consuming and give delayed information on the fraction composition. We present here the development of a versatile tool for fast screening and rapid detection of bioactive natural products from plant extracts: the on-line coupling of CPC-UV to HPLC-UV-MS/MS. The coupling of CPC and HPLC system, via a six position switching valve, reduces the time of complete fractionation procedure by direct on-line analyses of collected fractions and permits a guided fractionation step [3]. HPLC columns suitable for fast analysis, monolith and fused core columns, were evaluated to allow rapid analysis of compounds separated by CPC. Furthermore, the use of MS in tandem mode allows to get a direct structural identification of separated molecules. This strategy was applied to the fractionation and purification of bioactive compounds from berries and roots of sea buckthorn (Hippophae rhamnoides L., Elaeagnaceae), a Eurasian medicinal thorny bush, known to have various pharmacological effects [4].

Phytochemical profiling of Opopanax persicus Boiss.

Abstracts | 59th International Congress of the GA | 4th–9th September 2011, Antalya, Turkey

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Opopanax W. D. Koch is a genus of the Apiaceae family, with 11 species distributed throughout the Mediterranean basin and Iranica region (Iran, Afghanistan, Western Pakistan, Northern Iraq, Azerbaijan, Turkmenistan) [1]. With the exception of some phytochemical studies on O. chironium (L.) Koch growing in the Mediterranean and Balkan regions [2], the genus Opopanax has not been investigated from a chemical nor pharmacological viewpoint. We carried out a phytochemical profiling of O. persicus Boiss., an endemic species growing in parts of Turkey, Iran, Iraq and Transcausasia [1]. From the dichloromethane extract, a total of 20 compounds were isolated by medium pressure liquid chromatography (MPLC), vacuum liquid chromatography (VLC), and preparative and semi-preparative HPLC. Structure elucidation was carried out by on-line ESI-MS and PDA data, HR-ESI-TOF-MS and off-line 1D- and 2D-NMR spectra recorded in a 1-mm TXI microprobe. Compounds were identified as coumarins with predominantly linear and angular dihydropyranocoumarin scaffold which 10 are reported for the first time as new derivatives. The absolute stereochemistry of isolated compounds was determined by X-ray crystallography and CD spectroscopy. Some of the compounds determined moderate activity against Plasmadium falciparum K1 strain and Trypanosoma brucei rhodesiense (IC50s 3.6 to 6.9 µg/ml, and selectivity indices (SI) in L-6 cells of 5.7 to 25, respectively). Keywords: Opopanax persicus Boiss., phytochemical analysis, dihydropyranocoumarin, absolute stereochemistry, X-ray crystallography, CD spectroscopy

References:

Fungal co-culture as a new source of antifungal metabolites

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Microorganisms are a very rich source of secondary metabolites with antimicrobial potential [1]. In order to produce original metabolites from this source, strategies have to be developed to induce synthetic pathways triggered by genes that are often silent [2]. Nutritional or environmental stress can be used to activate these orphan pathways, in particular jasmonic acid, salicylic acid, abscisic acid and ethylene, can be involved in plant responses to stress [1]. Our attention was focused on volatile compounds emissions (VOCs) from Achillea collina Becker, a medicinal plant, exposed to biotic and abiotic stressing conditions. Headspace Solid-Phase-Microextraction-Gas Chromatography-Mass Spectrometry (HS-SPME-GCMS) "in vivo" method [2] was used to evaluate A. collina VOCs. Biotic stress was obtained by the infestation of A. collina plants with Myzus persicae or Macrosiphoniella millefolii, the generalist and specialist aphid species respectively. Mechanical stresses were obtained by applying a pressure to the plants using a specially designed equipment or by pricking the leaves with a needle. VOCs emissions are reported in Figure 1. As shown, some compounds were induced by both the biotic and abiotic stresses (eg. 1-Hexanol, Pinocarvone, α-Fenchene) while some other VOCs were specific to the type of stress applied. As an example Spathulenol was only induced by M. millefolii and β-Linalool was induced only by the mechanical damage. Aromadendrene, Terpineol-cis-β, Tetradecan-1-ol were only induced by the biotic stresses. A. collina shows a great plasticity in the VOCs biosynthesis, highly modulated by the external stimuli, a possible good model for future investigations at a molecular level.

Figures:

Figure 1: fungal co-culture on agar plates of Trichophyton rubrum (1) and Bionectria ochroleuca (2) showing long distance repulsion

Keywords: fungal metabolites, co-culture, confrontation, anti-fungals

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References:

Achillea collina response to biotic and abiotic stresses: a comparative evaluation of volatile emissions pathways

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Plants have evolved a wide range of mechanisms to cope with biotic and abiotic stresses. It’s suggested that hormone signaling pathways, in particular jasmonic acid, salicylic acid, abscisic acid and ethylene, can be involved in plant responses to stress [1]. Our attention was focused on volatile compounds emissions (VOCs) from Achillea collina Becker, a medicinal plant, exposed to biotic and abiotic stressing conditions. Headspace Solid-Phase-Microextraction-Gas Chromatography-Mass Spectrometry (HS-SPME-GCMS) “in vivo” method [2] was used to evaluate A. collina VOCs. Biotic stress was obtained by the infestation of A. collina plants with Myzus persicae or Macrosiphoniella millefolii, the generalist and specialist aphid species respectively. Mechanical stresses were obtained by applying a pressure to the plants using a specially designed equipment or by pricking the leaves with a needle. VOCs emissions are reported in Figure 1. As shown, some compounds were induced by both the biotic and abiotic stresses (eg. 1-Hexanol, Pinocarvone, α-Fenchene) while some other VOCs were specific to the type of stress applied. As an example Spathulenol was only induced by M. millefolii and β-Linalool was induced only by the mechanical damage. Aromadendrene, Terpineol-cis-β, Tetradecan-1-ol were only induced by the biotic stresses. A. collina shows a great plasticity in the VOCs biosynthesis, highly modulated by the external stimuli, a possible good model for future investigations at a molecular level.

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A validated HPLC method for standardization of the most active fraction of the antihyperglycemic drug Cleome droserifolia using bioactive markers

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The aqueous and ethanolic extracts of the aerial parts of Cleome droserifolia (Forssk.) Del. were assessed for their antihyperglycemic effects in male albino rats at the same dose level of the biguanide metformin (150 mg/kg body weight). Diabetes was induced intraperitoneally with a single dose of alloxan (150 mg/kg body weight) [1]. The blood glucose level was monitored after 2 and 4 weeks from zero time (Table 1). The four sub-fractions (n-hexane, chloroform, ethyl acetate and n-butanol) of the more active aqueous extract were tested at the same dose level. A validated RP-HPLC method for standardization of the most active ethyl acetate fraction (70% as potent as metformin after 4 weeks of oral administration) was developed. Three flavonoid glycosides; isorhamnetin-3-O-D-glucoside (F1), queretin-3'-methoxy-3-O-(4''-acetylrhamnoside) and kaempferol-4'-methoxy-3,7-dirhamnoside (F3) (were isolated from the ethyl acetate fraction and proved 70% as potent as metformin after 4 weeks of oral administration) was developed. Three flavonoid glycosides; isorhamnetin-3-O-D-glucoside (F1), queretin-3'-methoxy-3-O-(4''-acetylrhamnoside) and kaempferol-4'-methoxy-3,7-dirhamnoside (F3) (were isolated from the ethyl acetate fraction and proved 70% as potent as metformin after 4 weeks of oral administration) were evaluated. A standard validation of the method (linearity, repeatability, reproducibility, ruggedness, robustness and accuracy) were evaluated. A standard validation of the method (linearity, repeatability, reproducibility, ruggedness, robustness and accuracy) were evaluated. A standard validation of the method (linearity, repeatability, reproducibility, ruggedness, robustness and accuracy) were evaluated.

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Table 1. Antihyperglycemic effect of the different extracts of Cleome droserifolia

<table>
<thead>
<tr>
<th>Time</th>
<th>Group</th>
<th>M ± S.E.</th>
<th>% of change</th>
<th>M ± S.E.</th>
<th>% of change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic rats (Db)</td>
<td>non treated</td>
<td>243.7 ± 8.2</td>
<td>256.8 ± 9.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Db + metformin</td>
<td>257.3 ± 11.4</td>
<td>129.8 ± 4.3</td>
<td>49.5</td>
<td>13.2</td>
<td>68.2</td>
</tr>
<tr>
<td>Db + ethanic extract</td>
<td>256.8 ± 10.1</td>
<td>173.2 ± 6.2</td>
<td>32.5</td>
<td>141.9 ± 5.5</td>
<td>44.7</td>
</tr>
<tr>
<td>Db + aqueous extract</td>
<td>246.9 ± 7.8</td>
<td>214.3 ± 8.6</td>
<td>13.2</td>
<td>198.6 ± 7.1</td>
<td>19.5</td>
</tr>
<tr>
<td>Db + metformin fraction</td>
<td>251.9 ± 8.6</td>
<td>186.8 ± 7.4</td>
<td>25.8</td>
<td>138.9 ± 5.8</td>
<td>44.8</td>
</tr>
<tr>
<td>Db + ethyl acetate fraction</td>
<td>258.4 ± 7.1</td>
<td>187.4 ± 6.3</td>
<td>27.5</td>
<td>135.3 ± 4.1</td>
<td>47.6</td>
</tr>
<tr>
<td>Db + n-butanol fraction</td>
<td>255.8 ± 8.2</td>
<td>224.9 ± 8.4</td>
<td>13.3</td>
<td>203.7 ± 6.5</td>
<td>21.1</td>
</tr>
</tbody>
</table>

Extracts, fractions and the standard metformin were given at a dose of 150 mg/kg body weight. *Statistically significant difference from zero time at P < 0.01. M, mean; S.E., standard error (n = 6). Keywords: validation, bioactive markers, RP-HPLC, Cleome droserifolia, hypoglycemia References: 1. Eliasson SG, Samet JM (1969), Life Sci 8:493–498. 2. Abdel Motaal A, Ezzat SM, Haddad PS (2011) Determination of bioactive markers in Cleome droserifolia using cell-based bioassays for antidiabetic activity and isolation of two novel active compounds. PHYMED-D-11–00029R1 (under publication).

In vivo

Keywords: SPME, VOCs, Aphids, Achillea collina under biotic and abiotic stresses

VOCs induced by different stress conditions

Volatle emission pathways of induced VOCs of Achillea collina under biotic and abiotic stresses

Keywords: SPME, VOCs, Aphids, Achillea collina


Table 1. Antihyperglycemic effect of the different extracts of Cleome droserifolia
The most prominent determinant of acquired long QT syndrome is inhibition of the hERG potassium channel. Drug-induced QT prolongation can cause undesirable cardiac side effects and has led to several drug withdrawals in the past. In contrast to synthetic drugs, little is known about hERG channel inhibitors of natural origin. For assessing the risk of natural products on hERG channel inhibition, extracts obtained from frequently consumed spices, food, and medicinal plants were submitted to a broad in vitro screening. We established an HPLC-based activity profiling approach for this target by combining HPLC-microfractionation with on-line and off-line spectroscopy, and an automated two-micro-electrode voltage clamp assay with transfected Xenopus laevis oocytes. Among the extracts tested, the methanolic extract of the TCM herbal drug Evodiae Fructus (Evodia rutaecarpa [Juss.] Benth., Rutaceae) reduced the peak tail hERG current by 60.9 ± 6.9% at 100 μM. The most prominent determinant of acquired long QT syndrome is inhibition of the hERG potassium channel. Drug-induced QT prolongation can cause undesirable cardiac side effects and has led to several drug withdrawals in the past. In contrast to synthetic drugs, little is known about hERG channel inhibitors of natural origin. For assessing the risk of natural products on hERG channel inhibition, extracts obtained from frequently consumed spices, food, and medicinal plants were submitted to a broad in vitro screening. We established an HPLC-based activity profiling approach for this target by combining HPLC-microfractionation with on-line and off-line spectroscopy, and an automated two-micro-electrode voltage clamp assay with transfected Xenopus laevis oocytes. Among the extracts tested, the methanolic extract of the TCM herbal drug Evodiae Fructus (Evodia rutaecarpa [Juss.] Benth., Rutaceae) reduced the peak tail hERG current by 60.9 ± 6.9% at 100 μM. 

Potential inhibitors of chikungunya and dengue viruses isolated from Malagasy plants

Chikungunya virus (CHIKV) and dengue virus (DENV) are two emerging arboviruses. CHIKV has recently re-emerged, causing massive epidemics that have moved from Africa throughout the Indian Ocean to India and Southeast Asia. In humans, it is responsible for an acute disease, characterized by high fever, arthralgia and maculopapular rash. Regarding the dengue fever, it affects more than 50 million people annually. Increasing mortality and geographical expansion are the drastic changes noted in the recent epidemiology of the disease. No specific antiviral therapy is currently available, on the market, for these two diseases. A screen for cellular antiviral activity, towards selected Evodiae Fructus, led to the isolation of 400 plants from the Indian Ocean Islands. This screening has led to the selection of Flacourtia ramontchi L’Hér. (Salicaceae), a tree distributed in the south of Asia and in Madagascar. Fruits and seeds are used in folk medicine for the treatment of rheumatic arthritis, chlorea and dysentery. Eight new phenolic glycosides and one caffeic acid derivative, together with three known phenolic glycosides and one betulinic acid derivative were obtained by a bioassay-guided isolation from the stem bark. The phenolic glycosides have a salicin core structure; this core may be esterified with benzoic acid and/or 1-hydroxy-6-oxocyclohex-2-en-1-carboxylic acid, on the glucose moiety in 2', 3' and 4' positions and on the primary alcohol function of the salicyl alcohol moiety. Promising results were obtained on the dengue RNA polymerase inhibition assay and preliminary structure-activity relationships were deduced. CHIKV assays are in progress. Keywords: Flacourtia ramontchi, Salicaceae, phenolic glycosides, chikungunya, dengue, antiviral activity. This work was financially supported by Museum National d’Histoire Naturelle. 

**Toxicological risk assessment of Aristolochia species**

Aristolochia species are known to contain aristolochic acids, nitrophenanthrene derivatives responsible for their nephrotoxic and genotoxic effects [1]. There are numerous aristolochic acid analogues, including aristolochic acid I and II, present in raw and processed TCM products. The effect, however, was similar also in non-cancerous cells (IC50 > 20 μM). Common fatty acids were found in A. amurense, P. ternata and Alzibria jubjbris (Fabaceae). The fatty acids showed high PPARα and γ activation in a PPAR-luciferase reporter gene assay. Beside these known PPAR ligands, hydroxylated fatty acids, derived from linoleic acid via the lipoxigenase pathway, were isolated from A. jubjbris.(2) The influence of the hydroxyl groups on binding to the ligand binding domain (LBD) of PPARγ was studied in silico using the program LigandScout.(3) A pharmacophore model was generated based on available complexes of hydroxylated fatty acids with the PPARγ LBD. Thereby, part of the isolated trihydroxylated fatty acids and hydroxy-epoxy fatty acids were pre-

**Risk assessment of hERG channel inhibition by natural products**

New cerebrosides and hydroxylated fatty acids from TCM drugs

In recent years, increasing attention has been drawn towards the role of lipids in cell signalling pathways and regulation. Plants are a rich source of bioactive lipids. We, therefore, focused on (complex) lipids from plants used as traditional Chinese medicinal (TCM) drugs to assess their influence on cellular processes. Four new cerebrosides, which belong to the compound class of sphingolipids, were isolated and characterized from A. julibrissin. However, was similar also in non-cancerous cells (IC50 > 20 μM). Com-

**Potential inhibitors of chikungunya and dengue viruses isolated from Malagasy plants**

Potential inhibitors of chikungunya and dengue viruses isolated from Malagasy plants

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A rapid method for detection of alliinase activity in Allium, especially in the subgenus Melanocrommyum

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In the genus Allium about 800 species are currently known belonging to several subgenera [1]. Wild Allium species can be found on the northern hemisphere with a main habitat in Central Asia. The enzyme alliinase occurs in Allium plants catalyzing the cleavage of cysteine sulfoxides leading to typical odorous compounds [2]. Whereas the alliinases of common Allium species like garlic and onion [3] are well analyzed, the properties of other alliinases occurring in further Allium species have not been examined yet. Especially the species of subgenus Melanocrommyum do not express an alliinase like garlic, as can be shown on SDS-PAGE. The separation of alliinase from other proteins is sometimes difficult because of similar protein properties due to their size and probably also due to glycosilation. Therefore, a new method for direct alliinase activity detection has been developed. A crude alliinase preparation can be separated on a basic-native polyacrylamide gel. This method results in functional enzymes, separated into different spots. These protein spots can be cut out of the gel and screened for their alliinase activity. The L(-)S(-)-3-pyrrolylcysteine sulfoxide used as indicator turns into a red dye after the enzymatic cleavage by alliinase (Figure 1) [4]. Although the alliinase is still incorporated inside the gel matrix, a positive reaction can be detected after a few minutes (Figure 2). This new test allows an easy and quick detection of alliinase-like enzymes. Furthermore, the amount of sample needed is very small, allowing tests out of a single bulb.

Electrophoretic mobility in agarose gel as an isolating technique and correlation with synergistic cytotoxicity enhancement

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In previously published work, we reported that Saponinum album dramatically improves the inhibition of tumor growth by targeted toxins in mice in a synergistic way. Herein, we report a simplified slab gel based electrophoretic isolation technique to determine the highly effective fraction of Saponinum album with a relative electrophoretic mobility (Rf) of 0.44 from the mixture. In total, four different fractions were separated at a preparative scale, and evaluated by ESI-MS, HPLC and thin layer chromatographic analysis. Electrophoretic mobility and electrophrochemical properties of the different fractions of saponins from Saponinum album were set into relation to their ability to enhance the cytotoxicity of epidermal growth factor (EGF)-based targeted toxins. We here treated HER-14 cells, which are NIH-3T3 Swiss mouse embryo cells transfected with the human EGF receptor. Untransfected NIH-3T3 cells
served as control. The major bulk of Saponinum album (72.3%) (RF 0.62) migrated to the farthest and was found to be significantly ineffective (p < 0.05) in enhancing the cytotoxicity of the targeted toxin, while the second fraction (Rf 0.45) showed an enhancement factor of 9800-fold. The third (EM 0.30µ) had an enhancement factor of 3200, the fourth (RF 0.08) was again significantly ineffective (p < 0.05) in exhibiting any enhancement of cytotoxicity. This is the first report for the use of slab gel electrophoresis as a convenient isolation technique for saponins.


WORKSHOP III: Permanent Committee on Regulatory Affairs of Herbal Medicinal Products

WSIII 1 Quality of herbal medicinal products and food supplements – EU regulation and practical experience

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Herbs and herbal preparations have been playing an important role for both dietary and medicinal use from paleolithic hunter-gatherers to 21st century urban culture. People have always been aware that the variability of the properties of herbal materials depends, e.g., on the exact plant part, vegetal state, weather conditions, harvesting time and – essentially – the mode of preparation by, e.g., drying, peeling, cooking (or not), fermentation, treatment with inorganic substances, extraction etc., are not less important for the tolerability, digestibility or – in case of medicinal use – success of treatment than the choice of the plant itself. Over the past decades scientific findings have provided a rationale for what our ancestors had established on an empiric basis. We understand why, based on the absence/presence and dose of certain secondary compounds some plants/preparations are beneficial or harmful. Since both beneficial and potentially harmful properties of herbal ingredients in food supplements are being increasingly assigned to specific secondary compounds it is evident that certain standards are necessary in order to provide for adequate safety and effectiveness of such products. While legal limits have been established in the EU for a large spectrum of possible contaminants in food and thus food supplements including, e.g., pesticides, heavy metals or mycotoxins, no common standards exist for the overall quality assessment of herbal raw materials/preparations for food supplements. While rules established for the quality control of herbal medicinal products and their respective herbal raw materials/preparations are not transferable for various reasons, they provide orientation regarding practicable technical standards and methodology. A comparison will be given of the EU regulatory quality standards in both areas in the light of practical experience.

WSIII 2 Regulatory Options for (Traditional) Herbal Medicinal Products

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All over the world plants have been selected by people because of their healing properties. In Europe, first manuscripts describing medicinal plants were written more than two thousand years ago. Starting from this traditional use, which was often based on ready-to-use receipts, there was a development towards usage of finished herbal medicinal products in central Europe during the 20th century. In parallel with an increasing scientific knowledge on medicinal plants – which included also the identification of highly active purified compounds of plant origin – a common regulatory environment for all medicinal products has been established in Europe. The basic regulatory approach is to control the access to the market and to assess quality, safety and efficacy of medicinal products in order to improve public health and to facilitate supply with medicinal products. There is a clear European regulatory framework for medicinal products (directive 2001/83/EC as amended) providing basic definitions and offering two options to bring products into the market: i) registration of traditional herbal medicinal products and ii) marketing authorisation of herbal medicinal products. Beside applications at the national level there are also options for European procedures. A harmonised scientific evaluation in this field is resulting from the work of the Committee on Herbal Medicinal Products (HMPC) at the European Medicines Agency (EMA). The major tasks are development of Community Monographs and List Entries on herbal substances as well as relevant guidance. These activities will facilitate handling of procedures and contribute to the harmonisation of the market.

WSIII 3 Traditional Herbal Drugs and Botanical Dietary Supplements: The Italian Experience

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Multicomponent phytotherapeutic medicines are widely used in the European clinical practice and their registration is nowadays regulated by two distinct registration schemes, based on “well established use” and “traditional use for herbal medicines”, now both codified in Dir. 2001/83/EC. In this scenario, also in Italy it is today crucial that the preparation of botanical “Active Pharmaceutical Ingredients” (API) has to fulfill both GAP and GMP guidelines for what harvesting and cultivation of plants and industrial production is respectively concerned. The combination of these two guidelines is essential in order to guarantee quality and reproducibility of the API and also it allows to avoid the following risks: use of inappropriate classified material, pesticides or other harmful agent contamination, API degradation. Botanical derivatives (mostly multicomponent products such as extracts) are widely used in Europe also as ingredients for non-pharmaceutical products which are sold as food supplements in relation to the different national legislations. In Italy, the Ministry of Health has recently (March, 2011) initiated a procedure to transpose into law (as Annex 1 of a draft decree which regulates the use of dietary supplements different from vitamins and minerals) a list of admitted plants which can be utilized for the preparation of food supplements. The quality criteria for the preparation of these products are those requested by the European regulations on food derivatives. Nevertheless some Italian companies, such as Indena are strictly committed to keep the challenge for quality criteria of botanicals used for food supplements as close as possible to “phytotherapeutic medicines”.

WSIII 4 Botanical Food Supplements – Regulatory Situation in the EU

Coppen P

European Botanical Forum, Brussels, Belgium

Botanicals are used as components of both food supplements and medical products. The European legal framework explicitly allows both products to exist in parallel. Botanical food supplements need to be in conformity with the full food legislation, including safety provisions, manufacturing requirements and labeling. In addition, Member States have national rules to assure their safe use. Relating to the borderline, the European Court of Justice has established rules to judge if a product should be considered as medicinal or not. It has ruled that such a decision must consider individual products, taking into consideration all of the product’s characteristics. Furthermore, the definition of medicinal product by function should be interpreted restrictively and not cover substances that do not distinctly modify the way in which it functions, i.e. that do not have a therapeutic effect. In recent years the EC has put into place two new laws that may help the EU to progress towards harmonisation. It concerns the legislation on health claims under which a procedure and the legislation on addition of nutrients to foods, under which a procedure has been created to deal with emerging safety issues. The claims legislation does not accept traditional use as a valid parameter for the validation of a health claim, while traditional herbal medicinal law product does. This has now created a new situation that the European Commission is reflecting upon.
Herbal medicinal products are regulated in Europe under the drug law. If only health claims are stated, some may be considered as dietary supplements. For drug approval, efficacy, safety and quality have to be demonstrated. A special European regulation exists for traditional herbal medicinal products (Directive 2004/24/EC), which does not require clinical studies, but only 30 years of traditional use, out of them 15 years in Europe [1]. A committee for Herbal Medicinal Products (HMPC) at the European Medicines Agency (EMA) currently prepares a list of traditional and well-established herbal drugs/preparations/combinations which can be used for drug approval in the European Union. Also Compendium monographs of the Commission E of Germany or other recommendations that may be used for full marketing authorisations of well-established herbal medicinal products or simplified registrations are elaborated by this committee. However, Chinese herbs have not been considered so far.

Chemical Mechanism Research during Chinese Medicine Processing

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Chinese medicine processing is a traditional Chinese pharmaceutical technology, and it is also the main characteristic property that distinguish traditional Chinese medicine and natural medicine. There are complex chemical changes during the process of Chinese medicine processing, and these chemical constituents maybe the basis of clinical efficacy changes. To clarify the changes of the chemical constituents in Chinese medicine is the main purpose of the mechanism research of Chinese medicine processing. In recent years, many research institutions at home and abroad have done deep research in chemical mechanism during the process of Chinese medicine processing, and initially clarify the chemical reactions and chemical phenomena of Chinese medicine processing. The main chemical reactions occurred in the Chinese medicine processing are hydrolysis reaction, oxidation reaction, replacement reaction, isomerization reaction, decomposition reaction and so on. This paper reviewed the main achievements in the chemical mechanism research during the process of Chinese medicine processing, and prospected the research directions and future in the search of Chinese medicine processing. Key words: Chinese medicine processing; process reaction; chemical constituents; mechanism

Identification of PPARα agonists from natural sources

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The peroxisome proliferator-activated receptors (PPARs) have been in focus for more than a decade for the development of drugs to treat and/or prevent diseases associated with the metabolic syndrome (MS). The PPARs (α, δ, γ) are nuclear receptors (NRs) highly involved in lipid and energy metabolism and hence, targets for treating MS-related disorders. In particular PPARα that is a key regulator of insulin sensitivity is the target of many conventional drugs, although this may result in severe side effects. However, such side effects could be avoided using selective modulators or partial agonists instead [1,2]. Natural products have proven to be a valuable source of PPAR activators [3] of which some have demonstrated interesting partial agonist activities in vivo [4]. In our studies on identification of PPAR modulators from natural sources we used in vitro approaches such as bioassay-guided fractionations, structure-activity relationships, and pharmacophore-modeling [5,6,7,8]. This has led to the identification of new potential PPARα agonists from purple coneflower (Echinacea purpurea) (L.) Moench [5], a plant not traditionally used to treat MS. For traditional anti-diabetic remedies such as sage (Salvia officinalis L) and elderflowers (Sambucus nigra L) PPAR activating properties have been identified suggesting a mechanism of action involving these NRs [6,7]. This was also the case when a pharmacophore-driven approach led to the identification of novel PPARα partial agonists from mastic gum (Pistacia lentiscus L.). Hence, there is a large potential for finding modulators of these versatile NRs amongst natural products and hence important information about their mechanisms of action and their use as drug candidates. Keywords: Metabolic syndrome, PPAR, natural products, medicinal plants, bioassay-guided fractionation.

Plant derived therapeutics for the treatment of Type 2 diabetes is a progressive disorder whose pathophysiology consists of physiological changes in the pathophysiology of the improvement of insulin sensitivity for treatment of the metabolic syndrome or type 2 diabetes remains as a primary clinical strategy. Recent data, however, have questioned the safety of the current pharmacologic agents used to enhance insulin sensitivity. As such, alternative strategies, e.g., nutritional supplementation with nonconventional compounds, are now being practiced by a large number of patients and are frequently done so without the knowledge of the provider. Based on historical human use, there has been great interest in plant extracts (botanicals) as a source for nutritional supplements intended as adjunctive therapies for human diseases. Specifically, isolated compounds identified from plant sources, i.e. phytochemicals or bioactives, have served as a source for therapeutic agents for many diseases, including malignancy, infectious diseases and diabetes. Interestingly, the development of one of the most commonly used anti-hyperglycemic agents in the world today, i.e. metformin, can be traced back to a botanical source. However, the concern with most nutritional supplements, including those considered "natural" (e.g. botanicals) by the consumer, is the paucity of data in humans in regard to efficacy to improve metabolic abnormalities. Thus, there remains considerable controversy regarding the use of botanical supplements for human health. Despite the stated concerns for botanical supplements, there are a number of botanicals that have shown considerable promise for human use but they have to be carefully validated in controlled clinical trials.
With the toolbox of the "omics", nutritional science has become a new adventure in understanding the processes that make up mammalian metabolism in health and disease. Essentially unlimited when taken into studies in human cells in culture or in vivo studies in animal models, omics applications in human trials are limited by the availability of biosamples restricted to body fluids, blood cells or biopsy materials. Nutrigenomics research in the last years has delivered some important insights into mechanisms by which dietary constituents affect metabolism and health risks. I shall be presenting findings from human studies in which transcript-, proteome- and metabolite-profiling techniques have been applied – mainly in peripheral mononuclear cells and plasma/urine as biosamples. It also will include some studies on the effects of plant secondary components. Despite enormous efforts, genome-wide association studies as a top-down approach have so far only shown very weak effects of genetic heterogeneity in individual genes and their association with disease initiation or progression. Amongst the reasons that science crossed fi eld in identifying causal molecular links between diet and disease is that usually only a "snapshot" is taken when applying any of the profiling techniques. But, it is an intrinsic feature of metabo- lism that it is highly dynamic in time (acute and chronic adaptation) and space (within cell compartments or i.e. the interorgan metabolism). In the future, we therefore should fi rst determine the phenotypic response of mammals as a function of time and space and redefine the homeostatic control as a transient equilibrium. To obtain robust phenotypic alterations it therefore may be advised to challenge the biological system to drive it in a critical state. Such a "critical state" may in terms of nutrition be defined as a severe state of starvation or diets providing extreme nutrient compositions. I shall demonstrate fi ndings from a metabolomics application in humans with such defined challenges. Taken together, nutrigenomics has extended our knowledge on how diets or individual ingredients affect human metabolism on the background of a given genetic make-up, but it is far from delivering predictive parameters for personalized health and/or disease prevention.


WSV 2 The Application of Pesticides in the Production of Medicinal Plants in China

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Traditional Chinese Medicine (TCM) is getting more and more attention all over the world, due to its systematic approach and clinical effectiveness [1]. The quality of TCM determines the safety and efficacy of Chinese-made TCM products. However, the improper use of pesticides in the medicinal plant production has seriously affected the quality of TCM. In the early 1980s, Chinese scientists had already recognized this problem and put in an effort to study the problem. During that time, the main focus was mainly involved in the half life of organochlone and those pesticides that easily caused cumulative toxicity (e.g., hexachlorocyclohexane, DDT, quintozene, drin) in Chinese herbal medicines, herbal preparations, and Chinese patent products. Accordingly, “Pharmacopoeia of People’s Republic of China” (CP, the edition of 2000, the first section) set up the assay methods and residual limit of the organochlorine pesticide [2]. During the “9th Five-Year project”, the government organized Chinese medicine technology research key project “Standard should fi r the quality of Chinese Materia Medica “, which including a systematic way of Research and determination of 71 kinds of Chinese herbal medicines. This project not only improved the research level on the pesticides residues in TCM, but also made us have basic understanding the polluted situations of organic pesticides in the commonly used Chinese medicinal plants. In the “10th Five-Year project “, the project, “Researches on the detection and limited standards of 50 kinds of Chinese medicine “, was classified as a major task by the Ministry of National Science and Technology of China. On the basis of this project, we have completed these items, such as: “Detection methods of the pesticide residue of pyrethroid in TCM “; “Detection methods of the pesticide residues of organic phosphorus in TCM”. And those above have been oﬃ cially recorded in 2005 version of Chinese Pharmacopoeia. In recent years, the detection technology of pesticide residues in traditional Chinese herbal medicine has developed rapidly. Application of technologies such as GC, LC, GC-MS, LC-MS, CE-MS, and multiple-stage mass spectrometry techniques, greatly increase the qualitative capability, the detection sensitivity, detection limit and detection coverage. At present, some new analysis methods and techniques are making great progress, especially in the analysis of multi-pesticide residues for many types of pesticides, the analysis of multi-pesticide residues for the same type of pesticides, the analysis in the trial sample for new single pesticides, and rapid analysis and other aspects. According to GAP for TCM herb requirements, pesticide residues should comply with the Green Standards of Medicinal Plants and Pesticide Residue Practice and Economy of Ministry of Commerce of the P. R. China [3]. At present, China Pharmacopoeia (Edition 2010) supplemented the respective analytical methods of 9 organochlorines (OCs), 3 pyrethroids and 12 organophosphor-ous pesticides (OPs), which aim to standardize the cultivation of TCM herbs by means of the quality of pesticide residues in herbal medicines. In the international practice, the Pharmacopoeia stipulated the MRLs (maximum residue level) of 9 OCs including BHC, DDT and pentachloronitrobenzene (PCNB) for medicinal materials, among which only two were involved, Radix et Rhiizaon Glycyrrhizae and Radix Astragali, but no MRLs for pyrethroids and OPs have been established for all Chinese medicinal materials [1, 4]. Therefore, implementation of GAP for medicinal plant is an important measure to ensure the quality of TCM. Keywords: Traditional Chinese Medicine (TCM); Pesticide; Medicinal Plant Production; China References: 1. Zhang BC, Peng Y, Zhang Z, Liu HT, Qi YD, Liu S, Xiao PG. (2010) Planta Med; 76:1948 – 1955 2. Yang MH, Wang LN. (2008) World Sci Technol; 10:107 – 112 3. The Ministry of Commerce of the People’s Republic of China. (2004) Green standards of medicinal plants and preparations for foreign trade and economy of Ministry of Commerce of the P. R. China. WM/T 2 – 2004. 4. Guo Q, Lv X, Tan L, Yu BY. (2009) Chin J Nat Med; 7:210 – 216
Legal requirements for the control of contaminants in herbal medicinal products

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Herbal medicinal products require a pre-marketing approval like all other medicinal products and have to prove their quality, safety and efficacy. In order to guarantee optimum consumer and patient protection according to the European legislation. Taking into consideration the natural origin and potential environmental influences, special emphasis is put on tests for contaminants such as heavy metals, pesticide residues, mycotoxins and microorganisms. For heavy metals, the European Pharmacopoeia has set limits for lead, cadmium and mercury within the general monograph on herbal drugs. Similar rules are being developed for extracts. In this respect, a German industry's working group has collected a large amount of data on heavy metals occurring in herbal drugs. The respective data base has been evaluated and published [1]. For pesticide residues in plant material, the respective European Pharmacopoeia monograph sets limits for 70 substances. For further substances reference is made to Regulation (EC) 396/2005 on pesticide residues in food. For aflatoxins, specific limits exist for medicinal products which are comparable to the food area. With regard to microbiological purity of herbal medicinal products, the European Pharmacopoeia describes requirements for three different categories of these products as well as determination methods. Raw materials of herbal origin which are intended for use in food supplements have to fulfill the respective European legal requirements for the food area. Besides the above mentioned Regulation (EC) 396/2005 on pesticide residues, limits for further contaminants are described in Regulation (EC) 1881/2006, e.g. for mycotoxins or heavy metals. The United States Pharmacopeia (USP) is currently developing specific rules on heavy metals in dietary supplements of botanical origin. References: [1] Gasser U, Klier B, Kühn AV, Steinhoff B (2009) Current findings on the heavy metal content in herbal medicinal products. Pharmaeuropa Scientific Notes 2009 – 1:37 – 49.

Pesticide testing according to the European Pharmacopoeia (Ph.Eur.) – legal requirements and practical approach

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In 1996 the monograph "Pesticide residues (2.8.13.)" in herbal drugs has been implemented to the European Pharmacopoeia (Ph.Eur.). There were "definition", "limits", "sampling", "qualitative and quantitative analysis of pesticide residues" and a "Test for pesticides" described in the monograph. Reference to the publication in PHARMEUROPA Vol.17 No.1, January 2005 (Pesticide Residues in medicinal Drugs and Preparations) the Ph.Eur. Pesticide Expert Group has been mandated to update the monograph Ph.Eur 2.8.13. The revised monograph (Ph.Eur. 6.2) has been published in 2008. The revision includes the following changes: The new harmonised European Pesticide Regulation (EU 396/2005) replaced old European Directives (EC 76/895 and EC 90/642); the list of pesticides has been extended from 31 to 115 substances frequently observed in herbal drugs; limits have been set in view of toxicity and according to a 90% percentile approach; the formula for the calculation of pesticide limits in extracts and other herbal drug preparations has been modified; more details for method validation procedure has been given (cross reference to document SANCO/10232/2006); the method for determination of pesticides ("Test for pesticides") has been deleted. With the updated monograph a framework for quality control of pesticide analysis in herbal drugs has been given. For the frequently found 115 substances pesticides in herbal drugs and herbal drug preparation limits could be found or calculated easily. The allocation from product to limit of all other (not listed) pesticides according to the new harmonised European Pesticide Regulation (EU 396/2005) remains difficult. The scope of testing depends on the methods of analyses used. Using additionally new analytical techniques based on LC-MS/MS detection more than 500 substances could be detected analysing pesticide residues in herbal drugs.

Phenylenaloid glycosides: Naturally occurring apoptosis inducers

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Natural products have long been regarded as excellent sources for drug discovery given their structure diversity and wide variety of biological activities. Phenylenaloid glycosides are naturally occurring compounds of plant origin and are structurally characterized with a hydroxypyrene-thyl moiety to which a glucopyranose is linked through a glycosidic bond and esterified by a cinnamic acid moiety. There can be one to four sugars in their composition and cinnamic acid esterification generally occurs on a glucose directly bound to phenylethanoyl moiety [1,2]. To date several hundred compounds of this type have been isolated from medicinal plants and further pharmacological studies in vitro or in vivo have shown that these compounds possess a broad array of biological activities including antibacterial, antioxidant, antitumor, antiviral, anti-inflammatory, neuro-protective, hepatoprotective, immunomodulatory, and enzyme inhibitory actions[1,2]. In this study, we have investigated in vitro anticancer (cytotoxic) activity and structure-activity relationships of 10 different phenylethanoid glycosides against human and murine cancer cell lines, Hep-2 (human epidermoid carcinoma), RD (human rhabdomysosarcoma), and L-20B (transgenic murine L-cells), using MTT method [3,4]. Acteoside, forsythoside B, samioside and teucriside were exhibited significant cytotoxic activity against tested cancer cell lines in the concentration range of 8 – 50 µg/mL. To determine the selectivity of cytotoxicity, VERO (African green monkey kidney) cell line was used for the comparison and no cytotoxicity was determined. In addition, apoptotic cell death was observed in the histological analysis of tested cancer cell lines. Keywords: Phenylenaloids, cancer cells, cytotoxicity, structure activity relationship Acknowledgement: Activity studies were supported by Hacettepe University Research Foundation (Project No: 0302301010). References: 1. Funes L, et al. (2010) Chemistry and Physics of Lipids 163: 190 – 199. 2. Korkina LG (2007) Cellular and Molecular Biology 53(1): 15 – 25. 3.Saracoğlu I, et al. (1995) Biological and Pharmaceutical Bulletin 18(10): 1396 – 1400. 4.Saracoğlu I, et al. (1997) Fitosfera 68(5): 434 – 438.
A one-tube assay for four Hypericum species – PlantID
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The benefits of DNA-based identification methods for medicinal plant products have been shown – negligible amounts of starting material, high resolving power, increased taxonomic specificity, and fast results (1). However, the simultaneous detection of multiple species in one sample has not until now been possible. We report the design of PlantID for St John’s Wort (SJW) (Hypericum perforatum L.); a technique capable of detecting both the target species (SJW) and a number of likely adulterants in one sample using a multiplex PCR approach coupled to high resolution DNA fragment analysis. The method is based on the creation of fluorescently labelled amplicons of different lengths which can be resolved via capillary electrophoresis. Each amplicon confirms the presence of four Hypericum species: H. androsaemum L., H. atu- mo Boiss. & Orph., H. ascyron Siebold ex Blume and H. perforatum L. These amplicons are produced in a multiplex PCR, with all four reactions occurring simultaneously. The target species for design used in this study represent a worst case scenario, with only a few base differences between the ITS regions for each target. It is likely that a selection of target medicinal plants would not be as closely related, and would therefore have significantly more sequence differences. This would dramatically increase the number of species which could be detected in one assay. This technique has the power to both confirm the presence of expected plant material and detect adulterant material in one reaction. The method of design could be replicated for any other medicinal plant, and its problem adulterants.

Keywords: Molecular Identification, Hypericum perforatum, PlantID


SL4

Design and synthesis of natural product-based ligands with high affinity to the kappa-opioid receptor
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Psychoactive natural products play an important role in the discovery and development of new drugs for the treatment of central nervous system (CNS) disorders. Our studies are focusing on identification of plant metabolites responsible for CNS activity and designing new ligands with high affinity to CNS receptors. Salvinorin A, the most potent naturally occurring hallucinogen isolated from the plant Salvia divinorum, was identified as its principal molecular um

Keywords: Salvia divinorum, salvinorin A, kappa-opioid receptor, synthesis of new ligands, affinity, ligand-receptor interactions


SL5

Sesquiterpene Lactones and Pentamethoxylated Flavone from Artemisia kalbuda Boiss. & Buhse Rustayian A, Ezatzadeh E
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Artemisia is a genus of small herbs or shrubs found in Northern temperate regions. It belongs to the family Compositae (Asteraceae), one of the most bulky vegetal groupings, which comprises about 1000 genera and over 20000 species. Within this family, Artemisia is included into the tribe Astereae and comprises itself over 500 species. The 500 species of Artemisia are mainly found in Asia, Europe and North America. They are mostly perennial herbs and dominating the vast steppe communities of Asia. Asia seems to show the greatest concentration of species with 150 accessions for China, 174 in the ex U.S.S.R, about 50 reported to occur in Japan and 35 species of the genus are found in Iran, of which two are endemic: A. melanolapis Boiss. and A. kermanensis Pold. Artemisia species, widespread in nature, are frequently utilized for the treatment of disease such as malaria, hepatitis, cancer, inflammation, and infections by fungi, bacteria and viruses and this prompted us to conduct a phytochemical investigation of Artemisia kalbuda.

The aerial parts of Artemisia kalbuda afforded a germacranolide and guaianolide type sesquiterpene lactones together with a pentamethoxylated flavone. The structures were elucidated by spectroscopic methods, including 1D and 2D NMR analysis. Keywords: Artemisia kalbuda, Compo- sitae, Sesquiterpene lactones, Guaianolide, Germacranolide, Flavone

Keywords: Sesquiterpene Lactones and Pentamethoxylated Flavone from Artemisia kalbuda, Boiss. & Buhse Rustayian A, Ezatzadeh E

activity relationship. Furthermore, the five active compounds were generated for general anti-inflammatory effects. At a test concentration of 5 μM they significantly inhibited the TNF or LPS induced expression of IL-8 and E-selectin in human endothelial cells [4]. The observed FAD induction indicates a possible involvement of this nuclear receptor in the mechanism of the inflammatory regulation by these compounds. Keywords: farnesoid X receptor, Ganoderma lucidum, lanostane triterpenes, molecular modeling, virtual screening, natural products Ac-

S 10703/S 10711/S 10713 granted by the Austrian Science Fund (FWF). Re-


SL7 Biological Activities of Andrographolide and Some Semi-synthetic Nonbitter Andrographolides
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2Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand;
3Department of Microbiology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand; 4Department of Mic rodevelopmental cysteine sulphoxides, whereas the subgenus

Andrographis paniculata Nees, Acanthaceae, is known as the king of the bitter due to its main constituent, andrographolide. The activities of A. paniculata and andrographolide were antiviral, analgesics, antiinflammatories, antibacterials and anti-inflammatory [1]. Andrographolide is an ent-labdane containing an α-alkyliden-γ-butyrolactone moiety; two double bonds Δ8(17),Δ12(13); and three hydroxyls at C-3 (a secondary), C-19 (a primary), and C-14 (an allylic). The functional group/s responsible for bitterness of the compound is not yet confirmed, however, esterification of the free hydroxyls of the compound with short chain and long chain fatty acids resulted in diminishing of the bitterness as well as improving those claimed activities of A. paniculata. In our studies 14-acetylation increases the antibacterial against some Gram positive bacteria which resulted in the cell division of B. subtilis [2]. The 3,19-isopropylidene

SL8 Different effects of the herbal combination STW 5 in small and large intestine of rats
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SL10 Differentiation of Allium subgenera by cysteine sulfoxides and further amino acids
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The genus Allium (> 800 species) has been divided into several subge-

nera, sections, species and subspecies [1]. Cysteine sulfoxides are most prominent amino acid derivatives of this genus. These substances are susceptible for the enzyme allinase. Allinase reaction and further non-enzymatic steps do result in volatile sulphur compounds, which exhibit various bioactivities, e.g., antibiotic activities. The pattern of cysteine sulfoxides is rather characteristic for some subgenera. For instance, the subgenus Melanocrommyum is characterized by different heteroaro-
matic cysteine sulfoxides, whereas the subgenus Cepa typically shows rather high amounts of isoalliin. In the now presented study, amino acids as well as cysteine sulphonamides have been analyzed by their corre-
sponding o-phthalaldehyde (OPA) derivatives. Besides the above mentioned differences in the pattern of cysteine sulfoxides, also further amino acids show some relations between their amounts and subge-
nera. Most prominent are differences in the content of L-arginin in
different subgenus of the Allium. L-arginine is a valuable nitrogen storage compound in many angiosperms [2]. But this amino acid has been also found in some Allium species in high concentrations. As an example, the amount of L-arginine in Allium cepa L. was increased during the maturation process [3]. Further on, the amount of L-arginine in samples belonging to the subgenus Allium and Cepa was significant higher (p < 0.05) as the amount of L-arginine found in the subgenus Melanocormyrum suggesting different mechanisms of nitrogen storage. These differences should allow chemonomical classification of subgenera. Keywords: Allium, cystine sulphoxides, o-pthalaldialdehyde, amino acids References: 1. Fritsch RM et al. (2010) Phyton 45 – 200 2. Van Etten CH et al. (1963) J Agr Food Chem 11: 399 – 409 3. Schu-pan W, Schwerdtfeger E (1972) Qual Plant Mater Veg 21: 141 – 157

What can phylogeny tell us about chemical diversity?

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Plant secondary metabolites are produced and selected by evolution for their biological activity. Such natural products played a major role in traditional medicine and as leads for modern medicine. Only a small fraction of the Worlds biodiversity has been explored for chemical and biological activity. A correlation between phylogeny and biosynthetic pathways is often assumed and could offer a predictive approach enabling more efficient selection of plants for traditional medidine lead discovery. However, formal tests of correlations between phylogeny and chemistry are rare, and the potential predictive power is consequently unknown. As a case in point, we are exploring the Amaryllidaceae subfamily Amaryllidoideae sensu APG, which is known for subfamily specific alkaloids with activity in the central nervous system (CNS). Galanthamine registered for the treatment of Alzheimer’s disease was first isolated from the Caucasian snowdrop Galanthus woronowii Linsk. We present a phylogenetic hypothesis of the Amaryllidaceae subfamily Amaryllidoideae based on nuclear, plastid and mitochondrial DNA sequences of over 100 of the cleaning plant species, representing all tribes and geographical regions. All major lineages are now well supported and the extended sampling uncovered several genera as non-monophyletic, emphasizing the importance of using phylogenetics rather than classical classification for interpretation of character distribution. Alkaloid profiles and CNS-related bioactivity profiles are significantly correlated with phylogeny using formal tests. Relationships between phylogenetic and chemical diversity are further explored. The predictive power can be used to select candidate taxa for lead discovery and to make recommendations for traditional use. Keywords: Amaryllidaceae, phylogeny, chemical diversity, lead discovery Acknowledgements: This research was supported by a Steno grant (N272 – 07 – 0281) to NR from the Danish Council for Independent Research – Natural Sciences.

Determinant of bioactive curcinin in turmeric using magnetic iron oxide nanoparticles as solid phase extractors by PLP

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Curcumin, a derivative of Curcuma longa L., is used extensively in the food industry and researches have shown the health benefits of this compound [1]. In this work, a novel, simple and rapid method for extraction and determination of curcumin in turmeric was performed using magnetic iron oxide nanoparticles (MIONs) as solid phase extractor and HPLC. The unique properties of nanoscale materials offer excellent prospects for designing new methods and instrumentation for chemical analysis [2]. The MIONs were synthesized according to the method proposed by Laurent et al. [3]. The average size of nanoparticles was in the range of 90 nm which was determined by using atomic force microscopy (AFM). Figure 1. Extraction of curcumin is based on the interaction of Fe3+ curcinin complex on MIONs. Desorption of analyte was performed by NaOH solution containing methanol in order to dissolve the desorbed analyte. Various parameters affecting the extraction recovery such as: pH, volume and concentration of NaOH as desorbing reagent, and conditions for successful extraction.

Abstracts | 59th International Congress of the GA | 4th–9th September 2011, Antalya, Turkey
centration of Fe (+3) and percentage of methanol were investigated and optimized. These optimized parameters were: pH = 2.0, 1.5 mL of 0.2 M NaOH containing 30% methanol and 0.1 M of Fe (+3), respectively. The in-dra-day precision (R.S.D.) was 4.0% and inter-day R.S.D. was less than 7.0%. The preconcentration factor of 100 was achieved in this method. The proposed procedure has been successfully applied to the determination of curcumin in turmeric.

**Figure 1:** size distribution of MIONs determined by AFM

Keywords: Curcumin, turmeric, Magnetic iron oxide nanoparticles, Solid phase extraction, Determination, HPLC


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**SL15**

Southern Africa flora: a source of potential antimalarial compounds

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Southern Africa has a diverse botanical flora with numerous plants being used traditionally as phytomedicines. Only a minuscule number of these plants have been scientifically validated for their therapeutic potential as antimalarials and even fewer have had the active chemical compound/s isolated from them. In an attempt to evaluate our plant flora, numerous plants have been assessed for their antimalarial activity. As such plants were collected from diverse areas of Southern Africa and extracts and isolated compounds prepared. The antimalarial properties of the extracts/compounds were screened against Plasmodium falciparum using the tritiated hypoxanthine incorporation assay. The compounds were evaluated for their haemolytic and cytotoxic properties, and ability to chelate iron and inhibit haemoglobin formation. Cullation of several years of research reveals that Crinum bulbispermum (Burm.) Milne-Redh. & Schweick., Rosmarinus officinalis (L.), L., Breonadia salicina (Hoffm.) Hepper & J.R.L.Wood, Agathosma gnaphalocarpa (Miq.) C.C. Berg, Agathosma angustifolia for antimalarial potential and our results justify their traditional use. Acknowledgement: UK Commonwealth Scholarship Commission and the Rick-Cannell Travel Fund of the School of Pharmacy are acknowledged for funding. References: 1. World Health Organization (WHO) (2009) Fact sheet on Malaria WHO, Geneva, Switzerland. 2. Vaughan AM et al. (2008) Cell Microbiol 11: 506 – 520. 3. Fowler DG (2007) Zambian Plants: Their vernacular names and uses. Royal Botanical Gardens, Kew, UK.

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**SL16**

Evaluation of the in vitro antimalarial and malaria prophylactic activity of eight Ficus species

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Malaria is a serious health problem in Africa, where approximately 250 million new cases and one million deaths are recorded per year [1]. The absence of long-term prophylaxis and the emerging resistance to the current therapies highlights the necessity of new drugs for its eradication and control. Liver stage malaria parasites show an absolute requirement for type-II fatty acid biosynthesis (FAS-II) [2], rendering this pathway a good target for causal prophylaxis. In Zambia, the genus Ficus is traditionally used against malaria [3]. Hence, we collected the leaves, barks and roots of eight Zambian Ficus species and screened their crude methanol (CR-Me) extracts for in vitro activity against the multi-drug-resistant Plasmodium falciparum strain K1 using a modified [3]H-hypoxanthine incorporation assay. Malaria prophylactic potential of the extracts was also evaluated against three recombinant FAS-II elongation enzymes, FabI, FabG and FabZ, by spectrophotometric assays. All CR-Me extracts were active against P. falciparum, with F. ovata Vahl bark exhibiting the best activity (IC50 4.76 μg/mL). Most of the root extracts, particularly F. sycomorus L. subsp. gnaphalocarpa (Misq.) C.C. Berg, positively inhibited FabZ (96.6% inhibition at 1 μg/mL). The CR-Me extracts were then subjected to solvent partitioning to yield n-hexane, CH2Cl2 and aq. MeOH subextracts. Remarkable antiplasmodial potency was observed in CHCl3 extracts of four species (IC50 < 2 μg/mL). The leaf and root aq-MeOH subextracts were active against FabI and FabZ enzymes with inhibition rates >90% at 1 μg/mL. This is the first screening of Zambian Ficus species for antimalarial potential and our results justify their traditional use. Acknowledgement: UK Commonwealth Scholarship Commission and the Rick-Cannell Travel Fund of the School of Pharmacy are acknowledged for funding. References: 1. World Health Organization (WHO) (2009) Fact sheet on Malaria WHO, Geneva, Switzerland. 2. Vaughan AM et al. (2008) Cell Microbiol 11: 506 – 520. 3. Fowler DG (2007) Zambian Plants: Their vernacular names and uses. Royal Botanical Gardens, Kew, UK.

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**SL17**

Polyphenolic compounds from Clusiaceae plants: modulating angiogenesis and vascular endothelium

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Polyphenolic compounds have created an increasing interest for their potency about cardiovascular diseases for several years3,4. Nevertheless, most of this research had been focused on polyphenolic compounds such as flavanols (e.g. catechine from tea), anthocyanin (e.g. delphinidin from blueberry) and stilbenoids (e.g. resveratrol from grape). The present study was designed to screen the potent effect of polyphenolic compounds isolated from plants belonging to Clusiaceae family on endothelial cells. A huge number of polyphenols such as xanthones and coumarins have been identified from those species and some of them exhibiting various biological activities such as anti-inflammatory and antioxidant properties3,4. Their effect on endothelium, more particularly on angiogenesis, is not yet well-known. Firstly, we assessed the capacity of endothelium-depenent relaxation in mouse aortic rings involving nitric oxide production, isocapalonic acid (A2) and 2-depenylrhediabinonate (A2) are able to increase NO production on endothelial cells and to induce endothelium-dependent relaxation. Then, we investigated the effects of these compounds on in vitro and in vivo angiogenesis. We showed that A1 treatment promoted the formation of capillary-like network contrary to A2. Endothelial cell adhesion, migration and proliferation were decreased in presence of A2 whereas endothelial migration and proliferation were improved with A1 treatment. We could explain these results with the capacity of A1 to increase VEGF expression and for A2, to decrease ICAM-1 expression. Thus, the strategy used for the screening allows the detection of active molecules from Clusiaceae family that might be of therapeutic benefit in cardiovascular diseases. Keywords: Clusiaceae, angiogenesis, endothelium, xanthone, chromonane. Acknowledgement: We thank Angers...
New cytotoxic pregnane glycosides from Caralluma sinaica growing in Saudi Arabia


Cytotoxic activity of all compounds was assessed against the cell lines mentioned above using the MIT assay. Four compounds (3, 5, 6 and 12) showed moderate to potent cytotoxic activity. Compound 5 displayed the most potent cytotoxicity against gastric and colon adenocarcinoma cell lines (IC50 7.5 – 21.6 µM). The mode of action was revealed as induction of apoptosis. This research has demonstrated that Bangladeshi plants are an exciting source of bioactive novel compounds and as such a rich basis for ongoing drug discovery research. References: 1. Newman DJ and Craig GM (2007) J Nat Prod 70(3): 461 – 77.

Keywords: Caralluma sinaica, Asclepiadaceae, Pregnan glycosides, Cytoxic Acknowledgement: Acknowledgements: the authors thank Phi lippe Eugster and Dr. Laurence Marcourt for their help in the recording of the in vivo bioassay.

Natural products and related drugs are used to treat 87% of all categorised human diseases including cancer and immunological disorders

[1] This study reports on the isolation and characterisation of novel cytotoxic compounds from Bangladeshi medicinal plants. Following LC-MS metabolic profiling and cytotoxic screening of 16 Bangladeshi medicinal plants against normal mouse fibroblast (NIH3T3) and three human cancer cell lines (AGS, HT-29 and MDA-MB-435S), Acrodictum aureum L. was selected for further phytochemical and pharmacological investigations. A total of 13 compounds were isolated from this plant using SPE and reversed-phase HPLC. The structures of compounds were elucidated by NMR, MS and other spectroscopic methods. Three compounds (1, 2 and 5) were identified as novel natural products. Eight known compounds were isolated for the first time from A. aureum: di(2-methylheptyl) phthalate (3), (2S, 3S)-pterin C (4), (2R)-pterin P (7), tetracosane (6), quercetin-3-O-β-D-glucosyl-(6→3)-O-L-rhamnose (9), quercetin-3-O-L-rhamnose (10) and quercetin-3-O-L-rhamnose-7-O-β-D-glucoside (11), and patriscabratine (12). Two known flavonoids, quercetin-3-O-β-D-glucoside (8) and kaempferol (12) have previously been isolated from A. aureum. The cytotoxic activity of all compounds was assessed against the cell lines mentioned above using the MIT assay. Four compounds (3, 5, 6 and 12) showed moderate to potent cytotoxic activity. Compound 5 displayed the most potent cytotoxicity against gastric and colon adenocarcinoma cell lines (IC50 7.5 – 21.6 µM). The mode of action was revealed as induction of apoptosis. This research has demonstrated that Bangladeshi plants are an exciting source of bioactive novel compounds and as such a rich basis for ongoing drug discovery research. References: 1. Newman DJ and Craig GM (2007) J Nat Prod 70(3): 461 – 77.

Keywords: Caralluma sinaica, Asclepiadaceae, Pregnan glycosides, Cytoxic Acknowledgement: Acknowledgements: the authors thank Philippe Eugster and Dr. Laurence Marcourt for their help in the recording of the in vivo bioassay.

Natural products and related drugs are used to treat 87% of all categorised human diseases including cancer and immunological disorders

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Keywords: coulometric array detection for the measurement of specific analytes. During this presentation I will illustrate the capability of electrochemical detection (HPLC-ECD). Such compounds, come from diverse chemical classes and include phenols, polyphenols, aromatic amines, quinones, isoquinolines, indoles, thiones and conjugated polyenes. Although HPLC-ECD with a single amperometric working electrode offers the analyst excellent sensitivity and some degree of selectivity, this approach is limited as it cannot be used routinely with gradient chromatography, relatively few analytes are measured concurrently, and qualitative information is lacking. The coulometric flow-through graphite working electrode is both sensitive (~100% of an analyte will react) and maintenance free. When used in series, chromatographically co-eluting analytes can be resolved voltametrically. Such behavior can be used to identify and characterize an analyte in an analogous fashion to the use of spectral data from a diode array detector. Furthermore, the CoulArray® detector, with its array of up to sixteen serially placed coulometric sensors, is fully gradient compatible and can be used for either targeted or metabolomics studies. During this presentation I will illustrate the capability of coulometric array detection for the measurement of specific analytes in plant, animals and human tissues. I will also discuss the use of metabotile profiles/metabolomics with pattern recognition and how this can be used to study product and botanical adulteration, contamination and composition. Keywords: HPLC, electrochemical, botanicals, natural products, plasma, urine, adulteration, characterization.
Metabolomic profiling of saw palmetto products using proton-NMR spectroscopy and multi-variate analysis

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Saw palmetto products with an often poorly known and variable chemical composition are used in the treatment of Benign Prostatic Hyperplasia. Here we present a method for the metabolomic analysis of saw palmetto products using NMR spectroscopy and multi-variate analysis in order to determine if there are significant differences in metabolites of the given products and if marker compounds can be identified. Spectra were obtained on a Bruker 500MHz spectrophotometer. TOPSPIN was used for spectra acquisition and processing. Deuterated methanol and chloroform were selected as solvents. NMR spectra were transferred to AMIX. The spectra were divided into 251 regions and the signal intensity was integrated. Unscrambler was used for PCA analysis. The analysis showed that saw palmetto products can be grouped according to their metabolic profile. Multi-variate analysis showed significant variations between powders, soft extract and tincture products. The largest variation in any product tested was observed for a hexane extract. Oleic acid and caproic acid ethyl ester were identified as potential marker compounds. Additional information regarding TLC analysis and clinical outcomes was supplied by Andy Suter from Bioforce AG, Switzerland.

Variations in chemical content were identified using NMR spectroscopy, however, multivariate analysis of the products suggested that there was no significant difference in metabolites between the European extracts tested but differences were observed when compared to non-European products or products that used hexane as the extraction solvent. It is possible to identify marker compounds in saw palmetto using proton-NMR spectroscopy. Acknowledgement: Centre for Pharmacognosy and Phytotherapy, University of London, School of Pharmacy, UK Michael Heinrich, Mire Zioh, Mzalina Said, Andy Suter. References: [1] Blumenthal M (2003) The ABC Clinical Guide to Herbs. New York: Thieme: 309 [2] Scaglione F (2008) Pharmacology 82(4): 270 – 275

Herb-based functional foods: from laboratories to the market

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Consumption of alternative herbal folk medicine has had a tremendous increase in the last decade. A number of medicinal plants contain secondary metabolites which have many biologically active compounds. They are used against hepatic fibrosis, heart ischemia-reperfusion and proved to have antioxidant, antiinflammation, anti-mariaction, anti-tumor, anti-angiogenic and anti-inflammatory activities. Non-availability of quality planting materials, low germination, slow plant growth, disease and pest incidence are the main obstacles in conventional medicinal plant cultivation. In Taiwan, many economically important medicinal plants and herbs are produced using various explant materials by tissue culture technique to meet the increasing demand for their medicinal properties. Rapid multiplication through in vitro tissue culture can be advantageous for the continuous supply throughout the year. We have developed and standardized efficient, simple and rapid tissue culture regeneration protocols of many medicinal plants, optimized the conditions in green house and successfully established the regenerated plantlets in the field for the large scale commercial production. Availability of tissue culture protocol is the first step towards the development of the genetic transformation. Keywords: Medicinal plant, Tissue culture, Functional foods Acknowledgement: Thanks to the National Science Council of Taiwan for financial support

The molecular cloning of dihydroartemisinic aldehyde reductase and its implication in artemisinin biosynthesis in Artemisia annua

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A key point in the biosynthesis of the antimalarial drug artemisinin is the formation of dihydroartemisinic aldehyde which represents the key difference between chemotype specific pathways. This key intermediate is the substrate for several competing enzymes some of which increase the metabolic flux towards artemisinin and some of which – as we show in the present study – may have a negative impact on artemisinin production. In an effort to understand the biosynthetic network of artemisinin biosynthesis, extracts of A. annua L. flowers were investigated and found to contain an enzyme activity competing in a negative sense with artemisinin biosynthesis. The enzyme, Red1, is a broad substrate oxidoreductase belonging to the short chain dehydrogenase/reductase family with high selectivity for dihydroartemisinic aldehyde and valuable monoterprenoids. Spatial and temporal analysis of cDNA revealed Red1 to be trichome specific. The relevance of Red1 to artemisinin biosynthesis is discussed.

Safety evaluation of licorice consumption from dietary and phytotherapeutic sources

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Licorice (Glycyrrhiza glabra L.) contains the triterpenoid glycyrrhizic acid (GA), which has an intensely sweet taste and is used as a flavouring agent, mostly in confectionary products and tobacco. Licorice also has a long history of medicinal use and is listed in current editions of the British, European and Chinese pharmacopoeias. GA and its aglycone, glycyrrhetinic acid, competitively inhibit 11β-hydroxysteroid dehydrogenase type 2, which converts active glucocorticoids to their inactive metabolites. Mineral corticoid-like effects can result, giving rise to pseudoaldosteronism characterised by hypokalaemia, sodium retention, oedema and suppression of the renin-angiotensin-aldosterone system with resulting hypertension. Licorice-induced pseudoaldosteronism is well documented with most cases involving confectionary licorice. Although it is not possible to accurately identify a NOEL for GA most human studies suggest that a daily intake of more than 100 mg GA over a period of weeks is necessary to produce pseudoaldosteronism. In order to evaluate the potential risk associated with the consumption of Australian made confectionary licorice products and liquid extracts dispensed in phytotherapy, we determined the GA content of ten confectionary products, five liquid extracts and two licorice root samples by reverse-phase HPLC. All but one liquid extract contained GA in concentrations that could readily result in a daily intake in excess of 100 mg if taken in a therapeutic dose. In contrast, confectionary products contained GA in concentrations that varied from three orders of magnitude and only a few of them would provide 100 mg/d of GA if consumed in amounts up to 100 g daily. Keywords: glycyrrhizic acid; licorice; safety


The key point in the biosynthesis of the antimalarial drug artemisinin is the formation of dihydroartemisinic aldehyde which represents the key difference between chemotype specific pathways. This key intermediate is the substrate for several competing enzymes some of which increase the metabolic flux towards artemisinin and some of which – as we show in the present study – may have a negative impact on artemisinin production. In an effort to understand the biosynthetic network of artemisinin biosynthesis, extracts of A. annua L. flowers were investigated and found to contain an enzyme activity competing in a negative sense with artemisinin biosynthesis. The enzyme, Red1, is a broad substrate oxidoreductase belonging to the short chain dehydrogenase/reductase family with high selectivity for dihydroartemisinic aldehyde and valuable monoterprenoids. Spatial and temporal analysis of cDNA revealed Red1 to be trichome specific. The relevance of Red1 to artemisinin biosynthesis is discussed.
HPLC-ESI-MS analyses showed the presence of phenolic compounds. Comparison of these samples with the commercial product revealed the lack of the latter. DAD-ESI-MS analyses to identify the marker constituents of the different sample was subjected, after lyophilization, to direct 2D NMR and HPLC-MS profile, NMR profile, dietary supplement, adulterant, biosurfactant, characterization, antimicrobial activity, antibiotics, surface tension

In this study HPLC-DAD-ESI-MS, HPLC-ESI-MS n and 2D NMR techniques were applied for the quality control of a herbal supplement. Several reports were received by the pharmacovigilance system by patients who fainted after consuming “Olivis([Irquor])”, a dietary supplement for the integrative treatment for hypertension. Declared components of this product are extracts from Olea europaea L., Crataegus oxyacantha L., Fumaria officinalis L., Capsella bursa-pastoris (L.) Medik. “Olivis([Irquor])” sample was subjected, after lyophilization, to direct 2D NMR and HPLC-DAD-ESI-MS analyses to identify the marker constituents of the different Herbal Drugs. Comparison of the NMR and chromatographic profiles of the product with those of the marker compounds of the declared plant species, (such as oleuropein, protopine), showed the lack of the latter. Samples of the declared plant species were taken into consideration as well. Comparison of these samples with the commercial product revealed marked differences in their content. Further, HPLC-DAD and HPLC-ESI-MS analyses showed the presence of Rauwolfia sp. type indole alkaloids, while HPLC-ESI-MS analyses revealed the presence of reserpine. Parallel phytochemical fractionations led to the isolation of ajmaline. Quantitative studies showed that the content of reserpine in the product was in the therapeutic range and therefore responsible for the collapses of the patients; (ii) ajmaline prevailed against reserpine indicating that a Rauwolfia species other than R. serpentina was used. The present study shows the importance of extensive controls using combined analytical tools of the botanical products on the market to assure their quality and as a consequence their safety profiles. Keywords: HPLC-UV-DAD-MS profile, NMR profile, dietary supplement, adulterant, reserpine, ajmaline

Biosurfactants are surface active amphiphilic compounds that reduce the surface tension of liquids, thereby increase the miscibility of hydrophobic compounds. One of the isolate from oil contaminated site was tested for its ability to produce biosurfactants. The bacterium was identified on the basis of morphological and biochemical characterization and found to be Pseudomonas putida SOL-10. The isolate was tested for biosurfactant production under shake flask fermentation and found to be potent biosurfactant producer. The biosurfactant production was analyzed by surface tension and emulsification index (E24%) measurements. In this study the biosurfactant was produced by a newly isolated Pseudomonas putida SOL-10 at optimized conditions. The biosurfactant produced by the isolate reduced the surface tension of culture broth from 43.6 – 29.9 m.N.m -1 achieving a maximum biosurfactant concentration of 4.5 g/L after 72 hours of incubation. The biosurfactant also demonstrated a good antimicrobial activity as well as the capability to enhance the antimicrobial effect of some antibiotics. The current study describes the effect of biosurfactant with Ampicillin, Ciprofloxacin and Cefixime, against Escherichia coli, Bacillus subtilis, and Klebsiella pneumonia. The results demonstrated promising antimicrobial activity enhancing effect of the biosurfactant and suggested a possibility of the biosurfactant to be used with antibiotic formulations in order to increase the effectiveness of antimicrobials against multidrug-resistant pathogenic bacteria. Keywords: Pseudomonas putida, biosurfactant, characterization, antimicrobial activity, antibiotics, surface tension

According to the World Health Organization (WHO), the goal of ‘Health for All’ cannot be achieved without herbal medicines. While the demand for herbal medicines is growing in developing countries including Egypt, there are indications that consumers in developed countries are becoming disillusioned with modern healthcare and are seeking alternatives in traditional medicines. There is, therefore, an increasing consumer demand for herbal medicines in developed countries. Medicinal plants have been used as a source of remedies since ancient times in Egypt. Many plants are still used today in folkloric medicine and are sold at herbal vendors and shops. Egypt is characterized by abundant production of medicinal and aromatic plants that are exported all over the world and is considered as one of the most important sectors can be relied upon to increase the volume of Egyptian exports due to the growing global demand but several factors pose constraints to their entry into the international market and put them in a disadvantageous position. This lecture explores the situation of the Egyptian herbal drug industry, the economic value, the needs and recommendations for developing this important sector. Keywords: herbal medicine, Egypt, drug industry, challenges References: References 1. Batanouny K H (1999) Wild Medicinal Plants in Egypt (with contribution: E. Aboutabl, M. Shaban & F. Soliman). 2. Dagmar I (2006) International Trade in Medicinal and Aromatic Plants, Actors, volumes and commodities, Plants.- In: B. B., Crapeer, I.E. & Lange, D. (eds.); Medicinal and Aromatic Plants, 155 – 170. 3. Das M. (2005) Medicinal Plants: An Approach towards sustainable development, available at http://www.iamot.org/conference/index.php/ocs/4/paper/viewFile/467/18 4. Saleh NAM (2003) Phytochemistry 63: 239 – 241.

Black cohosh (Actaea racemosa Walter ex Steud.) is a frequently used herbal remedy for the treatment of mild climacteric symptoms. The plants active principle was extensively studied, but its mechanism of action remained unclear [11]. In this study the modulation of GABA-induced chloride currents (I_{GABA}) through GABA_A receptors by black cohosh extracts and isolated compounds was investigated. GABA_A receptors consisting of α_1, β_2 and γ_2 subunits were expressed in Xenopus laevis oocytes and potentiation of I_{GABA} was measured using the two-microelectrode voltage clamp technique. In a bioactivity-guided isolation procedure the positive modulation of I_{GABA} could be restricted to the terpenoid fractions, resulting in the isolation of 11 cycloartane glycosides, of which 4 compounds significantly enhanced I_{GABA} by more than 150% (p < 0.05). The strongest effect was observed for 23-O-acetyl-

Keywords: Artemisia annua, red1, dihydroartemisinic aldehyde, dihydroartemisinic acid, trichome, reductase, dehydrogenase, trichome, oxidoreductase References: Rydén A-M, Ruyter-Spira C, Osada H, Muranaka T, Kayser O, Bouwmeester H (2010) Planta Medica 76:1 – 6

Figure 1: Biosynthetic pathway of artemisinin and the activity of Red1
shengmanol-3-O-D-xylopyranoside (100 μM) enhancing GABA by 1692 ± 201%, while actein, cimigenol-3-O-D-xylopyranoside and 25-O-acetylcimigenol-3-O-L-arabinopyranoside were significantly less efficient (range of GABA enhancement at 100 μM: 250 – 357% ± 64%). 23-O-acetyleshengmanol-3-O-D-xylopyranoside which exhibited the greatest potentiation of GABA was additionally investigated in a mouse model. In this model, a decrease in motor activity was also observed as a measure of activity levels, with treatments containing 23-O-acetyleshengmanol-3-O-D-xylopyranoside indicating a potential effect on the central nervous system. This finding suggests that these compounds may have potential therapeutic applications in the treatment of neurological disorders.

Keywords: Aloe vera, Aloe ferox, herb-drug pharmacokinetic interactions, drug absorption enhancements, Caco-2, intestinal mucosa, buccal mucosa transport, modulation of GABAA receptors, and thereby allowing paracellular drug transport. These types of pharmacokinetic interactions can lead to side effects, but on the other hand may be employed in a controlled way to enhance absorption of poorly absorbable drugs. Keywords: In vitro transport, herb-drug pharmacokinetic interactions, drug absorption enhancement, Caco-2, intestinal mucosa, buccal mucosa transport, modulation of GABAA receptors, and thereby allowing paracellular drug transport. These types of pharmacokinetic interactions can lead to side effects, but on the other hand may be employed in a controlled way to enhance absorption of poorly absorbable drugs. Keywords: In vitro transport, herb-drug pharmacokinetic interactions, drug absorption enhancement, Caco-2, intestinal mucosa, buccal mucosa transport, modulation of GABAA receptors, and thereby allowing paracellular drug transport. These types of pharmacokinetic interactions can lead to side effects, but on the other hand may be employed in a controlled way to enhance absorption of poorly absorbable drugs. Keywords: In vitro transport, herb-drug pharmacokinetic interactions, drug absorption enhancement, Caco-2, intestinal mucosa, buccal mucosa transport, modulation of GABAA receptors, and thereby allowing paracellular drug transport. These types of pharmacokinetic interactions can lead to side effects, but on the other hand may be employed in a controlled way to enhance absorption of poorly absorbable drugs. Keywords: In vitro transport, herb-drug pharmacokinetic interactions, drug absorption enhancement, Caco-2, intestinal mucosa, buccal mucosa transport, modulation of GABAA receptors, and thereby allowing paracellular drug transport. These types of pharmacokinetic interactions can lead to side effects, but on the other hand may be employed in a controlled way to enhance absorption of poorly absorbable drugs.

In vitro transport, herb-drug pharmacokinetic interactions, drug absorption enhancement, Caco-2, intestinal mucosa, buccal mucosa transport, modulation of GABAA receptors, and thereby allowing paracellular drug transport. These types of pharmacokinetic interactions can lead to side effects, but on the other hand may be employed in a controlled way to enhance absorption of poorly absorbable drugs.

Herb-drug pharmacokinetic interactions include interferences of plant constituents with drug bioavailability by means of altered absorption, metabolism, distribution and/or elimination [1]. Although in vitro pharmacokinetic interactions are not always clinically significant in the in vivo situation [2], it may in many cases indicate potential important interactions that can influence the bioavailability of co-administered drugs. This work reports on the effects of extracts from different botanicals such as whole leaf extracts and gels (Aloe vera, Aloe ferox) [3], perms (Hyponix hemerocallidiforme), aerial parts (Sutherlandia frutescens, Aspalathus linearis) [4], fruit (Sclerocarya birrea, Psidium guajava, Dovyalis caffra, Prunus persica, Fragaria ananassa, Prunus domestica), and vegetative tubers (Daucus carota, Beta vulgaris) on in vitro drug transport as well as transport of phytoconstituents in crude extracts in comparison to purified compounds (Hoodia gordoni, Sceletium tortuosum) [5]. The mechanisms of drug transport alteration was determined for some of the botanicals by means of either measuring transport in two directions (to indicate efflux inhibition induction) or by measuring the transepithelial electrical resistance (to indicate opening of tight junctions).

The results showed that some of the plant extracts increased drug transport in the absorptive direction by decreasing drug efflux transporters, while others induced the efflux transporters. Some plant materials showed the ability to enhance drug transport by opening tight-junctions and thereby allowing paracellular drug transport. These types of pharmacokinetic interactions may lead to potential side effects, but on the other hand they may be employed in a controlled way to enhance absorption of poorly absorbable drugs. Keywords: In vitro transport, herb-drug pharmacokinetic interactions, drug absorption enhancement, Caco-2, intestinal mucosa, buccal mucosa transport, modulation of GABAA receptors, and thereby allowing paracellular drug transport. These types of pharmacokinetic interactions may lead to potential side effects, but on the other hand they may be employed in a controlled way to enhance absorption of poorly absorbable drugs. Keywords: In vitro transport, herb-drug pharmacokinetic interactions, drug absorption enhancement, Caco-2, intestinal mucosa, buccal mucosa transport, modulation of GABAA receptors, and thereby allowing paracellular drug transport. These types of pharmacokinetic interactions may lead to potential side effects, but on the other hand they may be employed in a controlled way to enhance absorption of poorly absorbable drugs. Keywords: In vitro transport, herb-drug pharmacokinetic interactions, drug absorption enhancement, Caco-2, intestinal mucosa, buccal mucosa transport, modulation of GABAA receptors, and thereby allowing paracellular drug transport. These types of pharmacokinetic interactions may lead to potential side effects, but on the other hand they may be employed in a controlled way to enhance absorption of poorly absorbable drugs.

When reflecting on past antimicrobial studies undertaken on medicinal plants, it is clear that those having the most impact have been when a targeted disciplinary approach has been adopted. In order to elaborate on this, past and present studies will be presented with different approaches (pathogen specific, ethnopharmacological correlations, combination studies, structure activity relationships, formulations etc) in order to achieve outcomes that address recommendations made from previous antimicrobial reviews [1,2,3,4]. Medicinal plant use by inhabitants of Maputaland, Zululand (Southern Africa) were undertaken, whereby the in vitro antimicrobial investigations against diseases associated with ethanol extracts, root, bark preparation, its salicin rich ethanol fraction and imipramine for toxicological endpoints.

In vitro transport, herb-drug pharmacokinetic interactions, drug absorption enhancement, Caco-2, intestinal mucosa, buccal mucosa transport, modulation of GABAA receptors, and thereby allowing paracellular drug transport. These types of pharmacokinetic interactions may lead to potential side effects, but on the other hand they may be employed in a controlled way to enhance absorption of poorly absorbable drugs. Keywords: In vitro transport, herb-drug pharmacokinetic interactions, drug absorption enhancement, Caco-2, intestinal mucosa, buccal mucosa transport, modulation of GABAA receptors, and thereby allowing paracellular drug transport. These types of pharmacokinetic interactions may lead to potential side effects, but on the other hand they may be employed in a controlled way to enhance absorption of poorly absorbable drugs. Keywords: In vitro transport, herb-drug pharmacokinetic interactions, drug absorption enhancement, Caco-2, intestinal mucosa, buccal mucosa transport, modulation of GABAA receptors, and thereby allowing paracellular drug transport. These types of pharmacokinetic interactions may lead to potential side effects, but on the other hand they may be employed in a controlled way to enhance absorption of poorly absorbable drugs. Keywords: In vitro transport, herb-drug pharmacokinetic interactions, drug absorption enhancement, Caco-2, intestinal mucosa, buccal mucosa transport, modulation of GABAA receptors, and thereby allowing paracellular drug transport. These types of pharmacokinetic interactions may lead to potential side effects, but on the other hand they may be employed in a controlled way to enhance absorption of poorly absorbable drugs.
The high impact of Natural Products (NP) in medicine is proven and well sustained [1]. NP are characterized by unique structural diversity and critical drug-like features which rank them as the most promising candidates for possible future drugs [2]. However, several factors constrain their development related mostly to the time-consuming and labor procedures required for their isolation, the high cost and the dereplication problem [3]. In the present work, we propose a structure-oriented approach using UHPLC-HRMS/MS (LTQ-Orbitrap) methodologies trying to release the entire procedure from repeated and useless isolation steps and pursuing the targeted determination of the possible bioactive compound. This approach is applied in crude extracts and is based on fast UHPLC methods, high resolution mass spectra and ms/ms accurate mass measurements. As a proof of concept, the Cameroonian tree *Amphimasia pierocarpoides* Harms (Leguminosae) was selected and flavonoids – isoflavonoids were chosen as the chemical class of interest. Based on a chromatographic (Rt, polarity) and spectrometric features (UV, accurate m/z, proposed ECs, RDB values and R/AS) as well as ms/ms spectra, the compounds of interest were defined and structurally elucidated. 12 of the 17 traced flavonoids were selectively isolated and characterized using 1 and 2D NMR techniques verifying our concept. Applying this approach, the identification of target-compounds is achieved early in the discovery procedure facilitating the dereplication of known compounds. Consequently much time required for the fractionation, isolation and purification is saved while the possibility of the discovery of novel structures and subsequently novel actives is elevated. References: [1] Newman DJ, Cragg GM (2007) J Nat Prod 70: 461 – 477. [2] Yuliana ND, Khabib A, Choi YH and Verpoorte R (2011) Phytother Res 25: 167 – 169. [3] Poterat O and Hamburger M, (2006) Current Organic Chemistry 10: 899 – 920.

### SL37

Monodimensional and comprehensive liquid chromatography linked to mass spectrometry for unravelling bioactive components in natural products

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There is considerable evidence nowadays that dietary flavonoids and other phenolic compounds may exert preventive and/or therapeutic effects in a role of human diseases. Despite the great interest in determining the role of phyto-nutrients as potential therapeutic agents, and the rising demand of natural sources with nutraceutic benefits, the antioxidant content of many medicinal plants and foodstuffs is unknown, making accurate estimation for human dietary consumption and the correlation to human diseases difficult. High resolution chromatographic techniques are considered as the first choice. It is thus crucial to find a key solution to obtain the identification power required for a comprehensive examination of all the possible bioactive components. Principal component analysis (PCA) and 2D correlation analysis (MC-2D) were applied to the multivariate data and the effective identification of target-compounds was achieved based on accurate mass measurements. As a proof of concept, the Cameroonian tree *Amphimasia pierocarpoides* Harms (Leguminosae) was selected and flavonoids – isoflavonoids were chosen as the chemical class of interest. Based on a chromatographic (Rt, polarity) and spectrometric features (UV, accurate m/z, proposed ECs, RDB values and R/AS) as well as ms/ms spectra, the compounds of interest were defined and structurally elucidated. 12 of the 17 traced flavonoids were selectively isolated and characterized using 1 and 2D NMR techniques verifying our concept. Applying this approach, the identification of target-compounds is achieved early in the discovery procedure facilitating the dereplication of known compounds. Consequently much time required for the fractionation, isolation and purification is saved while the possibility of the discovery of novel structures and subsequently novel actives is elevated. References: [1] Newman DJ, Cragg GM (2007) J Nat Prod 70: 461 – 477. [2] Yuliana ND, Khabib A, Choi YH and Verpoorte R (2011) Phytother Res 25: 167 – 169. [3] Poterat O and Hamburger M, (2006) Current Organic Chemistry 10: 899 – 920.

### SL38

Gas chromatography-mass spectrometry combined with mathematical chromatography as a powerful tool in the analysis of citrus fruits essential oils

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Citrus fruits essential oils are valuable natural products that are more popular nowadays in the world due to their effects on health conditions and their role in preventing and curing diseases [1]. Also, they have a broad range of applications in foods, perfumes, cosmetics and human nutrition. Gas chromatography-mass spectrometry (GC-MS) is the most important technique for the analysis of essential oils [2]. However, there are some fundamental problems in their analysis including baseline drift, spectral background, noise, low S/N, changes in the peak shapes and co-elution (overlapped, embedded peaks) [3]. Mathematical chromatography (MC) as a branch of chemometrics [4] attempts to develop new tools to handle these problems. In this work, first, we have extracted the essential oils of the peels of eighteen citrus fruits such as lemon, lime, mandarin, and grapefruit using hydrodistillation and then analyzed with GC-MS. Then, their signals were analyzed by MC. Using this strategy, the numbers of identified components were extended and quality of the results was improved significantly. As a positive consequence of using the proposed strategy human time and work are saved. Also, some new components were identified for the first time. In addition, we used our recently developed software, called MCRC Software for processing these techniques [5]. After resolving the volatile components in different samples, principal component analysis (PCA) was used for monitoring the pattern of volatile components in different samples. It is concluded that GC-MS/MC-PCA can open a new window to the comprehensive analysis of essential oils. Keywords: Chromatometrics, Mathematical chromatography, Essential oil, Gas chromatography-mass spectrometry, Citrus fruits Acknowledgement: Parastar H. would like to acknowledge the Iran National Elite Foundation for their support.

### SL39

Allium species of the subgenus Melanosorronymum are a rich source for cysteine sulfoxides

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The plant genus *Allium* (onions) is highly diverse. About 800 species are currently known belonging to several subgenera [1]. The main centre of distribution is the northern hemisphere, especially the area of South-west and Central Asia. Inside this area, the subgenus *Melanosorronymum* is most prominent. Metlin 1 seems to be a nearly ubiquitous cysteine sulfoxide for the genus *Allium* (Figure 1). Propii 2 is a minor compound, which was found in *A. ubipetens* R.M. Fritsch. The *L-(+)-S(3-pyrrolidyl)cysteine sulfoxide 3 is the major cysteine sulfoxide of *A. stipitatum* Regel (0.22%, related to the fresh weight of bulbs) as well as the closely related *A. altsismum* Regel (0.50%) [2]. It seems to be possible that the corresponding N-oxide 3a is also present in these plants. Most species of the subgenus do also contain the *L-(+)-S(3-pyrrolidyl)cysteine sulfoxide 4 [3]. High amounts of 4 were found in *A. jesdianum* Boiss. & Buhse subsp. remediorum R.M. Fritsch (0.52%), A. macleanii Baker (0.29%), A. tchimganicum (0.23%) and A. roseanum (0.20%). Highest amounts of marasmin 5 were detected in *A. suworowii* (2.25%). Compound 5 is also present in *A. altsismum* and *A. stipitatum*. Especially the latter species is widely used as vegetable, spice and traditional medicine.
Keywords: Allium, Melanocrommyum, A. stipitatum, A. altissimum, A. jesdanum, A. rosenorum, A. suworowii, cysteine sulfoxides, marasmin and Withania riebeckii.

The greatest antimicrobial activity was found by the methanolic extracts of Ballochia atro-virgata, Teucrium socotranum, Exacum affine, Eureiandra balfourii, Jatropha unicospra, Commiphora parvifolia, Dendrosicyos socotrana, Punica protopunica, Boswellia socotrana, Buxus hildebrandtii, Commiphora randtii, Balf.f., and Withania riebeckii Balf.f., 520 – 526


SL40

Anticancer, antimicrobial and antiviral potentials of selected medicinal plants from the Island Soqotra

Soqotra is considered the “jewel” of biodiversity in the Arabian Sea. Surveys have revealed that more than a third of the plant species of Soqotra are found nowhere else [1]. Fifty plants were collected, extracted with methanol and hot water and evaluated for their in vitro anticancer activity against three human cancer cell lines (A-427, 5637 and MCF-7) and for their antimicrobial activity against Gram-positive and Gram-negative bacteria as well as multiresistant Staphylococcus strains. Moreover, the antiviral activity of 25 plants has been assessed in two in vitro viral systems, influenza virus type A/MDCK cells and herpes simplex virus type 1/Vero cells, at non-cytotoxic concentrations. The methanolic extracts of Ballochia atro-virgata Balf.f., Buxus hildebrandtii Balf.f. and Withania riebeckii showed anti-influenza virus type A activity [3,4]. The methanolic extracts of Ballochia atro-virgata Balf.f., Boswellia longifolia Thul. & Gifri, Boswellia elongata Balf.f., Boswellia socotrana Balf.f., Buxus hildebrandtii, Commiphora ornifolia (Balf.f.), J.B.Gillett, Commiphora parvifolia Engl., Euca- clea divinorum Hiern, Euphorbia socotrana Balf.f., Jatropha unicospra, Kalanchoe farinacea Balf.f., Leuca semaushna Cortés-Burns & A.G.Mill., Leucas virgata Balf.f., Pulicaria stephanocarpa Balf.f., Punicia protopunica, Rhus thyrsiflora Balf.f., Teucrium socotranaum Viehr., Withania adenium and Withania riebeckii [3,4]. The methanolic extracts of Boswellia ameero, Boswellia elongata, Buxus hildebrandtii, Cissus hamaderemensis Radel.-Sm., Cleome socotrana Balf.f., Exacum affine Balf.f., Jatropha uni- costata and Kalanchoe farinacea showed anti-influenza virus type A activity with IC50 values ranging between 0.29 and 8.2 μg/mL [2,3]. The greatest antimicrobial activity was found by the methanolic extracts of Ballochia atro-virgata Balf.f., Balf.f., and hot water and evaluated for their in vitro anticancer activity against three human cancer cell lines (A-427, 5637 and MCF-7) and for their antimicrobial activity against Gram-positive and Gram-negative bacteria as well as multiresistant Staphylococcus strains. Moreover, the antiviral activity of 25 plants has been assessed in two in vitro viral systems, influenza virus type A/MDCK cells and herpes simplex virus type 1/Vero cells, at non-cytotoxic concentrations. The methanolic extracts of Ballochia atro-virgata Balf.f., Buxus hildebrandtii Balf.f. and Withania riebeckii Balf.f., 520 – 526


SL41

Toxicological evaluation of DAS-777 – a herbal preparation

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DAS-777 is a herbal preparation that contains the milled bark of Mangif- era indica L. and root of Carica papaya L. (1:1). It is used for the treat- ment of various ailments in Nigeria hence this toxicity assessment. In the acute toxicity study, DAS-777 (constituted in dH2O) was administ- ered to mice p.o. up to 10 g/kg and i.p. at 250 – 3000 mg/kg. Mortality within 24 h was recorded. In the chronic toxicity study, rats were orally treated for 90 days at doses of 80, 400 (therapeutic dose, TD) and 2000 mg/kg. Rats were weaned and observed for feeding and drinking habits. By 90 days, animals were sacrificed and blood samples collected for haematological and biochemical analysis. Organs were harvested for weight determination, antioxidants and histopathological assessments. DAS-777 did not produce any lethality administered p.o. up to 10 g/kg but the i.p. LD50 was 1122 mg/kg. At TD, DAS-777 produced significant changes (p < 0.05) only in body weight and food intake (1), ovary weight (†), neutrophils (†), HDL (†), and K+ (†), which were reversible. Histop- athological presentations were generally normal. Effects at the other doses were comparable to those at TD except for reversible changes in vivo antioxidants (†) in the liver, kidney and testes, liver enzymes (†) and sperm parameters (motility (†), count (†) and abnormality (†)). DAS-777 was found to contain tannins (3.26 ± 0.15%), saponins (2.32 ± 0.04%), phenols (1.31 ± 0.07%), flavonoids (0.54 ± 0.02%) and alkaloids (0.04 ± 0.01%) w/v. Findings in this study revealed that DAS-777 is rela- tively safe with the potential for enhancing in vivo antioxidant activity. However, possibly reversible side-effects include electrolyte imbalance and sterility in males. Keywords: DAS-777 herbal preparation, acute toxicity, chronic toxicity

SL42

The design of DNA barcode-specific PCR primers for medicinal plant authentication

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The DNA Barcode of Life initiative aims to obtain designated “barcode” sequences for every known species on Earth. Progress of plant DNA barcoding have cited medicinal plant authentication as one of the po- tential applications of barcode information. However, DNA sequencing of barcode regions may not be suitable for routine quality assurance testing of plant materials, which could contain mixtures of plant species and/or degraded DNA. The value of DNA barcoding in this arena may in fact lie in its role as a platform for the design of standardised DNA-based tests. We have studied three groups of plant species (Hypericum spp., Actaea spp. and Rhodolia spp.), each comprising a target commercial medicinal plant and known or potential adulterant species. The suitability of four “barcode” regions for the design of species-specific PCR pri- mers was determined; the designated plastid rbcl and matK barcode regions, the candidate plastid trnH-psbA spacer barcode region and the nuclear ribosomal ITS region. Problems were encountered with the plastid barcodes (low inter-specific variation of rbcl, lack of reliable generic primers for matK, repetitive sequences in trnH-psbA) and for all three groups of plants the nrITS region proved to be most appropriate for primer design. Targeting the nuclear genome also allows discrimination of hybrids that may not be detected using plastid barcodes. Whilst DNA barcoding may prove to have a role in plant species identification, the current choice of universal primers for all plant barcodes may not be ideal for the development of routine authentication tests. Keywords: authentication, DNA barcode, PCR primer, ribosomal ITS

SL43

Traditional use as a regulatory category – experiences in Europe

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Directive 2004/24/EC established a regulatory pathway for traditional herbal medicinal products in the European Union that allows the regis- tration and marketing without the standard clinical data packages on safety and efficacy as required for other medicinal products. Five main
criteria have to be fulfilled to guarantee safety in specified conditions: (1) indications suitable for self medication, (2) specified strength and posology, (3) appropriate route of administration, (4) a minimum 30 years period of traditional use and (5) sufficient data to prove that a product is not harmful in specified conditions and effects are plausible based on long-standing use and experience. Thus, the traditional use in the regulatory sense comprises well-defined restrictions that deviate from the concept of traditional use in sensu lato but also standard information given in ethnomedical research. The necessary documentation for compliance with the five criteria are presented using examples of herbal substances from monographs as published by the Committee on Herbal Medicinal Products at the European Medicines Agency. Typical issues of data generation and availability are demonstrated. It is further discussed why not all types of traditionally used medicines or derived preparations fit into the current regulatory framework – even if scientific and historic evidence is available. Conclusions are drawn on the opportunities and limits for use of ethnopharmacological research data for regulatory purposes.

**SL44**

**Sceletium tortuosum - an ancient treatment for modern CNS-related disorders**

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Members of the genus Sceletium (Mesembryanthemaceae) have been used for millennia as masticatories, for the relief of thirst and hunger, to combat fatigue, as medicines, and for social and spiritual purposes by San hunter-gathers (historically referred to as Bushmen) and Khoi pastoralists [1,2]. Recently, several formulations containing Sceletium have been commercialised and marketed to treat anxiety, stress and tension. In 2010 the South African Government granted the country’s first ever integrated export and bioprospecting permit to a local pharmaceutical company who will market Sceletium in a joint venture with a nutraceutical company in the USA. Based on the interesting ethnobotanical information and the upsurge in commercial interest we embarked on a comprehensive biosystematic and biopharmaceutical study of S. tortuosum and its major alkaloids, mesembrenone, mesembrenol, mesembrine and mesembranol. Wild S. tortuosum (L.) N.E.Br. plants (n = 150), sampled from different localities (n = 31) in the south western region of South Africa, were examined for the phychomelical variability using GC-MS. The potential effect of chemotypic variation on product formulation and quality assurance protocols will be discussed. In vitro permeation studies of the alkaloids and different crude plant extracts (water, methanol and enriched alkaloid acid-base extract) across porcine buccal, sublingual and intestinal mucosa were also conducted to predict the bioavailability of the alkaloids. Absorption across intestinal mucosa was highest, but buccal and sublingual absorption contributed to overall bioavailability. In addition to results generated, a general overview on the botany, chemistry and ethnopharmacology of S. tortuosum will be presented. Keywords: Sceletium tortuosum, alkaloids, bioavailability, quality control Acknowledgement: National Research Foundation, Tshwane University of Technology, R&A abator (Pretoria).


**SL45**

**Antidiabetic Effect of Juniperus oxycedrus subsp. oxycedrus Berries by Using Bioactivity Guided Fractionation**

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Juniperus species are widely used in traditional medicine for many purposes in Anatolia. According to ethnobotanical reports which were carried out in different parts of Turkey, berries of Juniperus oxycedrus L. subsp. oxycedrus, have been ingested as decoctions of berries have been taken as tea to lower blood glucose levels (1,2). The extracts of berries were given to normal, glucose loaded and streptozotocin induced dia- betic rats at 0.5 and 1 g/kg doses. Joso extracts were found to possess antidiabetic effect. Additionally, blood glucose levels of normoglycemic and glucose loaded rats were decreased by administration of ethanol extract. After 10 days administration to diabetic rats, blood glucose and malondialdehyde levels in kidney and liver were decreased significantly. Etanol extract was fractionated by successive solvent-solvent ex- traction. Sub-extracts were given to the diabetic rats. Results indicated that n-butanol sub-extract (740 mg/kg) were found to possess high anti- diabetic activity. n-Butanol sub-extract was fractionated by column chromatography and the active principles were isolated. Shikimic acid, fericulic acid glycoside and oleuropeic acid glycoside were found in the active fraction of the extract. Since shikimic acid was found as major component of the active fraction, it was administrated to diabetic rats at 15 and 30 mg/kg doses. After subacute administration of shikimic acid, some biochemical parameters and tissue antioxidant levels were also evaluated. Whereas insulin level was not elevated blood glucose levels were decreased significantly by shikimic acid (24%). Moreover, malon- dialdehyde levels in kidney tissues (63 – 64%) and liver enzymes were decreased by subacute administration of shikimic acid.

**SL46**

**Euphol, a novel cannabinoid agonist, prevents inflammatory and neuropathic persistent pain in rodents**

Dutra RR, Silva KB, Bento AF, Pazuck AF, Marcon R, Menniti FC, Metso EM, Mandelli JS, Calixto JB

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The cannabinoids have been considered a relevant target for pain management, especially in inflammatory and neuropathic states, are prevalent and debilitating diseases, which remain without safe and adequate treatments. Inflammatory and neuropathic pain was induced by carrageenan, CPA, PSNL, cancer/chemotherapeutic agent, PCE2 and PKCg agonist. Pro-inflammatory mediators were measured by immunohistochemistry, enzyme-linked immunosorbent assay (ELIS- A) and real time-PCR. Here, we reported that euphol exhibited pronounced and long-lasting oral analgesia in several rodent behaviour models of inflammatory and neuropathic persistent pain. These effects were markedly blocked by CB1 or CB2-selective antagonists and oligo-nucleotide antisense. Of note, cannabinoid receptor binding experiments showed that euphol directly bound with high affinity to both CB1 (Ki = 71.090 nM) and CB2 (Ki = 0.037 nM), being 1,880-fold more selective for CB2 receptors. Euphol at similar dose inhibited the levels/mRNA expression, either at the transcription level, of pro-inflammatory mediators and the myeloperoxidase activity. Our data indicate that euphol activate transcription factors, such as PPAR-γ and inhibiting both NF-κB and CREB activation, associated with the inhibition of COX-2 and PKCg expression, either at the spinal cord or the dorsal root ganglia level. Of relevance, euphol did not display significant central nervous system alterations. Acute toxicological studies carried out in rodents showed that euphol is safe and well tolerated. Therefore, euphol represents a novel orally active and safe natural analgesic for the management of inflammatory and neuropathic pain states. Keywords: Euphol, cannabinoid receptors, pain, inflammation Acknowledgement: This work was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), the Programa de Apoio aos Núcleos de Excelência (PRONEX), and the Fun- dação de Apoio a Pesquisa do Estado de Santa Catarina (FAPESC). All of Brazil. R.C.D., K.A.B.S., A.F.B., A.F.P. and R.M. are Ph.D. students in pharma- tocolgy receiving grants from CNPq.
SL47

Analgesics of a lupane-type triterpene as potential anti-HIV agents
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1 Instituto Universitario de Bio-Organica “Antonio González”, Universidad de La Laguna, Avda. Astrofísico Francisco Sánchez 2, 38206 La Laguna, Tenerife, Spain; 2 Centro Nacional de Microbiología, Instituto de Salud Carlos III, Ctr. Majedahonda a Pozuelo, 28220 Majedahonda, Madrid, Spain.

Infection with human immunodeficiency virus (HIV), the etiologic agent of acquired immunodeficiency syndrome (AIDS), continues to be ranked high on the list of the most important health issues facing the world. Although significant progress has been made since the introduction of highly active antiretroviral therapy, it has also led to increased adverse effects and the emergence of multidrug-resistant viral strains. Therefore, there is a need for new classes of drugs involving novel molecular mechanisms [1]. Many classes of natural products and some of their analogues have been tested for their anti-HIV activity [2]. In fact, modification of betulinic acid led to the discovery of bevirimat, a first-in-class drug candidate as a viral maturation inhibitor [3]. The goal of our study was to get new insights into the antiviral potential of lupane-type triterpenes that could inhibit HIV-1 replication and would be useful for the design of new drugs with clinical application [4]. Therefore, we prepared 17 derivatives based on the betulin scaffold, whose structures were determined by spectroscopic studies, and comparison with data previously published. The obtained data opened a promising activity at 10µM with replication inhibition percentages of 26% and 31%, respectively. A study of the influence of the substitution pattern on the lupane skeleton revealed that oxidation at C-3, acetylation at C-28 and modification of the isoprenoid moiety play an important role in the activity. Keywords: lupane triterpene; betulin analogues; anti-HIV agents Acknowledgement: We are indebted to the Agencia Canaria de Investigación, Innovación y Sociedad de la Información (C200801000049) project for financial support.

SL48

Hoodia gordoni: Quality control and biopharmaceutical aspects
Vermaak I1, Viljoen AM1, Hamman JH1
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Hoodia gordoni: Sweet is a popularly consumed commercially available weight loss product. Therefore, developing rapid quality control methods for raw material and products, and investigating key biopharmaceutical aspects of the perceived active ingredient P57, is of utmost importance. High performance thin layer chromatography (HPTLC) and vibrational spectroscopy coupled with chemometric analysis are attractive alternative quantification methods for P57. The in vitro transport of pure P57 and P57 from crude plant extracts across porcine intestinal and buccal tissue was also investigated. The HPTLC system produced good band separation including the P57 band [1] and linear calibration curves with good correlation coefficient (R2) values of 0.9766 – 0.9993 were developed for P57 quantification. For the NIR spectroscopy data, the partial least squares projections to latent structures (PLS) model with 2nd derivative pre-processing predicted P57 content with an R2 value of 0.9629 and a root mean square error of prediction (RMSEP) of 0.03% [2].

SL49

Application of liquid chromatography and mass spectrometry methods in pharmacognostic investigations of Traditional Chinese Medicines (TCM)
Yong Y1, Ge L2, Ping TC, Qiang KC, Zhen ZX3, Ying PS4
1 Natural Products Chemistry Department, & State Key Laboratory of Drug Research, & SIMM/CUHK Joint Laboratory for Promoting Globalization of Traditional Chinese Medicines, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu-Chong-Zhi Road, Zhangjiang High-Tech Park, Shanghai 201203, P. R. China; 2 School of Biomedical Sciences, Faculty of Medicine, & SIMM/CUHK Joint Laboratory for Promoting Globalization of Traditional Chinese Medicines, The Chinese University of Hong Kong, Hong Kong SAR, P. R. China

TCM has a long history to treat human diseases in China. In our investigations to depict medicinal functionalities of TCM using LC-MS related technologies, a diversity of new chemical structures including novel scaffolds were acquired. These molecular-oriented technologies have been integrated effectively into the pharmacognostic investigations of medicinal herbs. In our chemical study of Stemona species, a HPLC-MSn method was developed for the characterization of alkaloids with a pyrrolo[1,2-a]azepine A, B-ring core from S. saxorum Gagnep. based on the ESI-MS3 results of five reference compounds. 41 compounds were separated, of which 12 compounds (4 new) were identified as Stemona alkaloids with such a core. A practical HPLC method was designed to detect the content of shikimic acid, the start material of Tamiflu, in Chinese Illicium plants from 21 different species or habitats. The minor toxic anisatin and its analogues were also monitored by an UPLC-MS/MS method. Our results provided scientific evidences for safe usage of fruits of Illicium plants. Additionally, new series of sesquiterpenoids and phe- nonpropanoid flavonoid polymers were identified from these species.

SL50

Effect of Catechins Extracts of Green Tea on Quality Protection of Black Tea-bag During Storage
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1 College of Food Science and Technology, Science and research Branch, Islamic Azad University, Tehran, Iran; 2 Faculty of Sciences, Tehran University, Tehran, Iran

Green tea is one of the richest sources of antioxidants especially catechins, which are studied most because of their health benefits. These valuable compounds may lose their activity and oxidize to thearobigin during fermentation stage or black tea processing. The main objective of this research is applying antioxidant mixtures extracted from green tea on packaging material of black tea and determining the amount of anti-oxidants released from the tea bags by their immersion into boiling water. The green tea extracts prepared by a two-step extraction process in water at 50°C and 80°C in four individual time periods, i.e., 10 min, 10 min, 10 min, 10 min, 20 min, 20 min (1). Final extract from each process was analyzed by HPLC and DPPH tests and then sprayed on the tea bag packaging material. The activity and concentration of antioxidants (catechins) released from treated tea bags were investigated immediately and after 3 months storage. Sensory properties
of the tea beverage samples were also studied. The results (Tables 1, 2) show that the concentration of antioxidants increases by 66 – 76% as compared to control i.e., untreated black tea. Tea beverage samples were more astringent as compared to untreated black tea. Under different time/temperature conditions the best results were obtained by the sample extracted by a two-step process (20 min at 50 °C) and (10 min at 80 °C) with the highest concentration of antioxidants, lowest astringency and best stability during 3 months storage. Keywords: Green tea, Antioxidant activity, Catechins, Black tea bag References: 1. Labbe, D, et al. (2008) Food Chem. 111:139 – 143.

Table 1: Effect of extraction time on concentration/activity of the anti-oxidants released from treated tea bags immediately after preparation

<table>
<thead>
<tr>
<th>Extraction time (min)</th>
<th>Total antioxidant</th>
<th>Activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10,10</td>
<td>0.52 ± 0.02a</td>
<td>71.70 ± 0.55a</td>
</tr>
<tr>
<td>10,20</td>
<td>0.57 ± 0.05a</td>
<td>72.58 ± 0.57a</td>
</tr>
<tr>
<td>20,10</td>
<td>0.62 ± 0.07b</td>
<td>72.88 ± 1.26b</td>
</tr>
<tr>
<td>20,20</td>
<td>0.64 ± 0.01b</td>
<td>73.34 ± 0.35b</td>
</tr>
</tbody>
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Table 2: Effect of extraction time on concentration/activity of the anti-oxidants released from treated tea bags after 3 months storage

<table>
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<th>Extraction time (min)</th>
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<tbody>
<tr>
<td>10,10</td>
<td>0.44 ± 0.01a</td>
<td>67.70 ± 0.35a</td>
</tr>
<tr>
<td>10,20</td>
<td>0.49 ± 0.01a</td>
<td>66.67 ± 0.42a</td>
</tr>
<tr>
<td>20,10</td>
<td>0.56 ± 0.02b</td>
<td>70.54 ± 0.30b</td>
</tr>
<tr>
<td>20,20</td>
<td>0.58 ± 0.01b</td>
<td>67.83 ± 0.38b</td>
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The genus Hypericum, which comprises about 450 species of herbs, shrubs and trees, belongs to the family Clusiaceae (Guttiferae), formerly Hypericaceae. One of the topmost and commercially renowned species of the genus Hypericum is Hypericum perforatum L., commonly known as St. John’s Wort. Hypericum perforatum, is well known for its profound pharmacological activities as antidepressant, anxiolytic, antiviral, antimicrobial and wound healing. In this study, using HPLC-ED system, quantitative analysis of hypericin was carried out in different water-methanolic extracts of St. John’s Wort. Hot of water-methanol extract of St. John’s Wort was prepared. The herba (1 g) was powdered and extracted with water-methanol (1:1:1 10 mL). Afterward 1 mL of that extract was centrifuged, obtaining supernatant which was used for analysis. The standard was hypericin purchased from HWI Analytic GmbH, Germany. Mobile phase: methanol-acetonitrile, water, acetic acid (20+10+70+1), electrochemical detector with range 50 nA, potential + 0.84 V, filter, 0.02 Hz, flow rate, 1 mL/min and temperature 25 °C. Injection volume: 20 µL. Concentrations of standard were 0.1 µg/20 µL, 0.2 µg/20 µL, and 0.4 µg/20 µL. Retention time of hypericin in the standard solution and herbal extracts were 28.4 minutes. Limit of detection was 100 ng of hypericin. Amount of hypericin in extracts of St. John’s Wort was 25 – 26 mg/g dry weight. The very high content of hypericin in Bosnian St. John’s Wort give more importance to this plant as traditional medicine and best stability during 3 months storage. Keywords: Green tea, Antioxidant activity, Catechins, Black tea bag. References: 1. Labbe, D, et al. (2008) Food Chem. 111:139 – 143.

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</table>

Hops (Humulus lupulus L.) as medicinal plant is abundant in rare polyphenols. Besides so called bitter-acids important in brewing there are chalcones like xanthohumol and flavonanes. Particularly 8-prenylarabinogalactan, a flavanone is the most prominent prenylfavanone since it is known as a very potent phytoestrogen [1]. Besides 6- or 8-prenylarabinogaularabinogalactan, hop extracts yield a number of minor compounds related to each other by ring closure reactions, e.g. 6-prenylarabinogalactan is formed from desmethylxanthohumol, so are hop flavones. The potency of hop flavonoids to induce at least growth arrest in leukemic cell cultures is nearly equipotent and remarkable [2]. The question addressed in this work was if this effect would be observed even in neuronal neoplastic cells types since hop flavonoids may cross the blood brain barrier [3]. A commercial extract is available enriched in rare prenylfavanoloids and xanthohumol. It is prepared mostly from a residue of the CO₂ extraction process by solvent extraction. This material was used in a process of various chromatographic techniques to purify pairs of complementary chalcones and flavonanes with identical side chains. Xanthohumol and Isoxanthohumol predominantly represent such a pair to get some hint of a structure activity relationship. Growth inhibitors frequently induce differentiation in highly proliferating clones. Here some hop flavonoids appeared to be distinguished since they may have converted proliferation into differentiation. Acknowledgement: Wissenschaftliche Station für Brauerei in München e. V., Dr. Biendl Hallertauer Hopfenveredelungsgesellschaft m. B. References 1: Milligan SR et al. (2000) Clin Endocrin Metab 85: 4912 – 4915. 2. Diller RA et al., (2007) Planta Med 73: 755 – 761. 3. Butterweck V et al., (2007) Pharm Pharmacol 59: 549 – 552.

Evaluation of total phenolics, flavonoids and anti-inflammatory property of ethanolic extracts of Paulownia tomentosa var. tomentosa bark

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Trees containing polyphenols and flavonoids have been reported to possess strong anti-inflammatory activity [1]. As one chain of our systematically screening the potential bioactivities of Paulownia tomentosa (Thunb.) Steud. var. tomentosa (Scrophulariaceae), a medicinal hard wood grown native to China and widely used in folk remedies to treat various diseases including inflammatory [2], in the present study, we evaluated the anti-inflammatory property of P. tomentosa var. tomentosa bark. The anti-inflammatory activity was investigated by evaluating the inhibitory effect of ethanolic extracts of the tree’s bark against lipopolysaccharide (LPS)-induced nitric oxide (NO) production in murine macrophages cell line RAW264.7 [3]. Resveratrol, an excellent anti-inflammatory agent, was used as a positive control. The amount of total phenolic compounds (Approx. 180.8 mg/100 g dry sample) and total flavonoids (Approx. 39.6 mg/100 g dry sample) of bark were also determined by Folin Ciocalteu reagent and aluminium chloride (LPS)-induced nitric oxide (NO) production in murine macrophages cell line RAW264.7 [3]. Resveratrol, an excellent anti-inflammatory agent, was used as a positive control. The amount of total phenolic compounds (Approx. 39.6 mg/100 g dry sample) and total flavonoids (Approx. 39.6 mg/100 g dry sample) of bark were also determined by Folin Ciocalteu reagent and aluminium chloride method, respectively. Results of the investigation revealed that ethanolic extracts of the P. tomentosa var. tomentosa bark possessed significantly anti-inflammatory activity. Also, the activity was found to be concentration dependent. This work will provide ample opportunities for further investigation to develop high value added anti-inflammatory products from P. tomentosa var. tomentosa. Keywords: total phenolics, total flavonoids, anti-inflammatory property, Paulownia tomentosa var. tomentosa, bark. Acknowledgement: This work was financially supported by National Natural Science Foundation of China (NSFC, No. 31000279), Program for New Century Excellent Talents in University (NCTE 2010) and Natural Science Foundation of Tianjin City (No. 09JCYBJC 15800). References: 1. Pelzer LE et al. (1998) Farmaco 53: 421 – 424. 2. Si, CL et al. (2009) Holzforschung 63: 440 – 442. 3. Tewtrakul S et al. (2011) Ethnopharmacol 133: 63 – 66.
SL54

Mite growth regulatory activity of Blechnum chilense (Kaulf.) Mett
Hincapié CA1, Monsalve Z2, Parada K3, Lamilla C3, Marcon F4, Céspedes CL3
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The genus Blechnum has 13 common and well-distributed species in Chile [1]. B. chilense plants have been used for various purposes [2,3]. Tetranychus urticae Koch is highly polyphagous species with 1094 host plant species reported worldwide to date [3]. In this research, we report a phytochemical analysis of B. chilense and its mite growth regulatory effects. From the n-hexane fraction four phytoecdysones were isolated: ecdysone, ponasterone, shidasterone and 2-deoxycrustecdysone. We conducted a bioassay with T. urticae eggs placed on Phaseolus vulgaris L. leaf discs previously treated and the development stage was recorded every 24 hours during thirteen days. All treatments showed statistical significant differences regarding control in emerged and living adults. The EtOAc fraction at 250 ppm and the n-hexane fraction at 250 ppm and 100 ppm caused the greatest mortality of nymphs and almost the total mortality of the low quantity of adults that emerged (Fig.1). Our results show that even low concentrations of 10 ppm in both fractions affects the life cycle of T. urticae causing a significant decrease in its population. Studies on the identification and physiological role of ecdysteroids in mites are minimal [4], especially in the order Prostigmata. Our results suggest that early exposure of eggs and larvae to phytoecdysones from B. chilense may interfere in the natural ecdysteroid metabolism in T. urticae leading to the death of nymphs and adults. Our results suggest too that the deaths could be caused by starvation due to the deterrent effects of some phytoecdysones [5,6].

Figure 1: Percentage of T. urticae individuals in every stage treated with different fractions of B. chilense.

Keywords: Blechnum chilense, phytoecdysones, Tetranychus urticae

SL55

New rare atropisomers: structure elucidation, absolute configuration and antimicrobial activity
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Fungi are very known to produce polyketides, which are structurally a very diverse family of natural products with interesting biological activities and pharmacological properties. We report in this chemical investigation of two endophytic fungi, Stemphylium globuliferum and Talaromyces wortmanni. Several rare new atropisomers were isolated and identified, including homo- and heterodimeric bisantraquinones. The structure of isolated compounds were determined on the basis of one- and two-dimensional NMR spectroscopy and mass spectrometry. The absolute stereochemistry of the new compounds was established by means of TDDFT ECD calculations. Furthermore, the isolated compounds exhibited antibacterial activity against multi drug resistant strains of Staphylococcus aureus, Streptococcus pneumoniae, Enterococcus faecium and Enterococcus cloacae. In addition, the anti-fungal activity of the isolated compounds was measured against drug resistant strains of Aspergillus fumigatus, Aspergillus faeacalis, Candida albicans and Candida krasei. Interestingly, among the compounds isolated, only the new altersolanol N, tetrahydroaltersolanol B and altersolanol C were active against HV2 and HV8 human viruses. Keywords: Endophytes, Atropisomers, Spectroscopy, CD calculation, Antimicrobial activity

SL56

Advances of Infrared Spectroscopic Imaging and Mapping Technologies of Plant Material
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Fourier Transform Infrared (FTIR) spectroscopic imaging and mapping techniques have become essential tools for the detection and characterization of the molecular components of biological tissues [1]. These modern analytical techniques enable high-resolution molecular imaging of complex botanical samples [2]. Imaging and mapping are based on the absorption of IR radiations by vibrational transitions in covalent bonds and their major advantage is the acquisition of high spatial resolution molecular imaging of complex botanical samples [2]. Imaging and mapping are based on the absorption of IR radiations by vibrational transitions in covalent bonds and their major advantage is the acquisition of local molecular expression profiles, while maintaining the topographic integrity of the tissue by avoiding time-consuming extraction, purification and separation steps. All IR related information is recorded in a so-called “hyper-spectral cube” from which any ingredient relevant information can be selectively extracted with a resolution of 4 μm and transformed into an image as depicted for e.g. Urtica dioica in Figure 1. These new techniques enable global analysis of biological samples with highest spatial resolution and provide unique chemical-morphological information about the tissue status. With these non-destructive examination methods it is possible to get qualitative and quantitative information of heterogeneous samples. In this presentation recent applications of infrared spectroscopic imaging and mapping techniques of plant material are introduced and discussed. Keywords: Phytochem, tissue, instrumentation, data processing, transmission, reflection, hyper spectral imaging
Dipterocarpus vortex is a species of tree found in the family Dipterocarpaceae. The bark and leaves of this plant are used in traditional medicine, particularly for treating stomachache and fever. The fruit of this plant is rich in phenolic compounds, which are known for their antioxidant and anti-inflammatory properties.

**Figure 1:** Model of hyperbolic cube

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**References:**
- University Malaya Publication, Kuala Lumpur, pp. 1 – 356.

**SL58**

**SL58**

Potent inhibitory effects of anthraquinone compounds from Morinda officinalis on in vitro osteoclastic bone resorption

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The root of Morinda officinalis F.C. How has been shown to exert protective effects against bone loss in sciotic neuritis and ovariectomy rats [1 – 3], and anthraquinones from this plant may contribute to this activity [4]. In the present study, we investigated the effects of 1, 3, 8-trihydroxy-2-methoxy-anthaquinone (1), 2-hydroxy-1-methoxy-anthaquinone (2), and rubiadin (3) from the plant in vivo bone resorbing activity and mechanism. In the coculture system of osteoblast and bone marrow cells, the compound 1, 2 and 3 decreased the formation of bone resorption pit, the number of multinucleated osteoclasts, tartrate resistant acid phosphates and cathepsin K activity of osteoclasts within the dose range of 0.1–10 μmol/L (P < 0.01). Further, the compound 1, 2 and 3 at concentration of 1 μmol/L improved the ratio of mRNA and protein expression of OPG and RANKL in osteoblasts (P < 0.01 for compound 1 and 2; P < 0.001 for compound 3). In osteoclasts induced from bone marrow cells with MCSF and RANKL, the compound 1, 2 and 3 at concentration of 1 μmol/L enhanced the apoptosis of osteoclast (P < 0.01), disturbed the JNK and NF-κB signal pathway (P < 0.01), reduced the expression of calcitomin receptor (P < 0.05 for compound 1; P < 0.01 for compound 2 and 3) and carbonic anhydrate II (P < 0.05 for compound 1 and 3; P < 0.01 for compound 2). Therefore, the findings of the present study demonstrate that anthraquinones from Morinda officinalis is an inhibitor of bone resorption, and potentially explains some of the reported inhibitory effects on bone loss. Keywords: Morinda officinalis, anthraquinone, osteoclast, bone resorption, apoptosis

DIPA Grant from the Southeast Asian Regional Centre for Tropical Biology (SEAMEO-BIOTROP), Bogor, Indonesia.

Cimicifuga racemosa, osteoarthritis, osteoporosis

Keywords: (SEAMEO-BIOTROP), Bogor, Indonesia.

DIPA Grant from the Southeast Asian Regional Centre for Tropical Biology and in other countries.

ican Herbal Pharmacopoeia from information supplied by botanical inducts. This presentation reviews many of these quality control chal郡ious and illegal addition of active pharmaceutical ingredients (con-
tivated adulteration (EMA) – as well as the “spiking” of extracts with the disturbing trend of intentional adulteration – economically moti-
control measures, etc. Also, there have been persistent cases of inadver-
ical materials due to nomenclatural confusion, lack of adequate quality
diligence for raw material suppliers and manufacturers. In recent years
areas. Chemical complexity of botanicals requires added quality control

The antimicrobial activity of honey in relation to the composition of pollen (Bosnia-Herzegovina, W. Balkan)

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Honey has significant antimicrobial activity (AMA)[1, 2]. As the quality of honey depends on the composition of pollen, and it can expect a different AMA. Honey samples were collected from 12 different locations in the continental part of Bosnia. Honey samples were taken at the end of the season (September – October 2006.). Microscopic pre-
parations were made for the standard method of pollen analysis. For every sample 300 pollen grains were counted. Each pollen grain has been determined. Antimicrobial activity was tested by diffusion method at Muller-Hilton agar. For that clinical isolates of Staphylococcus aureus, Escherichia coli and Pseudomonas sp. were used. On the edge of prepared pools from strains of microorganisms placed 200 μl prepared honey. All 12 samples of honey caused high inhibition zone for Staphylococcus aureus (11 – 21 mm), eight samples of honey caused growth inhibition of Escherichia coli (11 – 13 mm) and three samples of honey caused a zone of inhibition at Pseudomonas sp. (12 mm). The highest antimicro-

Economically Motivated Adulteration of Botanical Raw Materials, Herbal Extracts, and Essential Oils in the Global Marketplace

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The trade of botanical ingredients for the production of herbal drugs and phytomedicines, dietary supplements, and natural cosmetics is global, with supply and quality issues in one geographical region affecting other areas. Chemical complexity of botanicals requires added quality control diligence for raw material suppliers and manufacturers. In recent years there have been numerous cases of accidental misidentification of bot-a
nical materials due to nomenclatural confusion, lack of adequate quality control measures, etc. Also, there have been persistent cases of inadvert-
tent contamination with heavy metals, agricultural chemicals, excessive microbial load, excessive solvent levels in extracts, etc. But there is also the disturbing trend of intentional adulteration = economically moti-
vated adulteration (EMA) – as well as the “spiking” of extracts with undisclosed lower-quality and lower-cost ingredients. This includes the spurious and illegal addition of active pharmaceutical ingredients (con-
tentional pharmaceutical drugs), e.g., sildenafil in dietary supplement products for erectile dysfunction and sibutramine in weight-loss pro-
ducts. This presentation reviews many of these quality control chal-

S.P. presents immunity-stimulatory effects of Aloe vera extracts on both chicken and fish. The main advantages of using the bioactive compound of Aloe vera are its immuno-stimulatory effect and its role in the protection of the host against different pathogens. The study was carried out on two species of fish (S. aurata and S. aurata) kept in controlled conditions. Aloe vera extracts were used as antigens and their effect on the immunity of the fish was investigated. The results showed that Aloe vera extracts had a significant effect on the immunity of the fish. The extracts were able to stimulate the production of immunoglobulins in the fish, which is an important factor in the protection of the host against different pathogens. The results showed that Aloe vera extracts had a significant effect on the immunity of the fish.

Immunostimulatory and protective effects of Aloe vera components against coccidiosis in broilers

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Present study reports immunostimulatory effect of Aloe vera L. extracts in chickens and their protection against coccidiosis. Study was divided into two experiments. Experiment-I was conducted for evaluation of immunostimulatory activity of Aloe vera extracts and experiment-II for protective efficacy against coccidiosis. Results of experiment-I revealed significantly higher (P<0.05) lymphoproliferative responses in chickens administered with ethanolic extract as compared to chickens adminis-
tered aqueous extract and control group. Microplate haemagglutination assay for humoral response on day 7th and 14th post primary and sec-
dary injections of sheep red blood cells (SRBC) revealed significantly higher (P<0.05) anti SRBC antibody (total Ig, IgG and IgM) titers in experimental groups as compared to control; although both the Aloe vera extracts showed no significant effects on the development of lympho-
organs. Results of experiment-II revealed maximum percent pro-	ection (60%) in chickens administered with aqueous extract as compared to ethanolic extract administered chickens (45%). Mean oocysts per gram of droppings in control group was significantly higher (P<0.05) as compared to chickens in both experimental groups. Chick-
en administered with aqueous extract (40%) showed severe lesions (3.0 – 4.0); whereas 55 and 75 percent severe lesions were recorded in ethanolic extract administered and control chickens, respectively. Daily weight gains from day 3rd-12th post-challenge were significantly high-
(P<0.05) in chickens administered with aqueous extract as compared to those administered with ethanolic extract and control. It was con-
cluded that Aloe vera may be potential and valuable candidate to stimu-
late the immune responses and can be used successfully in immunosup-
Efector of nutrient media strength on cardioactive glycoside accumulation in Digitalis lamarckii Ivan, an endemic medicinal species

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The effects of different strengths of Murashige and Skoog (MS) (1) medium on cardioactive glycoside accumulation in Digitalis lamarckii Ivan were investigated. D. lamarckii, commonly known as dwarf foxglove (yi卢sükotu in Turkish), is an Turkish endemic medicinal plant and belongs to the family of Plantaginaceae. The Digitalis species are biennial or perennial herbs containing important cardioactive compounds (glycosides) that are used to treat heart problems. D. lamarckii has been marked as valuable (VU) in the Red Data Book of Turkish Plants (2). Contents of five different cardenolides (namely, digoxigenin, gitoxigenin, lanatoside C, digoxin and digoxigenin, lanatoside C, digoxin and digitoxin) in the callus developed from hypoxyl explants, which were cultured for 10 days on different strengths of MS media supplemented with 33 sucrose, 0.5 ppm TDZ and 0.25 ppm IAA, were determined by HPLC. Three different strengths of MS media were tested: quarter-, half- and full-strength. Of the five cardenolides, only lanatoside C could be detected in the callus cultured on all medium strengths. The highest concentration of lanatoside C was observed on half-strength MS medium (690µg/g dry weight, dw), while the callus cultured on full- and quarter-strength media producing 231 and 332µg/g dw lanatoside C, respectively. In conclusion, the protocol described here is expected to have an important contribution to the future efforts for a large scale production of cardenolides in Digitalis species. Keywords: Cardenolide accumulation, Digitalis lamarckii, medium strength, Murashige and Skoog basal medium strength. References: 1. Murashige T, Skoog F (1962) Physiol Plantarum 15: 473; 2. Ekim T, Koyuncu M, Vural M, Duman H, Aytaç Z, Adıgüzel N (2000) Türkiye Bitkileri Kirmızı Kitabı. University Press, Ankara, Turkey

A Phase IIA and IIB clinical trial of a quantified extract of Nauclea poglobuinii stem bark against uncomplicated falciparum malaria

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In a clinical study of type IIA, the quantified 80% ethanol extract from the stem bark of Nauclea poglobuinii (Pobég.) E.M.A. Petit containing 5.6% strictosamide appeared to be effective in the treatment of uncomplicated falciparum malaria when taken for seven days, at a dose regimen of two 500 mg capsules three times daily for 3 days, followed by one 500 mg capsule three times daily for four days. No serious side effects were noted. The Phase IIA clinical trial was carried out according to the WHO 14 days test (WHO 2003) and the results revealed that all eleven patients were completely cleared of parasitaemia and fever on days 3, 7 and 14, except for one patient. In a Phase IIB study designed as a single blind prospective trial in 65 patients with proven P. falciparum malaria, the efficacy and safety of the extract was compared with an artesunate–amodiaquine (AS + AQ) combination as the second arm. Evaluated on the WHO criteria (WHO 2003), both treatments were effective in the treatment of uncomplicated malaria. AS + AQ appeared to be slightly more effective while the extract was better tolerated. All these results suggest that the quantified 80% ethanol extract from the stem bark of Nauclea poglobuinii should be considered as a candidate for a clinical trial Phase III. Keywords: Nauclea poglobuinii, quantified extract, clinical trial IIA, clinical trial IIB, malaria

Caribenolide revisited. Reisolation of caribenolide-I together with new congeners

Yamaguchi N, Sata T, Kobayashi J

The Cimicifuga racemosa (L.) Nutt. (CR) extract BNO 1055 and thereof purified 2 fractions have cartilage protective effects and prevent accumulation of fat tissue in ovarietomized (ovx) rats

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Estrogens exert beneficial effects in the bone, joint cartilage and prevent obesity. Their use however, bears several risks. Therefore, a search for non-estrogenic alternatives is going on. We showed previously that CR BNO 1055 has bone protective effects. Whether cartilage tissue is also protected and whether this can be attributed to less obesity was not yet studied and was a scope of the present study. Both groups were administrated orally over a period of 4 weeks with CR BNO 1055 and 2 thereof purified fractions (S and R) and estradiol (E2) fed animals served as additional controls. The thickness of the knee joint cartilage layer as well as the size of the Hoffa’s fat pad (a fat pad within the knee joint) was determined histomorphometrically. Thickness of the knee joint cartilage was lowest in the ox, highest in E2 and at intermediate values in the CR and S treated animals. The size of Hoffa’s fat pad was smallest in E2 and largest in ox animals and an intermediate size was determined for the CR and S treated animals. It is concluded that E2, CR BNO 1055 and S have chondroprotective effects which correlate with the size of Hoffa’s fat pad. The adipocytes in this fat pad are known to secrete cytokotoxic cytokines. Therefore, the lower size of the fat pad in E2 and CR treated animals may have resulted in less cytokine production with less chondrotrophic effects.

References: 1. Shi-mizu and coworkers. The structure was interpreted to be a 26-membered macrolide containing a 6-membered hemiacetal ring, a tetrahydropurpur ring, an epoxide, a ketone carbonyl, four C branches, and five hydroxyl groups. On the other hand, Kobayashi et al. reported the isolation of amphidinolide N [2] isolated from a symbiotic dinoflagellate Amphidinium species earlier than the report of caribenolide-I. The structure of amphidinolide N was elucidated to be the ring-opening form at the C-21-C-24 tetrahydrofuran ring, an epoxide, a ketone carbonyl, four C branches, and five hydroxyl groups. On the other hand, Kobayashi et al. reported the isolation of amphidinolide N [2] isolated from a symbiotic dinoflagellate Amphidinium species earlier than the report of caribenolide-I. The structure of amphidinolide N was elucidated to be the ring-opening form at the C-21-C-24 tetrahydrofuran ring for caribenolide-I. Caribenolide-I was reported to exhibit strong cytotoxic activity against tumor cell lines and in vivo antitumor activity. Amphidinolide N also showed extremely potent cytotoxic activity. Caribenolide-I as well as amphidinolide N would therefore also appear to be a promising anticancer therapeutic lead. Nevertheless, the scarcity of materials has prevented more detailed studies. Because stereochemistries of caribenolide-I and amphidinolide N have not determined yet, it is difficult to supply the sample by synthesis. In our investigation for anticancer drug leads from the Amphidinium dinoflagellates, we have isolated caribenolide-I together a three new caribenolide-I congeners from two benthic Amphidinium strains collected off Immunoe Island, Japan. In this symposium, we will discuss the isolation of these four compounds, structural relationship between caribenolide-I and amphidinolide N, and structure elucidation of these new compounds. References: 1. Bauer I, Maranda Y, Young K A, Shimizu Y, Fairchild C, Cornell L, MacBeth J, Huang S (1995) J Org Chem 60: 1084 – 1086. 2. Ishibashi M, Yamaguchi N, Sata T, Kobayashi J (1994) J Chem Soc, Chem Commun,1445 – 1446.
**Artemisia annua**: A New Medicinal Plant in the Egyptian Cultivation as a Source for Artemisinin

This study aimed to introduce *Artemisia annua* L. plant to the Egyptian cultivation and to achieve the technological package for its production under Egyptian conditions. The seeds were introduced from Germany and propagated. Several experiments were carried out during two successive seasons in different locations (clay loamy soil and sandy loam soil). The first experiment aimed to study the effect seasonal variation on growth, yield, essential oil and chemical composition using organic farming system under the Egyptian conditions. The essential oil content and essential oil yield of *A. annua* significantly increased with increasing plant age to reach their maximum values after 180 days after transplanting. The second experiment was carried out in loamy clay soil to study the effect of the mineral fertilization on the growth, yield and the active constituents of *A. annua*. The highest value of artemisinin was obtained from plants treated with 75 kg N/fed + 50 kg K/fed. The third experiment was carried out in loamy clay soil to study the effect of organic fertilizer and/or biofertilizer on the growth and active constituents of *A. annua*. The highest yield of Artemisinin was obtained from plants treated with 30 m³ compost/fed. without biofertilizer followed by the application of 30 m³ compost/fed. with biofertilizer. The fourth experiment was carried out to study the effect of soil type on the growth and active constituents of *A. annua*. Plants grown in sandy soil gave a positive increase in the essential oil yield and artemisinin content and yield. Feddan = 4200 m²

**Production and use of Artemisia annua (sweet wormwood)** against bacterial diseases in poultry stocks and its effect on food quality

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Necrotic enteritis (NE) in broilers is caused by *Clostridium perfringens* type A (CP) resulting in severe production losses and mortality. Present preventive treatments include the dietary addition of ionophores which may be banned in the EU before long. The plant *Artemisia annua* L. (AA) produces antimicrobial essential oil components (EOCs) [1] that could substitute the use of these antibiotics in poultry production. A study focused on improving the production of bioactive EOCs in AA by the application of 30 m³ compost/fed. The hexane extract containing EOCs showed the strongest inhibition (MIC = 170 ppm) confirming the potential use of AA EOCs as antimicrobial agents. This extract was incorporated in the diet of broilers applying a NE disease model. The treatment reduced the population of CP and the severity of the associated small intestinal lesions (p < 0.05). Furthermore, CP infected broilers fed the diet supplemented with AA hexane extract gained more weight than the control animals (p < 0.05). Healthy broilers were fed diets supplemented with dried AA material to ascertain that the palatability of the meat is not affected. Breast filets evaluated by a descriptive sensory analysis did not show any effect of the treatment on meat flavour/taste nor texture or appearance. Hence, AA extracts show promising results as antimicrobial additives in poultry diets. Keywords: *Artemisia annua*, necrotic enteritis, *Clostridium perfringens*, essential oil components, sensory analysis


**Artemisinin** is a sesquiterpene lactone isolated from *Artemisia annua* having an unusual endoperoxyl moiety which is essential for the activity when activated by iron [1]. In addition to its well established antimicrobial properties artemisinin has potent anticancer activities in a variety of human cancer cell types [2]. The cytotoxic effect of artemisinin is specific to cancer cells because transferrin-receptors are highly expressed on the surfaces of tumour cells and iron content is higher than in normal cells [3]. The aim of our work was to test the cytotoxic effect and stealth liposomes for passive targeting and transferrin-conjugated liposomes for active targeting loaded with artemisinin. Multilamellar vesicles were prepared according to the film hydration method; in order to reduce the dimensions of the vesicles, an high pressure homogenizer Emulsiflex C3® was used. Conventional, stealth and targeted liposomes were fully characterized by particle size, zeta potential, Pdl, drug entrapment efficiency and transmission electron microscopy. Coupling of transferrin to the targeted liposomes was obtained by amide bond between Tf and lipid linker and the average amount of transferrin conjugated to the liposome was quantified with bichonichonic acid (BCA). A preliminary study about the cellular uptake of conventional liposomes loaded with fluorescein sodium salt has been performed in K562 cells using flow cytometry analysis and fluorescence microscopy; the highest internalization of fluorescein sodium salt loaded liposomes was after 60 minutes of exposure. References: 1. Nakase I et al. (2008) Int J Pharm 354: 28 – 33. 2. Firestone GL et al. (2009) Expert Rev Mol Med 11: e32. 3. Effrert T et al. (2004) Free Radic Biol Med 37: 998 – 1009.

**Conventional, stealth and transferrin-conjugated liposomes for artemisinin delivery to cancer cells**

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Artemisinin is a sesquiterpene lactone isolated from *Artemisia annua* having an unusual endoperoxyl moiety which is essential for the activity when activated by iron [1]. In addition to its well established antimicrobial and antimalarial properties* artemisinin has potent anticancer activities in a variety of human cancer cell types [2]. The cytotoxic effect of artemisinin is specific to cancer cells because transferrin-receptors are highly expressed on the surfaces of tumour cells and iron content is higher than in normal cells [3]. The aim of our work was to test the cytotoxic effect and stealth liposomes for passive targeting and transferrin-conjugated liposomes for active targeting loaded with artemisinin. Multilamellar vesicles were prepared according to the film hydration method; in order to reduce the dimensions of the vesicles, an high pressure homogenizer Emulsiflex C3® was used. Conventional, stealth and targeted liposomes were fully characterized by particle size, zeta potential, Pdl, drug entrapment efficiency and transmission electron microscopy. Coupling of transferrin to the targeted liposomes was obtained by amide bond between Tf and lipid linker and the average amount of transferrin conjugated to the liposome was quantified with bichonichonic acid (BCA). A preliminary study about the cellular uptake of conventional liposomes loaded with fluorescein sodium salt has been performed in K562 cells using flow cytometry analysis and fluorescence microscopy; the highest internalization of fluorescein sodium salt loaded liposomes was after 60 minutes of exposure. References: 1. Nakase I et al. (2008) Int J Pharm 354: 28 – 33. 2. Firestone GL et al. (2009) Expert Rev Mol Med 11: e32. 3. Effrert T et al. (2004) Free Radic Biol Med 37: 998 – 1009.

**Biological and chemical study of two Indonesian marine endophytic fungi**

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In the search for biologically active natural products from Indonesian marine sources two strains of marine endophytic fungi could be isolated from the marine red alga *Kappaphycus alvarezii*. One strain (KT 30) could be identified as *Xylaria psidii*, the other one (KT 31) remains sterile and could not be identified till now. Ethylacacetate extracts from the culture medium displayed considerable cytotoxic activity against a urinary bladder carcinoma cell line with IC₅₀ values of 4 and 1.5 µg/ml respectively. Both strains were also obviously active to inhibit the growth of fish and human pathogenic microorganisms. Most remarkable is the strong antimicrobial activity of ethylacetate extracts against the gram-negative bacteria *Pseudomonas aeruginosa*, *Escherichia coli*, *Vibrio angillarum* and *Aeromonas salmonicida*. A new cyanomethoxy benzoic acid derivative was isolated from X. psidii KT30 and a new quinone derivative from two Indonesian marine fungi, algae and microorganisms. The new compound was characterized as 1,2,4-trihydroxy-3-[5-(quinoxalin-2-yl)ethoxy]benzoic acid. This compound showed cytostatic effects on the HT29 and the colon cancer cell line. In addition, it inhibited the growth of the gram-negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli* and the gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*.

Keywords: Indonesian marine fungi, algicolous, antifungal, cytotoxic Acknowledgement: We thank PD Dr. Marc Stadler (InterMedDiscovery, Dortmund, Germany) for identification of the *Xylaria* species.
Proanthocyanidins (PCs) are some of the most abundant polyphenolic substances in the plant kingdom. OPCs are an integral part of the human diet, found in high concentrations in fruits such as apples, pear, tea, hawthorn, grapes, and in chocolate. Due to potent antioxidant activity, PCs have been the subject of recent research, demonstrating anticarcinogenic, anti-inflammatory, antimicrobial, and vasodilatory properties, making them a potentially valuable therapeutic tool for the treatment of a variety of conditions. PCs are present in plants as complex mixtures of polymers with an average degree of polymerization between 4 and 11, usually in association with their composing flavan-3-ols. Structural diversity is possible by variation in hydroxylation pattern, stereochemistry at the three chiral centers, and the location and type of interflavan linkage. The most frequent basic units of proanthocyanidins are derivatives of flavan-3-ols: (+)-catechin, (-)-epicatechin, (+)-gallocatechin, (-)-epigallocatechin (EGC) and (-)-epigallocatechin gallate (EGCG). PCs, naturally occurring antioxidants widely available in fruits, vegetables, nuts, seeds, flowers and bark, have been reported to possess a broad spectrum of biological, pharmacological and therapeutic activities against free radicals and oxidative stress. Epicatechin, dimeric procyanidin B2 and B5, proanthocyanidin A2 and trimeric procyanidin C1 of *Crataegus sinaica* Boiss. and/or *Adansonia digitata* L. display potent antioxidant antiviral properties in vitro. References: 1. De Bruyne T, et al. (1999) Biochem Syst Ecol 27: 445. 2. Shahat FM, et al. (1996) Planta Med 62(1): 10 – 13. 3. Shahat A et al. (2002) Planta Med 68: 539 – 541. 4. Shahat A, Ahmed H, Hassan R, Hussein A (2008) Asian Pacific Journal of Tropical Medicine (Asian Pac J Trop Med) 1(3): 55 – 59

Over the past years, marine microorganisms have proven to be a prolific source of structurally interesting and biologically active natural products. Marine fungi in particular have attracted considerable interest due to the diversity in chemical structures and biological activities observed for their secondary metabolites. Chemical investigation of the crude extract obtained from the sponge-associated fungus *Aspergillus* sp., isolated from a specimen of the Mediterranean sponge *Tethya aurantium*, yielded new meroterpenoid metabolites of the austalide type, as well as new tryptoquivaline and fumiquinazoline alkaloids, in addition to several known compounds. The structures of the new compounds were unambiguously elucidated on the basis of extensive one- and two-dimensional NMR (1H, 13C, DEPT, COSY, HMQC, HMBC, and ROESY spectra) and mass spectral analysis. The absolute configurations of the new compounds were established by means of TDDFT ECD calculations. All compounds were evaluated for their cytotoxic activity by the MTT method against the murine cancer cell line L5178Y, as well as the human cancer cell lines K562, A2780, and A2780 cisR, where some of the isolated compounds exhibited moderate to pronounced cytotoxicity. Keywords: Aspergillus, marine fungi, Structure elucidation, absolute configuration, cytotoxicity
Phytosterols (PSs) are a group of naturally occurring steroid alcohols found in plants. There is considerable interest in PSs as dietary supplements as they are reported to lower cholesterol levels and also have a positive impact on cardiovascular diseases. However, recent research suggests that PS supplementation may aggravate atherosclerosis and lead to aortic valve stenosis. PSs are typically measured by gas chromatography (GC), but this approach is time-consuming since it requires saponification of the sample, several extractions, and derivatization. We developed a simplified method using reversed-phase, HPLC and charged aerosol detection (CAD). CAD is sensitive, has a dynamic range of >4 orders of magnitude, can measure any non-volatile species, and analytes show similar response independent of their chemical structure. Samples were prepared by simple dilution prior to analysis. Five PSs, campesterol, cholesterol, stigmasterol, beta-sitosterol, and stigmastanol, were resolved in <35 min. Calibration curves showed linear correlation coefficients >0.997. The LOD was <5 ng (on column). Analysis of red palm oil is used as an example. The method is simple to use, has good linearity and sensitivity, and is capable of measuring numerous PSs in plant extracts. This approach can be used to examine product purity, supplement content, and adulteration.

PA3 Simple and Direct Analysis of Falcarniol and other polyacetylenic Oxylipins in Carrots by Reversed Phase HPLC and Charged Aerosol Detection

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Food plants in the Apiaceae (formerly Umbelliferae) family (e.g., carrots, parsley and celery) contain a group of bioactive C17-polyacetylene compounds, sometimes referred to as the polyacetylenic oxylipins. These compounds have been shown to be highly toxic towards bacteria and fungi and to exhibit a diverse range of biological activities in mammals, with both beneficial (e.g., their cytotoxicity is proposed to reduce the risk of developing cancer) and detrimental (e.g., occupational allergic contact dermatitis). Three such compounds, falcarniol, falcarkinol and falcarindiol-3-acetate, natural pesticides produced by carrots in response to fungal diseases, have recently garnered a lot of media attention. Although falcarniols have a distinctive UV spectrum, the consequence of conjugated triple bonds, sensitivity tends to be poor due to the actual number of unsaturated bonds present in their structure. Measurement at 205 nm offers the best sensitivity; however, sample chromatograms tend to be very complicated due to the presence of many other compounds absorbing at this wavelength. Charged aerosol detection (CAD) is a “universal”, mass-based detector and offers excellent sensitivity, a wide dynamic range, and the advantage that all non-volatile analytes produce similar response, independent of chemical structure. Additionally, unlike UV detection, analytes need not possess a chromophore in order to be determined. We developed a simple reversed-phase HPLC-CAD method to rapidly screen for falcarniol, falcarkinol, and falcarindiol-3-acetate. The method was sensitive (LOQ ~5 ng on column) and reproducible, and the analysis was completed in 15 mins. Data from fresh, baby carrots and Queen Anne’s Lace (root, leaf, and flower) are presented.

PA4 Sensitive Analysis of Commonly Used Artificial and Natural Sweeteners Including Stevia and Their Impurities and Degradation Products

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Many of the recently commercialized sweeteners have increased potency, and therefore the amount of the active ingredient added to beverages and other food products is reduced. This is problematic. But, this has contributed to a need for sensitive analytical methods to quantify the active product and detect low levels of breakdown products and impurities. Such product characterization is required for quality and safety issues. Traditional HPLC-UV approaches are inappropriate as these compounds typically do not possess any chromophore. This work describes a number of HPLC-CAD methods that can be used to study common natural sugars (fructose, glucose, turanose, saccharose, trehalose, maltose, melezitose, and raffinose); artificial sweeteners (sucralose, aspartame, saccharin, and acesulfame K); and newly introduced products containing Stevia extracts (rebahudioside A and stevioside). These methods provide sensitivity at low (ng) levels with good reproducibility and accuracy, and correlation to the component concentrations. Stevia products were analyzed by Charged Aerosol Detection and UV; the CAD showed a greater than fivefold improvement in sensitivity over UV for all major components. Finally, the UHPLC methods developed showed a decreased run-time and an increased sensitivity for glucose, lactose, and sucrose. Typical limits of detection were found to be <500 pg (on column) for glucose and other mono- and disaccharides. HPLC-CAD is a very flexible approach to measuring sweeteners and overcomes many of the limitations of UV, RI, LC-MS, ELSD, and HPLC-pulsed amperometric approaches.
of this detector is illustrated using a variety of examples including: a global gradient method for determination of FSUs and FSAs in plasma; a gradient method for the analysis of carotenoid isomers in tomato; an isocratic method for the measurement of tocopherol and tocotrienol isomers in palm oil; and an isocratic method for the determination reduced and oxidized CoQ 9 and CoQ 10 in human plasma.

Iridoid and flavonoid patterns of the genus Veronica sect. Alisine subsect. Agrestis (Benth.) Stroh (Lamiaceae) and their systematic status

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In most taxonomic schemes Agrestis is considered as a subsection of the genus Veronica (1,2). The distribution of two iridoid and six flavonoid compounds in four Veronica L. Sect. Alisine subsect. Agrestis species (23 samples) from Iranian Natural populations was investigated. Veronica franciscpetea M. A. Fisch. and V. siaretenis E. Leh. were studied for these compounds for the first time. The iridoid and flavonoid patterns showed a good correlation with morphological and chemical features of these taxa. The studied species are closest together according to the flavonoid patterns: species containing quercetin derivatives (V. Persica Poir., V. polia Fr.) and species containing quercetin (V. franciscpetea, V. siaretenis). V. persica and V. polia are generally related in their morphology, however, V. persica can be distinguished from V. polia due to the occurrence of 6-0-isovanilloylcatap迟到. V. persica is an aggressive tetraploid species. Different opinions exist regarding its origin as an autopolyploid from V. polia (3). Acknowledgement: This research was supported by the project of the Ganjali University. References: 1. F. MA, Peev D (1995) Genus Pseudolysimachion Opiz. In: Kozhuharov St., supported by the project of the Guilan University.

Seed and mucilage yield of isabgol (Plantago ovata Forsk.) under salinity stress

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Salinity may adversely reduce the overall productivity of plants by inducing numerous abnormal morphological, physiological and biochemical changes. An experiment was conducted in 2010 in the Greenhouse of the University of Tabriz, to investigate isabgol (Plantago ovata Foks) performance under non-saline (control) and three saline conditions (4, 8, 12 dS/m NaCl). The experiment was arranged as completely randomized block design with three replications. Ten seeds were sown 1 cm deep in each pot filled with 800 g perlite. Salinity treatments were applied immediately after sowing. Tap water and saline solutions were added to the pots in accordance with the treatments to achieve 100% FC. After emergence, seedlings were thinned to keep four plants in each pot. During the growth period, the pots were weighed and the losses were made up with Hoagland solution. At maturity, plants from each pot were harvested and seed yield per plant was determined. Means of seed and mucilage yields per plant decreased with increasing salinity. However, seed yield per plant under 0 and 4 dS/m salinity were statistically similar. Mucilage percentage was not significantly affected by salinity stress. Thus, reduction in mucilage yield was attributed to deductions in seed yield per plant under high salinity treatments.

Composition of polysaccharides from aqueous extracts of some wound healing plants

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Plant polysaccharides represent ideal candidates for therapeutics with immunomodulatory and wound healing actions. Polysaccharides from several medicinal plants have been shown to exhibit immunomodulatory activities [1 – 2] and stimulate proliferation of keratinocytes and dermal fibroblasts [3 – 6]. In most cases, hot water extracts containing the water-soluble polysaccharides are used for the treatment of wounds [7] and also the isolated pure polysaccharides have been shown to exhibit immunomodulatory and wound healing activities [8]. AQuim has been to determine the monosaccharide composition of polysaccharides from aqueous extracts of selected medicinal plants traditionally used in Western Africa as wound healing agents [7] and to identify compounds which possess skin cell-promoting activities under in vitro conditions. Raw polysaccharides (RPS) were isolated from aqueous extracts of the selected plants by ETOH precipitation and dialysis (MWCO 3.5 kDa). TFA 2N hydrolyzed samples were analyzed concerning monosaccharide composition by TLC and HPLC-PAD (Carbo PacTM PA1 stationary phase). 3 of 11 plants contained substantial amounts (>3%) of cold-water soluble mucilages. RPS from 3 plants were characterized by high fucose contents, a polysaccharide normally not forming a big part of polysaccharides from higher plants. The high amount of galactose (32%) and arabinose (30%) in the hydrolyzed RPS of Parquejia nigrescens (Azel) Bullock probably gives an indication of presence of arabinogalactans. Glucan structure is reasonable for polysaccharides from Alstonia boonei De Wild. Further in vitro studies on influence of polysaccharides on skin cell physiology have to be initiated to establish exact structure-activity relationship. Acknowledgement: The authors thank Deutscher Akademischer Austausch Dienst (DAAD) for the fellowship awarded to C. Agaye. References: 1. Diao et al. (2003) J Ethnopharmacol 84: 279 – 287. 2. Ingjerdingen et al. (2006) Biomacromolecules 7: 48 – 53. 3. Deters et al. (2010) J Ethnopharmacol 127: 62 – 69. 4. Deters et al. (2008) J Pharm Pharmacol 60(2): 197 – 204. 5. Deters et al. (2005) J Ethnopharmacol 102(3): 391 – 399. 6. Deters et al. (2005) J Cell Physiol 202(3): 717 – 722. 7. Agaye et al. (2009) J Ethnopharmacol 125(3): 393 – 403.

Seasonal variation of kaurna-type diterpenes and cinamic acid derivatives in leaves of Mikania laevigata and Mikania glomerata cultivated under different shading conditions

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Mikania glomerata Spreng. and Mikania laevigata Sch.Bip. ex Baker are medicinal plants popularly named ‘guaco’, whose leaves are used to treat respiratory diseases, with coumarin (1) and kaurna-type diterpenes regarded as the bioactive constituents. The goal of the study was to undertake seasonal studies on the contents of chemical markers in leaves under different shading conditions. Species were cultivated under different levels of solar radiation and full sunlight. The leaves were collected in the middle of each season year. The contents of 1, o-coumaric (2), benzoylgrandifloric (3), cinamoylgrandifloric (4) and kaurnoic (5) acids were quantified in dried leaves of both species by RP-HPLC [1]. Significant differences were found in the contents of cinamic acid derivatives (1 and 2) and kaurna-type diterpenes (3, 4 and 5) for the evaluated harvesting periods and cultivation environments. o-Coumaric acid was solely detected in M. laevigata in concentrations bellow the limit of quantification (<0.045%), in plants under 80% shading, collected in the autumn. Both 1 and 2 were not detected in the analyzed samples of M. glomerata. The average concentration of coumarin reached its maximum (0.94%) in the summer, in plants growing under 80% shading. In general, both species presented higher amounts of the kaurna-type diterpenes in plants cultivated under sunlight, except for 3 in M. glomerata. Altogether, the obtained results point out that the highest content of coumarin is reached in M. laevigata cultivated under 80% shading, preferentially harvested in the summer, but with reduced levels.

PA10

HPLC-DAD analysis of chemical markers in leaves of Mikania laevigata and Mikania glomerata submitted to long-term storage

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Mikania laevigata Sch. Bip. ex Baker and Mikania glomerata Spreng., known in Brazil as guaco, are medicinal species widely used to treat respiratory affections. Stability analyses of vegetal drugs are crucial to assure the quality of derived products. The present study aimed at undertaking qualitative and quantitative analysis of chemical markers [coumarin (CO), o-coumaric (OC), kaurenic (KA), benzoylgrandifloric (BA) and cinnamoylgrandifloric (CA) acids] in dried leaves of M. laevigata and M. glomerata submitted to long-term storage. The plant materials were stored in a dark room with controlled temperature and humidity, and had their fingerprints analyzed three-monthly up to 18 months. Changes in chemical markers were evaluated by UV spectral purity of the peaks and by quantitative analysis of their contents (％w/w in dried leaves), employing an HPLC method previously reported by us [1]. The concentrations of the chemical markers did not vary significantly within the evaluated storage period (p > 0.05) for both species. In contrast, changes in BA, CA and KA peaks were detected for three-months stored samples of both species and CO peak, found only in M. laevigata, was detected after six months of storage, suggesting compound degradation.

The CO contents in M. laevigata samples ranged from 0.10 to 0.03％ to 0.12 ± 0.03％ and therefore fulfill the Brazilian pharmacopeical requirement established for the species (minimum of 0.1％ w/w), except for the 12-month sample (0.09 ± 0.03％) [2]. Therefore, the quality control of Mikania species should be based both on the quantification of the selected compounds and fingerprint analysis. Acknowledgement: FAPEMIG, CAPES and CNPq, for the financial support. References: 1. Bertolucci SKV et al. (2009) Planta Med 75:280 – 285. 2. Farmacopéia Brasileira IV (2005) Sexto Fascículo:292.

PA11

Bioavailability and pharmacokinetic of the Algerian propolis constituent naringenin in rats after oral administration

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Till now, a limited number of pharmacokinetics and bioavailability of propolis compounds studies have been performed. So, the absorption of orally administered Naringenin, an active component of Algerian propolis (14.2％), in rats has been studied to evaluate its pharmacokinetics and bioavailability in vivo in comparison with those of a standard solution of naringenin. Rats were given 100 mg/kg of body weight of aqueous Algerian propolis extract or 14.2 mg/kg of naringenin. Blood was collected from the retro-orbital sinus. Naringenin was quantified by coulometric detection using HPLC-UV system. In vivo pharmacokinetic study of propolis extract shows a good and rapid absorption from the gastrointestinal tract and reveal a high bioavailability. The serum concentration of naringenin from propolis was 17.45 nmol/ml, T_max = 60 min, the total clearance 9.35 ml/min, the area under the curve (AUC0 – 360) 32821 nmol/ml, and the volume of distribution (Vd) was 1949.15 ml. Compared to the standard naringenin, Algerian propolis constituent naringenin shows a better bioavailability and diffusion that may explain the antioxidant effects flavonoids extracted from propolis. References: 1. Harmon AW et al. (2004) Breast cancer Res Treat 85:103 – 110. 2. Hou YC et al. (2001) Planta Med 67:538 – 541. 3. Hsu SL et al. (2002) Life Sci 70:1481 – 1489.

PA12

Development of a rapid isocratic reverse phase-ultra fast liquid chromatographic method for determination of phenolic acids in fruits

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Some low molecular weight phenolic acids namely gallic, chlorogenic, protocatechuic, p-coumaric, vanillic and ferulic, are well-known in their health-promoting properties. Isocratic Ultra Fast Liquid Chromatographic methods (UFLC-DAD) for detecting these compounds are advantageous, due to their simplicity and economy of time and solvent usage. This paper aimed at the development of a rapid and comprehensive isocratic UFLC-DAD method for analysis of phenolic acids in Brazilian fruits mangaba (Hancornia speciosa Gomes) and umbu (Spondias tuberosa Arruda). Mobile phase compositions (different solvents A – Dihydrogen potassium phosphate, trichloroacetic acid and trifluoroacetic acid and different percentage of solvent B – B 8; 10 and 12% of acetonitrile) were combined with flow rates (0.4; 0.5 and 0.6 ml/min) in a statistical factorial design. Among the combinations tried, the trichloroacetic acid was found to be the best solvent "A" and 8% – 10% of acetonitrile as the best solvent B, and flow rate of about 0.6 ml/min as the best range of flow. Method presented limits on detection ranging from 0.014 to 0.094 μg and higher recovery percentages were observed to extraction with methanol-acetone (69.51 to 72.59 for protocatechuic acid and 69.58 to 126.31 for the chlorogenic acid). Chlorogenic acid concentrations in mangaba samples (62.93 μg/g) were higher than in umbu samples (8.49 μg/g). Linearity of detector responses (represented by the linear regression coefficient of plots), was higher than 0.999 for all phenolic acids. These results permitted to develop a rapid and practical method for phenolic acids determination in the tropical fruits of umbu and mangaba. Acknowledgement: We thank the INC/ CNPq (National Council for the Development of Science & Technology, Brazil) for the financial support received while the first and last co-authors thank CAPES for fellowships.

PA13

Development of an enzyme-linked immunosorbent assay using monoclonal antibody against asiaticoside

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ACKNOWLEDGMENT: The authors wish to thank Prof. Dr. H. Isbakan for his help in the preparation of the carbohydrates used. The monoclonal antibody (Mab) against asiaticoside (AS), the bioactive constituent of Centella asiatica (L) Urban was produced and characterized [1]. As immunogen, AS was conjugated to bovine serum albumin (BSA). In order to confirm its immunogenicity, the ratio of hapten in the AS-BSA conjugate was determined by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS). After immunization, hybridomas secreting MAbs against AS were produced by fusing splenocytes with the mouse myeloma cell line, SP2/0-Ag14[2]. After the screening, anti-AS MAb 2B4 was obtained. No cross-reactivity with other related triterpenoid glycosides was found except madecassoside which gave a 7.08% cross reaction value. Subsequently, a quantitative ELISA system for AS using the Mab was established and evaluated comparing with HPLC method. The assay was suitable for quantitating AS in the range of 0.78 to 50 mg ml⁻¹. The validation study showed that the method was precise, accurate and sensitive. The ELISA method described should prove useful as an analytical tool for quality control and standardization of medicinal plants and pharmaceutical products containing AS. Acknowledgement: The JSPE-NRCT Core University Program, Dr. Mayuree Tantisira from Chulalongkorn University of Sergipe, Brazil.

The monoclonal antibody (Mab) against asiaticoside (AS), the bioactive constituent of Centella asiatica (L) Urban was produced and characterized [1]. As immunogen, AS was conjugated to bovine serum albumin (BSA). In order to confirm its immunogenicity, the ratio of hapten in the AS-BSA conjugate was determined by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS). After immunization, hybridomas secreting MAbs against AS were produced by fusing splenocytes with the mouse myeloma cell line, SP2/0-Ag14[2]. After the screening, anti-AS MAb 2B4 was obtained. No detectable cross reactivity with other related triterpenoid glycosides was found except madecassoside which gave a 7.08% cross reaction value. Subsequently, a quantitative ELISA system for AS using the Mab was established and evaluated comparing with HPLC method. The assay was suitable for quantitating AS in the range of 0.78 to 50 mg ml⁻¹. The validation study showed that the method was precise, accurate and sensitive. The ELISA method described should prove useful as an analytical tool for quality control and standardization of medicinal plants and pharmaceutical products containing AS. Acknowledgement: The JSPE-NRCT Core University Program, Dr. Mayuree Tantisira from Chulalongkorn University.

The use of astringents in the cosmetic industry is widespread as tonic lotions, cleansers, deodorants and antiperspirants. Aluminium chloride, among others aluminium salts, is highly used as an astringent. Recently, the use of aluminium in cosmetics raised the concern about its safety to humans. Although the regulatory agencies worldwide assure the safety of this raw material, this issue has led the search for substitutes of aluminium salts to serve consumer needs. In the development of new raw materials it is wise to evaluate a priori the in vitro efficacy to address the extremely complex functional systems of living organisms. Colorimetric titration methods for evaluating tannins based on precipitation of bovine gelatin with 8 commercially available natural and semi-synthetic tannin containing extracts. The bovine gelatin is an especially good source of protein binding sites found in human skin. The tannins belong to the hydrolysable and condensed types present in 5 different plant species. ITC is a reliable and fast technique to evaluate important parameters like enthalpy, entropy, stoichiometry and association binding constant in an unique experiment for further decision support. All raw materials had the efficacy compared to aluminium chloride. The developed methodology has provided an useful tool for astringency evaluation of tannins and will illuminate the road towards better cosmetics.

**References:**

**PA16 Chemical composition and lipid fraction properties of Iranian pomegranate (Punica granatum L.) seeds**
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Department of Food science, Engineering and Technology, Faculty of Agricultural engineering and Technology, University of Tehran, Karaj, Iran.

The pomegranate seeds of four commercial varieties (Abanamahi (AB), Malas (MS), Pust Sefid (PS) and Shahvar (SH)) cultivated in Iran were evaluated in terms of quality properties including protein, oil, dietary fiber, mineral contents and fatty acid composition. Physicochemical properties and antioxidant activity of pomegranate (Punica granatum L.) seed oils (PSOs) also was determined. The oil antioxidant activity was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity. Results showed that PS had the highest oil (16.9%) and crude fiber (42.4%), and nutritional value (460.7Kcal/100g) among selected varieties. PS had the highest level of phosphorus (2766.3 mg/kg) and magnesium (2052.0 mg/kg), while the highest calcium (673.5 mg/kg) and potassium (3724.6 mg/kg) were related to SH. The main fatty acid identified by gas chromatography was punicic acid ranged from 72.07% for SH to 73.31% for MS (p < 0.05). The ratios of polyunsaturated/saturated and unsaturated/saturated fatty acids of PSOs were found to be between 9.174 and 9.540, and 10.325 and 10.861, respectively (p < 0.05). PSOs obtained processed acid (3.78 – 8.36% punicic acid), peroxide (0.39 – 0.48meq O₂/kg), iodine (216.9 – 220.3 g I₂/100g) and saponification (179.3 – 182.5 mg KOH/g) values. Also, refractive index in 25 °C, viscosity and density of PSOs varied from 1.461 – 1.527, 0.316 – 0.391 and 0.9911 gcm⁻³, respectively. The oil obtained from MS showed the lowest level of ortho-diphenols (ODC) and DPPH radical scavenging capability. The relationship between percentage of remaining DPPH and ODC of PSOs also illustrated high correlation among all varieties (R² = 0.98, p < 0.01).

**PA17 Stereoisomeric composition of two bioflavonoids from Larix sibirica**
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Nowadays the bioflavonoid complex of Larix is well-known and well-studied (1). Current work is devoted to detailed studies of structure and stereoisomeric composition of two major bioflavonoids from Larix sibirica Lede.: dihydroquercetin (taxifolin, CAS Nº 480 – 18 – 2, 2,3-dihydro-2-3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4-H-1-benzopyran-4-on) and dihydrokaempferol (aromadendrin, CAS Nº 480 – 20 – 6, 2,3-dihydro-2(4-hydroxyphenyl)-3, 5, 7-trihydroxy-4-H-1-benzopyran-4-on). Using preparative RP-HPLC both compounds were isolated from the extract of larix wood and purified to the > 99% of purity. Detailed analysis by RP- and chiral HPLC, UV, LC-MS and high resolution NMR spectroscopy allowed us to characterize these compounds as mainly 2R3R-isomers with small (up to 43%) content of 2S3R-enantiomers. So we can establish that Larix sibirica produces “high quality” optical pure flavonoids. References: 1. Kolesnik Yu et al. (2007) Abstr 55th Int Congr Ann Meet Soc Med Plant Res, Graz: 938.

**PA18 Absence of Penicillin-derivatives in preparations from Penicillium species used in homeopathic medicinal products**
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Homeopathic medicinal products produced from preparations of Penicillium chrysogenum (Notakehl), P. glabrum (Quentakehl), P. roquefortii (Fortakehl) or P. brevicompactum (Stolonkehr) have been used for over 30 years without reports of any serious or major adverse events. Safety studies and other available data gave no indications for immunotoxic or sensitizing effects. While some species of the genus Penicillium do produce antibiotic substances, they are unwanted by-products in the homeopathic use of active substances derived from the Penicillium species. The aim of this study was to check for the presence of antibiotic substances in these active substances. For the production of the Penicillium species active substance, the biomass yielded by fermentation is purified and then mechanically opened by a cell mill. The liquid phase is separated and the insoluble components are filtered. The filtrate then
undergoes multiple filtration and washing steps prior to sterile filtration and freeze drying. The resulting starting material is named “e volumine cellulæ (lyophil., steril.,)” [1] and is raised to homoeopathic potencies (D2, D4, D5). HPLC-ESI-MS data is presented to prove the absence of Penicillin-derivatives and precursors (Penicillin G, m/e 335 [M+H]+ and 6-Aminopenicillanic acid, m/e 217 [M+H]+) in used active substances. Whilst being found in the fermented culture broth of e.g. Penicillium chrysogenum, Penicillin G and 6-Aminopenicillanic acid are no longer present in the active substance after processing. During the manufacture of the active substances, unwanted antibiotic compounds are eliminated whilst preserving the products high quality. Acknowledgement: HPLC-ESI-MS analysis was taken out at Phytos GmbH & Co. KG in Neu-Ulm

Research work carried out in Sudan on the weed *Argemone mexicana* L. pointed to the potential of the plant as a source of larvicides against mosquito. Mosquito, *Anopheles amphibius*, is on focus in Sudan because of its role as a vector of several tropical diseases. The objectives of this study was evaluation or selection of the best solvent used for extraction of the alkaloids and identification of the alkaloids using spectroscopy as well as chromatographic methods. The result showed that ethanol was the best for extraction of *Argemone* leaf alkaloids both qualitatively and quantitatively; hexane the poorest while acetone and chloroform were in between. Ethanol was selected as the best organic solvent for extraction and different water dilutions were used (10%, 25%, 50%, 75%, and 100%). The three alkaloids were detected in all ethanol dilutions. The alkaloid quantity increased with the increase of ethanol %. In addition, pure ethanol extract was comparable to distilled water. The three alkaloids were detected in all ethanol dilutions. The alkaloid quantity increased with the increase of ethanol %. In addition, pure ethanol extract was comparable to distilled water. The three alkaloids were detected in all ethanol dilutions. The alkaloid quantity increased with the increase of ethanol %. In addition, pure ethanol extract was comparable to distilled water. The three alkaloids were detected in all ethanol dilutions. The alkaloid quantity increased with the increase of ethanol %. In addition, pure ethanol extract was comparable to distilled water. The three alkaloids were detected in all ethanol dilutions. The alkaloid quantity increased with the increase of ethanol %. In addition, pure ethanol extract was comparable to distilled water. The three alkaloids were detected in all ethanol dilutions. The alkaloid quantity increased with the increase of ethanol %. In addition, pure ethanol extract was comparable to distilled water. The three alkaloids were detected in all ethanol dilutions. The alkaloid quantity increased with the increase of ethanol %. In addition, pure ethanol extract was comparable to distilled water. The three alkaloids were detected in all ethanol dilutions. The alkaloid quantity increased with the increase of ethanol %. In addition, pure ethanol extract was comparable to distilled water. The three alkaloids were detected in all ethanol dilutions. The alkaloid quantity increased with the increase of ethanol %. In addition, pure ethanol extract was comparable to distilled water. The three alkaloids were detected in all ethanol dilutions. The alkaloid quantity increased with the increase of ethanol %. In addition, pure ethanol extract was comparable to distilled water. The three alkaloids were detected in all ethanol dilutions. The alkaloid quantity increased with the increase of ethanol %. In addition, pure ethanol extract was comparable to distilled water. The three alkaloids were detected in all ethanol dilutions. The alkaloid quantity increased with the increase of ethanol %. In addition, pure ethanol extract was comparable to distilled water. The three alkaloids were detected in all ethanol dilutions. The alkaloid quantity increased with the increase of ethanol %. In addition, pure ethanol extract was comparable to distilled water. The three alkaloids were detected in all ethanol dilutions.

**PA19**

**Effect of exogenous silicon and salt stress on germination and seedling establishment in Borago officinalis**

**Torabi F**

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**PA20**

**Effect of exogenous silicon and salt stress on germination and seedling establishment in Borago officinalis**

**Torabi F**

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**PA21**

**Cichoric acid content and antioxidant activity of commercially available Echinacea herbal medicinal products**

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Echinacea spp. are very well known medicinal plants with immunomodulatory activity. The purpose of this study was to investigate the phenolic content (cichoric acid and total phenol) and the antioxidant activity of seven medicinal products with *Echinacea purpurea* (L.) Moench commercially available in Croatia. The content of total phenolics was determined spectrophotometrically with Folin Ciocalteu reagent, while the content of cichoric acid was established by using isocratic RP-HPLC. Antioxidant activity was evaluated using the following techniques: radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical, reducing power and β-carotene-linoleic acid assay. The cichoric acid content varied greatly in products with a maximum of 1.6% (w/v) as well as the content of total phenols (0.03 – 16 mg/mL). In all the in vitro analyses the extracts demonstrated marked antioxidant activities. The activity in the reducing power assay correlated very well with the content of total phenols (r² = 0.995, P < 0.0001), while the activity in the other assays did not correlate neither with the amount of cichoric acid nor with that of total phenolics. It seems that some other substances might be responsible for the activity of the investigated preparations in those assays. The results suggest that cichoric acid, as unstable phenolic compound characteristic for *E. purpurea* products vary as expected. On the other hand, the total phenolic and antioxidant activity of investigated products are greatly influenced by other ingredients of products.

**PA22**

**Deglycosylation of individual flavonoids and flavonol containing plant extracts using human intestinal lactase-phlorizin hydrolase (LPH)**

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Lactase-phlorizin hydrolase (LPH, EC 3.2.1.123/62) is an apically-sorted glycoprotein of the small intestine. One of the two catalytic sites of LPH is responsible for hydrolyzing lactose, the main carbohydrate in milk. The second active site exhibits a broad specificity against substrates like phlorizin and glycosyl-4-acyl-derivatives [1]. Phlorizin, a dibenzochromene, belongs to the group of flavonoids often present in glycosylated forms. Lactase-phlorizin hydrolase (LPH, EC 3.2.1.123/62) is an apically-sorted glycoprotein of the small intestine. One of the two catalytic sites of LPH is responsible for hydrolyzing lactose, the main carbohydrate in milk. The second active site exhibits a broad specificity against substrates like phlorizin and glycosyl-4-acyl-derivatives [1]. Phlorizin, a dibenzochromene, belongs to the group of flavonoids often present in glycosylated forms. Lactase-phlorizin hydrolase (LPH, EC 3.2.1.123/62) is an apically-sorted glycoprotein of the small intestine. One of the two catalytic sites of LPH is responsible for hydrolyzing lactose, the main carbohydrate in milk. The second active site exhibits a broad specificity against substrates like phlorizin and glycosyl-4-acyl-derivatives [1]. Phlorizin, a dibenzochromene, belongs to the group of flavonoids often present in glycosylated forms. Lactase-phlorizin hydrolase (LPH, EC 3.2.1.123/62) is an apically-sorted glycoprotein of the small intestine. One of the two catalytic sites of LPH is responsible for hydrolyzing lactose, the main carbohydrate in milk. The second active site exhibits a broad specificity against substrates like phlorizin and glycosyl-4-acyl-derivatives [1]. Phlorizin, a dibenzochromene, belongs to the group of flavonoids often present in glycosylated forms. Lactase-phlorizin hydrolase (LPH, EC 3.2.1.123/62) is an apically-sorted glycoprotein of the small intestine. One of the two catalytic sites of LPH is responsible for hydrolyzing lactose, the main carbohydrate in milk. The second active site exhibits a broad specificity against substrates like phlorizin and glycosyl-4-acyl-derivatives [1]. Phlorizin, a dibenzochromene, belongs to the group of flavonoids often present in glycosylated forms.

The activity in the reducing power assay correlated very well with the content of total phenols (r² = 0.995, P < 0.0001), while the activity in the other assays did not correlate neither with the amount of cichoric acid nor with that of total phenolics. It seems that some other substances might be responsible for the activity of the investigated preparations in those assays. The results suggest that cichoric acid, as unstable phenolic compound characteristic for *E. purpurea* products vary as expected. On the other hand, the total phenolic and antioxidant activity of investigated products are greatly influenced by other ingredients of products.
Effect of static magnetic field on seed germination early growth and activities of some enzymes in *Melissa officinalis* seeds

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The objective of the present study was to investigate the effect of static magnetic field (0, 25, 50 and 75 μT) and exposure time (15, 30 and 60 minutes) on *Melissa officinalis* L. seed germination. Treatment of *Melissa officinalis* seeds in these magnetic fields increased the germination rate (GR), germination index (GI), germination rate coefficient (GRC), seedling length and seedling dry and fresh weight under laboratory germination tests. In germinating seeds, enzyme activities of α-amylase, dehydrogenase and protease were significantly higher in treated seeds in contrast to controls. The higher enzyme activity in magnetic-field-treated *Melissa officinalis* seeds could be triggering the fast germination and early vigor of seedlings. **Keywords:** Melissa officinalis, seed germination, test, α-amylase, dehydrogenase, protease and magnetic field **References:** 1) Balouchi HR and Sanary SAM (2009) International Agrophysics 23: 111–115. 2) Gholami A and Shahi F (2010) World Journal of Science, Engineering and Technology 62: 270–282. 3) Kavi PS (1977) Sci Cult 43: 405–406. 4) Podlesny J, Lenartowicz W and Sowinski M (2003) Zecz Probl Post Nauk Rohn 495: 399–406. 5) Vashishth A and Nagarajan S (2010) Journal of Plant Physiology 167: 149–156.

Phytochemical analysis of *Anthyllis hermanniae* - Leguminosae, and development of a sensitive UHPLC-HRMS/MS method for the rapid analysis of the phenolic content

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*Anthyllis* genus includes several species, very few of which are investigated from a phytochemical point of view. Previous works about the phytochemical analysis of the aerial parts of some species describe the isolation and structure elucidation of several glycosides of kaempferol, quercetin and other flavonoid aglycons [1–4]. In the present study, a detailed phytochemical analysis of the methanolic extract of the aerial parts of *Anthyllis hermanniae* L. is described, a species for which there are no previous data concerning its metabolite content. Applying several chromatographic techniques (VLC, LC, prep-TLC, HPLC, CPC), twenty-two secondary metabolites, belonging to categories of cyanogenic and benzoic acid derivatives, sterols, coumarins, isoflavons and flavonols were isolated and their structures were fully elucidated by means of UV-Vis, MS, HR-MS and NMR (18D spectra). Moreover, triglycosides of quercetin and kaempferol, which are new natural products, were isolated and unambiguously elucidated [5]. After the structure elucidation of the isolated metabolites, we tested all the isolated compounds with invitro assays for the development of a fast and sensitive method for the simultaneous characterization of the phenolic content of *A. hermanniae*. The analyses were performed on a UHPLC system hypenated with a hybrid-LITQ-Orbitrap mass spectrometer using ESI & AP CI ionization probes, in both positive and negative modes. The study of the full scan spectra together with the accurate MS/MS data enabled the identification of additional phenolics, with high confidence. This newly developed analytical method could be applied for the rapid identification of phenolics in other *Anthyllis* species, as well as in other Leguminosae plants. **References:** [1] Pistelli L et al. (2007) Nat Prod Res 21: 418 – 425. [2] Marco J et al. (1989) Phytochem 28: 1513 – 1516. [3] Adell J et al. (1988) Phytochem 27: 2967 – 2970. [4] Barbera O et al. (1986) Phytochem 25: 2361 – 2365. [5] Halabalaki, M et al. (2011) J Nat Prod In press.

Investigation of the existence of five major flavonoids in *Satureja sahendica* Bornm. and optimization of their extraction conditions using experimental design, solid phase extraction and HPLC

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*Satureja sahendica* Bornm. (SSR) (Lamiaceae) is an endemic species of Iran [1] and in traditional medicine is used as a rapid antidote for food poisoning [2]. In this work, chemometrics, solid phase extraction (SPE) and HPLC methods were used to investigate the existence of five major flavonoids including; myricetin, quercetin, luteolin, apigenin and kaempferin in (SSR) and to optimize the extraction conditions of detected flavonoids. The effects of five experimental factors including; percentage of ethanol, volume of extraction solvent, concentration of HCl, extraction time and temperature on the extraction recovery were investigated using a rotatable, orthogonal central composite design (CCD). Grid search method was used to find the optimum extraction conditions. The SPE was used to preconcentrate the presumably available flavonoids. The SPE parameters including; pH of loading solution, type and volume of elution solvent and break-through volumes were optimized. The preconcentrated extracts were analyzed by HPLC using a C18 column and methanol:0.5% phosphoric acid (60:40 v/v) with flow rate of 1.0 mL/min as mobile phase. Among the investigated flavonoids only quercetin, luteolin and apigenin were found in this species which showed two different patterns for extraction. Quercetin and luteolin were extracted using 20 mL of 68% aqueous ethanol containing 2.0 M HCl, refluxed for 30 minutes at 90 °C while apigenin was extracted sing 20 mL of 68% aqueous ethanol containing 1 M HCl, refluxed for 1 hour at 45 °C. Concentrations of quercetin, luteolin and apigenin were 10.20, 19.21, and 48.50 mg·Kg⁻¹, respectively. **References:** [1] Rechinger K (1982) Flora Iranica. Akademische Druck-und Verlagsanstalt, p 495–504. [2] Taherpour A, Maroofi H, Nasri F (2005) Inter J Appl Chem 1(1):57 – 61.

Accelerated Solvent Extraction: development of a representative extraction method from medicinal plants for cosmetic applications


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The first crucial step to discover new cosmetic plant active ingredients is the extraction before the analysis of plant materials. Accelerated Solvent Extraction (ASE) is a fast and automatic sample preparation technique which offers the ability to carry out sequentially multiple extracts (up to 24 samples). Then, cosmetic activities (enzymatic targets and skin cells) can be evaluated. For preliminary phytochemical investigations, an efficient and exhaustive method is traditionally used to extract polar plant
metabolites. The aim of our study was to define ACE conditions to obtain the most complete and representative extraction. In contrary, most of publications deal with the optimization of operational parameters to enhance the selectivity of extraction of only compounds of interest. The transposition of our conventional solvent-based extraction method (hydroethanolic reflux extraction, 1 h, ratio plant/solvent 10%) to ASE technology was performed with respect of amount of extracted material (dry matter, yield) and chemical composition. Among herbs selected, ASE extracts of Lepidium sativum Michx. and Scutellaria baicalensis Georgi extracts were obtained and analyzed by Thin-Layer Chromatography. The flavonoids content of the extract was identified by HPLC coupled with UV densitometric detection. The quantification of orientinin/ homoorientin and baicalin/baicaikalin was achieved to select the best ACE parameters (50 °C, 10 min, ratio plant/solvent 20%). In addition, the antioxidant activity of extracts was evaluated. The results showed that optimized ASE extracts were equivalent to conventional ones concerning phytochemical composition and antioxidative activity. To conclude, standardization of ASE extraction method is a powerful tool for rapid screening of new cosmetic plant active ingredients.

Determinantion of vitamin e (α,β-tocopherol) in canola oils by high performance liquid chromatography

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A method based on HPLC-UV-DAD coupled to an ESI-MS interface was developed for the determination of the constituents in the aqueous preparations of Viola odorata L. flowering tops. The assay was fast, simple and effective and permitted the quality control of the preparations. Aim of this work was to assess the qualitative and quantitative profile of the investigated preparations, with wide applications in food and cosmetic industry, and to propose a validated method for their quality control. HPLC-DAD-ESI-MS analyses supported by extensive preparative chromatographic investigations and NMR analyses revealed the predominance of complex flavonoid and flavonolignans. Eleven constituents were unambiguously identified. Main secondary metabolites were flavonol glycosides, principally derivatives of kaempferol. Flavonol tri- and tetracyclics were reported for the first time in the genus Viola. Their structure was confirmed by NMR analyses. The analytical method provided a good separation of the constituents. Good linearity of the calibration curves was achieved between 0.84 10-3 μg to 0.63 μg (r² > 0.9998). The assay was validated for LOD, LOQ, intra- and inter-day precision and accuracy. All validation criteria were fulfilled. The proposed qualitative method could be used as a starting method for the evaluation of Viola sp. preparations with further optimization case by case, according to the geographical variability. It is noteworthy that at the same time flavonoids baring different aglycones and number of sugar moieties are well separated. This is the first report of detailed analysis of the chemical composition of Viola odorata flowers.

Study of electrooxidation mechanisms and antioxidative properties of flavonoids and flavonolignans

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Flavonoids are a large group of naturally occurring polyphenolic compounds that are distributed in various plant organs. A wide range of the biological activities are attributed to flavonoids' antioxidative abilities. Flavonoids are benzo-γ-pyrone derivatives that can be divided into several groups according to their structural differences. Here we focused on quercetin, silybin and their derivatives which belong to the group of flavonols and flavonolignans, respectively. We described basic aspects of electrooxidation of flavonols and flavonolignans at a pyrolytic graphite electrode using cyclic and square wave voltammetry. Flavonols (quercetin, rutin and isoquercetin) and flavonolignans (silybin, 2,3-dehydroxy-silybin, 7-O-methylsilybin, 20-O-methylsilybin and isosilybin), were studied in an adsorbed state using ex situ (adsorptive transfer – Adapt) voltammetric methods. Under the given conditions, flavonols and flavonolignans are subject to a multistep oxidation. The potential of the oxidation of hydroxy groups and other substituents corresponds with antioxidative properties of studied polyphenols. Using ex situ voltammetry, the following order in antioxidant capacities was proposed: flavonols > 2,3-dehydroxy-silybin > silybin and its derivatives. The results provide a solid basis for further study of both the antioxidant and prooxidant parameters of polyphenolic compounds using voltammetric methods and extend our knowledge of their electrooxidation mechanisms. Acknowledgement: This work was supported by the Czech Science Foundation (Grant Projects No. P503/11/P312 and P301/11/0767), by the Ministry of Education, Youth and Sports (MSM 619895216). Lime flower is used worldwide for its sedative and antispasmodic properties. Traditionally it is used for migraine, hysteria, feverish colds, and for raised arterial pressure associated with arteriosclerosis and nervous tension [1]. Besides flavonoids, little is known for the rest of the phenolic content. In the present study extensive HPLC-DAD, HPLC-ESI-MS and HPLC-MS/MS analyses were undertaken in the aqueous preparations of Tilia platyphyllos Scop. flavonoids. An HPLC-DAD-ESI-MS method was developed and optimised for the quantitative determination of the constituents. Analyses of the ethanol extracts confirmed the predominance of flavonol glycosides and protocatechuic acid. In contrast, both decocation and infusion, which are nevertheless the traditional herbal preparations, were more complex, containing polar simple phenolics and low molecular weight procyanidins. The use of different HPLC col-
umns permitted a good separation of the constituents and enabled their quantitation. The method showed good linearity, \( r^2 > 0.9999 \) for tilisolide and catechin and 0.9986 for quercetin-3-O-glucoside, intra/inter-day variability (SIRSD 1.56 and 1.33) and real sample repeatability (SIRSD < 4.00). Preparative chromatographic investigations (Sephadex LH-20) and NMR analyses revealed the presence of procyanidin B4, while HPLC-MS/MS analyses enabled the identification of procyanidin trimers and tetramers. Overall, 20 constituents were detected and identified, belonging mainly to three classes of compounds: phenolic acid derivatives, condensed tannins and flavonol glycosides. Aqueous extracts contain a higher amount of procyanidins (strong chelating properties) than flavonoids and caution should be taken upon frequent use of the drug. This is the first report of detailed analysis of the chemical composition of Tiliae flos. References: 1. Barnes J, Anderson LA, Phillipson JD. (2007) Herbal Medicines, Pharmaceutical Press.
use. All extracts were subjected to chromatographic and spectrophotometric analyses for their chemical compositions. In conclusion, the 70% ethanol extract was found to be rich in anthocyanins and cyanidin-3-glucoside when compared with other extracts. References: 1. Motohashi N and Sakagami H (2000) Top Heterocycl Chem 16: 1 – 40. 2. Flora of Turkey and The East Aegean Islands, Vol 4, Edinburgh, University Press, pp. 541 – 543. 3. Davis PH (1972) Flora of Turkey and The East Aegean Islands, Vol 4, N and Sakagami H (2009) Top Heterocycl Chem 16: 1 – 40. 2. Kong JM, L., sweet or annual wormwood (in Chinese q/C239nghao:

\[ C_{236} \text{chrysosplenetin from... } \]

is the first report on the separation of the pair of isomers casticin and also reported. The best results in terms of efficiency of isolation were equipped with a prepacked normal phase silica column were performed. Briefly, the method consists of a pretreatment of the original extract between organic and aqueous layers and further purification of pared. The estimation of the antioxidant activities of water and methanol extracts of the leaf is formed on an Acquiting UPLC BEH phenyl column (100 mm x 1.0 mm, i.d., 1.7 µm) system by using a mobile phase containing acetonitrile and formic acid buffer solution (pH = 3.77) with 3% triethylamine (15:85) (v/v). (See Figure 1). Chromatographic separation is by an isocratic elution with the flow rate of 0.3 mL/min. Calibration graphs for both compounds in the linear concentration range of 5 – 40 µg/mL were obtained by using the relationship between the concentration and the peak area based on the detection at 340 nm. The validity of the UPLC method was done by analyzing the plant samples. The developed UPLC method was applied to the quantitative analysis of A. anulata consisting of rutin and chlorogenic acid and a good agreement was reported. The estimation of the antioxidant activities of water and methanol extracts of the leaf is based on thiobarbituric acid (TBA) assay in order to detect their lipid peroxidation. In this investigation, the significant activities were obtained from the water (IC\textsubscript{50} = 24.85 ± 6.33) and MeOH (IC\textsubscript{50} = 27.03 ± 6.64) extracts of R. anulata in the TBA test. Propyl gallate (IC\textsubscript{50} = 0.04 ± 0.18) was used as a positive control. References: 1. Bçhget L (2001) Turk Botany 25: 103 – 105.

PA36

Ultra-performance liquid chromatographic (UPLC) determination of the rutin and chlorogenic acid in the Ribe

\[ \text{anulata } \]

Bçhget (Grossulariaceae) which is an endemic species in Turkey [1]. Good chromatographic separation and determination were performed on an Acquiting UPLC BEH phenyl column (100 mm x 1.0 mm, i.d., 1.7 µm) system by using a mobile phase containing acetonitrile and formic acid buffer solution (pH = 3.77) with 3% triethylamine (15:85) (v/v). (See Figure 1). Chromatographic separation is by an isocratic elution with the flow rate of 0.3 mL/min. Calibration graphs for both compounds in the linear concentration range of 5 – 40 µg/mL were obtained by using the relationship between the concentration and the peak area based on the detection at 340 nm. The validity of the UPLC method was done by analyzing the plant samples. The developed UPLC method was applied to the quantitative analysis of R. anulata consisting of rutin and chlorogenic acid and a good agreement was reported. The estimation of the antioxidant activities of water and methanol extracts of the leaf is based on thiobarbituric acid (TBA) assay in order to detect their lipid peroxidation. In this investigation, the significant activities were obtained from the water (IC\textsubscript{50} = 24.85 ± 6.33) and MeOH (IC\textsubscript{50} = 27.03 ± 6.64) extracts of R. anulata in the TBA test. Propyl gallate (IC\textsubscript{50} = 0.04 ± 0.18) was used as a positive control. References: 1. Bçhget L (2001) Turk Botany 25: 103 – 105.

PA37

Rapid and efficient isolation of polymethoxylated flavonoids and artemisinin from Artemisia annua L. acetone extract Timoteo P, Wesself C, Ros G, Righesci C, Bila A

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Artemisia annua L., sweet or annual wormwood (in Chinese q/C239nghao: chrysosplenetin from...

\[ C_{236} \text{chrysosplenetin from... } \]

is the first report on the separation of the pair of isomers casticin and also reported. The best results in terms of efficiency of isolation were obtained by flash chromatography and to the best of our knowledge this is the first report on the separation of the pair of isomers casticin and chrysosplenetin from A. annua by flash chromatography. Acknowledgement: Acknowledgments: Hanze University Groningen, Institute for Life Sciences and Technology, Groningen (NE) in combination with Erasmus European Commission Education and Training for the fellowship to C. Wesself. References: 1. Bila et al. (2006) Phyto medicine: 487 – 493 2. Ferreira et al. (2010) Molecules 15: 3135 – 3170

PA38

Finding the most appropriate IR technique for plant species identification

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Quality control of herbal medicinal products is of extreme importance. Procedures for identification of plant species, such as biochemical analysis or macroscopic and microscopic examination of morphological and anatomical properties are time consuming and expensive[1]. A good alternative is infrared spectroscopy since it is rapid, easy to use, non-destructive and low-cost. Identification of species from dried whole leaf samples of pharmaceutically important Epilobium and Hypericum genera were investigated. To determine which infrared spectroscopy mode gives most informative spectra for plant species identification different modes of infrared spectroscopy were applied. These were diffuse reflectance, attenuated total reflectance (ATR), direct transmission of whole leaves, and KBr tablet transmission with comminuted leaves. First the informative wavenumbers were chosen by one-way analysis of variance. Afterwards the colinearity was reduced with principal component analysis. At last the species identification was determined with discriminant analysis. Best results were obtained with ATR and KBr tablet transmission. Still there were important differences between genera. ATR proved to be appropriate for discrimination among Epilobium species (accuracy of plant species identification was 98%), Epilobium species differ in distribution and morphology of trichomes on the surface of the leaves[2]. While for Hypericum species KBr tablet transmission proved to give best results (accuracy of plant species identification was 97%). Hypericum species differ in secondary metabolites that are accumulated in the interior of the leaves[3,4]. Results show that morphological properties of plant material should be taken into consideration when developing an infrared spectroscopy based method for identification of plant species.


PA39

Applicability of ultra- and nanofiltration for the concentration of medicinal plant extracts

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The present investigation revealed the potential benefits of ultra- and nanofiltration application in herbal extracts processing. Helleborus purpurascens Waldst. & Kit., Geranium Robertianum L. and Salvia officinalis L. were widely used in China for more than 2000 years for treating many disorders including malaria [1]. Polymethoxylated flavonoids and arte

\[ \text{mosinin, one of the main compounds presents in Artemisia annua } \]

L., are among the most promising natural products for antimarial and antancancer purposes [2]. In this work, three different chromatographic meth\ods, including a rapid and selective isolation method of the main poly-
methoxylated flavonoids (PMFs) – namely epius, the isomers castician and chrysosplenetin, artemetin and 5-OH-3,4,6,7-tetramethoxylavone – and artemisinin from A. annua acetone dried extract, have been compared. Briefly, the method consists of a pretreatment of the original extract between organic and aqueous layers and further purification of the richest extract in PMFs and artemisinin with Sephadex LH-20, silica gel normal phase column chromatographies and flash chromatography equipped with a prepacked normal phase silica column were performed. Quali-quantitative analyses of the main PMFs found in the extract were also reported. The best results in terms of efficiency of isolation were obtained by flash chromatography and to the best of our knowledge this is the first report on the separation of the pair of isomers castician and chrysosplenetin from A. annua by flash chromatography. Acknowledgement: Acknowledgments: Hanze University Groningen, Institute for Life Sciences and Technology, Groningen (NE) in combination with Erasmus European Commission Education and Training for the fellowship to C. Wesself. References: 1. Bila et al. (2006) Phyto medicine: 487 – 493 2. Ferreira et al. (2010) Molecules 15: 3135 – 3170
PA40

Profiling of African Mimosaceae for the rapid identification of new triterpenoid electrophiles

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Avicins are complex triterpenoid saponins isolated from an Australian Mimosaceae (Leguminosae), Acacia victoriae Benth. They are based on an acetic acid core, substituted by two glycosidic units and by a specific side chain at C-21 containing two monoterpene carboxylic acids and a quinovose moiety, conferring thereof particular electrophilic properties [1]. Avicins exhibit potent proapoptotic and anti-inflammatory activities, selectively inhibit the growth of tumor cells and thus appear as a new potential class of anticancer natural substances [2, 3]. In order to discover new avicins analogues and to identify the pharmacophore responsible for the activity, different African Mimosaceae species, including Acacia, Albizia, and Entada genera, were selected to screen their saponins content. Chemical profiling of saponin-enriched fractions, based on a LC-UV-MS/MS dereplication method and NMR experiments, has pointed out the presence of avicins analogues in the different studied species. Characteristic structural features of avicins consisting of an acetic acid aglycone, a tri- or tetra-saccharide moiety at C-28, a sugar side chain at C-21 containing two monoterpene carboxylic acids and a quinovose moiety, conferring thereof particular electrophilic properties [4]. The dereplication approach enabled to quickly identify the compounds of interest, and so to focus on their specific isolation and saving a considerable amount of time. Structures of the isolated triterpenoids will be unambiguously elucidated on the basis of extensive analysis of NMR experiments and mass spectrometry, and further submitted to bioassays to study in depth their mechanism of action. References: 1. Jayatilake GS et al. (2003) J Nat Prod 66: 779 – 83. 2. Haridas V et al. (2001) Proc Natl Acad Sci USA 98: 11557 – 11562. 3. Gaikwad A et al. (2005) Clin Cancer Res 11: 1953 – 1962 4. Notel O et al. (2011) Phytochem Rev, in press

PA42

Phytochemical study of plants and plant cell cultures of three Salvia species

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The genus Salvia L. is widely distributed cultivated in various regions all over the world because of its numerous biological and pharmacological properties. These positive effects have its source in the high diversity of their secondary metabolites and enable its application on pharmaceutical, cosmetics and food industries. The main activities namely adstringent, antibacterial, anti-inflammatory and antioxidative are effected for instance by the essential oils as well as phenolic acids, sterols and higher terpenoids. Beside cultivation parameters, the composition of these secondary metabolites is mainly affected by the species. According to their interesting spectrum of secondary metabolites, S. officinalis L., S. triloba L. and S. virgata Jacq. were selected for the induction of in vitro cultures as source for the production of new and valuable compounds. The cultivation of Salvia species by plant is influenced by various parameters like climate, geographical conditions and infestation by parasites, the application of plant cell and tissue cultures in vitro reveal a potential alternative. In this case cultivation can be conducted under defined and optimized conditions in a bioreactor without the need of herbicides. Different phytochemical methods including extraction, isolation and chromatography techniques were applied on both the plants and their in vitro cultures in order to compare their secondary metabolite production. Therefore GC/MS analysis was performed for the identification and structure determination of present secondary metabolites. The observed results and the experiments will be presented. Acknowledgement: This work has been supported by European Social Funds and the Free State of Saxony, project number 080938406.

PA41

Exploitation of HPTLC for methodology development: quantification, fingerprinting and partition coefficient determination

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High Performance Thin Layer Chromatography (HPTLC) is a simple, modern analytical densitometric analysis technique [1 – 5]. In the present study, HPTLC methods were developed, validated and compared to analytical techniques routinely used in the laboratory. The quantification of oleuropein in differently processed extracts of olive leaves was carried out. A calibration curve was created with oleuropein as standard and their content was determined with HPTLC. Quantification was achieved by UV detection at 240 nm and excellent linear behaviors over the investigated concentrations were observed. The results were statistically compared with the ones obtained from two well established techniques, namely HPLC and NMR, and proved to be precise and accurate. Furthermore, HPTLC was used for the documentation of the fingerprinting of Genista halacaysi Heldr, in order to detect and quantify the major compounds. The chromatograms allowed the identification of seven main constituents. Moreover, the possibility of elaborating HPTLC for the determination of the partition coefficients used in counter-current chromatography was examined. The obtained results were successfully applied for the purification of the target compounds of the afore-mentioned plant, indicating that the partition coefficients could effectively be determined with HPTLC analysis and not necessarily with HPLC. HPTLC provided reliable results in all the methods which were developed. It was shown to be sensitive, selective, repeatable, easy to handle, requiring low analysis time and less cost per analysis. Overall, HPTLC could be efficiently employed instead of expensive and time-consuming techniques. References: 1. Vahnhaelen-Fastre RJ et al. (2000) J. Chromatogr A 868: 269 – 276. 2. Yadav D et al. (2011) Sep Sci Technol 46: 286 – 291. 3. Rashmi et al. (2011) Pchcog J 3: 41 – 44. 4. Dhalwal K et al. (2010) J Med Plants Res 4: 1289 – 1296. 5. Plocharz P et al. (2010) J Chromatogr A 1217: 4868 – 4872.

PA43

Raman spectroscopy analysis of tobacco alkaloids

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Tobacco plants and products contain alkaloids, mainly nicotine, besides e.g., nornicotine, cotinine, and anabasine. The latter are also metabolites of nicotine produced in the liver by cytochrome P450. The aim of our research was in situ investigation of tobacco alkaloids directly in the plants as well as in some pharmaceutical products. Two-dimensional Raman maps of nicotine distribution were obtained with 1064 nm excitation wavelength, the spatial resolution of 50 – 200 µm and analyzed with the aid of quantum-chemistry calculations [B3LYP/6 – 31+G(d,p) and B3LYP/6-311G(d,p)] and B3LYP/aug-cc-pVDZ]. Additionally, calculations were performed for salts and protonated forms of nicotine. Distribution of nicotine was obtained by integration of characteristic bands of the compound over the measured surface. Acknowledgement: This research was supported by the Polish Ministry of Science and High Education (grant no. N204013635). Computational center “Cyfrowy” (Krakow, Poland) is acknowledged for CPU time.
Quantification of scopoletin in Artemisia annua L. using HPLC-ED
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Artemisia annua L. (Asteraceae) contains various phytochemical compounds such as monoterpeneols, sesquiterpenoids, flavonoids, coumarins, sterols etc. Scopoletin (7-hydroxy-6-methoxycoumarin) is coumarin compound mainly found in the species. Scopoletin (7-hydroxy-6-methoxycoumarin) is coumarin compound mainly found in the species. The genus Origanum L. is represented in Turkey by 26 taxa (23 species, 3 subspecies), 13 being endemic to Turkey. 50% of all 51 Origanum taxa, known in the World are distributed in Anatolia (12,3). This high rate suggests that the gene centre of Origanum is Turkey (4) 9 perennial Origanum plants from 23 location were collected from natural flora of Antalya. In order to analyse the total phenolics in Origanum (leaf and flowers) samples (2.5 g of powder material of each species were processed. The ethanol extraction was performed with 25 ml of 70% ethanol at room temperature for 24 h with a shaker. The total phenolic content was estimated by the Folin-Ciocalteu method. Ethanol extractions were centrifuged and 100 μl extracts were taken, nine hundred microlitres of water were added. 5 ml of 1:10 diluted Folin-Ciocalteu reagent and 4 ml of sodium carbonate (75 g/l) added to extracts. After 2 h of incubation in the dark at room temperature, the absorbance at 765 nm was measured. The total amount of phenolic compounds varied in the range of 56.1 – 109.1 mgGAE/g at the level of location and 61.6 – 98.6 mgGAE/g at the level of species. The highest value at the level of location was obtained from Origanum vulgare L. subsp. hirtum (Link) l.etw., in Kemer location. However, Origanum sylomaticum P.H.Davis, local endemic species had high values in all three locations and the highest average at the level of species. Origanum vulgare L. subsp. hirtum (Link) l.etw. and Origanum onites P.H.Davis had the lowest average at the level of species. References: 1. Ietswaart J H (1982) Origanum L. In: Davis, P.H. (Editor), Flora of Turkey and the East Aegean Islands, Vol. 7: 297 – 313, Edinburgh University Press, Edinburgh. 2. Davis PH, Mill R R and Tan K (1988) Flora of Turkey and the East Aegean Islands (Suppl. III), Vol 11, Edinburgh University Press, Edinburgh. 3. Güner A, Özhatay N, Ekim T and Başer K H C (2000) Flora of Turkey and the East Aegean Islands (Suppl. II), Vol 11, Edinburgh University Press, Edinburgh. 4. Baser K H C (2002) The Turkish Origanum species. In: Kintzios S E (Editor), Oregano: the genera Oregano and Lippia. Taylor & Francis, pp.109 – 126, London.
PA48

Natural product Marie-Lise database development for high-throughput phytochemical profiling of plant extracts
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Modern drug discovery is greatly based on the identification and structural characterization of new lead compounds, stemming from the huge diversity of natural plant chemicals. The process is tedious facing the complexity of plant metabolome, and the time-consuming steps of purification. In order to reduce the number of de novo purification and elucidations of chemical entities, an interesting strategy is to create a natural-product database. Using about two hundred natural compounds including alkaloids, steroids, terpenoids and phenolic compounds from commerce and our in-house chemical library (UMR 7200), we developed a database named “Marie-Lise” through a combination of HPLC-DAD and LC-QqTOF-MS/MS analysis using Galaxie and Masslynx softwares. High detection sensitivity and selectivity of these methods permits us to characterize: retention time, UV spectra, mass spectra, MS/MS datasets and metabolite specific calibration curve of the two hundred validated pure substances. This data matrix allowed us to build programs that permit to achieve high-throughput screening (HTS) of chemical structures in order to accelerate the discovery of new compounds or to identify and quantify known compounds of specific biological interest or toxicity from complex mixtures such as plant extracts or preparations.

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Combination of LC retention, high resolution TOF-MS information and web database search as dereplication tools in a chemotaxonomic study of Lippia spp
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Dereplication of natural products (NPs) in crude plant extracts represents a key process to rationalize bioactivity guided isolation procedures [1]. This process is efficient using LC-MS, notably with instrument specific MS/MS databases, but libraries of spectra have to be built or rebuilt from large collection of pure standards. In order to evaluate how far NPs presents a key process to rationalize bioactivity guided isolation procedures dereplication of natural products (NPs) in crude plant extracts represented, making it possible to perform quality control on preparations containing P. hysterophorus. Firstly, parthenin was isolated from the plant material in order to use this as reference. In this study the development and validation of a HPLC method for the determination of parthenin in the powdered plant material are presented, making it possible to perform quality control on preparations containing P. hysterophorus. Firstly, parthenin was isolated from the plant material in order to use this as reference material. During the method development, the extraction procedure, sample preparation, and HPLC conditions were evaluated and optimized. The final method was fully validated in terms of calibration model, precision, accuracy, and specificity. Based on these results, it was concluded that the developed HPLC method is suitable for the determination of parthenin using a single-point calibration. The calibration model was

References:
Liquid chromatography techniques for separation of flavonoids from Droseraceae plants

Flavonoids which are presented in insectivorous plants of the Droseraceae family have wide range of advantageous properties i.e. an antioxidant, anti-inflammatory and antimicrobial activities, anti-tumor activity was reported as well. The purpose of the research was to develop the most favourable conditions for liquid chromatographic separation and identification of myricetin and queretin in extracts of insectivorous plants. In the research a methanol and chloroform extracts of the Drosera binata Labill., Drosera capensis L., Droseraaliciae Raym.-Hamet and Dionaea muscipula Ellis cultivated in vitro were used. In the first stage of the research an optimal composition of the eluent for selective separation of the components included in the extracts was selected. The thin layer chromatography (TLC) in the reversed phase and hydrophilic interaction chromatography (HILIC) conditions was used for selection of optimal chromatographic systems. In the second stage of the research a high performance liquid chromatography (HPLC) in RP and HILIC optimal conditions was used for detailed characteristic of analysed mixtures. The results show that thin layer chromatography is helpful technique for pre selection components of the eluent to separation of the flavonoids from complex herbal matrix. In the paper there is reported about the pharmacological properties of these secondary metabolites.

Optimal conditions of naphthoquinones separation from carnivorous plants extracts using thin-layer chromatography and high performance liquid chromatography

Droseraceae plants are owned by the family of carnivorous plants, which in an unusual way adapt to environments poor in nutrients. Droseraceae obtain essential nutrients catching insects and other small invertebrates. Plumbagin, chloroplumbagin, ramâtecon and droseron are naphthoquinones, which could be found in leaves and shoots of plants from Droseraceae family. In the literature there are a lot of information about the pharmacological properties of these secondary metabolites. The purpose of this research was to develop optimal conditions for separation of naphthoquinones contained in extracts of insectivorous plants of the species Dionaea muscipula Ellis, Droseraaliciae Raym.-Hamet, Drosera capensis L. and Drosera binata Labill. The experimental studies have been divided into two stages, the first included study using thin layer chromatography (TLC) and the second using high performance liquid chromatography (HPLC) technique. Both the studies by thin layer chromatography and high performance liquid chromatography was performed in normal and reversed phase system. Optimal conditions of naphthoquinones separation using TLC and HPLC in normal and reversed phase system will be presented. Acknowledgement: State Committee for Scientific Research, Grant No N N405 3757 37. This research work was supported by the European Union in the framework of the European Social Fund. The system project of the Pomorskie Voivodeship "InnoDoktorant – Scholarships for PhD students, II edition".

Application of HPTLC-MS for the on-line identification of oxypregnan glycosides in Hoodia gordoni

Hoodia gordoni (Mass.) Sweet is a succulent plant from South Africa and Namibia which has been used by the indigenous people to suppress appetite. Oxypregnan glycosides (hoodigosides) are considered as active principles [1], HPTLC method has been previously developed for the fingerprint analysis and identification of extracts from Hoodia gordoni [2,3]. Ion-trap tandem mass spectrometry and liquid chromatography coupled with electrospray ionization time-of-flight mass spectrometry have been used for the identification of isolated steroidal glycosides in Hoodia gordoni [4]. Recently, an HPTLC-MS Interface became available, which semi-automatically can extract zones of interest directly from a TLC/HPTLC plate and can direct them into a LC-MS system so that mass spectra can be obtained [5,6]. Previously we have tested the HPTLC-MS Interface with analytical screening herbal drugs [7]. We now have investigated the HPTLC-MS Interface for the identification of hoodigosides in extracts of Hoodia gordoni. Extracts have been applied as bands onto HPTLC plates using an automatic TLC sampler. Separated zones were eluted from the plate by the HPTLC-MS interface using methanol, as solvent delivered by an HPLC pump at 100 μl/min. The interface was hyphenated to a Finnigan LCQ Deca XP Plus ion trap mass spectrometer equipped with an electrospray ionization (ESI) source. Hoodigosides M, L, U, O, E, F, J, N, and C could be identified on the basis of the mass spectra obtained by HPTLC-MS. Therefore, the HPTLC-MS interface is both a quick and powerful tool for the on-line identification of hoodigosides in TLC separations and can complement the classical TLC detection tools. References: 1. Vermaak J et al. (2011) Planta Med 77: 2596. 2. Widmer V et al. (2008) Planar Chromatogr (2008) 21(1): 21 – 26. 3. Rumalla Ch et al. (2008) J Planar Chromatogr (2008) 21(1): 21 – 26. 4. Avula B et al. (2008) Rapid Commun Mass Spectrom 22(10): 2587 – 2596. 5. Luftmann H et al. (2007) Rapid Commun Mass Spectrom 21: 3772 – 3776. 6. Reich E, Widmer V (2009) Planta Med 75(7): 711 – 718. 7. Bauer R et al. (2010) Planta Med 76: 1334.

Application of near-infrared spectroscopy (NIRS) as a tool for quality control in Traditional Chinese Medicine (TCM)

Traditional Chinese Medicine is becoming more and more popular all over the world. Novel analytical tools for quality control are highly demanded enabling analysis starting at breeding and ending at biological fluids including urine or serum. Compared to analytical separation methods (chromatography, electrophoresis) near-infrared spectroscopy (NIRS) allows analyzing matter of interest non-invasively, fast and physical/chemical parameters simultaneously. It can be used for the quantitative control of certain ingredients. In many cases identification can only be achieved by pattern recognition. Therefore, NIRS combined with cluster analysis offers huge potential to identify e. g. species, geographic origin, special medicinal formula etc (Figure 1). In the present contribution the fundamentals, possibilities of NIR applied in quality control of TCM are pointed out and its advantages and disadvantages are discussed in detail by several practical examples [1,2]. A Böchi FT-NIR spectrometer was used for recording. Cluster analyses and PLSD calibration models were generated with HeNiCal 4.21 and/or The Unscrambler. A Perkin Elmer 400 spectrometer in combination with a microscope with a nitrogen cooled MCT detector-array was used to acquire the hyperspectral images. NIR imaging is highly useful to judge the botany and morphology of the sample and allows visualizing the distribution of active plant

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ingredients. Stable PLS calibration models can be applied for quantitative determination of APIs, judgment of raw materials, during the production and the preparation of medicinal formulations. Cluster analyses are highly suitable for identifying falsification, species and geographic regions. Both methods in combination are applied to monitor the quality of patented formulations.

**Figure 1:** Flow diagram of NIRS application fields in TCM


**Study and comparison of the Pistacia atlantica Desf. oleoresins from Iran**

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**PA57**

Quantitative and qualitative analyses of rosmarinic acid in South African *Salvia* species using two chromatographic techniques

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The genus *Salvia* (Lamiaceae) encompasses about 900 species worldwide of which 26 are indigenous to southern Africa. The South African species are used in traditional medicines [1], as spices and tea due to its reported good anti-oxidant properties [2]. The anti-oxidant capacity of these plants has been ascribed to the presence of phenolic compounds such as rosmarinic acid (RA), caffeic acid, carnosic acid and carnosol [2]. HPTLC-densitometric and HPLC-UV chromatographic techniques were used for qualitative and quantitative analyses of RA in 18 methanol- chloroform extracts from sixteen *Salvia* species. Polynomial and linear regression analyses were used to estimate the amount of RA in solvent extracts by HPTLC-densitometric and HPLC-UV techniques, respectively. RA was identified in all the samples investigated and ranged from 13.1 µg/mg (S. stenophylla (Burch. ex. Benth.) to 113.0 µg/mg (S. murioides L Bolus)). The paired sample t-test showed no statistical significant difference in the estimation of the amount of RA in the solvent extracts using the two chromatographic techniques. A strong correlation (r²=0.93) was found between the estimation using the HPTLC-densitometric and the HPLC-UV calibrations. Acknowledgement: National research Foundation (South Africa); Tshwane University of Technology. South Africa National Biodiversity Institute (Pretoria). References: 1. Watt JM, Breyer-Brandwijk MG (1962) The Medicinal and Poisonous Plants of Southern and Eastern Africa. 2nd edition. E and S. Livingstone, Edinburgh. 2. Kamatou OPP et al. (2010) Food Chem 119: 684 – 688.

**PA56**

The genus *Salvia* (Lamiaceae) encompasses about 900 species worldwide of which 26 are indigenous to southern Africa. The South African species are used in traditional medicines [1], as spices and tea due to its reported good anti-oxidant properties [2]. The anti-oxidant capacity of these plants has been ascribed to the presence of phenolic compounds such as rosmarinic acid (RA), caffeic acid, carnosic acid and carnosol [2]. HPTLC-densitometric and HPLC-UV chromatographic techniques were used for qualitative and quantitative analyses of RA in 18 methanol- chloroform extracts from sixteen *Salvia* species. Polynomial and linear regression analyses were used to estimate the amount of RA in solvent extracts by HPTLC-densitometric and HPLC-UV techniques, respectively. RA was identified in all the samples investigated and ranged from 13.1 µg/mg (S. stenophylla (Burch. ex. Benth.) to 113.0 µg/mg (S. murioides L Bolus)). The paired sample t-test showed no statistical significant difference in the estimation of the amount of RA in the solvent extracts using the two chromatographic techniques. A strong correlation (r²=0.93) was found between the estimation using the HPTLC-densitometric and the HPLC-UV calibrations. Acknowledgement: National research Foundation (South Africa); Tshwane University of Technology. South Africa National Biodiversity Institute (Pretoria). References: 1. Watt JM, Breyer-Brandwijk MG (1962) The Medicinal and Poisonous Plants of Southern and Eastern Africa. 2nd edition. E and S. Livingstone, Edinburgh. 2. Kamatou OPP et al. (2010) Food Chem 119: 684 – 688.

**PB1**

Expression of amorph-4,11-diene synthase (ADS) gene in Iranian *Artemisia annua* L. genotypes

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Malaria disease is caused by unicell of *Plasmodium falciparum*. Nowadays, malaria has been reported in more than 100 countries. The artemisinin is a sesquiterpene that is produced by two pathways of isoprenoid and mevalonate in *Artemisia annua* L. Artemisinin is new and effective drug is widely used. *Artemisia annua* is annual plant and native to Asia and most probably China. In this study, six different genotypes *Artemisia annua* was collected from the province of Golestan. Amorpha-4, 11-diene synthase (ADS) promoter was analyzed by using different
cis-elements database of PLATcare, TRANSFAC and PLACE. The result showed that there are different cis-elements responding to plant hormones and abiotic stress in ADS promoter. We also identified two new putative transcription factors in EST library of Artemisia annua then studied the expression of ADS gene and three transcription factors by using real time PCR technique. The result showed that WRKY transcription factor had more important role than other transcription factors.

**PB2**

Development of NaCl-tolerant line in Tanacetum cinerariaefolium through shoot organogenesis of selected callus line

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Plants were regenerated successfully through shoot organogenesis of a NaCl-selected callus line of Tanacetum cinerariaefolium (Trevir.) Schultz-Bip developed through stepwise increase in NaCl concentration in MS medium. Increasing NaCl level concentration (0, 5, 10, 15, 20, 25, 30, 35, 40, 45mM) from low level to high level was found to be a better way to isolate NaCl-tolerant callus line, since direct transfer of callus to high saline medium was detrimental to callus survival and growth. Among different media and growth regulator treatments, MS medium containing 1 mgL-1 BA and 1 mgL-1 NAA or 1 mgL-1 BA, 2 mgL-1 NAA and 0.5 mgL-1 GA3 for shoot organogenesis in selected callus line and B5 medium supplemented with 2 mgL-1 NAA showed best response for root regeneration. As increasing NaCl concentrations (From 0 to 45 mM) the ability of shoot and root regeneration were decreased. The selected callus line showed significance increase in proline content and decrease in proline content. Based on growth performance and proline content (20 mM in callus line and 35 mM in shoot culture) could be considered as NaCl-tolerant line showing all positive adaptive features towards the salinity stress. Further studies about agronomic performance of obtained plants under saline soil condition are necessary for understanding to check the genetic stability of the induced salt-tolerance plants.

**PB3**

Stable Over expression of codeinone reductase gene in transgenic Papaver somniferum plant

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Papaver somniferum today is the commercial source of the narcotic angesics morphine and codeine. By conversion of codeineone and morphine to morphine, codeinone reductase is a key gene in metabolic engineering of isoquinoline alkaloids pathway. In this project, at first we optimised expression of gus gene in P. somniferum via Agrobacterium tumefaciens containing pBI121 plasmid. Encoding gene of COR enzyme was isolated using primers which design on the base of gene sequence of P. somniferum. Transgenic plant was developed through stepwise increase in NaCl concentration in MS medium. Increasing NaCl level concentration (0, 5, 10, 15, 20, 25, 30, 35, 40, 45mM) from low level to high level was found to be a better way to isolate NaCl-tolerant callus line, since direct transfer of callus to high saline medium was detrimental to callus survival and growth. Among different media and growth regulator treatments, MS medium containing 1 mgL-1 BA and 1 mgL-1 NAA or 1 mgL-1 BA, 2 mgL-1 NAA and 0.5 mgL-1 GA3 for shoot organogenesis in selected callus line and B5 medium supplemented with 2 mgL-1 NAA showed best response for root regeneration. As increasing NaCl concentrations (From 0 to 45 mM) the ability of shoot and root regeneration were decreased. The selected callus line showed significance increase in proline content and decrease in proline content. Based on growth performance and proline content (20 mM in callus line and 35 mM in shoot culture) could be considered as NaCl-tolerant line showing all positive adaptive features towards the salinity stress. Further studies about agronomic performance of obtained plants under saline soil condition are necessary for understanding to check the genetic stability of the induced salt-tolerance plants.

**PB4**

Accumulation of phytoalexins in potato tuber treated with plant extract

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One of the best and longest studied defense responses of plants to infection is the induced accumulation of antimicrobial, low-molecular-weight secondary metabolites known as phytoalexins. A role for these compounds in defense has been revealed through several experimental approaches. Samples of Olea europae L. leaves were collected from Béjaia in January 2009. The whole samples were dried in shade and crushed to fine powder. 20 g of dried powder of olive leaves were submitted to extractions which were carried out twice for 24 h with 400 mL of ethanol according to Ranalli et al. (2006). The total phenolic contents of the samples were determined with the Folin Ciocalteau reagent. The half tuber cultivars Desiree and Spunta, was treated by depositing 100 µl of one of the previously prepared phenolic extracts in the hole drilled with a cork as described by val et al. (2006). They were then inoculated with an inoculum of Pectobacterium atrosepticum (106 cfu/ml). The tubers were assessed after five days for the development of disease symptoms, and were used to evaluate production of phytoalexins The rate of phytoalexins in relation to cessation of pathogen development, quantification of phytoalexins at the infection site, of potato tubers treated with plant extract was studied. The results of the half-tuber inoculation treated by various plant extracts showed a remarkable reduction in the amount of rotted tissue. Evidence in support of phytoalexins in resistance as well some recent advances in phytoalexins biosynthesis are reviewed. Criteria for evaluating a role for phytoalexins in disease resistance are also discussed. Keywors: phytoalexins, potato, tuber, resistance.

**PB5**

Effect of Plant Growth Promoting Rhizobacteria (PGPR) on agronomic characteristic and root colonization in fennel

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In order to study the effect of Plant Growth Promoting Rhizobacteria (PGPR) on agronomic characteristic and root colonization in fennel (Foeniculum vulgare Mill.), an experiment was conducted in western of Iran in 2008 and 2009 growing seasons. The factors were Plant Growth Promoting Rhizobacteria (Azotobacter inoculation with and non-inoculated) and nitrogen application (0, 40, 80 Kg/ha-1). The treatments were arranged as factorial in a randomized complete blocks design with three replications. Results showed that the highest grain yield, umbrella per plant, biological yield and root colonization percent were obtained with Azotobacter treatment. Nitrogen application was significant affected on studies traits. The highest grain yield, biological yield and root colonization percent obtain by 80 Kg/ha-1. Interaction effect PGPR x nitrogen application was affected on grain yield and colonization percent. The highest grain yield and root colonization percent obtained Azotobacter x 80 Kg/ha-1 nitrogen.

**PB6**

Effect strains of Mycorrhiza on root characteristic and concentration of phosphate, iron and zinc in cumin (Cuminum cyminum L.)

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Symbiosis between plants and mycorrhizal fungus are very important for agriculture system and natural resource. In soil whit, less fertility mineral matter absorbed by Mycorrhiza can lead to improvement in growth that results increase in mycorrhizal plant resistance in comparison whit non-mycorrhizal plants in stress condition. In order to test the influence of the strains of Mycorrhiza on root characteristic and nutrients on Cuminum cyminum L. an experiment was conducted based on completed design with three replications in western of Iran in 2009 – 2010 growing season. Strains of mycorrhiza including of Glomus fasciculatum, Glomus etunicatum, Glomus mosseae and Glomus intraradices. In these research traits as: root length, root length, root length/root dry weight ratio, total dry weight of colonization and concentration of phosphate, iron and zinc. Results of variance of analysis showed that strains of mycorrhiza had significant affected on studies characteristic, as Glomus fasciculatum had the highest root colonization, root dry weight, root length/root dry weight ratio. Strains of Mycorrhiza had significant affect on absorption of nutrients. Glomus fasciculatum had a more ability in absorption of phosphate, iron and zinc other then strains of mycorrhiza.
Endophytic fungi constitute one of the most interesting sources of bioactive natural products. They are synergistic to their respective host and at least some of them are thought to play an important role in the host’s defence by producing secondary metabolites that protect the host from being attacked by pathogenic fungi [12]. Ten known compounds alatinone, emodin, w-hydroxyemodin, 2-Acetylatediquinolizin-4(3H)-one, chrysophanol, cyclo-L-Ala-L-Leu, cis-cyclo (pro, val), 2,3’-Dihydroborbicillin, meleguina, and uraci1 were identified from the EtOH-extract of a Crassip-s-petrole culture of the endophytic fungus Penicillium chrysogenum isolated from red alga (Liaogia viscid (Forsskål) C. Agardh) collected from the Egyptian Red Sea. The structures of the compounds were elucidated on the basis of comprehensive NMR spectral analysis (1H- and 13C- NMR, HRCLS, HSQC, HMBC) as well as mass spectrometry. The crude organic extract and some of the pure compounds showed moderate to strong antimicrobial activity. References: [1] Stierle A et al. (1993) Science 260: 214 – 216. [2] Strobel G et al. (1997) Aust J Bot 45: 1037 – 1042.

Derivatives of natural and aromatic compounds obtained by biotransformation constitute an important resource for natural pharmaceutical, fragrance and aroma ingredients or active compounds. Monoterpenes and their new metabolites are very important resources in such applications. Key words: β-phellandrene, biotransformation, monoterpenes

Hyoscyamine and scopolamine are medicinally important tropane alkaloids, which possess anticholinergic and central nervous system activities. They have well established therapeutic uses (in ophthalmology, cardiology, gastroenterology, etc.). For medicinal purpose, scopolamine is much more useful and valuable because of its higher physiological activity and fewer side effects. These natural substances are exclusively extracted from plants [14]. Several species from the family Solanaceae like the genus Atropa, Datura, Duboisia, Hyoscyamus, Scopolium produce these alkaloids. Atropa belladonna L. is a perennial herbaceous plant and most importantly commercial source of pharmaceutical tropane alkaloids in the family Solanaceae. Hyoscyamus 1 species in Iran; one of them is Hyoscyamus kurdicus Bornm. from Kurdistan province [2]. Hyoscyamine 6β-hydroxylase (h6h) is a bifunctional enzyme which catalyzes the last two oxidative reactions of tropane alkaloid biosynthetic pathway, converting hyoscyamine to scopolamine [3]. We expressed the h6h gene from A. belladonna in H. kurdicus, which caused over accumulation of scopolamine. For this purpose RNA was extracted from leaf disk of A. belladonna plants. cDNA was synthesized and amplified by specific h6h primers. PCR products were sequenced and constructed to pRH121 shuttle vector. H. kurdicus seeds were collected from its natural habitats in Kurdistan-Iran and cultured. Keywords: Hyoscyamus kurdicus, Atropa belladonna, scopolamine, h6h gene transformation Acknowledgement: This research was supported by the National Institute of Genetic Engineering and Biotechnology References: 1. Liu X et al. (2010) MedPlant Res 4(17):1708 – 1713. 2. Bahmanzadegana A, Sefidkona Fand Sonboli A (2009) Iranian J Pharmaceutical Res 8(1): 65 – 70. 3. Hashimoto T, Yun D-J and Yamada Y (1993) Phytochemistry 42:713 – 718 4. Yang Ch et al. (2011) Plant Omics Journal 4(1):29 – 33.

Mushroom polysaccharides offer a lot of hope for cancer patients and sufferers of many devastating diseases. A variety of polysaccharides from a number of mushroom varieties have been shown to enhance the immune system. Yield and functionally of polysaccharides are highly dependent on their culture conditions, such as different culture compositions and environmental parameter (1). In this study the effects of different Nutrient sources including: yeast extract, Mycological peptone, poly peptone, ammonium nitrate, ammonium sulfate and ammonium oxalate in two different media (Complex and synthetic liquid culture) were investigated. For the determination of polysaccharides produced by Agaricus blazei Murrill the total polysaccharides which were precipitated by absolute alcohol were weighed. The experiments showed that the highest growth and polysaccharide production were obtained when yeast extract used as nutrient source. The concentration of polysaccharide in both complex and synthetic media when yeast extract was used were similar. The lowest growth and productivity were also seen in medium containing ammonium sulfate.

In the present study, we evaluated genetic diversity between seventy one samples of olive (Olea europaea L.) germplasms (40 accesses of Iranian cultivars and 26 foreign cultivars) growing in Kermanshah province by Morphological, RAPD and ISSR markers. Morphological characters were compared to the molecular data obtained using RAPD and ISSR markers. Thirty-four RAPD primers and 8 ISSR primers amplified 412 and 118 polymorphic fragments, respectively. The dendrograms based on UPGMA cluster analysis using Jaccard’s similarity index for RAPD, ISSR markers and combined both markers include 7, 5 and 7 groups, respectively. The results indicated not a relationship between genetic diversity and different geographical regions in Kermanshah Province. This suggests that cultivar selection has occurred in different genetic pools and in different areas. The results of these analyses showed the existence of a genetic divergence between accessions and this diversity can be used in olive breeding programs. This study allowed us to analyze genetic diversity for further prospecting, to provide additional genetic information on the agronomic and quality characteristics of the olive varieties, and for introducing new olive accessions. Keywords: Genetic diversity, Olea europaea L., RAPD, ISSR, Kermanshah province.
Production of phytohormone auxin by rhizospheric cyanobacterium Leptolyngbya sp. MMG-1

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The genus Leptolyngbya is one of the most flourishing filamentous cyanobacteria in rice fields. Leptolyngbya sp. MMG-1 was isolated from the rhizosphere of rice plants. The strain was characterized morphologically by light microscopy and confocal laser scanning microscopy and later identified by 16S rDNA sequence analysis. The ability of this strain to synthesize the auxin like bioactive compound was demonstrated under various cultivating conditions. Auxin was extracted from the culture of Leptolyngbya strain MMG-1 and its identity was confirmed as IAA (indole-3-acetic acid) by thin layer chromatography (TLC) as well as by high performance liquid chromatography (HPLC) of a flavouring auxin L-tryptophan was required for IAA biosynthesis. Highly significant corre-
lation was recorded between the IAA secreted by the strain and the initial concentration of L-tryptophan in the medium as well as the incubation time. Leptolyngbya strain MMG-1 tended to accumulate more IAA than it released into the medium. The bioactivity of the secreted IAA was established by its effect on the formation of roots by Pisum sativum. There was a significant positive effect of the supernatant of cultures of BAP and IAA; callus produced 492.75 mg/g dry weight (dw) while re-
served when they were cultured on media containing combinations of sidered variations in patterns of total phenolics in the

In vitro regeneration and analysis of total phenolics in Ocimum basilicum L. (sweet basil)

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An efficient in vitro regeneration system via direct and indirect shoot organogenesis was developed from cotyledonal leaf and hypocotyl explants of Ocimum basilicum L., commonly known as a sweet basil, belonging to the family Lamiaceae. Sweet basil is used in traditional medicine as a culinary herb and a well known source of flavonoids and phenol-

terpenoids. Various types and concentrations (ranging from 0.1 to 3.0 mg/l) of plant growth regulators in different combinations (TDZ+IAA, BAP+IAA, KIN+IAA, TDZ+NA) were tested using Murashige and Skoog medium. The highest number of shoots (1.3 shoots per explant) was obtained from hypocotyl explants on medium supplemented with the growth regulators 6-benzylaminopurine (BAP) and indole-3-acetic acid (IAA). For rooting, regenerated shoots were transferred to auxin-containing media. After the rooting stage, healthy regenerants were transferred to pots for acclimatization process, through which almost all of them grew very vigorously, attained maturity and produced fertile seeds. We also ana-
lyzed variations in patterns of total phenolics in the in vitro cultured callus, regenerated plantlets from callus as well as in leaves of ex vitro plants by UV-spectrophotometer. The phenolic contents of regenerated plantlets and leaves of ex vitro plants were found very similar but con-

Enhanced glycyrrhizin production in Glycyrrhiza inflata hairy roots cultures using elicitation

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Glycyrrhiza inflata Batal (Leguminosae) has been used as a source of licorice. Licorice has had a high market demand due to its high medicinal value, whereas the licorice resources in the world regions are lim-

PB15

SPOTlight: Sustainable Production of Thapsigargin using Light – turning moss into a terpenoid producer

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Terpenoids is the biggest group of secondary metabolites among plants. Their accumulation in species belonging to the Apiaceae and Asteraceae family is the reason why several of these plants possess biological activities that are used in the treatment of various diseases [1,2]. Thapsigarin-gin’s ability to induce apoptosis by inhibiting the endoplasmatic calcium ATPase (SERCA) makes it a promising agent for the therapy of cancer. The development of a pro-drug targeted to prostate cancer cells allows its selective use [3]. Comparison with related sesquiterpenes for which biosynthetic enzymes have been identified enables us to propose an enzymatic pathway by which thapsigargin could be generated from farnesyl diphosphate via several intermediates [1]. Large scale HIGH Throughput Sequencing of expressed mRNAs from Thapsia species was undertaken to provide contig database of gene fragments, to date we have identified and cloned several genes of interest, which are un-
dergoing characterization. Our first targets are two sesquiterpene synthases. Secondly, we have cloned 12 P450’s in the CYP71 clade, which is believed to be involved in secondary metabolism. These are currently undergoing characterization in yeast. To optimize Physcomitrella as a production host for thapsigargin we aim at constitutively upregulate the expression of the enzymes involved in the biosynthesis of the isopentenyl diphosphate (IPP) biosynthesis [4]. These metabolic modifications will increase the pool of the terpenoid precursor IPP that is available for sesquiterpene biosynthesis. We aim at establishing the moss Physcomitrella patents as the system of choice for the production of all kinds of terpenoids. Acknowledgments: We would like to thank The Danish Council for Strategic Research for their financial support.

PB16

The challenges of Podophyllum tissue culture

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Podophyllotoxin is obtained from the rhizomes and roots of wild popu-
lations of Podophyllum hexandrum Royle. This is a low-growing plant with a long juvenile phase, making the availability of this natural pro-
duct limited (1). Demand for podophyllotoxin was created with the introduction of its semi-synthetic derivatives in cancer chemotherapy (2). The species is endangered in the Himalayan region (3) through over collecting and lack of organized cultivation (4). Efforts remain to facilitate the in vitro propagation (5, 6) of Podophyllum. In the present investigation on the tissue culture of *P. hexandraum*, seeds germinated within 35 to 40 days in moist, dark conditions, with in vitro grown seedlings being obtained either on 0.2 normal strength semi-solid B5 medium (7) or full-strength MS medium both lacking growth regulators. Although callus induction from root explants cultured on 0.5 normal strength B5 medium containing 1.0 mg/l 2,4-D, 1.0 mg/l BAP and 1.0 mg/l GA3 was slow, tissue became embryogenic after successive subcultures. Embryogenic cell suspensions were established in the dark from root-derived callus, cultured in liquid MS medium containing 2,4-D and kinetin, at 2.0 mg/l and 0.25 mg/l, respectively. Differentiation of somatic embryos and subsequent shoot formation occurred on either full-strength or half-strength MS medium with 0.45 mg/l BAP. Rooting of somatic embryo-derived plants was stimulated by the inclusion of 10^{-5} M lipo-oligosaccharide in the culture medium. A robust explant-to-plant transformation system for *P. hexandraum* will reduce pressure on wild resources and may offer an alternative source of podophyllotoxin production. Acknowledgement: To RHEA/CNPq and Funded for their financial support to CGS, which is greatly appreciated. References: 1. Choudhary DK et al. (1998) J Med Aromat Plant Sci 20: 1071 – 1073. 2. Ertürk E et al. (2004) Appl Microbiol Biotechnol 65: 504 – 519. 3. Aini S et al. (1997) Plant Genet Resour News 110: 20 – 34. 4. Nadeem M et al. (2000) Biol Conserv 92: 121 – 129. 5. Chalabrody A et al. (2010) Indian J Biotechnol 9: 217 – 220. 6. Silva CG (2000) Ph. D. Thesis. University of Nottingham, Nottingham, UK. 7. Heyenga AG et al. (1996) Plant Cell Rep 9: 382 – 385.

PB17

Another CAPS DNA marker for sex identification in jojoba seedlings

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Jojoba (*Simmondsia chinesis* (Link) Schneider) plant has immense economic value. Jojoba is grown for its oil, which is a unique liquid wax consisting of esters formed from acids and alcohols with chain lengths of 20 or 22 carbon atoms. Higher seed yield is obtained when well balanced male and female plants are established in jojoba orchard. However, the sex of jojoba plants cannot be determined with morphological characters until the plants reach reproductive maturity. DNA markers are commonly used for plant genetic studies [1,2,3,4]. Up-to-date several male and female specific DNA markers have been developed for sex identification in jojoba seedlings [5,6]. This study reports another cleavage-amplified polymorphic sequence (CAPS) assay, which easily identify male individuals. Genomic DNA samples of 16 male and 16 female jojoba plants growing in an orchard were extracted using a DNA extraction protocol [4]. A touch-down PCR approach was used to amplify genomic DNA of jojoba using *J818F* 5' AGGGGATAATAGGGCCGAAT-3' and *J818R* 5' ACCACAGAATGTTAGGAT-3' primer pair [6]. Amplified products were digested using Hind III restriction enzyme. Electrophoresis separation of the digested products as shown in Figure 1 indicated that male and female jojoba plant can be easily identified. CAPS marker reported in this study could be used in breeding studies and in the sex allocation of seedlings in seed orchard establishment.

![Figure 1: Male specific CAPS marker](image)

M. DNA size marker


PB18

Comparison of multiple DNA alignment algorithms for Labiatae molecular phylogeny inferences

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Multiple alignment of sets of nucleotide or amino acid sequences are usually required for phylogenetic studies for phylogeny inferences. There are several widely used programs for carrying out automatic multiple alignment of nucleotide sequences [1,2,3]. Since there exist different type of multiple alignment programs, the selection of proper alignment program is important for true phylogeny inferences. In order to compare different alignment algorithms implemented in five different multiple alignment programs (ClustalW, T-Coffee, MAFFT, Kalign and MUSCLE, all implemented in the Ugene software) we were used to align internal transcribed spacer sequences of 24 taxa of Labiatae family. MetaPIGA 2.0 software was used to obtain phylogenetic trees. Analysis parameters of MetaPIGA 2.0 were set to heuristic search using stochastic consensus pruning (metaCA). GTR model of likelihood rate test and other parameters were kept at default values of the program. Evaluations of the multiple alignment algorithms were based on bootstrap values and groupings at the genus level of consensus trees. Analyses indicated that phylogeny inferences were affected with the multiple alignment algorithms used. Among the tested multiple alignment programs, sequences aligned with MAFFT produced better phylogenetic tree. Further studies will be useful to reveal whether the use of different phylogeny programs and different gene sequences could overcome the effects of multiple alignments on phylogeny inferences. Acknowledgement: This research is supported by the Scientific Research Projects Coordination Unit of Akdeniz University. References: 1. Ince AG et al. (2005) Akdeniz Univ Ziraat Fak Derg 18: 157 – 162. 2. Helaers R, Milinkovitch MC (2016) BMC Bioinformatics 11: 379. 3. Ince AG et al. (2010) Genet Resour Crop Ev 57: 773 – 779.

PB19

Molecular authentication of Thai medicinal plant, *Vitex glabrata*, by PCR-RFLP

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Several species in *Vitex*, a genus in the family Labiatae, are medicinal plants used in folk remedy in many countries with different effects. *Vitex glabrata* R. Br. is used as anthelmintic, wound healing and sexual enhancer, which are different from the uses of other *Vitex* species. These effects are related to the presence of ecdysteroids in the plant [1] since it has high amount of 20-hydroxyecdysone (2). Crude drugs of many *Vitex* species have similar appearance, which lead to confusion and misuse. Therefore, an accurate authentication of *V. glabrata* is essential for medicinal purpose. In this study, PCR-restriction fragment length polymorphism (PCR-RFLP) based on the chloroplast matured K (matK) gene analysis was applied to identify *V. glabrata* from other *Vitex* species commonly found in Thailand. Among five *Vitex* species, a distinctive site recognize by a restriction enzyme HindIII in *V. glabrata* was found. A pair of new primers, VI1111mutKF and VI1516mutMK, was designed based on the sequence of *V. glabrata* to amplify a smaller fragment of 407 bp in length from genomic DNAs of the leaves of these five species. Only the PCR product of *V. glabrata* could be digested with HindIII into two fragments of 324 bp and 83 bp while the other species remained undigested. This result suggests that PCR-RFLP analysis is an effective and accurate method for authentication of *V. glabrata*. Acknowledgement: the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand. References: 1. Bóthóni M, Pongrácz Z (2005) Curr Med Res 12:153 – 172. 2. Werawattanametin K (1986) J Nat Prod 59(2):365 – 366.

PB20

Phenolic compounds and antioxidant capacity of chickpea seed

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Phenolic compounds have free radical scavenging abilities, antimutagenic and anticancerogenic activities and there is increasing interest for phenolics compounds in food, today [1,2]. In our work the content
and composition of phenolics compounds as well as their antioxidant activity of chickpea (Cicer aritinum L.) were examined. The chickpea seed were milled and the plant extracts were prepared by using 80% (v/v) ethanol. The total phenolics compounds content was determined based on a standard curve with chlorogenic acid concentrations covering the range from 50 to 1000 μmol and the antioxidant activity was determined by using DPPH radical scavenging capacity [3]. The composition of phenolics compounds was determined by HPLC analyses on an Agilent 1100 Series HPLC system, Agilent Eclipse XDB-C18 column and spectrophotometric detection in the UV region at 350 nm was used [4]. The phenolics compounds content was 8.37 μmol of chlorogenic acid per g of dried extract residue i.e. 0.33 μmol of chlorogenic acid per g of milled chickpea seed. Results of antioxidant capacity of investigated phenolic extracts showed the maximum DPPH radical scavenging capacity was 38% at extract’s concentration of 10.9 mg/ml and the extract’s concentration sufficient to obtain 50% of maximum scavenging capacity was 2.9 mg/ml. By using the HPLC analysis, chlorogenic acid (4.26%), hydroxybenzoinic acid “C1” (10.86%), 5-O-cafeoilshikimic acid (5.21%), kaempferol 3-O-7-D-glisulose (7.7%), kaempferol 3-O-rhamnoside (33.52%), kaempferol-7-O-rhamnoside (7.6%) and genkwanin 4-O-glucoside (4.59%) of phenolics compounds were found. In total sum, the content of kaempferol glucosides was the biggest (48.96%).


**Effects of CAMP modulators on flavonoid accumulation in cell cultures of Hypericum androsaemum L.**

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Hypericum androsaemum L. has been used in traditional medicine for its diuretic and hepatoprotective properties [1], which are attributed to the diverse flavonoids and phenolic acids found in this species. Cell suspension cultures established from hypocotyl-derived callus of H. androsaemum were reported [2] to accumulate low amounts of flavonoids, with maximum levels occurring on the 14th day of the growth cycle. More recently [3], it was shown that treatment of 11-day-old cultures for 72 h with 15 mM CaCl2 or 5 mM calcium ionophore A23187 increased considerably the accumulation of flavonoids and the activity of phenylalanine ammonia-lyase (PAL, a key regulatory enzyme of phenylpropanoid metabolism). Since adenylyl cyclases can be regulated by Ca2+, similar experiments were carried out in this study using three different modulators of intracellular cAMP: dibutyryl-cAMP (100 μM, a membrane permeable cAMP analogue), IBMX (100 μM, a CAMP phosphodiesterase inhibitor) and forskolin (20 μM, an adenylyl cyclase activator). The first two treatments induced a marked increase in both PAL activity and flavonoid content of cells, as compared to control cultures. Increased levels of flavonoids were also found in forskolin-treated cells, but in this case accompanied by an insignificant rise in PAL activity. Considered together, these results are in agreement with the involvement of cAMP signaling in flavonoid metabolism of H. androsaemum cell cultures. Acknowledgement: PCT and POCI/POFED for financial support. References: 1. Novais M et al. (2004) Ethnopharmacol 93: 183 – 195. 2. Paranhes A (2006) Planta Med 72: 1060 – 1061. 3. Paranhes A (2007) Planta Med 73: 1017.

**Towards the sustainable and continuous in-vitro production of active pharmaceutical ingredients from medicinal plants**

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Medicinal plants have been used for the past millennium to treat various conditions, especially in the oncology market. Despite an increasing interest in the translation of traditional knowledge of medicinal plants into clinical drugs, progress has been quite slow since the discovery of Paclitaxel and Camptothecin in the 1970’s. One aspect which could explain the limited number of complex molecules entering clinical trials and reaching the patient is the restricted supply chain of the plant raw material, thus limiting the availability of the Active Pharmaceutical Ingredients (API). Still today, most of the raw materials needed for the extraction of the active ingredients are harvested from cultivated or wild plant populations, posing a threat to the bioavailability of certain medicinal plants and strong variability in the yield of API. We have developed a new in-vitro propagation method based on the use of temporary immersion bioreactors that allows for the rapid and abundant generation of a leafy-biomass from transgenic plants (Michoux et al., 2010). This technology is now being applied to medicinal plants and the results will be discussed. This technology provides a unique opportunity for the sustainable production of complex APIs which require plant cell differentiation. References: Michoux F, Ahmad N, McCarthy J and Nixon PJ (2010) Plant Biotechnology Journal Online Nov 24.

Taxus baccata plants. Using submerged culture conditions on an industrial scale.

Mushrooms have become attractive as functional foods while they represent an untapped source for the development of drugs and nutraceuticals. Comparing with the established field cultivation, their submerged cultivation has significant potential importance for the effective production of biomass and valuable metabolites [1]. In this study, Ganoandra australis (Fr.) Pat. (strain ATHUM 4345, collected from Greece), which is a species of pharmaceutical interest [2] was investigated for maximum biomass production with enhanced dietary fiber and bioactive β-glucan content in a batch–stirred tank bioreactor. Specifically, when the optimized culture medium was tested in a 20-L stirred tank bioreactor, high biomass yields (10.1 ± 0.4 g/L) and productivity of 0.09 g L⁻¹ h⁻¹ were obtained. The yield coefficients for total glucan and dietary fibers on biomass formed were 94.9 ± 6 and 341.2 ± 12.3 mg/g mycelium dry weight, respectively [3]. Additionally, the biomass produced by the process described above, was lyophilized and finally extracted with ethyl alcohol/ethyl acetate (1:1). The obtained extracts were analyzed for carbohydrate content and the presence of triterpenoids using HPLC. The obtained results indicate that the low taxane levels in the roots could reflect the low transcript accumulation of the aforementioned genes in this part of the plant, although an active metabolism or translocation of taxanes to the aerial part could also be responsible. The high content of 10-deacetylbaccatin III and very low levels of baccatin III, together with the low mRNA accumulation of DBAT in the aerial part, suggest that this gene could control a limiting step in the taxane biosynthetic pathway in T. baccata plantlets grown for 1 year in vitro conditions.

Optimization of biomass production with enhanced bioactive compound content by the medicinal mushroom Ganoandra australis under submerged culture

References:


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Changes of taxane production and gene expression during the development of in vitro Taxus plant cultures

PB26

The production and accumulation of secondary metabolites in plants is always regulated by the expression of genes involved in their biosynthesis. There are very few reports about the regulation of the biosynthesis of the anticancer agent taxol and other related taxanes and the rate-limiting steps involved, especially during the development of Taxus plants. Using Taxus baccata L. plantlets grown in vitro for 1 year, our group has studied the relationship between the profile and production of taxanes and the expression of genes coding for enzymes that participate in early and later steps of taxane biosynthesis (TXS, DBAT, BAPT and DBAT′). Our results indicate a higher taxane content in the aerial part of the plantlets than in the roots, 10-deacetylbaccatin III being the most abundant taxane, with very low conversion to baccatin III and taxol. The mRNA accumulation of the studied genes was also higher in the aerial part than in the corresponding roots. Our results indicate that the low

Characterization and in vitro evaluation of a new chitosan-based propolis tooth varnish

PB28

Characterization and in vitro evaluation of a new chitosan-based propolis tooth varnish

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Propolis has been recently studied due to its cariostatic activity. Although its activity has been exhaustively demonstrated, there is no formulation commercially available using this agent in tooth care. Chitosan has been extensively studied due its film-forming properties. Recent studies had also shown that chitosan can adhere on tooth surface and has an effective inhibition effect on the initial adherence of oral bacteria onto human tooth surface. Chitosan-based propolis varnish was successfully developed and characterized by ATR-FTIR spectroscopy SEM, hydration potential, casting time and mucoadhesive properties. The formulation presented good tooth surface adherence and ability to form films very fast on tooth surface when compressed air was used as casting agent. Also, the varnish presented interesting hydration potential (Figure 2), which suggests that the formulation will not be easily removed from tooth surface by saliva. Propolis varnish has, also, shown antimicrobial activity against Streptococcus mutans (MD 8.67 ± 0.52) and Streptococcus sanguinis (MD 11.70 ± 2.11). Its cytotoxicity was made by direct contact with osteoblasts and evaluated by the MIT method. After 24 hours, the varnish reduced 20% of the cells, showing low toxicity (ISO 10993 – 5). The results were analyzed statistically by ANOVA in a significance of p < 0.05. Acknowledgement: The authors thank for the financial support to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível
Biotransformation of cis-jasmone by fungal strains
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Cis-jasmone, well known as a component of plant volatiles, is produced also by damaged plant vegetative tissues [1]. This natural ketone is considered to be the final product in the jasmonic acid biosynthetic pathway from linoleic acid [2]. This pale yellow, viscous liquid compound possesses strong jasmine fragrance and interesting biological activities. It is an activator of chemical defence in plants, causing the release of volatile semiochemicals e.g. bean plants, Vicia faba, treated with cis-jasmone showed a significant increase in the production of (E)-ocimene [3]. Cis-jasmone is also responsible for plant-insect interactions. The population of grain aphid Sitobion avenae (Fabricius) is reduced by cis-jasmone which plays a role of repellent [4, 5] while members of two families of insect parasitoids (Braconidae and Sarcophagidae) in hop (Humulus lupulus L.) cultivation are attracted by cis-jasmone [6]. In our study we focused on the biotransformations of cis-jasmone by fungal cultures: Pencillium, Abisida, Syncephalastrum, Botrytis, Aspergillus, Cun- ninghamella, Chaetomium, Didymosphaeria. Screening procedure led to the selection of fifteen microorganisms that have ability to biotransformation of cis-jasmone. Microbial transformations were used as a tool to obtain new biologically active oxiderivatives. Major product of biotransformations was 4-hydroxyjasmone which was formed in regio- and stereoselective process of hydroxylation.


PB31
Callus culture studies on Jasminum malabaricum - An endemic medicinal plant
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Jasminum malabaricum Wight belonging to the family Oleaceae is endemic to Western Ghats of India. It is a climber, with white flowers and fragrance and known for its ethno medical importance like antioxidant, antitumor, antiatherogenic properties [2,3]. One can expect that the ester derivatives containing both molecules can also possess the valuable biological activity and they can find an application as medicine for many diseases. Esterifications of CLA with betulin and betulinic acid were carried out with using of N,N-diacylhexylcarbodiimide (DCC) as coupling agent, in presence of 4-dimethylaminopyridine (DMAP) in dichloromethane for betulin derivatives or in pyridine for betulinic acid derivative respectively. The cytotoxic activity of betulin (1), betulinic acid (2), mixture of CLA and their derivatives (3a-c, 4) in vitro was determined by performing the MTT (3-(4,5-dimethylthia- zol-2-yl)-2,5-diphenyl tetrazolium bromide) cytotoxicity assay. Five different cell lines were used: P388 (mouse leukemia), CCRF (human leukemia lymphoblasts), CEM/C2 (camptothecin resistant derivative of the human T cell leukemia cell), HT-29 (human colon). The preliminary tests indicated that betulin (1), betulinic acid (2) and CLA are the most active agents against cancer cell lines studied. However the betulinic acid ester (4) showed comparable activity as CLA.


PB30
Synthesis and cytotoxic activity of new betulin and betulinic acid esters with conjugated linoleic acid (CLA)

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Novel ester derivatives of betulin (3a-c) and betulinic acid (4) with conjugated linoleic acid (mixture of isomers: 9t, 11t – 43.4%, 10t, 12c – 49.5%, other isomers – 7.1%) were obtained. Betulin (1) and betulinic acid (2) are natural lupane-type triterpenoids with numerous biological activities: anti-inflammatory, antifungal, antibacterial, antimalarial, antiviral, antitumour [1]. Conjugated linoleic acid (CLA) is a group of isomers of linoleic acid, found in milk of ruminants and bovine meat. CLA is known for their antioxidant, antitumor, antatherogenic properties [2,3]. One can expect that the ester derivatives containing both molecules can also possess the valuable biological activity and they can find an application as medicine for many diseases. Esterifications of CLA with betulin and betulinic acid were carried out with using of N,N-diacylhexylcarbodiimide (DCC) as coupling agent, in presence of 4-dimethylaminopyridine (DMAP) in dichloromethane for betulin derivatives or in pyridine for betulinic acid derivative respectively. The cytotoxic activity of betulin (1), betulinic acid (2), mixture of CLA and their derivatives (3a-c, 4) in vitro was determined by performing the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) cytotoxicity assay. Five different cell lines were used: P388 (mouse leukemia), CCRF (human leukemia lymphoblasts), CEM/C2 (camptothecin resistant derivative of the human T cell leukemia cell), HT-29 (human colon). The preliminary tests indicated that betulin (1), betulinic acid (2) and CLA are the most active agents against cancer cell lines studied. However the betulinic acid ester (4) showed comparable activity as CLA.

Fig. 1

Fig. 2

Jasminum malabaricum Wight belonging to the family Oleaceae is endemic to Western Ghats of India. It is a climber, with white flowers and fragrance and known for its ethno medical importance like antioxidant, antitumor, antiatherogenic properties [2,3]. One can expect that the ester derivatives containing both molecules can also possess the valuable biological activity and they can find an application as medicine for many diseases. Esterifications of CLA with betulin and betulinic acid were carried out with using of N,N-diacylhexylcarbodiimide (DCC) as coupling agent, in presence of 4-dimethylaminopyridine (DMAP) in dichloromethane for betulin derivatives or in pyridine for betulinic acid derivative respectively. The cytotoxic activity of betulin (1), betulinic acid (2), mixture of CLA and their derivatives (3a-c, 4) in vitro was determined by performing the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) cytotoxicity assay. Five different cell lines were used: P388 (mouse leukemia), CCRF (human leukemia lymphoblasts), CEM/C2 (camptothecin resistant derivative of the human T cell leukemia cell), HT-29 (human colon). The preliminary tests indicated that betulin (1), betulinic acid (2) and CLA are the most active agents against cancer cell lines studied. However the betulinic acid ester (4) showed comparable activity as CLA.
Development of low-Gly m Bd 30K(P34) allergen breeding lines using molecular marker in soybean
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Soybean (Glycine max (L.) Merr.) is an important source of vegetable oil and high protein. Use of soybean meal by the food industry is increasing, but severely limiting dietary choices and the quality of life of food-allergic individuals. Gly m Bd 30K (P34) is known as the main seed allergens in soybean-sensitive patients. The objective of this work was to determine the molecular basis of the low mutation of soybean P34 and to design molecular marker for the selection of the causative mutations for wild homozygous, heterozygous and mutant homozygous. We developed a co-dominant marker based on the sequence of Glyma08g12270 containing a four-base pair insertion at the P34 start codon. Also, we made a polyclonal antibody for investigation of P34 protein levels. Using a co-dominant marker and a polyclonal antibody, polymorphism and amount of protein for Glyma08g12270 were analyzed in F2 and F3 generation crossing PI 567476 and Hwanggumkong, Korean cultivar. To investigate the association of the P34 genotype with the P34 protein phenotype, segregating populations in F2 were developed from crossed Hwanggum and P1567476. For the 258 samples analyzed, the ratio of homozygous wild-type: heterozygous: mutant homozygous was 34.9:94:30 = 1:2:1 (test for goodness of fit by X2 analysis). As results, the polymorphism analysis was accustomed to a difference of protein level of wild homozygous, heterozygous and mutant homozygous. References: Bilyeu K et al. (2009) The plant Genome 2: 141 – 148 Joseph LM et al. (2006) Crop Sci 46: 1755 – 1763 Herman M et al. (2005) Plant Physiology 132: 36 – 43.

Effect of taxine B feeding on taxol production and cell viability in Taxus suspension cultures
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After several years, still the affair of paclitaxel (Taxol) and its analogs is one of the most stimulating subjects in anticancer remedy production studies [1]. Many reports have described that the yield of taxol is very low or sometimes not detectable in dedifferentiated cells such as callus tissues or suspension cultured cells. In order to obtain products in concentrations high enough for commercial manufacturing, many efforts have been made to stimulate or restore biosynthetic activities of cultured cells using various methods. Addition to the culture media of appropriate precursors or related compounds sometimes stimulates secondary metabolite production. This approach is advantageous if the precursors are inexpensive [2, 3]. To achieve a better understanding of the effect of taxoids addition to Taxus cell culture, an Iranian yew cell line growing 14 days in a selected growth medium was treated with concentration of Taxine B (a basic Taxoids with a C-4(20) Double Bond) in the range of 1.25 – 10 mg/l. The results showed that different concentration of taxine B can lead to various influence on taxoids production such as taxol (a taxoid with an oxetane Ring). Thus, taxine B caused to change the metabolite profiles of the biosynthesis of taxol preparation. Compared with an untreated control, all the taxol concentrations affected cell viability. References: 1. A. Ghassempour A et al. (2010) Chromatographia 72: 833 – 839. 2. Ketchum REB et al. (2007) Phytochemistry 68: 335 – 341. 3. Expo’sito O et al. (2009) New Biotechnol 25: 252 – 259.

Tissue culture studies on Semecarpus kathalekanensis an endangered medicinal plant
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Semecarpus kathalekanensis Dasappa & M.H.Swaminath is an evergreen tree with very large simple leaves, attains a height of about 30 m, belonging to the family Anacardiaceae is a critically endangered swamp tree and consists of major chemical compounds like phenols, biflavonoids and traditionally having high medicinal importance being used as an antimicrobial, antioxidant and anticancer. Hence considering its population, it is an endangered species and endemic to western ghats region of Shimoga district in India. The tissue culture of different plant parts was carried out on MS medium using different concentration of plant growth regulators in the culture tubes and the explants were incubated at 25 ± 20 C under 48 hrs photoperiod. Due its high phenolic content callus initiation was not occurred further isolation and identification of endophytic fungi from Semecarpus kathalekanensis plant was performed. References: 1. Dasappa & Swaminath MH (2000) Indian For 126: 78 – 82 2.Vasudeva R, Raghu HB, Dasappa, Uma Shaanker R & Ganeshiah KN (2001) Population structure, reproductive biology and conservation of Semecarpus kathalekanensis: A critically endangered freshwater swamp tree species of the Western Ghats. In Forest Genetic Resources: Status, Threats and conservation Strategies (Eds Uma Shaanker, R., Ganeshiah, K.N., and Bawa, K.S.) 211 – 223 (Oxford & IBH, New Delhi), 3.Murashige T, Skoog F (1962) Physiol plant 15:473 – 9.

Metabolic engineering: an effective approach for optimal production of secondary metabolite compounds
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Plants have a limited capacity to produce secondary metabolites in natural environmental conditions; however, recent developments in genetic engineering and recombinant DNA technology have had a great impact on their production. Metabolic engineering is considered as an efficient tool towards achieving higher level of the secondary metabolites. Globally, metabolite engineering is widely used for increasing the content of secondary metabolites or even producing novel medicinal compounds. Iran has a high level of genetic and phytochemical variability for different types of medicinal plants. Knowing the biochemical pathways and manipulating these native plants improve their commercial production. Consequently, the research group has started large-scale experiments on native medicinal plants such as different species of genus Artemisia, Cannabis, Papaver, and Salvia. We aim to increase and/or decrease and change metabolites by using anti-sense and RNAi technologies, isolation and transformation of related genes, promoter analysis, and changes in regulatory gene expression. Such studies would definitely provide a great chance to improve the production of secondary metabolites in these plants and to better understand the novel genes involved in bio-synthetic pathways.

Biotransformation of trans-farnesol by Ceriporiopsis subvermispora
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Terpenoids which are included in various plants are important for the food, animal feed, cosmetics and pharmaceuticals industries greatly. However they are expensive due to low concentration and difficult isolation. Therefore biotransformation of terpenoids could be an alternative way to produce them. In this study, whole cell of Ceriporiopsis subvermispora was used in biotransformation as biocatalyst. C. subvermispora is a naturally occurring fungus able to remove phenolic compounds than other compounds. Farnesol is a sesquiterpene which is an acyclic ses-
Hydnocarpus pentandra Buch-Ham (Flacourtiaceae) is an endangered species from Western Ghats region of India has been exploited traditionally against leprosy, rheumatic pain and inflammation. The callus cultures were established from leaf explants on MS media supplemented with sucrose and varying amounts of auxins and cytokinins. The callus initiation was observed with 2,4-D and NAA followed by incubation at 25 ± 2°C under photoperiod of 16 hrs.

**Hydnocarpus pentandra Buch-Ham (Flacourtiaeaceae) an endangered spe-
cies from Western Ghats region of India has been exploited traditionally
against leprosy, rheumatic pain and inflammation. Ten endophytic fungi
were isolated from the plant which are rich source of novel organic
metabolites. The present study focuses on the screening of sage suspen-
sion cultures on growth and metabolic activity. Different suspension
cultures from different species were cultivated in the modern Respiratory Activity Monitoring System (RA-
MOS, Hitce Zang, Germany). It allows the measurement of the oxygen
transfer rate (OTR) at shake flask scale and in parallel. Using the OTR the
growth properties of the cultures have been detected. Samples were
taken at the starting point, in the exponential growth phase and the
stationary phase. Ethanolic extracts were analysed with HPLC to quan-
tify the production rates of the target substances OA and UA. Using GC/MS the metabolite spectra were investigated to search for other high valuable substances and receive information about the metabolic activity of the cultures. All these data are important for the subsequent culture optimisation. Acknowledgement: This work has been supported by a PhD fellowship from the German Academic Exchange Service and a grant from the Max Buchner Research Foundation. References: [1] Duz- bak P et al. (2006) Nat Prod Rep 23: 394 – 411.

**Screening of sage suspension cultures for triterpenic acids and other metabolites**

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Two pharmacologically interesting substances present especially in sage species are oleanolic acid (OA) and ursolic acid (UA). These triterpenic acids possess several biological activities like hepatoprotective, anti-inflammatory, antimicrobial as well as anticancer properties [1]. A biotechnological way to produce valuable biological-active substances is the cultivation of cell suspension cultures in bioreactor systems. Before up scaling a suspension culture to bioreactor level an important step is to establish stable suspension cultures and screen them for their potential. Interesting features of suspension cultures are the size of the cell aggregates, the growth and the production rates of target metabolites. The present study focuses on the screening of sage suspension cultures on growth and metabolic activity. Different suspension cultures from S. officinalis L., S. triloba L. and S. virgata Jacq. were cultivated in the modern Respiratory Activity Monitoring System (RAMOS, Hitec Zang, Germany). It allows the measurement of the oxygen transfer rate (OTR) at shake flask scale and in parallel. Using the OTR the growth properties of the cultures have been detected. Samples were taken at the starting point, in the exponential growth phase and the stationary phase. Ethanolic extracts were analysed with HPLC to quantify the production rates of the target substances OA and UA. Using GC/MS the metabolite spectra were investigated to search for other high valuable substances and receive information about the metabolic activity of the cultures. All these data are important for the subsequent culture optimisation. Acknowledgement: This work has been supported by a PhD fellowship from the German Academic Exchange Service and a grant from the Max Buchner Research Foundation. References: [1] Duzbak P et al. (2006) Nat Prod Rep 23: 394 – 411.

**Biotransformation of Cycloartane-Type Sapogenols, Cycloastragenol and Cyclocanthenol, by Cunninghamella blakesleeanae NRRL 1369**

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Cycloastragenol is a cycloartane-type sapogenol found in Astragalus species. It is a minor metabolite mainly present in the roots of the plant and possesses very interesting pharmacological activities [1, 2]. The bio-

**Effects of aqueous extracts of Urtica dioica L. leaves on lifespan of Caenorhabditis elegans**

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transformation of cycloastragenol by the fungus Cunninghamella blakei-sleena was investigated previously by our group [3]. Inspired by the diversity of the transformed products, further studies were carried out on cycloastragenol (CG) and another secondary metabolite, named cyclobetancogenol (SKG). The biotransformation process was conducted in two scales; analytical scale and preparative scale. One-stage fermentation protocol was followed where the saponins were fed to the biotransformation media 72 hours after the inoculation. In the analytical scale, both submerged (30 °C, 200 rpm) and surface (30 °C) culture conditions were tested, taking 2 mL samples for 3 weeks for the evaluation of the chemical profiles, followed by preparative scale studies by using 500 mg of SKG and 1000 mg of CG. Incubation period was continued with centrifugation, extraction with ethyl acetate and n-butanol, and evaporation under vacuum. The isolation and purification studies performed on the extracts yielded total of 13 metabolites, 10 from CG and 3 from SKG. Structures of the isolated metabolites were elucidated by 1D- and 2D NMR techniques, and LC-MS analyses. The major products obtained from each sapogenol Cb.CG_MF_01 and Cb.SKG_MF_01 have the same tetra-cyclic steroidal framework with a primary alcohol substitution at C-11 position, encountered for the first time in microbial transformation studies [3]. Acknowledgement: ARS Culture Collection, TUBITAK (1087654 – 1095345). References: 1. Bedir E et al. (2000) Biol Pharm Bull 23: 834 – 837. 2. Valenzuela HF et al. (2009) Immunol 182: 90.30. 3. Kuban M et al. (2010) Org Lett 12: 4252 – 4255.

Antioxidant capacity of phenolic phytochemicals from peel of apples, pears, plums, red and white grapes
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Polyphenolic phytochemical extractions from peel of apples, pears, plums, red and white grapes were performed using 70% ethanol and 0.9% NaCl (7:3) with ultrasound assistance and extracts were analyzed for total phenolics, flavonoids, and antioxidant capacity. In the tested fruit the highest content of flavonoids was found in the red grapes (4.337 ± 0.218 mg/g), followed by the pear and the plum (3.333 ± 0.035 and 3.108 ± 0.157 mg/g), which have almost identical content, and the white grapes and the apples which again have an approximately equal content of flavonoids (2.126 ± 0.039 and 2.076 ± 0.096 mg/g). The antioxidant capacities of analysed fruit extracts were assayed for antioxidant activity by DPPH radical scavenging and total reducing power. The percentage of neutralize DPPH radicals can be reported by EC50 value or by the concentration of extract required to neutralize 50% of DPPH radicals. The lowest EC50 value, and therefore the highest antioxidant activity was in the extracts of red grape, followed by the extracts of pear, apple, white grape, and plum. The highest content of phenolic compounds were in extract of the red grape (12.884 ± 0.444 mg/g), than followed by the pear (9.590 ± 0.31 mg/g), the plums (9.296 ± 0.268 mg/g), the apples (3.676 ± 0.135 mg/g), and the lowest content was in the extract of the red grape (3.009 ± 0.161 mg/g). Acknowledgement: This work was supported under the projects No.O1.4501 by the Ministry of Science of the Republic of Serbia. References: 1. Halliwell B (1997) Nutr Rev 55: S4-S52. 2. Aquino R et al. (2002) J Ethnopharmacol 79: 183 – 191. 3. Xu B, Chang SKC (2007) J Food Sci 72: S159-S166.

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Malva neglecta Wallr. is a perennial plant with high mucilage content, expectorant and cough-suppressing actions. The leaves and flowers of M. neglecta and some Malva species are used in traditional phytotherapy. Ultraviolet radiation in sunlight has diverse effects on humans, animals, plants and microorganisms. UV can cause damage to membrane by excitation of UV-B receptors, resulting in generation of reactive oxygen species and ultimately oxidative burst. Consequently organisms need to protect against UV damage and repair UV-induced lesions. Antioxidants are an important group of medicinal preventive compounds as well as being food additives inhibiting detrimental changes of easily oxidizable nutrients. Polyphenols are commonly found in both edible and non-edible plants and they have been reported to have multiple biological effects, including antioxidant activity. In the present research callus cultures from leaf explants of M. neglecta were initiated in vitro, and their capacity to produce UV absorbing compounds was analyzed, after 90 minutes exposure to UV. The results showed that the levels of apigenin and delphinidin decreased after illumination with UV-B and UV-C, while Malvidin increased in UV-B and UV-C exposed Malva cells. The results demonstrate that polyphenols play important role in UV protection of Malva cells. References: Deters A et al. (2010) J Ethnopharmacol 127: 62 – 69. Hosseini Sarghein, Carapetian J and Khara J (2008) International Journal of Botany 4: 486 – 490. Jansen Marcel AK, Hectors K, O’Brien N M, Guisez Y, Potters G (2008) Plant Science 175: 449 – 458. Li J, Ou-Lee TM, Raba R, Amundson RG, and Last RL (1993) The Plant Cell 5: 171 – 179. Zacchini M, de Agazio M (2004) Plant Physiology and Biochemistry 42: 445 – 450.

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Induction of changes in secondary metabolites and essential oils of Calendula officinalis L. by methyl jasmonate
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In this study, effect of Methyl jasmonate as a chemical elicitor on the secondary metabolites and essential oils of Calendula officinalis L. shoot were evaluated. The plants were grown in hydroponic conditions in Hogland nutrient solution and were treated with 50 and 100μM methyl jasmonate. The results showed that lignin content were increased significantly in treated plants. In comparison, the content of total wall associated phenolic compounds and anthocyanins and flavonoid of shoots of treated plants decreased. However, there was no significant difference in membrane lipid peroxidation rate of treated plants and control. In the essential oils of C. officinalis shoots, α- Cadinol was the major constituent. A sesquiterpene compound, α-Muurolene, with anti fungal properties was induced in those plants treated with methyl jasmonate, therefore, this chemical elicitor can be suggested for inducing changes in isoprenoid biosynthesis pathway and special phytoalexins production. References: 1. YukimeYume, Tabata H, Higashi Y, Hara Y (1996) Nat Biotechnol 14: 1129 – 1132. 2. Kim DG, KimYJ, Lee SH, Lee (2005) Plant Biol 48(3): 298 – 303. 3. Gazim ZC, Rezende CM, Fraga SR, Callistus MC, Zapata de Espinosa JL, de Souza MS, de Matos MF (2010) J Food Sci 72: S159-S 166.

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Secondary Metabolites from Phomopsis amygdali, an Endophytic Fungus Isolated from Hazelnut (Corylus avellana)
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Endophytes are microbial entities that live within living tissues of plants. In most cases their relationship with the host plant is symbiotic and probably mutualistic. Many are capable of synthesizing bioactive compounds that have been proven useful for novel drug discovery. The early literature reports that species of Phomopsis isolated from plants produce different bioactive metabolites. The main aim of the study was to isolate endophytes from different parts of hazelnut, to extract bioactive secondary metabolites and then to elucidate their structures. Different plant materials including the roots, branches and leaves were collected from BlackSea region of Turkey and surface sterilized with 3% sodium hypochlorite (NaOCl). The outer layers removed with a sharp, sterilized blade and cut into pieces. Small pieces of the inner tissue were placed on the surface of potato/dextrose/agar (PDA) medium and incubated at 28 °C. Subsequently 7 fungal species was isolated and grown in 1 L flask containing 250 ml of Multi Extract broth medium and cultured at 150 rpm at 28 °C for 21 days in a rotary shaker. Then the fermentation broths were extracted with chloroform. The chloroform extracts were screened for their cytotoxic activities by MTT method. Based on the activity results, the isolate L1, identified as Phomopsis amygdali, was
selected for further studies. After large scale fermentation and purification studies, a new metabolite (LPF) together with a known compound, named (-)-pestalotin (LPF) were obtained. The structure of the new metabolite was elucidated as (R)-4-butoxy-6-((S)-1-hydroxybutyl)-5,6-dihydro-2H-pyran-2-one by the extensive use of 1D- and 2D NMR.

Figure 1


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Biotechnological process for obtaining bioaromas from leaves of cashew apple trees

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The cashew tree (Anacardium occidentale L.) is native to mainland Central and South America belonging to the family Anacardiaceae. The cashew apple fruit and nut are widely used and appreciated for its flavor quality. The aim of this work was to obtain bioaromas produced from the fermentation of the leaves of the cashew tree. Fermentations were performed at room temperature for 48 hours in three conditions: natural (leaves only), leaves in sterile distilled water and leaves in a solution containing 10% glucose. Every 24 hours of processing, sensorial (by a panel of five trained judges), microbiological (total mesophilic bacteria, molds and yeasts) and chromatographic (extraction by dynamic headspace method) analysis was performed on fermented products. Sensorial analysis revealed the presence of sweet fruity aromas caracterizing citrus and green odor notes in all samples. The citric note of aroma was more prominent in all three types of fermentation without significant difference between them. However, the aroma was less intense in the fermentations realized with green leaf alone and it was significantly different (p < 0.05) between the other fermentations. Furthermore the production of bioaromas generated after 24 hours led to an increase of total bacteria and fungi to the order of 102 CFU/ml. A large number of volatile compounds belonging to esters, terpenes, ketones and aldehyde classes were identified in the fermented products.

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Galbanum (Ferula gummosa Boiss.) is a valuable medicinal-industrial plant native to Iran which is at risk of extinction due to irregular and overharvesting from natural habitats. The objective of the study was to investigate the growth response of zygotic embryos of galbanum, originated from center of Iran, in in vitro conditions. The results however, showed that zygotic embryos of galbanum had not a suitable germination in in vitro conditions. However, the best treatment for in vitro germination of embryos was ½ MS (MS with ½ macro elements) medium supplemented with 0.3 mg l⁻¹ GA₃. The results also showed the embryos had good callus production in ½ MS medium (MS with ½ macro elements) supplemented with 2 mg l⁻¹ BA and 10 mg l⁻¹ NAA. In this study, the results showed that although applied treatments did not lead to normal germination of zygotic embryos of galbanum in in vitro conditions, but these treatments were able to force zygotic embryos into the callus production phase with good quality and quantity. The pH of primary media for zygotic embryo germination which was adjusted before adding plant growth regulators, can be effective on good callus production. Nevertheless, more experiments are needed to reveal the effect of pH on calllogenesis in galbanum plant. References: 1. Bernard F et al. (2007) Pakistan Journal of Biological Sciences 10: 1977 – 1983 2. Irvani N et al. (2009) Plant Cell, Tissue and Organ Culture 100: 293 – 299 3. Tafreshi RS et al. (2008) Iranian Journal of Medicinal Plants 27: 71 – 81

PB47

Assessment of somaclonal variation in Daucus anthropophila plants regenerated from long-term callus cultures using AFLP

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Somaclonal variation may be defined as tissue-culture-induced variation that has relevance in the micropropagation of endangered germplasms. It has been studied in some plant species, but only a few studies have reported on the assessment somaclonal variation in medicinal plants using molecular markers. Daucus anthropophila Boiss. is an endangered medicinal herb belonging to the Apiceae family. In this study somaclonal variation in plants regenerated from long-term cultured calluses of Dicusas anthrophi was characterized using amplified length polymorphism profile. Genomic DNA was double-digested with two Bgl II and MseI and the digested fragments were ligated to double stranded adaptors appropriate with the Bgl II and MseI restriction sequences. Results revealed that banding patterns were different between various explants in different subcultures. A total of 112 polymorphic fragments were scored, with an average of 22.4 fragments per primer combination. Results also showed that this method is reliable and effective way of assessing somaclonal variation in tissue culture-derived plants. Acknowledgement: The research was supported by funds received from the Institute of Medicinal Plants, Karaj, Iran

PB48

Responses of zygotic embryos of galbanum in in vitro conditions

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PB49

Conservation and multiplication of an endangered medicinal plant – Caralluma arabrica – using tissue culture

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Caralluma arabrica N.E.Br. (Asclepiadaceae) is a succulent, perennial herb that grows in arid regions, in West Asia and in the Middle East, including Oman and the United Arab Emirates. This plant is highly valued for its medicinal properties, and is commonly used in the preparation of traditional medicine for the treatment of diabetes, liver ailments, and painful and inflammatory conditions (1). Pharmacological studies revealed that C. arabrica extract has anti-nociceptive, anti-gastric ulcer, cytoprotective, and anti-inflammatory properties (1, 2). Unfortunately, this plant is facing considerable pressures which threaten its survival. Therefore, the development of a protocol for the propagation of C. arabrica in vitro is of high importance for the conservation of this species and its commercial cultivation. Plant regeneration via organogenesis was initiated for C. arabrica using stem segments excised from young shoots and used as explants for in vitro culture. Stem explants were cultured on Murashige and Skoog (MS) (3) medium containing different concentrations of kinetin and indol-3-acetic acid (IAA). Preliminary results showed that differentiation of adventitious shoots was initiated within 5 weeks of culture on a medium containing 1 mM Kinetin and 3 mM IAA. Root induction was obtained on half-strength MS medium containing Indol-3-butryic acid. Further investigation is underway to establish optimal culture conditions for the regeneration of this important medicinal plant. References: 1. Zakaria et al. (2001) J. Ethnopharmacology 76(2): 155 – 158 2. Zakaria et al. (2002) Pharmaceutical Biology 40(3): 225 – 230 3. Murashige, Skoog (1962) Physiol Plant 15: 473 – 497
In vitro study of callus induction and regeneration in Iranian Cichorium intybus
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Cichorium intybus L. belongs to the Asteraceae family and is one of the medicinally important plant that contains some useful secondary metabolites such as bitter sesquiterpene lactones, coumarins and flavonoids. This plant is conventionally propagated through seeds. In this research, in vitro culture of Iranian Cichorium intybus was studied, thus an experiment was laid out as a completely randomized design (CRD) in a factorial arrangement with there replications that investigated factors were different preparation of plant growth regulators (NAA and BAP) and explants ( hypocotyls and cotyledon). The results indicated that significant and non-significant differences among levels of explants and other factors for callus induction and plant regeneration, as we observed the highest percentage of callus formation from MS medium supplemented with 1.5 mg/l NAA and 2 mg/l BAP. Mean comparisons showed the best explant was cotyledon versus hypocotyls for the traits and maximum of regeneration recorded for MS medium with 0.4 mg/l NAA and 5 mg/l BAP.

The spondyloarthropathies (SpA) are a group of rheumatic diseases that affect the peripheral joints and the axial skeleton. The aim of this 6 months intervention study was to assess the efficacy and safety of a research preparation based on a combination of silymarin and selenium on healthy men with a prostate specific antigen (PSA) level lower than 2.0 µg/L. In this double-blind, placebo-controlled pilot study, a total of 55 participants were randomized to either treatment with 570mg silymarin, and 240 µg selenium as selenomethionine per day (n = 26) or placebo (n = 29). Baseline clinical and demographic characteristics were comparable. Outcome measures were changes in the International Prostate Symptom Score (IPSS), quality of life score, safety clinical chemistry and hematology parameters, serum selenium, PSA and testosterone levels, antioxidant status, transrectal ultrasound prostate volume, urinary flow rate, ultrasound estimated postvoid residual urine volume at baseline and 180 days. The results showed statistically significant differences between treatment and control groups for the following parameters: decreased PSAtoe value, improved selenium level, IPSS, quality of life score, urination parameters including voiding parameters-rate of urine flow (Qmax), average flow (Qave), total volume V, and prostate volume and postvoid residual urine volume (RV). There was no effect on blood testosterone level. Overall the treatment was well-tolerated with no adverse effects. In conclusion, the chosen combination of silymarin and selenium proved effective and may be beneficial for the maintenance of prostate health in men. Acknowledgement: Financial support from the Czech Ministry of Education, Youth and Sport (Grant No. MSM 619859216) is gratefully acknowledged. References: 1. Vladař A et al. (2010) Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 154(3): 239 – 244
the highest mortality rate. When the mortality of different solvent extracts was compared, the maximum (P<0.05) mortality was recorded at a concentration of 90 ppm of ethanol extract of S. nigrum. Extract of mature leaves of S. nigrum exhibited molluscicidal activity followed by S. sinaicum and the less was S. villorum. The study provides considerable scope in exploiting local indigenous resources for snails’ molluscicidal agents. Acknowledgement: The authors thank all the participants who shared their time for working on this study. References: Ahmed AH, Kamal IH, and Ramzy RM (2001) Journal of the Egyptian Society of Parasitology, 31(3): 843 – 852. Massoud AM and Habib FS (2003) Journal of the Egyptian Society of Parasitology, 33(2): 585 – 596.

**Efficacy of Punica granatum extract on in vitro and in vivo control of Trichomonas vaginalis**

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**Trichomoniasis vaginalis** is now an important worldwide health problem. Metronidazole has so far been used in treatment, but the metronidazole resistant strains and unpleasant adverse effects have been developed. Treatment of patients with metronidazole refractory vaginal trichomoniasis constitutes a major therapeutic challenge and treatment options are extremely limited. The last 7 years have seen over seven times as many publication indexed by Medline dealing with pomegranate (Punica granatum L.) than in all the years preceding them, because of this, and in vitro anti-trichomoniasis vaginalis activity of *P. granatum* was compared, the maximum (P<0.05) mortality was recorded at a concentration of 16 ppm of the ethanol extract (in vitro and in vivo) gave very promising results. Acknowledgement: The authors thank all the participants who shared their time for working on this study. References: ¹Abdel Hady NM, El-Sherbini GT, Morsy TA (2008) Egypt Soc Parasitol 38(3): 1024 – 5 ²Adams LS, Zhang Y (2010) Cancer Prev Res (Phila Pa) 3(1):108

**Plasma oligoelements levels in pediatric cohort of spondyloarthropathy**

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The importance of trace elements in chronic inflammatory diseases is related to their cofactor role in immune system functions and in different metabolic processes in articular tissues. Spondyloarthropathies (SpA) are a group of rheumatic diseases linked by common pathology, including inflammatory back pain and different degrees, the growth of microorganisms (15 – 20 mm). Furthermore, the methanolic extracts of aerial parts of plants were evaluated in different concentrations according to the disk diffusion method by using *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium*, *Streptococcus fecalis* and *Klebsiella pneumoniae* and all the extracts were compared with standard antibiotic discs like vancomycin, ampicillin and chloramphenicol. The methanolic extracts of *Stachys byzantina* and Rosmarinus officinalis were shown to inhibit, to different microorganisms (15 – 20 mm). Furthermore, *Stachys byzantina* showed significant antimicrobial activity against *Staphylococcus aureus* resistant to vancomycin. The other plant extracts had shown lower antibacterial activity in comparison with standard antibiotics. This study showed that some medicinal plants that were used in folk medicine significantly *Stachys byzantina* could be comparable with antibiotics and potential sources of new antimicrobial agents.

**Evaluation of the protective effect of Urtica dioica leaf extract on Beta cell islet langerhance of diabetic rats**

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Herbal medicine is a complementary way to improve the health. The traditional Iranian Medicine introduces many plants for treatment. This study investigates the anti-diabetic effects of Urtica dioica L. leaves that were introduced as anti-diabetic plant in the traditional Iranian medicine. The animal was made diabetic with intra tail vein injection of 50 mg/kg STZ (streptosotocine). Animals with fasting blood sugar > 250 mg/kg were considered as diabetic. One group of diabetics were treated with Urtica dioica leaf extract (1 ml/kg/day intra peritoneal). After one month, animals were decapitated to take the blood sample and pancreas tissue. Tissue was observed by histologist. The tissue parameters were studied in both diabetic and experimental group. Blood glucose in treated group decreased from 400 ± 54.2 mg/kg to 87.9 ± 11.9 mg/kg whereas no change was observed in diabetic group. In diabetic group, necrosis and infiltration of mononuclear cells were produced in plenty. Capillaries, islet cells, Beta cells and secretary vacuoles were damaged while in treated group the necrotic tissues was repaired and infiltration of mononuclear cells were a bit. Beta cells increased and secretory vacuoles were appeared. The number of capillaries, undifferentiated cells also increased. *Urtica dioica* repairs pancreas tissue and improves its function. This may lead to increase insulin secretion and Urtica Dioica direct influence in decreasing blood sugar. References: ¹Kawatali G et al. (2003) J Ethnopharmacol 84(2 – 3): 241 – 245 ²Farzami B et al. (2003) J Ethnopharmacol 89: 47 – 53 ³Kumar V et al. (2003) Basic Pathology, the Pancreas 7 ed, Saunders; pp: 635 – 657

**Antibacterial activity of some medicinal plants against antibiotics**

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In this study, the antibacterial activity of 6 species of Lamiaceae family: *Mentha spicata* L., *Mentha aquatica* L., *Stachys byzantina* K.Koch, *Marium vulgare* L., Rosmarinus officinalis L. and Melissa officinalis L. were investigated. The methanolic extracts of aerial parts of plants were evaluated in different concentrations according to the disk diffusion method by using *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium*, *Streptococcus fecalis* and *Klebsiella pneumoniae* and all the extracts were compared with standard antibiotic discs like vancomycin, ampicillin and chloramphenicol. The methanolic extracts of *Stachys byzantina* and Rosmarinus officinalis were shown to inhibit, to different microorganisms (15 – 20 mm). Further, *Stachys byzantina* showed significant antimicrobial activity against *Staphylococcus aureus* resistant to vancomycin. The other plant extracts had shown lower antibacterial activity in comparison with standard antibiotics. This study showed that some medicinal plants that were used in folk medicine significantly *Stachys byzantina* could be comparable with antibiotics and potential sources of new antimicrobial agents.

**How does long term exposure to base stations and mobile phones affect human hormones profile?**

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This study is concerned with assessing the role of long-term exposure to high frequency non-ionizing electromagnetic radiation (RFR) emitted either from mobile phones or from base stations. One hundred volunteers from different areas in Egypt exposed to radio frequency non-io-
ning electromagnetic radiation emitted from mobile phones or from base stations for period extended to six years suffered tangible effects on the pituitary – adrenal axis. Volunteers were divided into three subgroups according to non-ionizing radiation exposure: according to the time of exposure to RFR per day. In addition to negative control subjects of compatible age ranges, sex and socioeconomic status. Volunteers’ plasma ACTH and serum cortisol levels were measured. Also thyroid hormones were detected for all individuals. In addition, each of their serum prolactin, progesterone and testosterone level were measured to detect the different relations between all these seromarkers in all volunteers who expose to non-ionizing electromagnetic radiation emitted either from mobile phones or from base stations. Authors have previously found no association between self-reported illness symptoms and the exposures to microwave radiation emitted by mobile phones or electromagnetic fields in general. Other major sources [1]. The thyroid gland is one of the most exposed vital organs and may be a target for electromagnetic radiation. It has been established that even a small change in circulating thyroid hormone levels is sufficient to alter the brain functions [2].

Keywords: base stations, mobile phones, long-term exposure, electromagnetic radiation, ACTH, cortisol, thyroid, prolactin, progesterone, testosterone

**Topic D: Cultivation and Breeding**

**PD1** Response of seed priming on seed germination and seedling growth in basil

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In order to evaluate the effect of priming on seed germination and seedling growth in basil (Ocimum basilicum L.), an experiment was conducted based factorial in randomized complete block design with three replications in the west of Iran. Priming factor including: witness, KCL 2% and priming time role played in plant germination. Among treatments priming factor including: 0, 3, 5, 7 and 9 hours. Results showed that Priming was affected on radicle length, radicle dry weight, stem let dry weight, germination percentage and speed germination. KCL 2% had better results due to negative osmotic adjustment. Priming had significant affected on radicle length, radicle dry weight, stem length and stem let dry weight. Seed priming in 7 hours had positive affected on all studied traits. The highest radicle length, radicle dry weight and stem length obtained from 7 hours. According to the results Priming and priming time played in plant germination. Among treatments KCL 2% and 7 hours had an important role in germination. Keywords: Seed priming, Basil, Germination, Ocimum basilicum

**PD2** Effect of plant growth promoting rhizobacteria (PGPR) on the healthy and productivity of soybean plant

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A pot and field experiment were conducted to evaluate some rhizobacteria namely *Pseudomonas fluorescens*, and *Bacillus subtilis*. The pot experiment was executed to evaluate probable suppressive effect of rhizobacteria as bioagents against *Magnaporthe oryzae*, *Fusarium solani* and *Sclerotium rolfsii* under artificially infested soil. Results showed that co-inoculation of soy bean with rhizobacteria led to a significant decrease in pre- and post-emergence damping-off caused by all pathogens under investigation. In addition to increase the nodulation status, growth, N-content and pod yield plant under uninfested or infested soil. Field experiment were carried out in El-Sharqia governorate to evaluate the promotive and suppressive disease effects of rhizobacteria on nodulation, plant growth and yield of soy bean. Results showed that the inoculation with rhizobacteria led to a significant increase in the nodulation status, shoot dry matter and N-content after 15, 45, 75 days of planting. Moreover, the co-inoculation of *Bacillus subtilis* with *Rhizobium* sp. Salient superiority in suppressive disease. The obtained results explained that the synergy between rhizobacteria (*Bacillus subtilis, Pseudomonas fluorescens* and *Rhizobium* sp.) considered the efficient microorganisms to save the protection against the phytopathogens and promote the nodulation and symbiotic nitrogen fixation leading to a high quality yield of soy bean.

**PD3** Effect of plant density and application rates of vermicompost on essential oil content and composition of Balm (*Melissa officinalis L.)*

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In order to investigate plant density and application rates of vermicompost on essential oil content and composition of Balm, the experiment was conducted for 6 months in Dineh phytomedic company in 2010. This experiment was carried out in complete randomized block design with 3 replications at three plant densities (8, 8 and 10 plant/m²) and four application rates of vermicompost (0, 5, 10 and 15 ton/ha). In floral imitation, plants harvested and essential oil were extracted by water distillation. The essential oils were analyzed by GC and GC/MS. The results showed a significant difference (%1) among plant densities and application rates of vermicompost on essential oil yield, maximum amount of essential oil obtained from 10 plant/m² and 10 ton/ha vermicompost consumption. Identification of essential oil components showed that plant density had no effect on essential oil composition but some compounds of the oil decreased with more application of vermicompost, whereas some other compounds increased with most application of vermicompost.

**PD4** Study the effect of different levels of phosphobiofertilizer’s inoculation on some traits of *Anethum graveolens* L. in Rudhen

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It is well known that in nature, a considerable number of microorganisms (e.g. bacterial species), mostly those associated with the plant rhizosphere, are able to exert a beneficial effect upon plant growth. Therefore, their use as biofertilizers or control agents for agriculture improvement has been a focus of numerous researchers for a number of years. The use of phosphobiofertilizer can in general significantly increase P uptake by the plant. To inspect the influence of phosphobiofertilizer, we used 6 shapes of phosphobiofertilizers inoculation, included: (B seeds inoculated, B top dressing, E seeds inoculated, E top dressing, E-B & control) of Iranian (B) and non-Iranian (E) microorganisms, on hight of plant, yield of seed and essential oil production of the plant named experimental Dill (*Anethum graveolens* L.) in the form of factorial on the basis of Randomized Complete Block design in three replication was conducted in Rudhen university (Kolyak state). Results showed that in all traits, There was a significant difference between utilization of both types phosphobiofertilizers and other treatments. Acknowledgement: The authors are grateful to Dr. Baghi and Dr. Seyed Hadi for their scientific support to the present project.

**PD5** Effect of different nitrogen and phosphorus application on qualitative a quantities characteristic of beebalm

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In order to test the effect of nitrogen and phosphorus fertilizers on qualitative and quantitative characteristic of beebalm, an experimental was carried out using factorial with randomized complete block design with three replication in Ilam, Iran in 2009 – 2010 growing season. Experimental factor including different of nitrogen fertilizer (70, 100 and 130 kg/ha) and phosphorus fertilizer (50, 70 and 90 kg/ha). Results showed that nitrogen fertilizer was affected on essential oil content, essential oil yield, plant height, number of tillering, stem diameter, root length, root weight and shoot ratio. The highest essential oil yield, plant height, number of tillering, stem diameter, root length was obtained 130 kg/ha nitrogen application. Phosphorus fertilizer affected essential
oil content, essential oil yield, plant height and root weight. The highest essential oil yield, plant height, number of tillering, stem diameter, essential oil yield and root weight was obtained 70 kg/ha phosphorus application. Interaction effect nitrogen phosphorus application was affect on essential oil yield and plant height. The highest and the lowest essential oil yields were obtained 130 nitrogen and 70 kg/ha phosphorus application, respectively.

**PD6**
Effect of salinity on essential oil content and composition of *Agastache foeniculum* Kuntze

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Water and soil salinity on the environmental agents limit plant growth and its productivity in Iran. Anise Hyssop (*Agastache foeniculum* Kuntze) is a perennial aromatic plant, belonging to the family Lamiaceae. The essential oil of Anise Hyssop was used for antimicrobial, antimycotic, antifungal, anti-oxidant, anti-inflammatory, gastroenterical and respiratory disorders. For the first time in Egypt, the plant was cultivated under systematic agriculture regime to estimate the nitrogen doses for the best plant growth. Three nitrogen doses were applied (N1 (33.5), N2 (50.25) or N3 (67) kg N/ fedden = 4200m2). Nitrogen fertilization had significant effects on most of agronomic parameters studied. Plant height (cm), number of branches per plant, plant fresh and dry weight (g) increased with the increase in nitrogen fertilization. At the highest level of N2 (67) kg N/ fedden the best growth attributes, although the differences were not significant in most harvests (cuttings) between the rates of N2 and N3. On the other hand, oil content was not influenced by nitrogen fertilization in all harvests.

**PD7**
Allelopathic effect of cumin (*Cuminum cyminum* L.) on seed germination of three weeds

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The allelopathic effects of cumin (*Cuminum cyminum* L.) were evaluated on seed germination of velvet flower (*Amaranthus retroflexus* L.), fix-weed (*Descurainia sophia* L./Webb ex Prantl) and wild oat (*Avena fatua* L.), in laboratory using the aqueous extracts of dried powdered of cumin and made soda. This experiment was conducted in a randomized complete blocks design with four salt treatments including 0 (control), 50, 100, 150 and 200 mM NaCl and four replications in green house. Some parameters such as content and composition of essential oil were modulated. The results showed that salt stress had significant effects on estimated parameters. Salinity decreased the fresh and dry weight of leaves and shoots, herbage yield and the amount of essential oil. In the composition of essential oil β-pinene, myrcene, anisaldehyde and α-bourbonene increased and the content of linalool and methyl chavicol decreased. High salinity (100 and 150mM) destroyed the plants.

**PD8**
Growth, yield and essential oil content of *Marrubium vulgare* as affected by three levels of nitrogen fertilizer

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*Marrubium vulgare* L. (Lamiaceae) is a perennial herb commonly known as “White horehound” and grows wild in the Egyptian desert and commonly distributed in Europe, North and South America, the Mediterranean district and Western Asia. The plant is used in the folk medicine of several countries for the treatment of a variety of diseases, including inflammatory, gastroenterical and respiratory disorders. For the first time in Egypt, the plant was cultivated under systematic agriculture regime to estimate the nitrogen doses for the best plant growth. Three
Effect of sowing date and seeding levels on quantitative and qualitative yield of chamomile (Matricaria recutita) study conducted. The experimental design was split-plot in the basic of randomized complete block design with three replications. Main plots consisted of three sowing dates (6 Nov, 5 Mar, and 4 Apr) and sub-plots included three seeding levels (0.2, 0.4 and 0.8 g/m²). On the basis of the results, highest plant (47.4 cm), the most number of flowers and the most percentage of essential oil (0.59 and 5.62 percent respectively) and essential oil yield (0.79 g/m²) was obtained from the plots were sown on 6 Nov but the most photosynthetic rate (28.32 mmol/m²/s) and dry flower yield (749.1 and 175.1 g/m²) was obtained from the plants were sown on 5 of Mar. On the basis of the results of their interaction, highest plant (49.7 cm), the most yield of fresh and dry flower yield of seeds (0.97 g/m²) was obtained from the plants were sown on 5 of Mar. According to the results, the most suitable sowing date in Mashhad condition is 5 of Mar with 0.4 g/m² seeds. The results showed that all characteristics were superior in sun drying and berry picking off methods, except pH (Table 1). It has been reported that the amounts of anthocyanin was reduced at low light level [2], whereas the others reported that increasing light reduced it [1]. This differences related to plant, variety, location, season and growth phase [3]. Anthocyanines are more stable at high pH [4], Since in sun drying and berry picking treatments, amounts of acidity and anthocyanin were increased and pH was decreased, it seems that this pigments in seedless barberry could maintain with applying these treatments. Our results indicated that sun drying and berry picking off methods were more effective to improve qualitative properties of seedless barberry. References: 1- Bergvist J et al. (2001) 2- Dokoozlian N K, Klewer WM (1996) Soc.Hort Sci 121: 869 – 874. 3- Juyishi A N et al. (2005) Int J Food Prop 8: 221 – 232. 4- Inami O et al. (1996) J Agric Food Chem 44: 3000 – 3006. 5- Shamsa F et al. (1999) Ethnompharmacol 64: 161 – 166, 6- Timberlake CF, Bridle P (1982) Distrubution of anthocyanins in food plant. Anthocyanin as food colors. London: Academic Press.
Assessment diversity and cultivation potential of Coridothymus capitatus (L.) Reichenb. fil growing wild in Jordan

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Coridothymus capitatus (L.) Reichenb. fil. is a medicinal and aromatic plant growing wild in Jordan and locally known as Za’atar Farisi. The phenotypic diversity and potential cultivation study comprised fifteen wild populations of Coridothymus capitatus, one wild population of Thymbra spicata and two Thymbra. spicata landraces. The investigated wild populations of Coridothymus capitatus showed various degrees of phenotypic variation based on the characters under investigation. Significant variations were obtained for quantitative characters, the coefficient of variation (CV%) ranged from 12.60% to 39.20%. The average estimate of Shannon’s diversity index (H’) was 0.58. The genetic distance among pairs of populations was low. Coridothymus capitatus populations introduced for cultivation showed a good stand and potential toward producing dry herbage yield (3046 kg/ha). Cultivated populations showed phenotypic variation in the investigated traits. The results of this study indicate that a broad range of genetic variation exists among populations of Coridothymus capitatus collected from wild habitats in Jordan, and among Thymbra spicata populations. Seeds of Coridothymus capitatus and Thymbra spicata were conserved (ex situ) in seed bank and in the field bank. The results obtained pave the road for a potential commercial and large-scale cultivation and oil production from Coridothymus capitatus species. References: Faleiro L et al. (2005) J Agric Food Chem 19: 8162 – 8168. Goren A et al. (2003) J Biosci 28: 687 – 690. Haddad N and Turk M (2002) Medicinal and herbal plants cultivation. Ministry of Agriculture, National Center for Agricultural Research and Technology Transfer (NCARTT), Conservation of medicinal and herbal project preparation grant, Global Environment Facility (GEF), Amman, Jordan. Morales R (1996) Lamiales newsletter 4: 6 – 8. Morales R (1986) Biocosme Mésogéen 6:205 – 211. Morales R (1986) Ruizia 3: 1 – 324.

Saffron is a spice derived from the flower of the saffron crocus (Crocus sativus L.) Together with the styles stalks, which connect the stigma to their host plant the dried stigmas are used in cooking as a seasoning and colouring agent is native to Southwest Asia. Saffron also contains a carotenoid dye, crocin, which imparts a rich golden yellow hue to dishes and textiles. In order to the saffron variation in saffron under irrigation regimes, an experiment was carried out using a randomized complete blocks design with three replicates at Iran in 2010. The factors including irrigation regimes (control, irrigation interrupted from stem elongation stage, irrigation interrupted from flowering stage) were studied. The flower yield in saffron increased under control irrigation into interrupted irrigation but saffanal variations was increased under interrupted irrigation into control irrigation. The findings may give applicable advice to medicinal and aromatic plants researchers for management and concern on water strategy and estimate of irrigation carefully.
showed an antinociceptive effect in a dose-dependent manner as measured by writhing test as a model of visceral pain. Furthermore to reveal the antinociceptive mechanisms of *Bunium persicum*, we examined the effect of opioidergic, serotonergic, and histamine receptor antagonists on *Bunium persicum*-induced antinociception. Intraperitoneal (i.p.) pre-treatment with naloxone, chlorphenermine and cimetidine attenuated inhibition of the pain response induced by *Bunium persicum*. However, cyproheptadine did not affect inhibition of the pain response induced by *Bunium persicum*. Our results suggest that *Bunium persicum* shows an antinociceptive property in writhing test. Furthermore, antinociception of *Bunium persicum* may be mediated by opioidergic and histaminergic H1 and H2 receptors.

**PE3**

Essential oils composition and antioxidant properties of three *Thymus* species

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The essential oils of three wild-growing *Thymus* species (*Thymus kotschyanus* Boiss. & Hohen., *Thymus eriocalyx* Ronniger) Jalas. and *Thymus daenensis* Celak subsp *lancifolius* (Celak) Jalas. collected from west of Iran during the flowering stage, were obtained by hydrodistillation and analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC-MS). Under the optimum extraction and analysis conditions, 44, 38 and 38 constituents (mainly monoterpenes) were identified in *T. kotschyanus*, *T. eriocalyx* and *T. daenensis* subsp. *lancifolius* which represented 89.9%, 99.7% and 95.8% of the oils, respectively. The main constituents were thymol (16.4 – 42.6%), carvacrol (78.6 – 52.3%) and γ-terpinene (3.0 – 11.4%). Antioxidant activity was employed by two complementary test systems namely 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and β-carotene/linoleic acid systems. Antioxidant activity of polar sub-fraction of *T. daenensis* subsp *lancifolius* was found to be higher than those of the *DPPH* assay while non-polar sub-fraction of *T. eriocalyx* has most antioxidant activity in β-carotene/linoleic acid test (19.1 ± 0.1 µg/ml and 96.1 ± 0.8% inhibition rate, respectively).

**PE4**

Effect of heating on *Zataria multiflora* and *Cinnamon zeylanicum* essential oils for the evaluation of their antiradical activities by using 2,2′-diphenyl-1-picrylhydrazyl (DPPH)

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Oxidation of lipids, which occurs during raw material storage, processing, heat treatment and further storage of final products, is one of the basic processes causing rancidity in food products, leading to their deterioration. Since application of natural antioxidants may be one of the technically simplest ways of reducing fat oxidation, the present study was designed to survey the effect of heating on antiradical property of *Cinnamon zeylanicum* and *Zataria multiflora* Shirazi essential oils. The essential oils were heated in three temperatures (100, 140, 180 °C) for 1, 2, 3 hours and the antiradical property was compared with samples before heating. The antiradical property was evaluated by using DPPH† assay. All the data were analysed by SPSS software (version11.5). In the ambient conditions, EC₅₀ of *Zataria multiflora* and *Cinnamon zeylanicum* essential oils were 4026.67 ± 2.2 and 2605.01 ± 3.7 ppm, respectively. According to the results, different behavior of essential oils, based on different heat conditions, were observed. In conclusion, the essential oils under study exhibited good antiradical properties and might be efficiently used to control lipid oxidation during food processing. Keywords: essential oil, *Cinnamomum zeylanicum*, *Zataria multiflora*, DPPH assay References: 1. El-Baroty GS et al. (2010) African Journal of Biochemistry Research 4: 167 – 174. 2. Jayaprakasha G K, Negi P S, Jena B S and Rosa L. (2007) Food Composition and Analysis 20: 330 – 334. 3. Kulisic T, Radonic A and Katalinic V (2004) Food Chem 85: 633 – 640. 4. Marinova E M and Yanishlieva V N (1996) Food Chem 58: 245 – 248. 5. Moranguu B et al. (2007). Agric Food Chem24: 10022 – 10027.

**PE5**

Effect of Salt Stress on Growth and Essential Oil of *Matricaria chamomilla* L.

**Dadkhah A**

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A pot experiment based on complete block design was carried out to investigate the effect of salinity on growth traits and essential oil content of chamomile (*Matricaria chamomilla* L.). Four levels of salinity including control (0 mM), 50, 150 and 250 mM NaCl and CaCl₂ in 5:1 molar ratio were used. Result indicated that increased salinity caused reduction in plant height, number of branches per plant, number of flowers per plant. Increased salinity also significantly decreased plant dry weight, flower dry weight and essential oil content. The highest values of growth traits such as number of flower per plant, flower dry weight and essential oil content were observed under control condition (non-salinity stress). The effect of salinity on flower dry weight is greater than other traits. Flower dry weight of plants at low (50mM) level of salinity was decreased 12.2% compared to control (non-stressed plant) while essential oil content increased 18.2% at the same salinity concentration. At the highest level of salt stress (250mM) flower dry weight and essential oil content was decreased by 79.8 and 45.5% compared to non-stressed plants, respectively. Number of flower per plant was decreased by 16.1 and 69.2% at lowest (50 mM) and highest (250 mM) salinity concentration respectively. Salinity affects flowering time of plants. Flowering time of non-stressed plants started 50 days after plant transplanting while flowering time of plants treated by 250 mM salinity started 64 days after seedlings transplanting to pots. Acknowledgement: I would like to express my appreciation for research deputy of the Ferdowsi University of Mashhad for financial support. The author is grateful to Mr Hamid Eskandari BSc student of medicinal plant production for his excellent assistance.

**PE6**

Effect of Salt on Germination and Seedling Growth of Four Medicinal Plants

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This experiment was conducted in germinator in order to study the effects of water potential on seed germination, rate of germination and seedlings growth of four medicinal plants (*Coriandrum sativum* L., *Portulaca oleracea* L., *Daucus carota* L. and *Zataria multiflora*). Four water potential including distilled water as control (0), -0.37, -0.59 and -0.81 Mpa which made by different salts (NaCl, CaCl₂ and NaCl+CaCl₂ in 5 to 1 molar ratio). The experiment was carried out based on completely randomized design with six replications. Results showed that the effects of water potential, type of salt on germination percentage, rate of germination, root and shoot length were significant. With decreasing water potential, germination percentage and rate of germination declined but the response of plant were different. Germination of *Portulaca oleracea* was not affected by decreasing water potential whereas others significantly decreased. The effect of salt composition was significant on rate and percentage germination. The percentage of germination at lower water potential (-0.37 MPa) which made by NaCl + CaCl₂ significantly was higher than the same water potential made by only NaCl and CaCl₂. Although, percentage and rate germination of *Portulaca oleracea* were not affected by different water potential, seedling growth of *Portulaca oleracea* significantly decreased. Acknowledgement: I would like to express my appreciation for research deputy of the Ferdowsi University of Mashhad for financial support.

**PE7**

Effects of Different Level of Nitrogen and Phosphorous Fertilizers on Yield Quantity and Quality of *Matricaria recutita*

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In order to investigate the effects of different levels of nitrogen and phosphorous fertilizers on qualitative yield and some quality components of German chamomile (*Matricaria recutita* L.), a factorial experiment was based on a randomized complete block design with four replications was carried out in the Medicinal Plant Research Farm of Shirvan College of Agriculture in 2007. Nitrogen had three levels (0, 100, 200 kg/ha as source of urea) and phosphorous also had three levels including 0, 30, 60 kg/ha triple super phosphate. The results showed that application
of nitrogen and phosphorous fertilizers to the soil imposed a significant effects (p < 0.01) on plant height, number of branches per plant, number of flower per plant and flower yield. The main effects of nitrogen fertilizer appeared in improvement of vegetative growth of plants. Plants treated with 200 kg nitrogen per hectare and 60 kg phosphorous per hectare significantly produced the highest flower yield per square meter. Fertilizer did not affect the percentage of chamazulene. However, plant treated with 200 kg nitrogen and 60 kg phosphorous produced highest essential oil and chamazulene per square meter due to higher flower yield. Acknowledgement: I would like to express my appreciation for research deputy of the Ferdowsi University of Mashhad for financial support.


PE8

Enantiomeric composition of α-pinene in essential oils of leaves and unripe cones of Juniperus communis L.
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The leaves and unripe cones of common juniper (Juniperus communis L.) were sampled from 11 habitats across Lithuania. The essential oils of leaves and cones from 110 samples (+tree) were separated by hydrodistillation in a European Pharmacopoeia apparatus during two hours. The enantiomers of α-pinene were separated by the chiral-phase capillary GC and identified by matching their retention times to the optically pure analytical standards. The average content, the min-max values and the variation coefficient of (1R)-(+)α-pinene were 74 ± 1%, 7–92% and 16.4% in leaves and 69 ± 2%, 0–96% and 24.9% in unripe cones, respectively; the average content, the min-max values and the variation coefficient of (1S)-(-)α-pinene were 26 ± 1%, 8–93% and 45.8% in leaves and 31 ± 2%, 4–100% and 55.7% in unripe cones, respectively. It was established that the most of samples of leaves and unripe cones of the studied J. communis individuals were rich in (1R)-(+)α-pinene, while (1S)-(-)α-pinene dominated in 2.7% samples of leaves and 10.8% those of unripe cones only. However, the (1S)-(+)α-pinene absolutely predominated in leaves of two J. communis individuals. The absolute predominance of the (1R)-(+)α-pinene was not detected neither in leaves nor in unripe cones. Acknowledgement: This research was funded by a grant (No. MIIP-56/2010) from the Research Council of Lithuania.

PE9

Antifungal activity and chemical composition of Mentha cervina L. essential oils
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Mentha cervina L. is a very aromatic plant with a characteristic flavour, which can be found on some regions from central eastern and south of Portugal. In the present paper, we analysed the chemical composition of essential oils from fresh and dried leaves of M. cervina and its antifungal activity against strains of Aspergillus niger, Penicillium sp. and Fusarium oxysporum isolated from soils. M. cervina was collected in Almaceda – Vila Velha de Ródão, Central Eastern of Portugal during the flowering period. Yellowish essential oils were obtained in a yield 1.1% and 1.8% (v/w) to fresh and dry plant, respectively. Major component of the oils was identified as pulegone (78.0 and 80.4%). Both essential oils of M. cervina at the doses of 10 μl, inhibited totally the growth of the tested fungi. Doses of 5 μl of each essential oil also showed activity against the fungi strains used in this work, in particular against Penicillium sp. Taking into account the high level of pulegone observed in both essential oils and the antimicrobial activity of this compound reported by Duru [1], these results suggest that this compound could be the main responsible component for the antifungal activity observed. References: 1. Duru M E, Ozturk M, Ugar A, Ceylan O (2004) J Ethnopharmacol 94: 43–48.

PE10

The effect of drying temperature, storage and distillation times on the essential oil content and composition of anis hyssop (Agastache foeniculum var. chaubardii)
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This paper deals with the effect of different drying temperatures, storage and distillation times on the essential oil content and composition in anis hyssop (Agastache foeniculum Kuntze). The treatments were two levels of temperature (ambient temperature and 40°C), two levels of material storage time (immediately after drying and after 9 months), and two levels of distillation time (2 and 4h). The treatments were arranged in factorial design in base of Complete Randomized Design (CRD) with three replications. The findings show that essential oil content was declined with increasing temperature degree and storage time. Distillation time did not have a significant effect on the composition of the essential oil extracted from anis hyssop.

References:

PE11

Chemical constituents of the essential oil of Ferulago carduchorum Boiss. et Hausskn. and Levisticum officinale Koch
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The volatiles obtained by hydrodistillation and methanol extraction of the aerial parts of Ferulago Boiss. et Hausskn. and Levisticum officinale Koch, two Umbelliferae species of Iran, were analyzed by GC and GC/MS. The oil and the extract obtained by hydrodistillation and extraction of F. carduchorum were characterized a high amount of monoterpene hydrocarbons (93.8% and 70%, respectively). The main components of the oil and extract were (2)-β-ocimene (12.7% and 20.5%), terpinolene (13.1% and 6.0%), α-Phellandrene (12.7% and 8.3%) and β-Phellandrene (10.9% and 8.8%), respectively. The water distilled oil and methanol extract of the air-dried Levisticum officinale, were also both rich in monoterpene (85.8% and 52.9%, respectively). In the oil, α-terpi- nene acetate (40.5%) and β-Phellandrene (16.7%) were the main constituents, whereas in the extract, β-Phellandrene (23.0%), naphthalene (20.6%) and γ-terpinene (12.1%) were the major components. References: 1- Basar KHC, Koyuncu M and Vural M (1998) J Essent Oil Res 10: 665 – 666. 2- Masoudi S, Rustaiyan A and Ameri N (2004) J Essent Oil Res 15: 143 – 144. 3- Sedaghat S, Khausoos M, Masoudi S, Larijani K and Rustaiyan A (2002) J Essent Oil Res 14: 447 – 448.

PE12

Major volatile compounds of 50 Thymus taxa naturally grown in Antalya region of Turkey
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A large number of medicinal and aromatic plant species naturally grown in the Mediterranean Basin of Turkey contain secondary metabolites that are used in the food, pharmaceutical, cosmetic, and pesticide industries [1 – 5]. This study used 50 taxa consisting of 9 species or sub-species of the genus Thymus grown wild in Antalya area in the Mediterranean part of Turkey to determine their volatile compounds. The major constituents may suggest that the volatile constituents noted in six taxa of Thymus longicaulis C.Presl subsp. chaubardii (Boiss. & Heldr. ex Rchb.f.) Jalas var. chaubardii were borneol, nerol, geraniol, thymol, γ-terpinene, p-cymene, camphene, 1,8-cineole and linalool. The main components of these taxa of T. sylvestris Boiss. subsp. sylvestris var. rosularis (Borbas) Jalas...
were α-pinene, 1,8-cineole, β-caryophyllene, α-terpineol and interme-
diol. Three taxa of *T. sylvestris* Boiss. subsp. *sylvestris* var. *daviesii* Ron-
niger contained 1.8-cineole, ρ-cymene, β-caryophyllene, nerol, α-terpi-
neol and geranial as major constituents. Eight taxa of *T. sylvestris* Boiss. subsp. *sylvestris* var. *sylvestris* mainly contained myrcene, 1,8-cineole, lina-
lool, (E)-nerolidol, ρ-cymene, α-terpineol, carvacrol and hexadecanoic acid. Nine taxa of *T. zygoides* Griseb. var. *lycanthus* (Celak) Rönninger contained ρ-terpineol, ρ-cymene, bornene, thymol and carvacrol. Three taxa of *T. leucotrichus* Hal. var. *austromatriculatus* Jalas contained α-pinene, camphene, myrcene, camphor, linalool, bornyl acetate, thymol and car-
vacrol. Three taxa of *T. cherlerioides* Vis. var. *isauricus* Jalas contained 1.8-
cineole, ρ-terpineol and ρ-cymene. Six taxa of *T. revolutus* Celak contained 1.8-cineole, ρ-terpineol and ρ-cymene. Nine taxa of *T.
cifices* Boiss. & Balansa mainly contained 1.8-cineole, linalool, α-terpi-
neol, ρ-cymene, camphene, α-terpineol, camphene, ρ-cymene and bornene. The occurrence of volatile compounds among clearly indicated that Thymus taxa show significant inter-
and-intra-specific variations. 

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**Classification of 63 Origanum taxa based on microsatellite markers and essential oil composition**

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A large number of aromatic plant species naturally grown in the Medi-
terranean basin of Turkey contain and produce essential oil [1]. In this study 63 taxa of eight Origanum species grown in the Mediterranean region of Antalya, Turkey were DNA typed using microsatellite markers, and oil compositions of these taxa were determined using method de-
picted in [2,3,4,5]. All the 8 Origanum species were separated from one another according to classical taxonomic groups using DNA markers. Individuals of two *O. vulgare* L. subsp. hirtum (Link) l.etsw., two *O. mar-
jorana* L. two *O. syllectum* P.H.Davis and two *O. saccatum* P.H.Davis taxa could not be differentiated in the DNA typing studies. There were high levels of similarities between a dendrogram obtained from DNA markers and oil composition types among the taxa studied. *O. bilgeri* P.H.Davis consisted of two chemotypes (carvacrol oxide and alpha-
thujene) and they were clearly separated by DNA analyses. *O. husnien-
baseri* H.Duman, Aytac et A.Duran was also separated from other species and it was the only species containing trans-sabinene hydrate. Taxa collected from Elmali location of *O. onites* L. were linalool types and they were distinctly separated from other individuals from within the species. *O. majorana* consisted of two chemotypes (carvacrol and linalool). In conclusion, present study indicated that chemotypes could be identified using DNA markers. Thus, DNA markers developed in this study could be used in the identification of species in herbal mixtures, selecting the individual plant for desired oil compositions and the most importantly these DNA markers are valuable in Origanum improvement programs. 


**PE13**

Rhizomes of ginger (*Zingiber officinale* L.) are widely used ingredients in food technology, pharmaceutical and cosmetics industries. The charac-
teristic aroma and taste of ginger as well as medicinal effect is attributed to essential oils and oleoresins. In this work, the microwave distillation kinetics, yield and composition of the essential oil obtained at different dry plant material-to-water ratios (1:10, 1:15 and 1:20) and microwave powers (350W, 450W and 600W) were examined. The oil yield strongly depend on the dry plant material-to-water ratio and the applied micro-
wave powers (350W, 450W and 600W). The highest oil yield (1.23 mL/kg) was achieved at dry plant material-to-water ratio of 1:20 and microwave power of 600W. The essential oil composition was identified using GC-MS techniques. There are no significant differences in the chemical composition of the oils obtained at different microwave distillation conditions. Acknowledgement: This work was supported under the projects OI 172047 by the **Ministry of Science and Technological Development Republic of Serbia**.

**PE14**

Chemical composition of the essential oil from leaf, root and fruit of *Diplotenia damavandica*, an endemic species of Iran Yousefnejad F1, Amin C2, Salehi Sormaghi M1, Khorasani M1, Mohammadi S1, Tasharofi N2

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*Diplotenia damavandica* Mozaff., Hedge & Lamond (Apiaceae), which is locally named “Koza” is an endemic species of Iran and grows in Dama-
vand area in Tehran province (1). Fresh aerial parts of koza are used as flavoring agent for local dairy products. Our previous studies showed that this plant is a rich source of furanocoumarins like xanthotoxin and angelicin (2). In this study the essential oil of the leaves, roots and fruits of the plant obtained by hydrodistillation and analyzed by GC and GC/MS. Average yields of essential oil were 1.5%, 2.5%, 0.3%, for leaves, roots and fruits respectively. The most abundant components of the leaves were trans-cinna-
mene (22.5%), α-phellandrene (19.0%), linalool (7.3%) and cis-cimene (6.8%). The root essential oil was characterized by high amounts of α-phellandrene (20%), α-pinene and α-terpineol (12%). The main components of the fruits were α-phellandrene (17%), ρ-terpineol (16.9%), limonene (13.2%) and α-terpineol (11.2%). References: 1. Mozaffarian V (2007) Flora of Iran. Research Institute of For-
Essential oil variability and trichomes morphology from Lavandula pedunculata (Mill.) Cav. grown at Mata Experimental do Escaroupim (Portugal)

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The Experimental Forest of Escaroupim [Mata Experimental do Escaroupim (MEE), Salvaterra de Magos, Portugal], is a protected forest area with over 175 years and under the Total Forestry Regime since 1901. Lavandula pedunculata (Mill.) Cav. is an aromatic herb commonly growing in the Iberian Peninsula [1], and frequent in the understory of the MEE Pinus, Quercus, Ulmus and Eucalyptus spp. forests. In the present work, the essential oils and trichomes morphology from flowering aerial parts of two populations of L. pedunculata collected in two years were evaluated. The essential oils were isolated by hydrodistillation, and analyzed by GC and GC/MS [2]. The indumentum of L. pedunculata field grown plants was studied by LM and SEM, according to [3]. L. pedunculata essential oils were obtained in an average yield of 2% (v/w). Thirty six components were identified, representing 97 – 99% of the total essential oils, which were obtained in an average yield of 2% (v/w). cis-Verbenol (traces-5%), camphor (1 – 5%) and limonene (traces-4%) were also relatively abundant. Previous studies also showed essential oils fenchone-, 1,8-cineole- and camphor-rich [2,4,5]. L. pedunculata showed a morphologically complex indumentum of i) non-glandular uni- and bi-cellular unbranched trichomes and multi-cellular branched trichomes of the stellate type; ii) peltate and capitate trichomes, the last with different morphological types; iii) multi-cellular branched stellate type with only glandular arms and iv) multi-cellular branched stellate type with both glandular and non-glandular arms. These results are in agreement with a previous study on the indumentum of L. pedunculata field grown and in vitro-grown plants [5]. Acknowledgement: Telmo Nunes, Paula Pires, A. Sofia Borges, Prof. Ana Monteiro. References: 1. Morales R (2010) Flora Iberica, Lavandula. Vol. XII. Reai Jardim Botanico. CSIC. Madrid 2. Matos F et al (2009)J Essent Oil Res 21; 327–336. 3. Antunes T, Severino-Pinto J (1991) Flora 185: 65–70. 4. Zuzarte M et al (2009) Chem Biodivers 6: 1283–1292. S. Zuzarte, M et al (2010) Ind Crops Prod 32: 580–587.

Comparative assessment on efficiency and compounds of Ferula gummosa Boiss. essential oils at two different harvesting areas of Alborz mountains in Iran

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Ferula gummosa Boiss. from Umbelliferae family (Baridje in Persian and Galbanum in English) is one of the most important medicinal plants of Iran mountains area which has industrial applications, too. The northern steeps with 2000 – 4000 altitude and soils of well drained and deep, rich of humus and different quantity of lime are the best growth region for Galbanum, as ecological investigation have been shown. Galbanum exudes sap-like exudate which contains 60, 65 – 75% resins and 5 – 30% carbohydrates, roughly1. It must be noticed that no alkaldoids andphenol compounds have been found; except some trace of saponin and tannin. Non-carbohydrate portion of Galbanum can be extracted with ethanol. Incineration is used to determine the quality of exudates, results in ash content that must not exceed 10%. In this study, the percentage and essential oil components of Galbanum harvested from Tehran and Semnan provinces were investigated. The oleo-gum-resin was submitted to steam distillation. The essential oil was distilled at temperature of 0.865 – 0.890, was analysed using GC/MS, α-pinene, β-pinene, myrcene, δ-3 carene, limonene, fenchyl acetate and guaiol identified, among which limonene and fenchyl acetate ranked highest and lowest percentage, respectively2. The identified compounds have significant differences at the two locations, that means at southern slope of Alborz in Tehran, essential oils yields 2.16 – 2.44%, whereas 1.23% was assigned to southern slope in Semnan. Acknowledgement: We are grateful to the International Desert Research Centers, University of Tehran for financial support of this work. References: [1] Zargar A (1986) Medical Plant, Vol 2, University of Tehran Press, Tehran. p 598 (in Persian). [2] Mossaffar V (1987) Umbelliferae Family, Plants in Iran. Researches Institute of Forests and Rangeland press. Tehran (in Persian).

PE18

Essential oil composition of Artemisia spicigera C. Koch

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he genus Artemisia, which comprises about 400 species, belongs to the Asteraceae family and is the largest of flowering plants [2]. Most representatives are shrub. Artemisia spicigera (Koch) is a member of this family (Asteraceae) and from fodder and medical point of view is an important plant [4]. In this research the aerial parts of plant were collected from Azerbaijan province (Iran). The dry material of plant was subjected to steam distillation in Clevenger apparatus and a light yellow residue obtained. The essential oil was submitted to column chromatography. Gas chromatography was done and careful analysis by GC and GC/MS of essential oil from Artemisia spicigera allowed us to identify most components (99.01%). The chromatogram showed the presence of approximately 17 compounds. The results of analysis revealed the presence of camphor (24.6%), 18-cineole (23.3%), β-thujone (20.7%) and α-thujone (17.1%) as major components in this plant. The essential oil, having a very strong odor and acid taste, is described as neurotoxic due to the high thujone content [3]. The composition of the volatile oil in Artemisia spicigera varies widely according to geographical location, climate, day length, soil type and cultivar [1]. References: 1.aleskerova AN et al. (1986) Khim Prir Soedin (1):116–117. 2. Bremer Boiss. et al. (1986) Flora Iberica, Lavandula. Vol. XII. Real Jardín Botánico. CSIC. Madrid 2. Matos F et al (2009)J Essent Oil Res 21; 327–336. 3. Antunes T, Severino-Pinto J (1991) Flora 185: 65–70. 4. Zuzarte M et al (2009) Chem Biodivers 6: 1283–1292. S. Zuzarte, M et al (2010) Ind Crops Prod 32: 580–587.

Lemon thyme (Thymus x citriodorus (Pers.) Schreb) is widely grown as ornamental aims in Turkey and has no natural distribution. It's a hybrid of Thymus vulgaris L. and Thymus pulegioides L. Compared with other Thyme species, Thymus citriodorus has a herbaceous oblique structure and rich yellowish oil. By means of this oil, it was firstly cultivated in a field experiment and its yield and quality parameters recorded in semiarid conditions. Field trials were conducted in the Central Research Institute for Field Crops, Ankara, at complete randomized block design with four replications during the years of 2009 and 2010. Total yield of green herb, drug herb and drug leaf was 3769 – 4707 kg/da, 1193 – 1475 kg/da and 742.3 – 844.2 kg/da, respectively. The essential oil of Rosmarinus officinalis, representing 98.5% of the oil, was equal with 200 μg/mL of BHA (P > 0.05), but flowers oil of Thymus kotschyanus exhibited, the main component of which linalool (20.2%), carvacrol (24.5%) and trans-caryophyllene (8.6%) were reported as the main constituents. Though most Thymus species are thymol containing, the main component of Thymus citriodorus essential oil was determined as ‘geraniol’ with lesser amount of lemon scented citral (geranial + neral) (from twenty-one constituents). Keywords: Thymus x citriodorus, essential oil, geraniol, citral
The effects of different drying methods (natural method, oven and microwave) on drying time, essential oil content and composition of Savory (Satureja hortensis L.); this experiment was carried out during 2008. The experimental design was completely randomized block design with three replications and treatments were: two temperatures: 50° and 70°C, six microwave powers: 100, 180, 300, 450, 600 and 900 w and drying in shaded and sunny area. The drying process was continued until the mass of the sample reduced to a moisture content of about 0.10 on a dry basis or 10% on a wet basis. The results indicated that different treatments of drying had a significant effect on drying time and essential oil content. Minimum and maximum drying times (4.5 minutes and 96 hours respectively) obtained at 900 w microwave power and drying in shaded area. The maximum essential oil content (3%) obtained at drying by 70°C and drying in shaded area and minimum content (0.9%) obtained at drying in sunny area. 100 and 300 w microwave powers had average content of essential oil (2/3%). Maximum carvacrol content (63.9%) obtained at 300 w microwave drying. Maximum γ-terpine content (28.2%) obtained at drying by 70°C that it had little difference with 50°C, 100 and 300 w. According to the results, because of reduction of drying time and suitable essential oil content and composition in drying by low microwave powers, these methods counseled. References: 1. Szumny A et al. (2009) Food Engin 97(2): 253–260. 2. Soysal Y (2004) Food Engin 89(2): 167–173.

Chemical composition variation in essential oils of Calamintha hispidula (Boissier and Reuter) Maire, endemic in North-eastern Algeria

The essential oils from three samples at the full blossom stage of wild Calamintha hispidula Boiss. and Reuter. M. native of the mountain of Texanna, Algeria, (one sample taken at the altitude of 625 m south facing and the other two at 526 m and 620 m north facing), have been extracted by hydrodistillation and analysed by GC-MS. The oil yileds were 1,06%, 0,59% and 1,49%, respectively. The main oil essential constituents were isomethrone (68,8%, 66,2% and 57,9%), pulegone (18,1%, 15,1% and 22,2%) and piperitone oxide (16,6, 6,6% and 22,2%), respectively. The variation in the yields of essential oils was considerable between the two altitudes 526 m, 620 m north facing. Isomethione was found to be the major constituent in all cases. Acknowledgement: The authors are grateful to Mr Gérad de Bélar Lector at the University of Annaba for species identification and writing assistance, and Mr Desdous Abderrachid for the GC/MS analysis.

Investigation on Development of Zein Antimicrobial Edible Film and Essential oil of Zataria multiflora Boiss. on canfora Boiss, on calamintha enteritiidis, Listeria monocytogenes, Escherichia coli and Staphylococcus aureus

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Active packaging is a type of packaging that can control or react to things arranged inside (2). An antimicrobial Active Packaging is made by incorporating antimicrobial agents in food packages. Zataria multiflora Boiss. is a plant that belongs to the Lamiaceae family and grows in the mountains. It is a traditional medicinal plant in Iran and has been used to treat some gastrointestinal symptoms. This study was carried out during 2008. The experimental design was completely randomized block design with three replications and treatments were: two temperatures: 50° and 70°C, 100 and 300 w. According to the results, because of reduction of drying time and suitable essential oil content and composition in drying by low microwave powers, these methods counseled. Chemical composition variation in essential oils of Calamintha hispidula (Boissier and Reuter) Maire, endemic in North-eastern Algeria...
only in Iran, Pakistan and Afghanistan (1). The antimicrobial activities of the plant are also well established against a wide variety of bacteria (3). In this study zein is based on the films that contain essential oil of 
Zataria multiflora (0, 0.5, 1, 2, 3 and 4 w/v) and glycerol (30 v/v). The objective of this study was to determine the effectiveness of the edible film in inhibiting 
Salmonella enteritidis, Listeria monocytogenes, Escherichia coli 0157: H7 and staphylococcus aureus. The bacterial strains were transferred on the surface of nutrient agar plates using sterile swabs, and then discs of zein films containing different percent of the essential oil of the zataria multiflora put on the surface of the plates. After that incubated overnight at 37°C. Finally after 24 hours the inhibitory zones was measured with calliper. Overall, there was a decline in the test organisms that was generally increased in the presence of the Zataria multiflora Bois' essential oil. The mentioned bacteria showed significant reductions in bacterial survival in the higher extract concentration. To conclude zein antimicrobial edible film and Zataria multiflora essential oil might help to decrease the risk of food borne illness related to these microorganisms. References: 1. Ali MS et al (2000) Phytochemistry 55: 933–936. 2. Del Nobile MA et al. (2009) Journal of Dairy Research 80: 1–6. 3. Palmer A et al (2001) Food Microbiology 18: 463–470.

**PE30**

**Chemical composition, Antioxidant and Mosquito larvicidal activities of essential oils from Tagetes filifolia, Tagetes minuta and Tagetes elliptica from Peru**

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Developing of new pesticides from botanical pesticides has been attempted in the past [1]. Botanical pesticides are safe to use in different propose and IPM program [2]. Melia azedarach L. (Meliaceae) is characterized by containing allelochemicals compounds, with a high level of bioactivity against insect. In this study M. azedarach seeds were collected from Kerman, Iran. The seeds were milled and powdered mechanically using commercial electrical stainless steel blender to extract their oil. The experiments were directed to determine the effect of different solvents on insecticidal activity of seed oil of M. azedarach on 3 – 4-day-old individuals of Aphis fabae Scopoli. The oil was extracted with different solvents. The solvents were included acetic acid, aceton and n-Hexane. All experiments were conducted by topical test bioassay in laboratory, at 25 ± 1°C temperature, relative humidity of 60 ± 10% and 16 hours of artificial light at an intensity of about 4000 lux. Water and DMSO (Dimethyl sulfoxide) were used as control treatments. The results indicated that in the concentration 80 μl/ml, the mortality of A. fabae treated with n-Hexanic seed extract of M. azedarach after 24 hours, was more than 72%, while it was 40.75% and 60% in A. fabae treated with acetic acid and aceton, respectively. Seed extract of M. azedarach respctively the mortality of A. fabae treated with n-Hexanic seed extract of M. azedarach was significantly higher than acetic acid seed extract of M. azedarach. It could be concluded that some plant extracts may be applicable as a safe insecticide to aphid's control. References: 1. Isman M (2005) Biopesticides of Plant Origin. Lavoisier Tech & Doc. Paris. 2. Raja N et al. (2001) Stored Prod Res 37: 127–132.

**PE31**

**Chemical composition of the oil of Cicuta virosa L. from Kazakhstan**

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The present work is a part of our ongoing research into the volatiles of Apiaceae species. A well-known toxic plant, water hemlock, Cicuta virosa L. is widely distributed in the temperate regions. Herbal parts of C. virosa were collected in Karkaraly Mountain (Kazakhstan) during blossoming in 2009. The essential oil (yield 0.1%) was obtained by hydrodistillation using a Clevenger type apparatus. Chemical composition of the oil was analyzed by GC-FID and GC/MS methods. The oil was found to be rich in sesquiterpenic hydrocarbons. In this study, -farnesene (22.7%), α-humulene (5.4%), humulene epoxide II (5.9%), caryophyllene oxide (3.4%), germacrene D (3.2%) and (Z,E)-α-farnesene (3.6%) were detected in the major constituents. Among the monoterpenes myrcene (7.8%) was detected in high percentage. Fatty acids were represented mostly by hexadecanoic acid (8.4%). To the best of our knowledge, the essential oil of C. virosa from Kazakhstan was not investigated previously.

**PE32**

**Sulphur containing volatiles of Barbarea vulgaris W.T. Aiton from Kazakhstan**

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Aim of the present study was the investigation of the chemical composition of Barbarea vulgaris W.T.Aiton volatiles. The plant material was collected in Akmolinskaya and Karagandskaya provinces of Kazakhstan in 2009. Different plant parts (flower, leaf and herb) were subjected to microdistillation to obtain the volatiles. The volatiles were then analyzed by GC-FID and GC/MS methods. The most of samples were rich in sulfur containing compounds. Methyl (methylthio) methyl disulfide (14.5%), dimethyl trisulfide (11.2%), dimethyl sulfide (3.4%) were detected in the herb of B. vulgaris from Akmolinskaya province. Chemical composition of the flower and leaf volatiles was found to show some differences. Isopropyl isothiocyanate (36.7%) was the main constituent in flower, while phytol (25.7%), hexadecanoic acid (9.3%), hexahydrofarnesyl acetone (7.4%), dodecanic acid (5.5%) and isopropyl isothiocyanate (5.3%) were detected in the leaf. It should be noted that another sample of B. vulgaris from Akmolinskaya province was found to be very different in volatile composition. Namely, borneol (20.3%), camphene (13.5%), bornyl acetate (8.0%) and germacrene D (5.4%) were the major constituents. In the flower and leaf of B. vulgaris from Karagandskaya province, methylthyl cyanide (50.5% and 12.9%), 3-butenyl isothiocyanate (15.6% and 43.8%) and isothexyl cyanide (4.2% and 0.9%) were the major constituents.
volatile compounds. To the best of our knowledge, the volatiles of B. vulgaris from Kazakhstan were not investigated previously.

### Essential oil composition of different populations of Thymus caramanicus

Jalas growing wild in Iran Bigdelo M, Nazari V, Aminian F, Younesian M and Ravanpay K. A Faculty of Agriculture and Natural Resources, University of Tehran, Karaj, Iran; 2Department of Agriculture, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran; 3Department of Research and Development (R&D), Khorraman Pharmaceutical Co., Khoramabad Industrial City, 68135 – 579, Khoramabad, Iran

Thymus species are well known as medicinal plants because of their biological and pharmacological properties. Thymus caramanicus is an endemic species of Iran. Essential oils of the air-dried aerial parts of seven populations of Thymus caramanicus collected from different locations of Iran were isolated by hydrodistillation with a yield of 0.41 – 2.9% (w/w). The essential oils were analyzed by combination of GC and GC-MS techniques. Oxygenated monoterpenes were the main group of constituents in all samples, carvacrol (24.9 – 97.6%), Thymol (25.6 – 36.9%), p-cymene (2.9 – 16.1%) and γ-terpinene (1.3 – 8.1%), represented as the major compounds.

### Antioxidant activity of the essential oils of five species of the family Lamiaceae

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Species in the family Lamiaceae are praised medicinal and aromatic plants. They are used against various inflammations, stomach problems, as expectorant, as well as spices [1]. The aim of the present study was to evaluate the antioxidant activity of essential oils from wild growing herbs in South and South-East in Serbia: Hyssopus officinalis L., Origanum vulgare L., Satureja kitakulbii Wierzb. ap. Heuff., Nepeta nuda L., and Thymus serpyllum L. The essential oils were isolated by hydrodistillation in a Clevenger-type apparatus. The resulting essential oil was dried over anhydrous sodium sulfate, filtered and stored at +4 °C in a well-filled, airtight container, protected from light, until the analysis. Two antioxidant assays, scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) [2] and FRAP (Fe(II) Reducing Antioxidant Power) assay [3] were used to evaluate antioxidant activities, were used as standard. Essential oil from Thymus serpyllum L. exhibited the highest antioxidant activity (EC50 = 0.69 ± 0.14 µM), while the lowest activity was determined for Hyssopus officinalis L. oil (EC50 = 4750 ± 162 µM). Compared with vitamin C (EC50 = 0.04 ± 0.05 µM) all essential oils were of lower DPPH antioxidant activity. In the FRAP assay, the reducing power decreased in the following order: Thymus serpyllum L > L-ascorbic acid > Origanum vulgare L > Satureja kitakulbii L > Nepeta nuda L > Hyssopus officinalis L. Our results confirm that the traditional use of medicinal and aromatic plants in mitigating oxidative stress is an initiator of many diseases. Acknowledgement: This work was supported by the Ministry of Science and Technological Development, Republic of Serbia projects OI 172047. References: 1. Anon. (2004) PDR for Herbal Medicines, 3rd Ed., Thomson PDR at Montvale, USA. 2. Choi CW et al. (2002) Plant Sci 163 (6): 1161 – 1168. 3. Benzie IF et al. (1996) Anal Biochem 239: 70 – 76.

### Essential oil composition of Drosario assidii

D. assidii Alava, D. flabelifolia Boiss. and D. anethifolia (DC.) Boiss. of which the first two are endemics plants (1,2). There are few reports on analysis of the essential oils from Drosario species. The fresh aerial parts and fruits of Drosario assidii were collected in May and September 2007 from Dehbabekly of Kerman Province respectively and Voucher-Specimens were deposited at the Herbarium of the Research Institute of Forests and Rangelands (TARI) in Tehran, Iran. Hydrodistilled essential oils from the aerial parts and fruits of this plant were analyzed by GC and GC/MS, yielded 0.2% and 1.1% (v/w) respectively. Twenty four 82.2% components of the aerial parts and Thirty five 97.9% compounds of the fruits oils were identified. The main constituents of the oil of aerial parts on HPS-MS column were α-pinene (14.5%), decanal (10.1%), decanolic acid (10.4%), β-myrcene (6.1%), benzyl acetate (4.5%), dodecanal (4.1%), E,E-α-farnesene (3.3%) and limonene (2.57%). The major components of fruits oil on DB-1 column were verbolen + myrtrenal (71.0%), allo-ocimene + δ-3-carene (4.0%), caryophyllene acetate (3.3%), β-caryophyllene (3.0%), trans-pinocarvyl acetate (2.8%) and β-pinene (2.2%). The amounts of monoterpenes of aerial parts and fruits were 29.9% and 87.7% respectively. Fruits oil was rich of oxygenated monoterpenes (78.6%). Sesquiterpenes were minor compounds in the oils of this plant. The oil of the aerial parts comprised 43.8% of non-terpenoid compounds constituting the dominant portion of the oil. References: 1-Cherking KH (1987) Ducrosia, In: Flora Iranica, Umbelliferae. No.162. Eds., K.H. Rechinger and I.C. Hedge, Akademische Druck and Verlaganstalt, Graz, Austria, pp. 471. 2-Mozaffariany V (1996) A Dictionary of Plant Names. Farhang Moaseri Publishers, Tehran.

### Whitenining effect and antioxidant activity of essential oils from Cryptomeria japonica

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The aim of this study was to investigate whitening effect and anti-oxidation effect by determining the tyrosinase inhibition activity, DPPH radical scavenging activity and superoxide dismutase (SOD) like activity of essential oil from Cryptomeria japonica D.Don. Essential oil of C. japonica was extracted by steam distillation of Clevenger-type. Essential oil of C. japonica was divided into crude oil and 6 fractions by column chromatography and thin layer chromatography. Crude oil and 6 fractions of C. japonica essential oil inhibited the oxidation of L-tyrosine and L-di-hydroxyphenylalanine (L-DOPA) by mushroom tyrosinase. In tyrosinase inhibitory activity of essential oils of C. japonica, the activity was effective at the fraction A (L-tyrosine: 86.76%, L-DOPA: 88.45%) and B (L-tyrosine: 87.53%, L-DOPA: 85.03%). In examination of anti-oxidation activity, the C. japonica essential oils were determined using the free radical and the stable reductant, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and pyrogallol. Fraction B (44.11%), C (86.91%) and D (44.40%) were highly effective of DPPH radical scavenging, and in examination of SOD like activity, fraction B (96.19%) was appeared as extremely high effective. Fraction B of C. japonica essential oil, effective fraction of whitening and anti-oxidation activity, was mainly consisted of bornyl acetate and nezukol, which are terpenoids having hydroxyl group. These compounds were inhibition of acting on tyrosinase in melanin biosynthesis and block up DPPH radical scavenging and anti-oxidation by supplying hydrogen. Thus, they can apply for cosmeceutical material as functional raw material containing highly active compounds for whitening and anti-oxidation. References: 1. Blois MS (1958) Nature 181: 1199 – 1200. 2. Brand-Williams et al. (1995) LWT-Food Sci Technol 28(1): 25 – 30. 3. Kuliscic T et al. (2004) Food Chem 85(4): 633 – 640. 4. Marklund S et al. (1974) European J Biochem 47(3): 469 – 474. S. Yagi A et al. (1986) Planta Med 52: 517 – 519.
Salvia is the largest genus of the family Lamiaceae with ca. 900 species distributed around the world. Its centre of origin is considered to be south west and central Asia (1). *Salvia* is represented in Turkey by 97 species including 4 subspecies and 8 varieties. The rate of endemism in Turkey is 52.5% with 51 species (2). The genus *Salvia* fruticosa Mill. has a wide distribution in Turkey and its dried leaves are sold in local markets for consumption as herbal tea and dried leaves are exported especially to European Countries. We have distilled essential oils from samples collected from 20 localities by water distillation and analyzed them by GC and GC/MS techniques. Oil yields in the samples varied between 2.0% to 3.0% and the main components were characterized as 1,8-cineole (20.7% to 46.9%), β-caryophyllene (6.0% to 16.9%) β-pinene (5.3% to 11.3), and camphor (2.8% to 17.5%). References: 1. Hedge I C (1992) A Global Survey. World Med 7 – 17. 2. Ipek A, Gurbuz B (2010) Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi, 19 (1 – 2): 30 – 35.

**Variation in the essential oil composition of the fruits of *Vitex agnus-castus***

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*Vitex agnus-castus* L. (Lamiaceae) is widely distributed in Turkey, mainly in coastal areas of the West and the South West. The fruits are used in folk medicine to treat illnesses and the essential oil obtained from the fruits is known as a substitute for kkeik oil in Turkey (1). Essential oil compositions of the fruits of *Vitex agnus-castus* collected from 5 different regions, Balıkesir (Altınoluk) 1, Muğla (Bodrum) 2, Antalya (Manavgat) 3, Edirne (Enez) 4 and Zonguldak (Devrek) 5 were analyzed by GC and GC/MS to document the variability in their composition. The results indicate chemovariability in oils of the fruits sourced from different sites. There are also marked differences in contents of the major constituents. Sample 2 and 3 contains 1,8-cineole [17% (1), 13.2% (2)], sabine [12.8% (2), 12.1% (3)], β-caryophyllene [12.7% (2), 11.4% (3)] and bicyclogermacrene [11.0% (2), 12.1% (3)] as major constituents respectively. Sabine (15.4%), 1,8-cineole (17.3%) and bicyclogermacrene (12.1%) are the major constituents together with (Z)-b-farnesene (13.5%) in the sample 1. The highest content of bicyclogermacrene (22.1%) is shown in sample 4. Sample 5 is the only one which contains α-pinene (10%) as the main constituent. References: Reference: 1) Baytop T (1999) Türkiye’de Bitkiler ile tedavi (Therapy with plants in Turkey), 2nd ed. Nobel Tıp Kitabevleri Ltd. Istanbul

**Compositions of the essential oils of Centaurea aphrodisaea, *C. polycyma, C. ahyolopelis* and *C. iberica***

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**Phoenix dactylifera L. essential oil: Chemical composition, antimicrobial and insecticidal activities***


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*Phoenix dactylifera* L. (Arecaceae), is very common in the Arabian Peninsula. The essential oil of *P. dactylifera* from the spathe was obtained by hydrodistillation. The oil was subsequently analysed by GC and GC/MS, simultaneously. Overall, 16 components were characterized representing 99% of the oil. 3,4-Dimethoxytoluene (73.5%) and 2,4-dimethoxytoluene (9.5%) were found as the main components characterized representing 99% of the oil. 3,4-Dimethoxytoluene (73.5%) and 2,4-dimethoxytoluene (9.5%) were found as the main components. First the antimicrobial activity of the essential oil was determined using the broth microdilution method against various human pathogens, where a low inhibitory range was observed (MIC 1000 μg/ml). The oil was evaluated further for antifungal activity against the strawberry anthracnose-causing fungal plant pathogens *Colletotrichum acutatum* and *Gloeosporioides* using the direct overlay bioautography assay. As a result the essential oil showed no antifungal activity at 80 and 160 μg/spot concentrations compared to standard antifungal agents. In addition, the oil was subsequently investigated for its insecticidal properties against *Aedes aegypti*. The oil showed repellent activity against the yellow fever mosquito *A. aegypti* using the “cloth patch assay” with 0.051 mg/cm², however, the oil exhibited weak activity against the mosquito’s first instar larvae in a high throughput bioassay and adult topical assay. As a conclusion, the Phoenix essential oil and other fractions may have potential against parasites for further evaluation.
Structural and developmental studies on secretory epidermis in *Rosa damascena* Mill. petals

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*Rosa damascena* Mill. is one of the most important *Rosa* species, for its wide application in perfumery and cosmetics, and as a valuable natural drug possessing diverse effects (1). Therapeutic activities of *R.damascena* oil and water have been reported both in traditional Iranian medicine and modern pharmacological studies (2). The essential oil is secreted by epidermal cells in petals of *Rosa* species, but no histological data is available on the mode of secretion in this genus. The present research has focused on the secretory structure in *R.damascena* petals in relation to flower development. Flowers were collected at four successive ontogenic stages from Shiraz, south western Iran. Petals were fixed with glutaraldehyde and osmium tetroxide, dehydrated in acetone and embedded in resin. Semithin sections were stained with toluidine blue. The two petal surfaces were distinguishable from the stage with the emerging intensely colored petals. They differed by the shape and content of the epidermal cells. The upper epidermal cells revealed a very dense cytoplasm, and numerous small vacuoles in half open flowers. Their polyphenolic content diminished during flower development. At full bloom, the vacuolar polyphenolic compounds had totally disappeared and only a few cells had a dense cytoplasm. Structural features of the epidermal cells suggest the petals of the half open flowers to be in the most active secretory phase. Further analytical studies will correlate these histological data with the essential oil secretion process during *R.damascena* flower development. References: 1. Libster (2002) Delmar’s Integrative Herb Guide for Nurses. Delmar’s Thomson Learning. Albany. 2. Rakishandeh et al. (2007) Iranian Journal of Pharmaceutical Research 6: 193 – 197.

Quality profile of *Chios mastic* (masthi) essential oil

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Mastic oil is the essential oil of mastic (masthi), the resin of *Pistacia lentiscus* L. var. chia Duham (Anacardiaceae), uniquely produced in the Greek island of Chios. It is a valuable product with a small annual yield (~300 kg), as well as mastic itself. Its characteristic odor and its established beneficial activities [1,2,3,4] have created an increased demand in cosmetics industry, leading to a high purchasing price (~$2,500/kg), and an adulteration danger. Therefore, the determination of mastic oil quality profile, considering the factors that potentially contribute to it (resin origin, storage time), could be used to afford an unambiguously labeled product. For this purpose, several mastic oil samples of different local origin, as well as different resin and oil storage time were analyzed with chiral GC-MS in order to set content limits for its compounds. Locality has little influence in the proportions, while mastic storage time before distillation clearly affects them, as the differences in the major compounds’ contents show (α-pinene increases from 69.7% for samples distilled 2 months after harvest to 78.9% after 9 months, and myrcene decreases from 19.5% to 11.1%, respectively). The effect of mastic oil storage time was smaller (differences of < 3% within 1 year), but the analysis of a 40-year-old sample revealed a dramatic drop in myrcene content, appointing it as the most characteristic criterion of mastic oil aging. Content limits for all mastic oil compounds were determined, within which a mastic oil sample should lay in order to be characterized as authentic and non-aged. References: 1. Magiatis P et al. (1999) Planta Med 65: 749 – 751. 2. Paraschos S et al. (2007) Antimicrob Agents Chemother 51: 551 – 559. 3. Heo C et al. (2006) Korean J Med 71: 354 – 361. 4. Louttrari H et al. (2006) Nutr Cancer 55: 86 – 93.

Trichomes in *Echium amoenum* Fisch & Mey petals: A micromorphological survey

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*Echium amoenum* Fisch & Mey (Boraginaceae) grows widely in the northern highlands of Iran. Its perennial E. amoenum flowers have long been used for their anxiolytic, sedative, anti-inflammatory and analgesic effects in Iranian folk medicine (1). Phytochemical studies revealed a variety of substances which rosmarinic acid and flavonoids showed antioxidant activity in humans (2). Inhibition of humoral antibody synthesis has also been reported (3). All the published studies on *E. amoenum* have concentrated on the therapeutic uses and/or the phytochemical analyses. No botanical data is available and the secretory structure has not been reported so far. This study was carried out to provide elements on the morphology and localization of the secretory structure, with regard to floral development. *E. amoenum* flowers were collected from Ghazvin, at four developmental stages. Petals were double fixed in glutaraldehyde and osmium tetroxide, dehydrated in acetone, air dried in hexamethyldisilazane, coated with gold and viewed under the scanning electron microscope. The youngest floral buds were densely covered with protective non glandular trichomes of different length. The same trichomes covered the outer epidermis of the young petals emerging from the sepal. Short stalked capitate trichomes, with one globose secretory head were observed between the protective hairs. During development, the number of non glandular trichomes decreased, but capitate trichomes increased in number, reaching their maximum in 3.5 cm long petals at full bloom. Further histochemical studies will elucidate the variable nature of the secreted material, as well as the phases of the secretion process. References: 1. Zargari A (1996) Medicinal Plants, vol. 3. Tehran University Publications. 2. Ranobar A et al. (2006) eCAM 3(4): 469 – 473. 3. Amirghofran Z et al. (2000) Iranian Journal of Medical Sciences 2(3 & 4): 119 – 124.

References:


**PE45**

Composition and antioxidant activities of the essential oil of *Murraya paniculata* leaves growing wild in Cubans mountain

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*Murraya paniculata* (Linn.) Jack, syn. *M. exotica* Linn, known as orange jessamine, belongs to the family Rutaceae and is commonly grown in gardens is commonly grown in gardens for its glossy green foliage and large clusters of fragrant flowers (1). This plant has been used in ethnomedicine. Infusion of the leaves and flowers of *M. exotica* is tonic and stomachic. It is said to be aromatic, refrigerant, digestive, and beneficial in rheumatic fever, coughs, giddiness, hysteria, thirst, and burning of the skin (2,3). The essential oil was obtained by hydrodistillation, were analyzed by gas chromatography-mass spectrometry (GC-MS). The antioxidant activity was evaluated using several in vitro studies. The results showed that the essential oils tested differed in their chemical compositions although there is coincidence in the most abundant constituents. The analysis of *Murraya paniculata* volatile oil showed the presence of compounds identified, accounting for 95.1% of the total amount. The major compound of both oils was found the Caryophyllene (30%), with the other compounds in lesser amounts. The antioxidant activity has shown good activity for the inhibition of primary and secondary oxidation products in crude *Cucurbita* oil added at the concentration of 0.02% which were evaluated using peroxide, thiorbituric acid, p-anisidine values. Moreover, it was further supported by complementary antioxidant assay in linoleic acid system, comparable with synthetic and natural origin antioxidants (butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), carvacrol and timol). References: 1. Roig JT (1974) Plantas medicinales, aromáticas o venenosas de Cuba. Ed. Científico-Técnica. La Habana. 2. Jiangsu New Medical College (1977) Dictionary of Chinese Herbal Medicine; Shanghai Science & Technology Press: Shanghai, China. 3. Pery LM (1980):Medicinal Plants of East and Southeast Asia: Attributed Properties and Uses; MIT Press: Cambridge.

**PE46**

Chemical composition of essential oil collected in Paty do Alferes (Brazil)

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Brazil presents the highest floristic genetic diversity of the world. Among the native plants of Brazil is *Ocimum seliro Benth.,* an herbaceous annual plant of Lamiaceae family. This medicinal specie has been used as anti-diarrheic, antispasmodic and anti-inflammatory and these properties have been observed in pre-clinical tests. Leaves of *Ocimum seliro* were collected in a private property in Paty do Alferes district (Rio de Janeiro State) in 11/2010, and dried at room temperature (28 °C + – 2°) at shade conditions. Essential oil was obtained by hydrodistillation (Clavenger-type apparatus) for 4h and analyzed by GC-MS (Shimadzu, QP 5050-DB-5 capillary column – 30 m x 0.25 mm x 0.25 μm). Carrier gas was Helium (1.7ml/min); split ratio: 1:30. Temperature program: 50 °C, rising to 180 °C at 5 °C/min, 180 °C, rising to 260 °C at 10 °C/min. Injector temperature: 240 °C and detector temperature: 230 °C. Identifications of chemical compounds were made by matching their mass spectra and Kovat’s indices (IK) values with known compounds reported in the literature. In the essential oil were found 16 chemical compounds. The major compound characterized was methyl-chavicol (35.3%), followed by trans-caryophyllene (1.8%), germacrene-D (2.9%), bicyclogermacrene (3.3%), germacrene B (0.5%) and spathulenol (0.6%). Anethole was not observed in this essential oil. This results showed that this quercetin is similar to the one observed by Martins [1], founded in Viçosa- Minas Gerais State (Brazil) References: [1] Martini ER (1998) in Ming et al. Plantas Medicinais, Aromáticas e condimentares: avanços na pesquisa agronômica. UNESP. Botucatu. p. 97 – 126.

**PE47**

Antimicrobial activity of the essential oils and non-polar extracts from leaves and flowers of *Tithonia diversifolia* against cariogenic bacteria

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Bacteria of the Streptococcus and Lactobacillus genera, such as *S. salivarius, S. mutans, S. mitis, S. sobrinus, S. sanguinis, S. salivarius* and *L. casei* are the main microorganisms responsible for human dental caries. As part of our ongoing project on the prospection of natural products with anti-cariogenic potential, we have investigated in this work the antibacterial activity of *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae), which is used in the Brazilian medicine folk as anti-inflammatory [1]. The minimum inhibition concentration (MIC) values of the essential oils of flowers (EOF) and leaves (EOI), and their respective n-hexane extracts were determined by using the broth microdilution method. The n-hexane extracts of leaves and flowers, and the EOI were inactive against the panel of selected bacteria, having displayed MIC values from 1000 to 2000 μg/mL, whereas the EOF exhibited moderate activity (MIC = 250 μg/mL) against *S. mitis, S. sanguinis, S. sobrinus* and *L. casei.* The lowest MIC value was obtained for the EOI against *S. mutans* (MIC = 125 μg/mL). β-caryophyllene (0.04%) was found to be the major constituent in the EOI, however it has been reported to exhibit weak activity (MIC = 500 μg/mL). Further studies to verify the occurrence of possible synergistic effects between β-caryophyllene and other minor constituents in the EOI are in progress. Acknowledgement: FAPESP (Proc. 2000/09491 – 1); CAPES, CNPq. References: [1] Owosile VB et al. (2004) Ethnopharmacol, 90: 317 – 321.

**PE48**

Antibacterial effects of the essential oil from of *Rosmarinus officinalis* against some pathogenic antibiotic-resistant *Staphylococcus aureus* strains

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*Rosmarinus officinalis* L. (Labiatae) is an evergreen shrub native in Mediterranean region which has been used as condiments mainly with meat dishes. It has been cultivated in many herbal gardens also in Korea and used for herbal remedies and etc. To develop the new natural antibiotics from the plant resources against antibiotic-resistant *Staphylococcus aureus,* the antibacterial activities of the essential oil compounds compared to current antimicrobial drugs. As the results of the experiments, the *Rosmarinus oil* showed significant inhibiting activities against most of the tested strains with minimum inhibiting concentrations (MICs) between 0.5 – 16.0 mg/ml. Potential synergism was identified when antibiotics were combined with the oil. The activity of erythromycin, norfloxacine, or oxacilin, against antibiotic-susceptible and also against antibiotic-resistant strains of *S. aureus* was enhanced significantly by combination with *Rosmarinus* oil and its main component, 1,8-cineole with fractional inhibition concentration indices (FICIs) between 0.26 and 1.00. In conclusion, the combination of *R. officinalis* essential oil or 1,8-cineole with antibiotics could be used to reduce the effective dosage of antibiotics and to modulate the resistance of *S. aureus* strains. Acknowledgement: This work was supported by National Research Foundation of Korea Grant funded by the Korean Government (2010 – 07 – 1-M020 – 0006). References: 1) Shin S (2005) Kor J Pharma 36: 252 – 256 2) Shin S (2010) Yakhu Hweji 54: 122 – 125.
Chemical composition and antibacterial activity of the essential oil of *Achillea filipendulina* (Asteraceae)

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There are 19 *Achillea* species available in the Iranian flora and 7 of them are endemic. Various part of different species of the genus *Achillea* are widely used in folk medicine due to numerous pharmacological properties, such as antimicrobial, antiinflammatory, antiallergic and antioxidative activities. The antimicrobial activities of the essential oils and various extracts from several *Achillea* species have been reported before. As far as our literature survey confirms, the antimicrobial activities of leaves and flowers essential oil of *Achillea filipendulina* Lam. (Asteraceae) have not been studied previously. The chemical composition and antibacterial activity of the essential oils of the leaves and flowers of *Achillea filipendulina* were investigated. Essential oils were isolated by hydrodistillation and analyzed by GC and GC-MS. Overall, 95.3% and 92% of the constituents were characterized for the leaf and flower oils. The main components of the leaves and flowers were: 18-cineole (172 – 190%), chrysanthenyl acetate (18.5 – 19.3%), respectively. The antibacterial activity of the essential oils against seven gram positive and gram negative bacteria was investigated and high antibacterial activity was observed. The highest activity was observed against *Staphylococcus aureus* (ATCC 6538), *Salmonella typhimurium* (CCM 5445), *Escherichia coli* (ATCC 25922), *Candida albicans* (ATCC 10231), *Candida parapsilosis* (ATCC 22219), *Candida glabrata* (ATCC 29903), and *Candida tropicalis* (ATCC 22013). The highest inhibition zone was determined against *Staphylococcus aureus* (ATCC 6538) and *Salmonella typhimurium* (CCM 5445). The essential oil exhibited no repellent activity at the highest concentration tested, 0.375 mg/cm². Our research into exploring the repellent properties of the essential oil may be useful for the production of new natural repellents for personal protection and control of mosquito bites, and ultimately to reduce the incidence of mosquito-borne illnesses.

Antimicrobial Activities in Cultivated *Origanum vulgare* subsp. *hirtum* Populations of Different Origin

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*Origanum* L. genus belongs to Lamiaceae (Labiatae) family. It has anti-microbial activities on high rates. Especially *Origanum vulgare* L. has very efficient antimicrobial activities because of the high essential oil content. The main components of the essential oil are carvacrol, thymol, γ-terpinene and p-cymene. This study was conducted in order to determine antimicrobial activities in cultivated *Origanum vulgare* subsp. *hirtum* populations of different origin, and to correlate harvest times with antimicrobial activity. Species of bacteria tested were *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Streptococcus faecalis* ATCC 29212, *Bacillus cereus* CCM 99, *Salmoneella typhimurium* CCM 5445, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* and *Salmonella typhimurium* CCM 5445. Species of fungi tested were *Candida albicans* ATCC 10231, *C. tropicalis* RSRK 665, *C. krusei* ATCC 6258, *C. parapsilosis* ATCC 2219, *C. dublinensis* CD 36, *Aspergillus fumigatus* NRRL 2990, *CLSI* (The Clinical Laboratory Standards Institute) broth microdilution method was used for the determination of MIC (Minimum Inhibition concentration). MIC was determined according to the CLSI M27-A2 for Candida species, CLSI M38-A microdilution for Aspergillus fumigatus and CLSI M2-A7 microdilution for bacteria.

Effect of potassium nutrition on essential oil of *Calendula officinalis* L. flowers

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Effects of potassium (K) by rates of 0.00 (control), 115.5, 220.0, 288.7 and 346.4 kg ha⁻¹ on essential oil (EO) extracted from marigold (*Calendula officinalis* L.) flower heads were investigated by GC and GC/MS. The highest accumulation of EO (0.29±0.0095 g plant⁻¹) was recorded at the treatment of 173.2 kg ha⁻¹ compared with control treatment (0.13±0.015 g plant⁻¹). 28 constituents were identified in the EO. The main constituents (α-cadinol, α- and γ-cadinene) increased with K level increased. The highest amounts of main constituents (α-cadinol (33.11%), α-cadinene (18.41%), and γ-cadinene (9.99%) produced from the 346.4 kg ha⁻¹ treatment compared with other control treatments. Alcohols are the major constituents of the heavy oxygenated compounds (HOC) of *Calendula* EO. α-Cadinol represents the highest concentration among the alcohols. It indicates that *Calendula* EO grown under K belongs to the α-cadinol chemotype.

Composition of Artemisia abrotanum and *A. pontica* Essential Oils and Their Repellent Activity against *Aedes aegypti*


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Mosquito-borne diseases such as malaria, encephalitis, and yellow, Dengue, and Rift Valley fevers are diseases that result in significant morbidity and mortality in humans and livestock globally. Currently, the development of natural product-based insecticides and repellents are under exploration to increase and improve our ability to protect humans from mosquito bites, and ultimately to reduce the incidence of mosquito-borne illnesses [1]. We have undertaken a collaborative research project to discover new natural compounds for personal protection and control of mosquitoes. *Artemisia abrotanum* L. leaves have reportedly been used as a moth and insect repellent. Therefore, we evaluated *Artemisia abrotanum* and *A. pontica* L. essential oils for mosquito repellent activity against *Aedes aegypti*. *Artemisia* oils obtained by hydrodistillation of aerial parts were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The main *Artemisia* oil constituents were as follows: A. abrotanum: 32.6% l- cineole, 13.5% borneol, 10.2% 3,4-pentadienol-9-ox and 8.0% p-cymene; A. pontica: 35.6% artemisia ketone, 30.1% a-thujone, 22.3% l- cineole and 3.7% a-thujone. *Artemisia abrotanum* oil showed repellent activity down to a minimum effective dosage of 0.219 mg/cm² (±0.143) using cloth patch assay. Whereas A. pontica oil exhibited no repellent activity at the highest concentration tested, 0.375 mg/cm². Our research into exploring the repellency of specific compounds in the *A. abrotanum* oil will continue and be expanded to include other mosquito vectors and pesticide resistant mosquito strains. Acknowledgement: This study was supported by a grant from the Deployed War-Fighter Protection (DWP) Research Program and the U.S. Department of Defense through the Armed Forces Pest Management Board (AFHPMB), and by a grant from the Mississippi Agricultural and Forestry Experiment Station. References: 1. Hoel D et al. (2010) Wingbeats 21(1): 19 – 34.
Fresh flowers of Rosa damascena Miller var. trigintipetala Dieck cultivated in Taif, Saudi Arabia are the source of Taif Rose Oil. The oils were sourced from two dealers in Riyadh, Saudi Arabia in 2011. They were analysed by GC and GC/MS. Both oils gave a similar profile with quantitative differences. The main components characterized were citronellol (23–28%), geraniol (14–20%), nonadecane (11–16%), nerol (6–11%), linalool (8%) and heneicosane (7%) resp.

**Application of vibrational spectroscopy in the quality assessment of Buchu oil obtained from two commercially important Agathosma species (Rutaceae)**

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Agathosma species (Rutaceae) are medicinal shrubs used traditionally to treat renal and chest ailments1. In addition, Buchu oil (from two South African species; Agathosma betulina (P.J. Bergius) Pillans and Agathosma crenulata (L.) Pillans) is an important ingredient in flavour and fragrance formulations2,3. The use of vibrational spectroscopy as possible alternatives to conventional chromatographic techniques for the rapid and inexpensive assessment of ‘buchu oil’ was investigated. Samples of A. betulina (55) and A. crenulata (16) were collected from natural populations and cultivation sites in South Africa. The essential oil was scanned on NIR, MIR and Raman spectrometers. The spectral data was processed using orthogonal partial least squares discriminate analysis (OPLS-DA). Using GC-MS, the vibrational calibration models were developed for the MIR, NIR and Raman spectral data to predict the major compounds in ‘buchu oil’. The results showed that OPLS-DA technique is a useful tool in the differentiation of Agathosma species using a non-targeted approach. Identification of wave regions that contain peaks separating the two species was possible. The PLS calibration model developed using MIR data was the best with R2X = 0.96; R2Y = 0.88 and Q2Ycum = 0.85 for the quantification of six oil constituents. The model showed high predictive power for six-diosphenol (R2 = 0.97), isomethone (R2 = 0.97), menthone (R2 = 0.90), limonene (R2 = 0.91), pulegone (R2 = 0.96) and diosphenol (R2 = 0.85). These results illustrate the potential of NIR spectroscopy as a rapid and inexpensive alternative to predict the major compounds in commercially important buchu oil. Acknowledgement: The financial support of National Research Foundation (SA), Tswane University of Technology and Jagiellonian University is gratefully acknowledged. S. Chicken Naturals (Cape Town) are thanked for logistic arrangements to source plant material. References: 1. Van Wyk B-E, Wink M (2004) Medicinal plants of the world, Briza publications, Pretoria. 2. Simpson D (1998) Scott med j 43:189 – 191 3. Turpie JK, Heydensych BJ, Lamberty SJ (2003) Biol Cons 112: 233 – 251.

**PF1**

Phytochemical properties of *Aspilia africana* leaf

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Aspilia africana (Pers.) C.D.A Adams is used in herbal medicine for the perceived presence of some bioactive components in the leaves. Scientific reports suggest that different crude extracts of the plant contain specific bioactive constituents that could have varied effects on its biological activities. This study evaluated the phytochemical activities in the meal; aqueous, chloroform and ethanolic extracts of *A. africana* leaves. The anti-microbial activity of the four samples of *A. africana* were tested on nine micro-organisms of six bacteria and three fungal strains using the agar well diffusion technique. Results of the phytochemical screening and subsequent quantification revealed the presence of high amount of some bioactive compounds; saponins, tannins, alkaloids, flavonoids, terpenoids and phenols, but the absence of steroids (leaf meal and all extracts), phytobutamin (chloroform and ethanolic extracts) and cardiac glycoside (ethanolic extract) in the *A. africana* leaf products. Though the chloroform leaf extract had higher concentrations (P < 0.05) of these phytochemicals, significant (P < 0.05) improvements were observed in the chemical composition of the aqueous and ethanolic extracts. The anti-microbial activities observed indicated that biological activities were dependent on the types of extractants and the concentrations of principles present such as alkaloids and tannins. These activities were comparable to those obtained for ampicillin and gentamicin. The results of this study suggest that *A. africana* leaf products may contain some pharmacological properties. An ethnobotanical survey of medicinal plants used by traditional healers in Durban, South Africa

**PF2**

A comparative analysis of two medicinal plants used to treat common skin conditions in South Africa

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Infectious dermatological diseases are a common occurrence in southern Africa. Plants showing dermatological properties are highly sought after due to their ability to stop bleeding, speed up wound healing and to soothe skin exposed to burns. An attempt was made to validate the use of *Haworthia limifolia* Marloth and Aloe excelsa A.Bergen against microbial properties from extracts of leaves against five gram positive, four gram negative bacteria and six species of fungi. All gram positive bacteria were inhibited by both the ethyl acetate and acetone extracts for leaves of *H. limifolia*. However, only one gram negative bacteria was inhibited by the same extracts. Ethyl acetate extract of *A. excelsa* was only effective against three gram positive bacterial whilst acetone extract was effective against all bacteria except for *Shigella sonnei* and *Entero bacter aerogenes*. Both ethyl and aqueous extracts of *H. limifolia* and *A. excelsa* showed antifungal activity. *H. limifolia* extracts showed greater antibacterial activity than *A. excelsa* whilst *A. excelsa* showed greater antifungal activity than *H. limifolia*. Use of either species as traditional medicine will therefore depend on the type of infection or condition presented by the patient.

**PF3**

Evaluation of Some Medicinal Plant Extracts against Neuroinflammation Characterizing Alzheimer’s Disease in Experimental Rat Model

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Due to various disturbances in the environment is increasing the proportion of patients with respiratory diseases. A very high proportion are obstructive lung disease in relation to climate changes [1]. Especially children suffer from asthma and bronchitis. In chronic respiratory disease than the classic drugs, often used in various herbal medicines. Many of them used since ancient times in traditional phytotherapy, particularly in high altitudes [2, 3]. In order to find effective herbal means in the prevention and treatment of respiratory diseases are carried out ethnobotanical research in high mountain region of Bosnia and Herzegovina. It has been known that medicinal plants are used in the folk medicine is the basis for the work of traditional healers and herbalists and herb sellers. The use of wild medicinal plants in the traditional therapy of respiratory diseases in high mountain region of W. Balkan is used by traditional healers in Durban, South Africa

**PF4**

The use of wild medicinal plants in the traditional therapy of respiratory diseases in high mountain region of W. Balkan

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The main purpose of the present study is to evaluate the role of *Salvia triloba* L. and *Ruta graveolens* L. extracts in management of neuroinflammatory insults characteristic for Alzheimer’s disease in rat model. Male Sprague Dawley rats were classified into five groups: (1), control group; (2), AD group which was orally administered with aluminum chloride in a dose of 17 mg/kg b. wt. daily for one month; (3), AD group which was treated with rivastigmine in a dose of 0.3 mg/kg b. wt. daily for three months; (4), AD group which was treated with total extract of the aerial part of *Salvia triloba* daily for three months and (5), AD group which was treated with total extract of the aerial part of *Ruta graveolens* daily for three months. Scores ACh and brain AchE activity, Bcl2, NF-xb and CRP were estimated. Histological investigations of brain sections of all studied groups were also carried out. The results showed that administration of ACl3 resulted in significant elevation in AChE, NF-xb and CRP levels accompanied with significant depletion in ACh and Bcl2-
Bioactivity Guided Evaluation of Antinociceptive and Anti-inflammatory Properties of Cnestis ferruginea Vahl ex DC (Connaraceae)

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Cnestis ferruginea Vahl ex DC (Connaraceae) (CF) is a shrub widely used in traditional African Medicine (TAM) for the treatment of various painful and inflammatory conditions. This study sought to isolate, identify and investigate the anti-inflammatory and antinociceptive activity of the active constituents of CF through bioassay guided fractionation. The crude methanolic root extract of CF was sequentially fractionated into four sub extracts (chloroform, ethylacetate, n-butanol and the remaining aqueous fraction). The aqueous fraction having shown significant inhibition of inflammation and pain was subjected to fractionation through successive column chromatography on silica gel 60 – 120 mesh, eluted with a gradient of CHCl3 -MeOH. Sixty five fractions were collected; fractions with similar TLC profile were grouped into seven major fractions (1 – 7). Fraction 4 being most active in bioassay was rechromatographed to yield CF-2 and CF-5. The effect on inflammatory mediators was studied in rat astrocytoma cells (C6), nitrite release in cell lysate were monitored. The results showed that rats treated with GOV dose dependently exhibited significant (p < 0.05) decrease in levels of ALP, ALT, AST, GGT, LDH, cholesterol, creatinine, triglycerides, urea and MDA and substantially significantly (p < 0.05) increased the albumin, total protein, catalase, GPx, GSH, GST and SOD levels when compared to the toxin control rats. The data from this study suggest that the triherbal formulation possess hepatoprotective and nephroprotective potential against D-galactosamine induced hepatic and renal toxicity in rats, thus providing scientific rationale for its use in traditional medicine for the treatment of liver diseases.

PF7

W9, a medicinal plant from the pharmacopeia of the Eastern James Bay Cree, exhibits anti-diabetic activities in two mouse model of diabetes

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Aboriginal populations are particularly at risk for developing type 2 diabetes mellitus and its complications. In Canada, diabetes prevalence for these populations is at least three times higher than that of the general population. W9 has been identified among species used by the Cree of Eeyou Istchii of northern Quebec to treat symptoms of diabetes. In a previous study, the ethanolic extract of W9 enhanced glucose uptake in C2C12 muscle cells via stimulation of AMP-activated protein kinase (AMPK) pathway. In this study, we investigated the in vivo effect of this plant in two mouse models of type 2 diabetes. In the first one, KK/AY mice received W9 extract in drinking water (1% for 10 days). In the second model, C57BL/6 mice were fed a high fat diet (HFD; ~35% lipids) for 8 weeks until they became obese and insulin resistant (diet-induced-obesity; DIO). Treatment then began by adding W9 extract to HFD at 3 different concentration (125, 250 and 500 mg/Kg) for another 8 weeks. In both models, W9 significantly decreased glycemia, strongly tended to decrease insulin levels, and this was accompanied with reduced fluid intake in the KKAy model. This correlated with either a tendency or a frank increase in GLUT4 content and activation of the AMPK and/or Akt pathways in skeletal muscle. W9 treatment also improved hepatic steatosis by decreasing hepatic triglyceride levels and significantly activating the AMPK and Akt pathways. The results of the present study confirm that W9 represents a culturally relevant treatment option for Cree diabetics. Acknowledgement: This work was supported by a Team Grant from the Canadian Institutes of Health Research (CIHR Team in Aboriginal Antidiabetic Medicines) to P.S.H. and I.T.A. and was conducted with the consent and support of the Cree Nation of Mistissini.

PF8

Evaluation of the Hepatoprotective and Antioxidant Activities of an Indigenous Triherbal Formulation from South Eastern Nigeria using Wistar Albino Rats

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A triherbal formulation prepared from a mixture of the leaves of Conronema latifolium Bent., Ocimum gratissimum L. and Vernonia amygdalina Delile (GOV) was evaluated for hepato-nephro protective and antioxidant properties against D-galactosamine-induced hepatic and renal toxicity in Wistar albino rats. The triherbal fraction was subdivided into seven groups of seven animals each. Two control experiments were setup which included normal rats treated with D-galactosamine and normal rats that received only distilled water. Three groups were treated with different doses of GOV extract (2000, 4000 and 8000 mg/kg b.wt) and some standard hepatoprotective drugs such as Liv 52 and silymarin for 13 days prior to intoxication with D-galactosamine. The activities of serum liver enzymes, concentrations of some biochemical analytes, effect on hematological parameters and antioxidant status were monitored. The results showed that rats treated with GOV dose dependently exhibited significant (p < 0.05) decrease in levels of ALP, ALT, AST, GDH, cholesterol, creatinine, triglycerides, urea and MDA and substantially significantly (p < 0.05) increased the albumin, total protein, catalase, GPx, GSH, GST and SOD levels when compared to the toxin control rats. The data from this study suggest that the triherbal formulation possess hepatoprotective and nephroprotective potential against D-galactosamine induced hepatotoxicity in rats, thus providing scientific rationale for its use in traditional medicine for the treatment of liver diseases.

PF9

The most useful herbs of Traditional Iranian Medicine prescribed for Insomnia

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Insomnia is a highly prevalent condition, and due to ongoing demand from patients suffering with this condition, new pharmacological treatments are actively being sought. 1Insomnia is a well-known disorder in Iranian Traditional Medicine (ITM). Scholars of ITM described insomnia in their manuscripts precisely. According to ITM references, Insomnia is an excess and abnormal form of awakening. Various causes such as bilious temperament, pain, Indigestion, melanotic swelling around the brain and fever will result in insomnia. 2Herbal therapy was the major treatment prescribed by Iranian physicians. Six Iranian ancient medical texts i.e. Canon of Medicine (Avicenna 980 – 1037 AD), Alhavi (Razes 865 – 925 AD) Tohfat ul-Mo,menin (Mo,men tonekaboni 17th century), Makhzun ul-Adiva (Aghili 18thcentury), Ekhtitarat Badli, (Ansari 1329 – 1404 AD), and Al-ahnia An-Hahyegh el-adiva (Heravi 11thcentury) were studied for the treatment of insomnia. Subsequent to our study, the herbal medicines were listed and scored based on the frequency of their prescriptibility. Moreover, the effort has been taken to provide the best scientific name for each plant. This study showed that Papaver somniferum L., Crocus sativus L. and Lactuca sativa L. were the most frequent herbs mentioned in ITM prescriptions and in conclusion they can be introduced as new anti insomnia herbal medicines for clinical researches. Keywords: Insomnia, Herbal, Traditional Iranian Medicine References: 1. Richey SM, Krystal AD (2011) Pharmacological Advances...

PF10 Molluscidal Potential of the Fruit Pericarp of Blighia unijugata Baker Against Biomphalaria pfeifferi

Blighia unijugata Baker ( Sapindaceae ) is a small to medium-sized tree up to 30 m tall widespread in tropical Africa. The leaves are eaten as vegetable and various part of the tree are considered to have sedative and analgesic properties and are used in traditional medicine for the treatment of rheumatism, kidney pain and muscular stiffness. The macerated twigs, leaves, flowers and fruit as a fish poison by the coastal people in Nigeria and there is a high correlation between plants employed as fish poison or soap substances and molluscicidal activity. Powdered pericarp was macerated with 50% ethanol, filtered and the filtrate concentrated to dryness under vacuum to yield 10.30 g of the dried extract and out of this 9.80 g was dissolved in water and partitioned between ethyl acetate, butanol and water to give 2.77 g of ethyl acetate, 2.81 of Butanol and 3.35 of water fractions respectively Snails for the experiment were collected from streams that have not been subjected to either synthetic or poison or soap substances and molluscicidal activity. They were allowed to acclimatize in the laboratory for two weeks before use. The methods of Al-Zanbagi et al. (1) and Truiti et al. (2) were modified and used. The crude ethanolic extract has LC50 of 15pm and for the fractions ethyl acetate was the most active with LC50 for two weeks before use. The methods of Al-Zanbagi et al. (1) and Truiti et al. (2) were modified and used. The crude ethanolic extract has LC50 of 15pm and for the fractions ethyl acetate was the most active with LC50 of 7.6pm while butanal fraction had a LC50 of 15 ppm and water fraction was the least active with LC50 of 25pm. Efforts are being made to isolate the active compounds from each fraction. Keywords: Blighia unijugata, fruit pericarp, crude extract, fractions, Biomphalaria pfeifferi


PF11 Antibacterial and anticancer activity of kaurenoic acid from root bark extract of Annona senegalensis

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The antibacterial [1] and anticancer [2] activities of extract of Annona senegalensis Pers. (Annonaceae) have been reported. Bioactive-guided fractionation of the methanol-methylene chloride root bark extract of A. senegalensis afforded a potent antibacterial ethyl acetate fraction (EF) which on further fractionation, gave two active sub-fractions F1 and F2. F1 yielded a lipophilic liquid component while F2 on purification, precipitated a white crystalline compound, ASI, that was characterized by proton NMR and X-ray crystallography. F1 was analyzed using GC-MS to obtain 6 major constituents: 1-dodecanol, 1-alk-1-en-18-1-oic acid, 1-Naphthalenemethanol, 6,6-dimethyl-bicyclo[3.1.1]hept-2-en-2-ethanol, 3,3-dimethyl-2-(3-methylbuta-1,3-dienyl) cyclohexanol-1-methanol and 3-hydroxyandrostan-17-carboxylic acid. Agar well diffusion method, using a 0.5 McFarland standard, was employed to obtain the MIC’s for F1 and ASI. The MIC’s against clinical isolates of Bacillus subtilis in µg/ml of the EF, F1 and ASI were 180, 60, and 30 respectively. However, ASI exhibited appreciable activity against Staphylococcus aureus with an MIC value of 150µg/ml while F1 was active against Pseudomonas aeruginosa with an MIC value of 40µg/ml. The standard agent ciprofloxacin exhibited MIC values of 0.28, 1.18 and 3.6µg/ml against S. aureus, Ps. aeruginosa and Type II capitate trichomes with a single basal and stalk cell and an oviod head cell. Histochemical and phytochemical studies showed that essential oils of a terpenoid nature were present in the head cells of glandular trichomes. Flavonoids, triterpenoids, tannins, saponins, fixed oils and fats, phenolics and cardiac glycosides were also detected in a crude ethanolic extract of the leaves using phytochemical test methods. Acknowledgement: The National Research Foundation (South Africa) is gratefully acknowledged for the funding of this research.


PF12 Microscopic and histochemical characterization of leaves of the medicinal plant Ocimum obovatum

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Ocimum obovatum E. Mey. subsp. obovatum var. obovatum has been valued for its hair restorative properties for decades on the African continent. The member of the Lamiaceae is also traditionally prescribed as a remedy for infantile abdominal cramps and a hot water extract of the leaves is used to treat epigastric conditions in children. Commonly known as ‘cat’s whiskers’, the aromatic plant can be seen growing along the KwaZulu-Natal coastline and the Western Cape of southern Africa. Ocimum obovatum is also common in Zimbabwe and Swaziland as well as northern and west Africa [1]. The medicinal properties of the plant are attributed to the essential oils supposedly produced and secreted by appendages on the foliar surfaces referred to as trichomes [2]. Traditional light and electron microscopy studies revealed the presence of two types of glandular trichomes and one type of non-glandular trichome across all stages of leaf development. The glandular trichomes were classified as large, four-celled peltate trichomes and smaller capitate trichomes. The latter were further classified into two subtypes; Type I capitate trichomes with a single basal cell and two head cells and Type II capitate trichomes with a single basal and stalk cell and an oviod head cell. Histochemical and phytochemical studies showed that essential oils of a terpenoid nature were present in the head cells of glandular trichomes. Flavonoids, triterpenoids, tannins, saponins, fixed oils and fats, phenolics and cardiac glycosides were also detected in a crude ethanolic extract of the leaves using phytochemical test methods. Acknowledgement: The National Research Foundation (South Africa) is gratefully acknowledged for the funding of this research.


PF13 Effect of drying methods on the antioxidant activity of Anacardium occidentale L. (Cashew)

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Studies on the drying characteristics of Cashew leaves are scarce in the literature; particularly the traditional sun drying properties as well as oven drying properties of plants are not adequately investigated. The aim of the work was to determine the sun, oven, shade dried and fresh leaves drying characteristics of Cashew and to compare the effect of the same on the antioxidant property of the extracts. The extracts of leaves exposed to various drying conditions were prepared using various solvents. The phytochemical tests were carried out for various constituents of the extracts in order to ascertain the presence/absence of various phytoconstituents. The effect of drying conditions on the antioxidant activity of the extracts, the extracts were evaluated for their antioxidant effect by DPPH. Assay, Greiss assay and determination of total phenol content.
The antihyperglycaemic and antihyperlipidaemic effects of *Raphia hookeri* root extract on alloxan induced diabetic rats

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The antihyperglycaemic and antihyperlipidaemic effects of *Raphia hookeri* G.Mann & H.Wend. (RH) root extract used for diabetic treatment were evaluated against alloxan diabetic rats. Adult rats weighing 150 ± 10 g were fasted for 18 hrs and induced with diabetes for three days using alloxan monohydrate (150 mg/kg body weight). Animals with blood glucose level > 250 mg/dl were used. Diabetic rats were divided into four groups and treated as follows: groups 1 – 3 received graded doses of RH (50, 100 and 200 mg/kg) by gavage; group 4 - glibenclamide (10 mg/kg); groups 5 and 6 as normal and diabetic controls. Each comprised of 5 rats. Blood was collected at days, 0, 3, 5, 7, 9, 11, 13 and 15 and analyzed for glucose by oxidase method; Lipid profile, by modified enzymatic procedure; Insulin assay, using Diagnostic Automation Kit 2 and glycosylated haemoglobin (HbA1c).

PF15

Ethnobotanical survey and antifungal activity of plants identified for the management of opportunistic fungal infections in HIV/AIDS patients in the Amathole District of the Eastern Cape Province, South Africa

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In a study to document plants used to treat opportunistic fungal infections (OFIs) seen in HIV/AIDS patients in the Eastern Cape, South Africa, ethnobotanical information was obtained through questionnaires and conversations with 22 traditional healers and 101 HIV/AIDS patients. Thirty two plant species, belonging to 26 families, were identified as being used for this purpose. Two frequently used plants, *Artncis arctooides* (Katoe) and *Morus alba* (L. leaf in normal and diabetic rats. Diabetes was induced by injection of streptozotocin (STZ, 45 mg/kg) intraperitoneally. All diabetic rats were divided into 5 groups, each of which was orally received one of the following; vehicle, glibenclamide and leaf extract (150, 300 and 600 mg/kg) for 12 days. The results showed that the leaf extract at the doses of 300 and 600 mg/kg significantly (p < 0.05) reduced blood glucose levels in diabetic rats. Moreover, the study of *Morus alba* leaf extract in decreasing of acute hyperglycemic effect was undertaken by oral glucose tolerance test (OGTT) which revealed that the leaf extract could not reduce blood glucose levels in acute hyperglycemia in both diabetic and normal rats. The histological examination of pancreas

The objectives of this study were to evaluate the hypoglycemic effects and histological changes of pancreatic islets cells in diabetic and normal rats

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The study of hypoglycemic effects of the *Morus alba* L. leaf extract and histology of the pancreatic islets cells in diabetic and normal rats

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The objectives of this study were to evaluate the hypoglycemic effects and histological changes of pancreatic islets cells after receiving water extract of *Morus alba* L. leaf in normal and diabetic rats. Diabetes was induced by injection of streptozotocin (STZ, 45 mg/kg) intraperitoneally. All diabetic rats were divided into 5 groups, each of which was orally received one of the following: vehicle, glibenclamide and leaf extract (150, 300 and 600 mg/kg) for 12 days. The results showed that the leaf extract at the doses of 300 and 600 mg/kg significantly (p < 0.05) reduced blood glucose levels in diabetic rats. Moreover, the study of *Morus alba* leaf extract in decreasing of acute hyperglycemic effect was undertaken by oral glucose tolerance test (OGTT) which revealed that the leaf extract could not reduce blood glucose levels in acute hyperglycemia in both diabetic and normal rats. The histological examination of pancreas...
showed that all doses of leaf extract could recover the damaged islet cells in diabetic rats in a dose dependent manner. The pancreatic islets of diabetic rats receiving the extract were larger and the cells within the islets were rounder and less congestive when compared to diabetic control rats. Conclusion, the results of this study suggested that Morus alba leaf extract could reduce blood glucose levels and improve the histological features of pancreatic islets in diabetic rats. Acknowledgement: This research was financially supported by Faculty of Medicine, Thammasat University Research Fund.

The present study was conducted to evaluate the hepatoprotective effect of the Anethum graveolens L. extract on paracetamol-induced hepatic damage in rats. Thirty rats were divided into 5 groups of 6 rats. Group I served as control that received 5% tween 80. Group II and III were pre-treated with 5% tween 80, for 7 days before 3 g/kg BW of paracetamol was administered on day 8 whereas group IV received N-Acetyl Cysteine (NAC) at 2 hours after paracetamol administered. Groups IV and V were pre-treated with 1 g/kg and 500 mg of the plant extract respectively for 7 days following by 3 g/kg BW of paracetamol on day 8. The extract was examined for antioxidant effects by DPPH radical scavenging activity while the biochemical parameters such as serum Aspartate transaminase (AST), Alanine transaminase (ALT) and histopathological examination were determined at the end of the experimental period. The result showed that the ethanolic extract has the antioxidant activity with the EC50 value by DPPH radical scavenging activity is 97.66±5.65 µg/ml. Moreover, the ethanolic extract has a hepatoprotective action by decreasing the AST and ALT levels in Group IV and V. Moreover, hepatotoxicity was observed in rats treated with paracetamol alone whereas these were reduced in the pre-treatment with ethanolic extract and NAC treatment rats. In conclusion, the ethanolic extract of Anethum graveolens L. has an antioxidant activity and exhibits hepatoprotective effect against paracetamol-induced hepatic damage in rats. Acknowledgement: This research was financially supported by Faculty of Medicine, Thammasat University Research Fund.

The effects of ethanol extract of Raphia hookeri seed on exogenous testosterone and estradiol induced benign prostatic hyperplasia in adult male rats

Mbak AM1, Okparaebo Connective Tissue, Nigeria

The activity of Raphia hookeri G.Mann & H.Wendt. (RH) seed extract used locally in the treatment of benign prostatic hyperplasia (BPH) was investigated on exogenous induced prostatic enlargement. Adult male rats weighing 200 ± 10g/kg were induced with BPH by exogenous administration of testosterone and estradiol in staggered doses (three times a week) for three weeks (1). The induced animals were divided into five groups. Group 1 and 2 received the extract at 50 and 100 mg/kg body weight by gavages for forty five days; group 3- fenantisteride (0.1 mg/kg); group 4- was left untreated for forty five days; group 5- (negative control) was sacrificed immediately after induction. Group 6- received the extract (100 mg/kg) and the steroid hormones simultaneously while group -7 was normal control. Prostate specific antigen (PSA) and testosterone levels were determined from blood serum. The oxidative activity, Catalase (CAT), Superoxide dismutase (SOD), Lipid peroxidation and glutathione (GSH) were assayed as described by Rukumii et al.(2). The result showed significant decrease (P < 0.05) in PSA level in RH treated compared to the negative control. There was also decrease in testosterone level in RH treated. The levels of CAT and SOD (Table 1) in RH treated were comparable to normal. However, GSH showed comparably higher level to normal while the extract peroxidative activities decrease slight. Prostatic tissue morphology of the extract treated (Fig. 1) showed extensive shrinkage while hypertrophy of prostate tissue occurred in the untreated (Fig. 2). RH effectively reduced enlarged prostate mass, lowered PSA and testosterone levels and also exhibited anti-oxidative activity. Acknowledgement: Prince Musibau Si- kiru, herbalist, Ogun State. Nigeria assisted with the plant material. References: (1) Bernoulli J (2008) An experimental model of prostatic inflammation for drug discovery. Medica – Odontologica. (2) Rukumii K et al. (2004) J Pharm Sci 72(2): 274 – 283.
pounds from fraction D will be identified and their antiinfective and antiinflammation properties will be investigated.

### Table 1: Antinociceptive activity for petroleum ether fraction of Mitrigina calabura

<table>
<thead>
<tr>
<th>Fraction (mg / kg)</th>
<th>First phase (mg / kg)</th>
<th>Percentage</th>
<th>Second phase (mg / kg)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (300)</td>
<td>79.67 ± 1.585</td>
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<td>145.67 ± 2.246</td>
<td>0.44</td>
</tr>
<tr>
<td>B (300)</td>
<td>74.83 ± 1.922</td>
<td>5.8</td>
<td>137.83 ± 2.056</td>
<td>5.8</td>
</tr>
<tr>
<td>C (300)</td>
<td>57.16 ± 2.056</td>
<td>41.1</td>
<td>86.16 ± 1.831</td>
<td>12.1</td>
</tr>
<tr>
<td>D (300)</td>
<td>27.60 ± 2.418</td>
<td>81.4</td>
<td>27.10 ± 1.778</td>
<td>81.4</td>
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<tr>
<td>E (300)</td>
<td>42.16 ± 3.381</td>
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<td>77.33 ± 1.537</td>
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<td>74.33 ± 1.429</td>
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<td>134.83 ± 2.428</td>
<td>1.7</td>
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</tbody>
</table>

### PF22

**Protective effect of Polygonum odoratum L. on acetaminophen-induced liver injury in rats**

This study aims to investigate the protective effect of the Polygonum odoratum L. on acetaminophen-induced liver injury in rats. 30 male Sprague Dawley rats were divided into 5 groups. Group I, II and III were given 5 g/kg of food and group IV and V were given 500 and 1000 mg/kg body weight/day of the plant extract, respectively for 7 days. On day 8th, animal in group II-V were received acetaminophen (3 g/kg body weight) and group II also received N-Acetyl Cystein (NAC) 400 mg/kg body weight at 2 hours after acetaminophen administrated. All animal were sacrificed on day 9 th and blood samples were collected for the determination of Aspartate transaminase (AST), Alanine transaminase (ALT), Malondialdehyde (MDA) and nitrite formation. AST, ALT, MDA and nitrite levels were significantly higher in rats treated with acetaminophen alone compared to normal control (p < 0.05). Pretreatment of the animal with the extract and administration of NAC significantly reduced oxidative stress and liver injury induced by acetaminophen as shown by reduction of MDA, nitrite levels, AST and ALT levels compared to the rats treated with acetaminophen alone (p < 0.05). In conclusion, the result of this study demonstrated that pretreatment with the ethanolic extract of Polygonum odoratum can ameliorate oxidative stress and liver injury induced by acetaminophen. Acknowledgement: This research was financially supported by Faculty of Medicine, Thammasat University Research Fund.

### PF23

**Ethnobotanical study of medicinal plants used by local Bedwins in the Badia region of Jordan**

An ethnobotanical study was conducted as a part of the local knowledge study which was carried out in 2010. The target participants were the Livestock owners in the arid Jordanian Badia region. The objective of the study is to document the traditional knowledge in using wild plants in treating health problems in order to conserve this valuable knowledge from loss; to identify the key plant species used and to calculate the Informant Consensus Factor (ICF) for the health disorder categories. The data was collected from interviewing 70 participants, 21% of them were females. The interviews were face to face and few focus groups were conducted. A questionnaire that helps in the data gathering was prepared, video recording was taken to show the procedures that the local communities were using in their process. A total of 48 plant species are used by local Bedwins for medicinal purposes, the majority of those are native to the study area, e.g. Artemisia judaica Lour., Citrullus colocynthis (L.) Schrad., Dacrosia anethifolia Boiss., Ecballium elaterium (L.) A.Rich., Paronychia spp and Rheum palmatum Linnaeus. The study showed that the plant species with the highest use value is Artemisia herba-alba. Moreover, the highest value of Informant Consensus Factor (ICF) was scored for Jaundice disease (0.87) followed by Gastrointestinal disorders (0.86) and dental disorders (0.81). This may indicate the high incidence of these diseases and the lack of dental care services in the rural areas.

### PF24

**Current status, role and challenges of traditional medical practitioners involved in management of diabetes mellitus in Nigeria**

Patients suffering from Diabetes mellitus in Nigeria have resulted to consulting Traditional Medical Practitioners (TMPs) to manage their health conditions. There are no available data on the role and status of traditional medicine practice in the management of the disease in the country. A study was initiated in 2009, to document this in six geographical zones of the country. Data was collected through oral interviews in the local languages of over 90 practitioners and responses documented in a specially designed questionnaire along with prior informed consent form and entered into a database. This paper intends to highlight the results obtained which include collection of over 80 recipes in various forms, (mostly of plant based), preliminary screening which yielded 4 most active recipes along with pharmacognostic standards, adequate referral system of the practise, low percentage of women involved in the practise, inadequate educational background of practitioners, good understanding of disease diagnosis, inadequate record keeping and improved shelf life of products. Challenges include need for training on standardization methods of their products and practise, establishment of botanical gardens due to dereosterization, establishment of clinics and more opportunities for product resgistration. These results are required to aid the promotion, standardisation and integration of the practice into National Health Care System. Acknowledgement: The authors wish to acknowledge the STEPB Project of the World Bank for award of Research Grant for this study as part of a larger study.

### PF25

**Ethnobotanical Evaluation of Some Medicinal Plants in Eskisehir, Turkey**

An ethnobotanical survey was made to collect information by means of oral and written questionnaire about the use of medicinal plants in Eskisehir with the assistance of herbal markets. A list of medicinal plants and their reported folkloric uses was compiled during the survey. Information regarding latin name, common name, part(s) used, medicinal uses, recipe preparations of plants. According to the survey 49 plant taxa were members of Aquifoliaceae (1 taxon), Apiceae (1 taxa), Asteraceae (4 taxa), Caprifoliaceae (1 taxon), Compositae (2 taxa), Brassicaceae (1 taxon), Equisetaceae (1 taxon), Ericaceae (1 taxon), Fabaceae (1 taxon), Ginkgoaceae (1 taxon), Poaceae (2 taxa), Juglandaceae (1 taxon), Labiatae (5 taxa), Lauraceae (1 taxon), Loranthaceae (1 taxon), Lycopodiaceae (1 taxon), Malvaceae (5 taxa), Oleaceae (1 taxon), Onagraceae (1 taxon), Plantaginaceae (1 taxon), Primulaceae (1 taxon), Ramunculaceae (1 taxon), Rosaceae (6 taxa), Rubiaceae (2 taxa), Theaceae (1 taxon), Tilicaceae (2 taxa), Urticaceae (1 taxon), Zingiberaceae (1 taxon), Zygophyllaceae (1 taxon). Most of the remedies were prepared from single species. The highest percentage of plants were obtained from Turkey (about 85.7%). The highest number of taxa were used for cardiovascular-cholesterol (22 plants) disorders, diuretic (20 plants), gastrointestinal (15 plants), respiratory (12 plants) and diabetic (14 plants) illnesses. Keywords: Ethnobotany, survey, medicinal plants, Eskisehir

### PF26

**In vitro and in vivo antioxidant activities of the leaves of Chrysophyllum albium**

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Chrysophyllum albium G. Don. (Sapotaceae) which is distributed in Nigeria is used for the treatment of yellow fever, malaria, diarrhoea, vaginal disorders, etc [1]. The study was aimed at investigating the antioxidant...
properties using in vitro and in vivo models. The effect of 1,1-diphenyl-2-picrylhydrazyl (DPPH) antiradical activity on ethanol, petroleum ether, ethylacetate, butanol, and water fractions of C. abindum was determined. The ethylacetate fraction was purified in column chromatography which led to the isolation and characterization of a myricetin rhamnside [2]. The structure was elucidated by NMR and mass spectroscopic techniques. Furthermore, ethanol extract was administered to five groups of eight rats per group. The positive control animals were administered with vehicle on the first four days, and with the vehicle and CCl4 on the fifth, sixth and seventh day [3]. The animals in the treatment category were respectively administered (by gastric intubation) with 500, 1000 and 1500 mg/kg bw of extract & distilled water for the first four days, and with distilled water, extract and CCl4 on the last three days. Animals were anaesthetized and blood samples were collected for some antioxidant assays. Petroleum ether fraction showed the least antiradical activity (4057.5 ± 809.6 g/kg) while ethyl ether fraction exhibited the highest activity (414.4 ± 92.0 g/kg). Myricetin rhamnside also exhibited an excellent radical scavenging activity (314.1 ± 60.2). C. abindum exhibited significant (p < 0.05) differences on the activity of malondialdehyde, catalase, and reduced glutathione. The plant therefore possesses anti-inflammatory activity, inhibiting chemically induced inflammation. 

Acknowledgement: Department of Pharmacology, Faculty of Basic Medical Sciences, American University of Beirut, Beirut, Lebanon; “Department of Biology, Faculty of Arts and Sciences, American University of Beirut, Beirut, Lebanon; “Department of Biology, Ahyman University, Faculty of Science-Deir el-Balahand Ellouar, Lebanon

Linalool is the major component of coriander-sativum seeds. We have recently reported [1] a 100%-decrease in the viability of HepG2 treated with 2 μM linalool. No effect was observed with other cell lines. Linalool resulted in a decrease in the ATP and GSH levels; increase in ROS; and inhibition of ETC-complexes I and II. ROS are known to affect level of UCP2 and ANT. Recent report showed Leukemias cells treated with linalool induced apoptosis mediated by P-53 up-regulation [2]. We investigated the effect of linalool on 10 μM –hepatocytes, variation in UCP2, ANT and P53 expression in HepG2 and 10 μM-hepatocytes. Viability of 10μM-hepatocytes (3), treated with varying concentration of linalool was determined using MTT assay. Expression of P53, ANT, & UCP2 in 10μM-hepatocytes was compared to those of HepG2 cells, using western blotting and was expressed relative to GAPDH. We report that: a) 10μM-hepatocytes were not sensitive to linalool treatment; Comparing 10μM-hepatocytes to HepG2 cells, a 250 fold of linalool concentration was needed to demonstrate a 100% b) Increase in P53 expression was obtained in HepG2 cells whereas P53 was not detected in 10μM –hepatocytes; c) Decrease regulated of the expression of UCP2 in HepG2 cells. Linalool effect is specific to HepG2 cells but had no significant effect on 10μM- hepatocytes. There is a role of P53, and the mitochondrial proteins ANT and UCP2 in rendering HepG2 cells more sensitive. Bio-transformation into toxic metabolites of linalool by HepG2 cells, but not 10μM-hepatocytes, may not be disregarded. Acknowledgement: Medical Practice Plan and University research Board at the American University of Beirut References: 1. Usta J et al. (2009) Chem- Biol Interact 180: 39 – 46. 2. Gu Y et al. (2010) Toxicology 268: 19 – 24 3. Shahfer J et al. (2005) Assay Drug Dev Technol 3(1):27 – 38. 

The aqueous root extract of Aristolochia ringens (Vahl.) prevents chemically induced inflammation

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Aristolochia ringens Vahl Aristolochiaceae belongs to a family with many medicinal uses, but also reported toxic (1). Based on its use in traditional medicine, the anti-inflammatory activity of the aqueous root extract of Aristolochia ringens (AR) (10 – 100 mg/kg p.o.) was evaluated using the carrageenan and egg albumin induced rat paw oedema (2); formaldehyde induced arthritic inflammation (3) and xylene induced mouse ear oedema methods (2). AR (10 – 50 mg/kg) produced a dose-dependent decrease in rat paw oedema in the carrageenan and egg albumin induced in rats at time intervals studied. The maximum inhibitory effects of AR (50 mg/kg), 57.1% and 65.6% in both experiments were comparable to the 57.9% and 63.9% of standard drugs, indomethacin and dicyclofenac (10 mg/kg p.o.) respectively. AR (10 – 50 mg/kg) also dose dependently inhibited the arthritic paw oedema induced by formaldehyde over the 10 day period of study. The maximum inhibition by AR (50 mg/kg) (50%) was greater than the 40.8% inhibition by dicyclofenac (10 mg/kg i.p.). AR (10 – 50 mg/kg) also produced a significant (p < 0.05) dose dependent inhibition of mouse ear oedema, with a peak effect at 50 mg/kg of 84.78%, which was greater than the 65.21% inhibition by dexamethasone (1 mg/kg). No mortality was observed in 24 hours, with AR (up to 100 g/kg p.o.), but an LD50 of 453 mg/kg was obtained with the intraperitoneal route of administration in mice. Results suggest that the aqueous root extract of Aristolochia ringens possesses antiinflammatory activity, inhibiting chemically induced inflammation. 


Medicinal plants and their traditional uses in Kabylia (Algeria): an ethnobotanical survey

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This study aims to assess ethnobotanical knowledge in Kabylia, focusing on the use of traditional medicinal plants, at eight rural municipalities in the department of Tizi Ouzou. This region has remained relatively isolated and agro-industrial development is not led to a significant decline in traditional practices, including the use of plants in traditional medicine. Ethnobotanical information was gathered using a questionnaire among herbalists, traditional healers and local populations in the study area. Overall, 98 vascular plants were identified and recorded, a large majority of them live in a wild habitats (forests and wetlands, especially), with the exception of 6 crops. They belong to 48 families, the most represented are the Lamiaceae (13 species) and Asteraceae (12 species). The many diseases listed in the survey are grouped into 10 major disease groups. The most pathologies treated are those of the digestive system (40 plants), skin diseases (29), circulatory system (24) and respiratory system (21). In contrast, the visual system, too precious, is treated with a single plant (Ocimum basilicum L.). The toxicity of some herbs used over the 10 year period of study. The maximum number of medicinal plants are often multipurpose plants (food, flavor, feed, veterinary, crafts, etc.). Moreover, 31 of these wild plants yet still have an interest in food for rural populations. Finally, a large majority of medicinal plants used in Kabylia are also known for their therapeutic properties in the Mediterranean basin. For example, 73.5% of the plants of this study are cited by the project Rubia. References: Gonzalez-Tejero MR et al. (2008) Ethnopharmacol 116: 341 – 357. 

Evaluation of anti-fertility of Lawsonia inermis L. (Lythraceae) roots found in Kaduna State, Nigeria

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Traditional contraceptive practices and use of medicinal plants is a common occurrence in Kaduna State, Nigeria [1]. The use of Lawsonia inermis L. (Lythraceae) roots was evaluated for antifertility activity. Extraction of the plant root and phytochemical studies of the extract were carried out according to methods described Evans [2]. Apparently healthy female and male Wistar rats were used. Pre-implantation and mating ratio using methods of Ambali et al. [3] were carried out. Determination of implantation sites was by method of Cavieres et al. [4], determination of Corpora Lutea was the method described by Armanazi et al. [5] and the effects of extract on weight of ovaries were also determined. It was observed that the extract effect on contractility of isolated rat uterus was less than oxytocin. There were loss of implantation sites and decrease in body weight. The number of implantation sites showed dose-response relationship significantly (P < 0.05) among
Assessment of wound-healing, anti-inflammatory and antioxidant activities of *Helichrysum graveolens* (Bieb.) Sweet

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*Helichrysum graveolens* (Bieb.) Sweet is used for the treatment of jaundice, as a wound-healing and diuretic agent in Turkish folk medicine. In order to prove the claimed utilization of the plant, effects of the extracts and the fractions were investigated by using the in vivo linear incision and circular excision wound models. Antioxidant and anti-inflammatory activities, which are correlated to wound healing activity, were also evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay and the acetic acid-induced increased vascular permeability model, respectively. The methanolic extract, which demonstrated potent activities, which are correlated to wound healing activity, were also evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay and the acetic acid-induced increased vascular permeability model, respectively. The methanolic extract, which demonstrated potent effect on above-mentioned models, was then subjected to successive solvent extraction with n-hexane, chloroform, ethyl acetate and n-butanol. Each solvent extract was also applied on the same experimental models. The results of the histopathological examination also supported the outcome of both incision and excision wound models. Bioassay-guided fractionation, and thorough phytochemical analysis led to the determination of active principle/s.

Pakistan Medicinal Plants traditionally used for wound healing therapy studied for activity against resistant *Staphylococcus aureus* strains Høller Fc1, Sloved HC2, Gázman A1, Melgard P1

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Plants traditionally used for wound healing therapy by the Huilliche people in Chile were investigated for their activity against a selection of *Staphylococcus aureus* strains. *S. aureus* is a frequently encountered pathogen in skin infections and ethnomedicinal knowledge on treatment of infected wounds may prove valuable in the search for anti-staphylococcal compounds. 30 plant samples of 24 species were collected in the Valdivian rainforest west of Osorno in Chile. Material was extracted with three different organic solvents and antibacterial activity against susceptible and resistant *S. aureus* was evaluated. An agar-overlay diffusion assay and a MIC-determination were utilized for comparative purposes. Total phenolics and tannins were determined and antibacterial contribution of the tannins evaluated. Extracts of 19 species were active against susceptible *S. aureus* at 100 µg extract. At the same concentration 16 species showed activity against resistant *S. aureus*. Extracts without tannins rendered only six samples active. The MIC-determination showed antibacterial activity of 20 extracts on all eight strains, and the highest effect was 64 µg/ml. Species *Aristotelia chilenensis* (Mol.) Stuntz, *Baccharis magellanica* (Lam.) Pers., *Baccharis sphaerocephala* in *Hauck et Arn.*, *Berberis buxifolia* Lam. and *Crinodendron hookerianum* Gay being among the most active. Activity against multidrug resistant *Vanthida* strain was remarkable with 36 active extracts. The results support Huilliche traditional knowledge, and the hypothesis that their wound healing plants are potential sources of anti-staphylococcal agents. These results will form the basis for a selection of plant species for further investigation of new antibacterials in the fight against resistant pathogens. Acknowledgement: Robert Leo Skov, SSI, the National Reference Center in Denmark, for strains and helpful advice. Arjif Onder and Betül Asar for technical support and Sara Nleam for valuable knowledge during plant collection.

**PF31**

In vitro antiplasmodial activities and cytotoxicity of water extracts of *Piper rostratum* Roxb., *Sida rhombifolia* Linn. and *Tilia cordata* (Coeber.) Diels

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This study aims to evaluate the in vitro antiplasmodial activity and cytotoxicity of *Piper rostratum* Roxb., *Sida rhombifolia* Linn. and *Tilia cordata* (Coeber.) Diels, herbs traditionally used to treat malaria in Thailand. The water extracts of these Thai medicinal plants were tested for their antiplasmodial activity by assessing their ability to inhibit the uptake of [3H] hypoxanthine into the multidrug-resistant strain *Plasmodium falciparum* K1. The antiplasmodial activity was expressed by the concentration that inhibited 50% of parasite growth (IC50). Cytotoxicity of the extracts was determined on Vero cells, and the Cytotoxicity Index (CI = IC50 on Vero cells/IC 50 on *Plasmodium falciparum* K1) was calculated to evaluate the safety of tested extracts. *Tilia cordata* (Coeber.) Diels. was the only one of three plants that showed the in vitro antiplasmodial activity (IC10 = 43.43 ± 0.90 µg/ml) with good Cytotoxicity Index (CI = 5.92) whereas *Piper rostratum* Roxb. and *Sida rhombifolia* Linn. did not show this activity. Further study is needed to evaluate an in vivo antiplasmodial activity of *Tilia cordata* (Coeber.) Diels. extract. Acknowledgement: This study was supported by The Annual Government Statement of Expenditure for Thammasat University.

**PF32**

Chilean medicinal plants traditionally used for wound healing therapy studied for activity against resistant *Staphylococcus aureus* strains

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Plants traditionally used for wound healing therapy by the Huilliche people in Chile were investigated for their activity against a selection of *Staphylococcus aureus* strains. *S. aureus* is a frequently encountered pathogen in skin infections and ethnomedicinal knowledge on treatment of infected wounds may prove valuable in the search for anti-staphylococcal compounds. 30 plant samples of 24 species were collected in the Valdivian rainforest west of Osorno in Chile. Material was extracted with three different organic solvents and antibacterial activity against susceptible and resistant *S. aureus* was evaluated. An agar-overlay diffusion assay and a MIC-determination were utilized for comparative purposes. Total phenolics and tannins were determined and antibacterial contribution of the tannins evaluated. Extracts of 19 species were active against susceptible *S. aureus* at 100 µg extract. At the same concentration 16 species showed activity against resistant *S. aureus*. Extracts without tannins rendered only six samples active. The MIC-determination showed antibacterial activity of 20 extracts on all eight strains, and the highest effect was 64 µg/ml. Species *Aristotelia chilenensis* (Mol.) Stuntz, *Baccharis magellanica* (Lam.) Pers., *Baccharis sphaerocephala* in *Hauck et Arn.*, *Berberis buxifolia* Lam. and *Crinodendron hookerianum* Gay being among the most active. Activity against multidrug resistant *Vanthida* strain was remarkable with 36 active extracts. The results support Huilliche traditional knowledge, and the hypothesis that their wound healing plants are potential sources of anti-staphylococcal agents. These results will form the basis for a selection of plant species for further investigation of new antibacterials in the fight against resistant pathogens. Acknowledgement: Robert Leo Skov, SSI, the National Reference Center in Denmark, for strains and helpful advice. Arjif Onder and Betül Asar for technical support and Sara Nleam for valuable knowledge during plant collection.

**PF33**

Total phenolics content of the ethyl acetate extract of *Salvia tomentosa*

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Salvia generally including flavonoids is one of the largest genera in Lamiaceae family and this genus has 900 species. It is composed of 88 species in Turkey and mostly benefited in the treatment of skin infections, colds, stomach ache, headache and tuberculosis. In the present study, aerial part of *Salvia tomentosa* Mil., was used and extraction procedure was applied to its ethyl acetate extract. Yield was obtained as percentage (%). In addition, it was investigated for its total phenol content (TPC) and performed by Singleton and Rossi method with a few modifications. Gallic acid was used as standard and results were expressed as micrograms of total phenolics including of extract as gallic acid equivalents (GAE). In conclusion, its TPC was found as 103.75 ± 4.32 GAE µg/mg of extracts. Each datum was calculated as a average of duplicate measurements acquired from at least three separate experiment sets. According to this result, when its total phenol content was compared with other Salvia species, *S. tomentosa* showed higher TPC than that of other Salvia species. References: 1. Kelen M, Tepe B (2008) Bior-esources Technology 99: 4096 – 4104. 2. Kivrak I, Dur ME, Öztürk M, Mercan N, Harmandar M, Topçu G (2009) Food Chemistry116: 470 – 479. 3. Singleton V L, & Rossi J A (1965) American Journal of Enology Viticulture 16:144 – 158.
Proteomics study in Pueraria mirifica Cathershawat W1, Jungsukcharoen J2, Sangvanich P3, Chokchaichamnankit D1, Srissomap C1
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Proteomics are commonly used in direct protein study of tissues, cells and living organisms for functional component analysis. This technique is widely applied in biological science because it provides more information on living systems than the genomics approach. Proteomics are applied by researchers for medical proteomics, pharmaceutical proteomics and plant proteomics. The principles of proteomics comprise of 4 main steps: protein separation, protein identification, protein quantitative and protein analysis. Application of this technique in soybean (Glycine max) has established reference map in nodule cytosol in which 69 glycoysis enzymes have been found [1]. In soybean leaf, a total of 71 unique proteins are identified [2]. High levels of flavonoid in soybean leaf are confirmed to be sensitive to UV-B at the proteomics level [3]. Since very few proteomics study is established in herbal plants, we thus will focus on the proteomics study of Pueraria mirifica Airy Shaw & Suvath. or kow Ka-Krua", an indigenous Thai medicinal plant is traditionally consumed for the treatment of menopausal symptoms bong to legume family the same as soy bean. The plant phytosterogens and their estrogenic activity have long been investigated. This will enable an investigation into the key proteins related with metabolite production in the Thai herbal plant tissues. Acknowledgment: Thailand Research Fund DBGS180025, Department of Biology, Department of Chemistry, Department of Biotechnology, Laboratory of Biochemistry, Chulabhorn Research Institute References: 1. Oehlfe NW, Sarma AD, Waters JK, Emerich DW (2008) Phytochemistry 69: 2426 – 2438. 2. Xu C, Garrett WM, Sullivan J, Caperna TJ, Natajaran S (2006) Phytochemistry 67: 2431 – 2440. 3. Xu C, Sullivan J, Garrett WM, Caperna TJ, Natajaran S (2008) Phytochemistry 69: 38 – 48.

PF36
Phytoconstituent of Petroleum Ether Extract of Atriplex lindleyi Moq. aerial Part and Its Hepato-renal protection
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The work aimed the detailed description of the lipoidal profile and hepato-renal protective effect of Atriplex lindleyi Moq. aerial part against bromobenzene (BB) intoxication in rats. Column chromatography of petroleum ether (60 – 80) extract and GC/MS analysis of the unsaponifiable matter and fatty acid methyl esters were qualitatively and quantitatively investigated. Oxygenated and non-oxygenated hydrocarbons, phenolic, steroidal and triterpenoidal compounds were identified in the petroleum ether extract. GC/MS analysis of fatty acid methyl ester led to the identification of 20 compounds. In vivo examination of the petroleum ether extract against bromobenzene (BB) intoxication using heparcin as a reference drug was included five groups of albino rats were selected in this study. Group1: normal control group. Group 2: ip injected with BB (460 mg/kg b.wt) two times/week for three weeks, group 3: received oral doses of plant extract (150 mg/kg b.wt) at the same time and duration of BB injection. Group 4: served as group 3 and treated with heparcin (Medical Union pharmaceuticals company, Egypt) (100 mg/kg b.wt.) as a reference drug. Group 5: received plant extract only. The Drastic changes observed after BB intoxication in liver function enzymes (AST, ALT and ALP), hepatic cell organelles marker enzymes (SD, LDH, G-6-Pase, AP and 5’-nucleotidase), kidney disorder parameters (creatinine and urea) and certain antioxidants (glutathione and superoxide dismutase). Treatment with petroleum ether extract improved all biochemical parameters under investigation as well as the histopathological chromatogram of liver and kidney. The petroleum ether of A. lindleyi contains bioactive compounds exhibiting hepato-re nal protective effect. References: 1. El-Sharaky AS, Newairy AA, Kamel MA, Eweda SM (2009) Food Chem Toxicol 47(7): 1584 – 90. 2. Shaker E, Mahmoud H, Mnaa S (2010) Food Chem Toxicol 48(3): 803 – 6. 3. Saed O, Fuldner S, Khalil K, Aazaib H, Kaisis E, Saad B (2008) Evid Based Comp Med 3(4): 421 – 428.

PF37
In vitro effect of purified plumbagin of Plumbago indica against motility of Parapharyngodon cervi Saowakon N1, Kuekthai P2, Changklungmoo N2, Lorsawwanarat N3, Sobhon P4
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The crude extract of Plumbago indica L. has been used as Thai traditional medicine for treating digestive tract disorders [1]. Reportedly, the effect of purified plumbagin of P.indica inhibited the motility of Caenorhabditis elegans and infective cercariae stage of Schistosoma mansoni [2]. However, the anhelminctic activity of plumbagin on P.cervi has not been studied. Therefore, this work aimed to investigate the effect of purified plumbagin of P.indica root on adult P.cervi was evaluated after incubating parasite in M-199 medium containing plumbagin in the serial concentrations of 10 fold dilution of 100 μg/ml, for 3, 6, 12 and 24 h, relative motility assay and histopathological changes. It was found that the complete inhibition of worm motility and subsequent mortality was observed at 100 μg/ml, in early 3h observation. The motility of P.cervi was progressively decreased since 3 to 12h exposure, and few activity of P.cervi was observed at 24h exposure at the concentration 0.1,1 and 10.0 μg/ml, respectively. Observation under the stereo-microscope, adult P.cervi appeared brown to black color, numerous blebs and peeling of tegument after 12h exposure. Light microscopic observation showed the numerous blebs, erosion and desquamation of tegument of P.cervi. They also revealed theecal epithelial cell lining detachment. Although, the previously report that crude extract of Arctocarpus lakanoo Roeb. affected on P.cervi [3], but motility stage is higher than plumbagin. These results suggest that plumbagin of P. indica could be against the motility of adult stage of P.cervi better than A.lakanoo. Acknowledgement: This work was supported by the Thailand Research Fund (Senior Research Scholar Fellowship to Prof. Prasert Sobhon), Mahidol University and research grants from Suranaree University of Technology. References: [1] Wuttidhammaved W (1997) The Encyclopedic of Medicinal Plants of Thailand: The Thai Traditional Medicine, The O-dien Store Publishing Co., Ltd, Bangkok, Thailand (Published in Thai), 681. [2] Atjanasuppat K, Wongkham W, Meepowpan P, Kittakoo P, Sobhon P, Bartlett A, Whitfield P (2009) Exp Parasitol 122(4):289 – 98. [3] Saowakon N, Tansing T, Wanchanond C, Chanakul W, Reutrakul V, Sobhon P (2009) Exp Parasitol 122(4):289 – 98.

PF38
 Diospyros lotus L. fruit extract protects G6PD-deficient erythrocytes from hemolytic injury in vitro and in vivo: prevention of favism disorder
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Favism is a life-threatening hemolytic crisis that can result from the ingestion of fava beans by susceptible individuals who have G6PD enzyme deficiency (1). The aim of this study was to evaluate the protective effect of Diospyros lotus L. fruits extract against the hemolytic damage induced by fava beans extract in both G6PD enzyme-deficient human and rat erythrocyte in vitro and in vivo models. In vitro model, venous blood samples were obtained from five subjects with known G6PD deficiency, which was also confirmed with standard techniques. Erythro-
cytotoxic hemolysis was induced by fava beans extract in the presence and absence of Diospyros lotus fruits extract. The hemoglobin release in the supernatant, as well as the value of the hematoцит was determined by recording optical density at 540 nm in a spectrophotometer and microhematocrit, respectively in vivo. Model, G6PD enzyme deficiency was induced in rats by intraperitoneal (i.p.) injection of DHEA (Dehydroepiandrosterone) (100 mg/kg), a specific G6PD enzyme inhibitor, for 35 consecutive days (2). Then the animals were pre-treated with different doses of Diospyros lotus (500, 750, 1000 mg/kg) by oral administration for seven consecutive days after induction of G6PD deficiency. Rats were administered orally on the seventh day with Vicia faba beans extract (40 mg/kg b.w.), the blood was removed for evaluation of its value of erythrocyte hemolysis and hematoцит after one hour. Our results have shown that Diospyros lotus fruits extract with an antioxidant activity has protective effect against hemolytic damage induced by fava beans extract in both G6PD-deficient human and rat erythrocytes.

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PF39

Ethnopharmacological evaluation of male contraceptive efficacy of Dendrophoe falcuta in albino rats

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Search for male-antifertility agent in plants remains a potential area of investigations. Though, antispermatogenic activity has been reported in some plants, only few are reported. Therefore, present study was undertaken to evaluate the contraceptive-efficacy of Dendrophoe falcuta (L.f.) Ettingh. in male albino rats as reported in folk remedies1. Shaded dried stems of D. falcuta were extracted in methanol and then fractionated with different solvents. A part of isolated-fractions were also processed for various phytochemical-techniques to identify active constituents. Spectral studies revealed that it has one compound in major quantity i.e. kaempferol, a natural flavonol. Male rats of the group were given water, kaempferol rich fraction at 50 mg/rat/day for 60days. On day 61st animals were autopsied and testes, epididymides, seminal vesicle and ventral-prostate were dissected out and weighed. Sperm motility and density were assessed. Biochemical and histological analysis were also performed. A marked reduction in weight of testes, epididymides, seminal vesicle and ventral prostate was observed. The sperm motility and density were significantly reduced. The histoarchitecture of testes revealed degenerative changes in the seminiferous-tubules and arrest of spermatogenesis at the stage of round-spermatids. A marked alteration in Leydig cell differentiation was also noticed. Serum-testosterone was greatly reduced. The results showed that the studied extracts presented high phenolic and flavonoid content values and possessed a good ability to scavenge free radicals. Both plant extracts had a significant dose-dependent effect on the growth of mouse fibroblasts (NCTC clone 929 cell line) growth, as well as their antioxidant capacity using TEAC and ORAC methods. The effect of the two plant extracts on mouse fibroblasts (NCTC clone 929 cell line) growth, as well as their antioxidant protective effect against hydrogen peroxide-induced cell damage were also investigated. The degree of fibroblast growth and protection against hydrogen peroxide damage was maximized by Neutral Red and LDH assays. The results showed that the studied extracts presented high phenolic and flavonoid content values and possessed a good ability to scavenge free radicals. Both plant extracts had a significant dose-dependent effect on the growth of mouse fibroblasts (NCTC clone 929 cell line) growth, as well as their antioxidant protective effect against hydrogen peroxide-induced cell damage. Treatment of the cells with Arnica montana and Urtica dioica for 24h prior to 0.05 mM hydrogen peroxide increased the cell viability from 52% in hydrogen peroxide treated cells to more than 80%. The results obtained in cell culture correlated well with the antioxidant potential of the plant extracts. Our data indicate that the studied plants may be useful as agents for skin diseases caused by oxidative stress.


PF40

Popular medicinal plants in Iran for the treatment of Gl disorders

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This paper is a collection of plant samples and their derivative products (gum and extracts) that have been used traditionally for Centuries to treat Gl disorders as it has been mentioned in Iranian old reference books Considering today’s applications of these medicinal plants, reveals the great value & effect of traditional medicine in Iran. Of course, Gl disorders, according to ancient texts in Iran, include: different types of abdominal pains due to flatulence and gastritis types, Constipation, diarrhea, stomach cramps, which can be classified according to the traditional treatments and their comparison with the current one. These plants can include: Labiatae (Mentha spicata L., Zataria multiflora Boiss.), Compositae (Matricaria chamomilla L.), Umbelliferae (Carum carvi L., Pimpinella anisum L.), Zingiberaceae (Zingiber officinale Roscoe, Curcuma Longa L.), Anacardiaceae (Pistacia lentiscus L., Pistacia atlantica Desf.). etc.

We selected 2 – 3 sample, from each family. Most important parts of these plants are their leaves with 35%, then their fruits about 30%, roots & Rhizomes with the lowest standing 10%, and the whole plant is about 20%. Of course, gums and other parts of plant such as flowers, with the very low percentage, are also used in the treatment of Gl disorders. In the case of this leaves, the leaves are consumed as boiling in the first stage and taking into distillates (aqueous and alcoholic extract) in the second stage Most of these plants are used as carminative and antispasmodic and sometimes anti diarrheal ones, and in comparison with chemical drugs (Pan- topazole, Dicyclomine, Sucralfate, Magnesium Hydroxide) they have the same or sometimes better effects. Acknowledgement: Farsam H. Amin GH of School of Pharmacy, Tehran University of Medical Sciences

PF41

Antioxidant potential of Arnica montana and Urtica dioica hydroalcoholic extracts on mouse fibroblasts in vitro

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The use of medicinal plant derivatives is an alternative to conventional medicine to treat diseases associated with oxidative stress [1 – 3]. In this study, we have determined the total phenolic and flavonoid contents of the hydroalcoholic extracts obtained from two Romanian traditional medicinal plants: Arnica montana and Urtica dioica and we assayed their antioxidant activity using TEAC and ORAC methods. The effect of the two plant extracts on mouse fibroblasts (NCTC clone 929 cell line) growth, as well as their antioxidant protective effect against hydrogen peroxide-induced cell damage were also investigated. The degree of fibroblast growth and protection against hydrogen peroxide damage was maximized by Neutral Red and LDH assays. The results showed that the studied extracts presented high phenolic and flavonoid content values and possessed a good ability to scavenge free radicals. Both plant extracts had a significant dose-dependent effect on the growth of mouse fibroblasts (NCTC clone 929 cell line) growth, as well as their antioxidant protective effect against hydrogen peroxide-induced cell damage. Treatment of the cells with Arnica montana and Urtica dioica for 24h prior to 0.05 mM hydrogen peroxide increased the cell viability from 52% in hydrogen peroxide treated cells to more than 80%. The results obtained in cell culture correlated well with the antioxidant potential of the plant extracts. Our data indicate that the studied plants may be useful as agents for skin diseases caused by oxidative stress.

PF42

In vitro effect of Myrrh extracts on the viability of Schistosoma mansoni larvae
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Schistosomiasis, a parasitic disease caused by trematode flatworms of the genus Schistosoma, represents a growing concern in the Sub-Saharan Africa, where up to 80% of the population is infected. Mirazid®, a commercial drug obtained by combination of two solvent extracts of Myrrh, the oleo-gum-resin from the stem of Commiphora molmol Engl. ex Tschirch (Burseraceae), is marketed in Egypt since 2001 as an alternative treatment for schistosomiasis [1]. However, recent independent studies question its efficacy. All experiments conducted with Myrrh so far are either in vivo tests or clinical trials, but no in vitro data is available. In order to shed light into controversy around Myrrh, two commercial Myrrh samples (from S. Africa and M. East) were extracted and/or combined as described in Mirazid®; first with petroleum ether (A), subsequently with MeOH (B) by hydrodistillation to yield volatile oil (c) for myrrh (8.5% parts of resin + 3.5% parts of volatile oil) [2–4]. They were also extracted with MeOH and then partitioned between hexane, CHCl3, and aqueous MeOH. As the combination ratios of the extracts A+B in Mirazid® are unclear, extracts A/B were combined in simple ratios. In the medium throughpout visual S. mansoni larval assay, all ipophiilic extracts, the combinations, and the essential oils showed moderate, but different activity (IC50 7.18–32.69 μg/mL). The extracts and the essential oils were also different phytochemically (by TLC, H NMR, GC-MS). This study shows that Myrrh has antischistosomal potential, but the origin of the plant material and extraction method is of importance. Reference: 1. Badran et al. (2001) Pharm Bio 39: 127–131. 2. Mansour A et al. (1998) Parasitol Int 47: 105–113. 3. Sheir Z et al. (2001) Am J Trop Med Hyg 65: 700–704. 4. Abdel-Hay MH et al. (2002) Spectroscopy Lett 35: 183–197.

PF43

Evaluation of four traditional Romanian medicinal plants as wound healing agents
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Achillea millefolium L., Hyssopus officinalis L., Equisetum arvense L. and Echinacea purpurea (L.) Moench, are four medicinal plants traditionally used in Romania for the treatment of skin disorders and wound healing. In order to support their use for new plant derived products the aim of this study was to evaluate their potential to stimulate the wound healing process. We investigated the four herbal extracts by HPLC-MS for the presence of polyphenolic compounds, we also assessed the antioxidant activity by the DPPH photometric method, BHT was used as positive control and by Sircol assay we evaluated the rate of soluble collagen activity by the DPPH photometric method, BHT was used as positive control. The HPLC-MS analysis revealed the presence of antioxidant compounds, phenolic acids and flavonoids such as: rosmarinic acid, chlorogenic acid, caffeic acid, luteolin–7-O–D glucoside, apigenol 7-O-glucoside, rutin and apigenin. High antiradical capacity was detected in ethanolic extracts and flavonoids and phenolic acids may be reasonable. Collagen excretion was increased in culture medium of fibroblasts treated with herbal extracts when compared with untreated cells. At the highest concentration of herbal extract, the highest collagen synthesis was observed which was almost 2 times higher as compared to the synthesis of untreated cells. The current study explains the medicinal utility of these plants due to their antioxidant activity and to their ability to stimulate the collagen synthesis, activities that would accelerate the wound healing process. Acknowledgement: This study was supported by Project PN II 62071 References: 1. Huang Hj, Cheng Hj (2005) Bot Bull Acad Sin 46: 99.

PF44

Study of apoptosis induction effects of traditional remedies and quality control strategies
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Traditional medicine is an important part of health care system in Vietnam [1]. Nevertheless, lack of scientific and therapeutic evidences as well as quality control system limit its development. Many anticancer products used in cancer therapy act by inducing apoptosis in cancer cells [2]. In addition to chromatographic analysis with standard compounds, biological response fingerprinting are suggested for quality control of traditional formulations [3]. We investigated five traditional remedies reported in traditional pharmacopoeia as having anticancer effects. We determined apoptosis inducing capacity and cell cycle arrest of these remedies and their components on HeLa cells by DNA fragmentation assay, fluorescence microscopy, caspase activity assay and flow cytometry-based method. We showed that the modified remedy “Hoang Lien Giao Do Thang” (HLGDT) caused cell death by inducing apoptosis, independently of caspase-3 activation. Three components, Coptis sinensis Franch, Scutellaria baicalensis George and Phellodendron amurense Rupr have higher cytotoxicity than the whole remedy on HeLa cells. Microarray data analysis performed on HeLa cells treated with HLGDT for 24 and 36 hours showed differential, increased or decreased, expression of 408 genes. Some overexpressed genes – DDIT3, TRIB3, FAM129A, STC2, GDF15, SERPINE2 were reported as involved in ER-stress. Expression level of these genes was confirmed by real-time RT-PCR. Real-time RT-PCR amplification of these genes are further used to set up biological fingerprints. Baicalin and berberin were used as chemical fingerprints through chromatographic analysis. These fingerprints could be considered for quality control purposes of the remedy. Acknowledgement: These work was supported by grants from the Department of Science and Technology – Ho Chi Minh City. We are grateful to Prof. Sangho Lee and the Microarray platform from Sungkyunkwan University for microarray analysis. We acknowledge the financial support from the Microarray platform from Sungkyunkwan University, Ho Chi Minh City. We are grateful to Prof. Sangho Lee and the Microarray platform from Sungkyunkwan University for microarray analysis. The current study explains that Myrrh has antischistosomal potential, but the origin of the plant material and extraction method is of importance. References: 1. WHO (2002). WHO traditional medicine strategy 2002 – 2005. 2. Fulda S (2010) Planta Med 76(11): 1075 – 9 3. Chavan P, Joshi K, & Patwardhan B (2006) eCAM: 1 – 11

PF45

Evaluation of the effects of Parinari curatellifolia seed and Anthocleista vogelli root extracts individually and in combination on postprandial and alloxan-induced diabetes in animals
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Parinari curatellifolia Planch. ex Benth. seed and Anthocleista vogelli Planch.roots extracts mixture (1:1) have been used locally for the treatment of diabetes. The postprandial effects were evaluated on albino rats (20) randomly distributed into four groups. Each received orally 500 mg/kg of the extract mixture, P. curatellifolia and A. vogelli respectively and the control 0.5 ml (2% w/v) acacia solution. Blood glucose levels were monitored at 30, 60, and 120 min. intervals as described by Ogbonnia et al. (2001) Pharm Bio 39: 127–131. Twenty five diabetic albino rats with plasma glucose>= 200 mg/dl were randomly divided equally into five groups and treated orally for 30 days as follows: Groups I, II and III received orally 500 mg/kg body weight of the mixture of P. curatellifolia and A. vogelli respectively while group IV received glibenclamide 600μg/kg body weight (2), while V diabetic control received 0.5 ml acacia solution. Results showed a significant reduction (p<0.05) in postprandial plasma sugar level after 30 min in all treatments. Also significant reductions (p<0.05) in the plasma glucose, LDL-cholesterol, AST and ALT levels, and increase in HDL-cholesterol were observed in the treated diabetic groups. These results support the use of these extracts in traditional medicines for the treatment of diabetes. The liver and pancreas sections of animals treated with the extract mixture showed marked necrotic changes while pancreatic tissue of diabetic untreated animals showed more severe necrosis of beta cells which formed mass of amorphous eosinophilia. The glibenclamide treated animals showed
spots of necrotic changes; otherwise they had predominantly viable beta cells. The results showed that the extracts and mixture had both good hypoglycaemic activity and beneficial effects on cardiovascular risk factors.


PF46

In vitro screening of selected medicinal plants against Schistosoma mansoni larvae
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Schistosomiasis, caused by various members of the trematode flatworms (Schistosoma species) is the second most important parasitic disease next to malaria. Resistance and low susceptibility towards praziquantel, the only available schistosomicidal drug, urge the search for new drugs. This study aimed at assessing the in vitro schistosomicidal effects of several medicinal plants traditionally used for the treatment of schistosomiasis or other helminths [1]. The crude MeOH extracts of selected plants Artemisia abisinthium L. and A. abrotanum L. (aerial parts) Phytolacca dodecandra L. (roots), Curcuma longa L. (roots), Zingiber officinalis Roscoe (roots), Panax ginseng L. (peels, fruits and the aqueous extract of the fruits) and Citrus reticulata Blanco (peels) were tested against juvenile worms (schistosomulae) of S. mansoni by using the standard visual larval assay. All crude extracts, except P. ginsengum and C. reticulata exhibited significant antischistosomal effect. The highest activity was displayed by C. longa (IC50 = 4.06 µg/mL), followed by A. abrotanum and Z. officinalis extracts with IC50 values of 11.1 µg/mL and 11.73 µg/mL respectively. In the next step, the crude MeOH extracts were subjected to a liquid–liquid partitioning scheme between aqueous MeOH, hexane and CHCl3 and the subextracts were retested in the same assay. Generally, the MeOH extracts of the selected plants contained more complex constituents (mostly phenolic compounds which might account for the observed activity. Four organic solvents (n-hexane, dichloromethane, ethylacetate, and 70% ethanol) were used for the sequential extraction. Decoction of each plant material was prepared according to traditional use. Different species of mycobacteria, namely, M. smegmatis, M. smegmatis, M. avium, M. avium, and M. tuberculosis were employed to screen extracts by broth microdilution method. The cytotoxicity against human macrophages from the mononcytic TH-1 cells was also evaluated. Overall, n-hexane extracts of Maerua edulis Gilg & Gilg-Ben. and Securidaca longepedunculata Fresen, ethyl acetate extract of Tabernaemontana elegans Stapf and dichloromethane extract of Zanthoxylum capense (Thunb.) Hary., were found to possess considerable activity against M. bovis BCG and M. tuberculosis H37Ra with MIC = 15.6 – 62.5 µg/mL. Tabernaemontana elegans ethyl acetate extract displayed strong activity against M. tuberculosis H37Rv (MIC 15.6 µg/mL) as well as potent cytotoxic effects in THP-1 cells (IC50 < 4 µg/mL). Based on 1H NMR spectroscopic analysis, major compounds in both Maerua edulis and Securidaca longepedunculata n-hexane extracts were linear chain unsaturated fatty acids. Zanthoxylum capense dichloromethane extract contained more complex constituents (mostly phenolic compounds), and the prominent compounds in ethyl acetate extract of Tabernaemontana elegans were identified as indole alkaloids. Keywords: Antimycobacterial activity, medicinal plants, Mozambique, tuberculosis


PF47

Changing of some elements during phenological stages in Nitraria Schobieri L
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Nitraria Schobieri L. from Nitrariaceae as pharmaceutical, industrial and fodder plants can tolerate harsh environmental conditions especially sandy and saline soil. This plant is natural sources of some important chemical compounds like schoberine nitarine, dihydroxynitrane, e- nitrateamine, nitroxine and etc. More than 25 Mha of salt affected land exists in Iran and this plant can offer an economic and practical alternative towards achieving saline land and water resources in order to produce natural chemical components for pharmaceutical industries. Changing in some elements (Nitrogen, Phosphor, Sodium, Potassium, and Calcium) during flowering and forming seed stages (2009) were investigated. Samples of the aerial part of Nitraria Schobieri collected in the Kashan region were dried, grained and analyzed in Laboratory. The results showed that elements level indices values including P, K, Ca and Na were significantly different among phenological stages but Na did not show any difference between two stages. Decreasing in P, K and N showed chemical component of this plant could change by sequence of phenological stages and finally these changes affect on quality of fodder and medicinal properties.

PF48

Phytochemical characterization of antimycobacterial crude extracts from medicinal plants traditionally used in Mozambique
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A number of medicinal plants have long been used by traditional healers to treat tuberculosis and related diseases in Mozambique [1,2]. The present study was aimed at evaluating selected medicinal plants for their in vitro antimycobacterial activity, and reveal the main classes of compounds which might account for the observed activity. Four organic solvents (n-hexane, dichloromethane, ethylacetate, and 70% ethanol) were used for the sequential extraction. Decoction of each plant material was prepared according to traditional use. Different species of mycobacteria, namely, M. smegmatis, M. smegmatis, M. avium, M. avium, and M. tuberculosis were used to screen extracts by broth microdilution method. The cytotoxicity against human macrophages from the mononcytic TH-1 cells was also evaluated. Overall, n-hexane extracts of Maerua edulis Gilg & Gilg-Ben. and Securidaca longepedunculata Fresen, ethyl acetate extract of Tabernaemontana elegans Stapf and dichloromethane extract of Zanthoxylum capense (Thunb.) Hary., were found to possess considerable activity against M. bovis BCG and M. tuberculosis H37Rv with MIC = 15.6 – 62.5 µg/mL. Tabernaemontana elegans ethyl acetate extract displayed strong activity against M. tuberculosis H37Rv (MIC 15.6 µg/mL) as well as potent cytotoxic effects in THP-1 cells (IC50 < 4 µg/mL). Based on 1H NMR spectroscopic analysis, major compounds in both Maerua edulis and Securidaca longepedunculata n-hexane extracts were linear chain unsaturated fatty acids. Zanthoxylum capense dichloromethane extract contained more complex constituents (mostly phenolic compounds), and the prominent compounds in ethyl acetate extract of Tabernaemontana elegans were identified as indole alkaloids. Keywords: Antimycobacterial activity, medicinal plants, Mozambique, tuberculosis


PF49

Genome wide expression analysis of the effect of WWCSW, a traditional Korean herbal formula, on rat intracerebral hemorrhage
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Woo-woang-chong-shim-won (WWCSW) is a traditional Korean herbal formula which is commonly used for treating patients with hypertension, arteriosclerosis, coma and stroke in China and Korea. WWCSW is composed of various kinds of chemical components, it would be difficult to isolate major components having pharmaceutical effect. Therefore, high throughput screening systems such as microarray analysis is essential protocol to elucidate the molecular effects of herbal extract on animal disease model. In this experiment, we measured the effect of WWCSW on ICH in rat using microarray technology. ICH was induced by injection red total RNA was isolated. Hierarchical clustering was implemented using CLUSTER and TREEVIEW program, and for Ontology analysis, GOSTAT program was applied in which p-value was calculated by Chi square or Fisher's exact test based on the total array element. WWCSW-treatment restored the gene expression
Isoflavonoid biosynthesis in Pueraria mirifica leaves

Pueraria mirifica Airy Shaw & Suvatap. is a Thai indigenous herb with long-term consumption among Thai menopausal women for menopausal treatment. Researches in the tubers of this plant are mostly focused on their estrogenic potency and application to human health. The tubers are sources of active ingredients including the potent estrogenic miroestrol and deoxymiroestrol, and also isoflavonoids, however, the plant tubers showed limited growth rate. Isoflavonoids are the abundance secondary metabolites in P. mirifica [1, 2] which play important roles in estrogenic effects [3] in animal assays, especially daidzein and genistein are potent anti-cancer, including breast cancer [4]. This group of chemistia is also needed for dietary supplement and cosmetic products. The plants produce a lot of leaves during their growth and development. In this study, the leaves were collected for 12 consecutive months and tubers were collected for every 4 months. The leaves were dried and extracted for isoflavonoids in the absence of chlorophyll for HPLC analysis together with the dried tubers. The analysis revealed that plant leaves contain significant amount of isoflavonoids. Thus it would imitate not only on isoflavonoid extraction industry but also on specialized products derived from these chemicals. In addition, proteomics approach is interesting in our study which resulted in finding some interesting appearance proteins in the plant tubers. Acknowledgement: Thailand Research Fund DBC5180025. References: 1. Cherdshewasart W, Sriwatcharakul S, Malaivijitnond Cherdshewasart W, Sriwatcharakul S (2007) Biosci Biotechnol Biochem 71: 2527 – 2533. 3. Cherdshewasart W, Sriwatcharakul S, Malaivijitnond Cherdshewasart W, Sriwatcharakul S (2007) J Pharm Biomed Anal 43: 428 – 434.

Larvicidal and antimalarial activity of some Zulu medicinal plants

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Pueraria mirifica Airy Shaw & Suvatap. is a Thai indigenous herb with long-term consumption among Thai menopausal women for menopausal treatment. Researches in the tubers of this plant are mostly focused on their estrogenic potency and application to human health. The tubers are sources of active ingredients including the potent estrogenic miroestrol and deoxymiroestrol, and also isoflavonoids, however, the plant tubers showed limited growth rate. Isoflavonoids are the abundance secondary metabolites in P. mirifica [1, 2] which play important roles in estrogenic effects [3] in animal assays, especially daidzein and genistein are potent anti-cancer, including breast cancer [4]. This group of chemistia is also needed for dietary supplement and cosmetic products. The plants produce a lot of leaves during their growth and development. In this study, the leaves were collected for 12 consecutive months and tubers were collected for every 4 months. The leaves were dried and extracted for isoflavonoids in the absence of chlorophyll for HPLC analysis together with the dried tubers. The analysis revealed that plant leaves contain significant amount of isoflavonoids. Thus it would imitate not only on isoflavonoid extraction industry but also on specialized products derived from these chemicals. In addition, proteomics approach is interesting in our study which resulted in finding some interesting appearance proteins in the plant tubers. Acknowledgement: Thailand Research Fund DBC5180025. References: 1. Cherdshewasart W, Sriwatcharakul S, Malaivijitnond Cherdshewasart W, Sriwatcharakul S (2007) Biosci Biotechnol Biochem 71: 2527 – 2533. 3. Cherdshewasart W, Sriwatcharakul S, Malaivijitnond Cherdshewasart W, Sriwatcharakul S (2007) J Pharm Biomed Anal 43: 428 – 434.

Methanolic extracts of 4 different populaion of three species of Salvia (Salvia fruticosa Mill. 20 samples, Salvia pungens Mill. 5 samples and Salvia tomentosa Mill. 15 samples) were analyzed for their antioxidant properties. Samples were collected from different natural ecological areas in Marmara Region in Turkey. The antioxidant capacity (TAC) was investigated with the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and expressed as trolox equivalents (TE). The amount of total phenolics was determined by using Folin-Ciocalteu method and Flavonoid contents in the extracts were determined by a colorimetric method. The TAC values of the spices ranged from 288. 57 to 3608.32 μmol (TE)/100 g dw. The total phenolic and flavonoid content ranged from 488.07 to 5277.97 mg of gallic acid equivalents (GAE)/100 g dw and 664.03 to 4046.77 mg of catechin equivalents (CE)/100 g dw, respectively.
**PF54**

An investigation of the contents of phenolics, flavonoid compounds and antioxidant activity of some wild mushrooms

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The antioxidant activity and properties of 12 wild mushrooms (Lactarius piperatus (L.) Pers., Lactarius piperatus Fr., Lactarius chrysorrheus (Pat.) Maire, Laccaria laccata (Scop.) Fr. > Laccaria laccata (L.) Pers., Lactarius deliciosus (L.) Gray, Lactarius salmonicolor R. Heim & Leclair, Cantharellus cibarius Fr., Hydnum repandum L., Picoa lefebvrei (Pat.) Maire, Ramaria aurea (Schaef.) Quel., Lactarius semian- guifluus R. Heim & Leclair, Craterellus cornucopioides (L.) Pers., Laccaria laccata (Scop.) Fr.) collected from Turkey were evaluated. Their metha-nolic extracts were used to determine antioxidant capacity (TAC), total phenolics and flavonoids. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities were measured to evaluate flavonoid content of the extracts and expressed as trolox equivalents (TE). The amount of total phenolics was determined by using Folin-Ciocalteu method and flavonoid contents in the extracts were determined by a colorimetric method. Wild mushrooms were found to be high in antioxidant phyto-chemicals, such as phenolics (575.10–2156.40 mg GAE/100 g DW), flavonoids (103.01–346.53 mg CE/100 g DW). The TAC values of the spices ranged from 525.32 to 1693.85 μmol (TE)/100 g DW. and the antioxidant activity was found to vary in the order: Hydnum repandum > L Ramaria aurea (Schaef.) Quel. > Lactarius salmonicolor R. Heim & Leclair > Crater- ellus cornucopioides (L.) Pers. > Lactarius deliciosus (L.) Gray > Lactarius piperatus (L.) Pers. > Picoa lefebvrei (Pat.) Maire > Tricholoma caligatum (Viv.). Ricken > Amanita caesarea (Scop.) Pers. > Cantharellus cibarius Fr. > Laccaria laccata (Scop.) Fr. > Lactarius semian-guifluus R. Heim & Leclair.

**PF55**

Identification of proteins in preparations of Candida species used in homeopathic medicinal products

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Preparations with Candida albicans and C. parapsilosis have been safely used as active substances in isopathic remedies for over 30 years. Iso-pathy is a special kind of homeopathy \(^{[1]}\). Water-soluble fractions after cell mill treatment and different purification steps are obtained from both yeast and named e volumine cellulae (lyophil., steril.) \(^{[2]}\). D 3 to D 5 potencies of these starting materials are used e.g. for the treatment of eczema or mycotic skin disorders (marketing authorizations for dif-ferent dosage forms of Albicansan and Pefrakehl in Switzerland). The proteins were then identified by LC-MALDI mass spectrome-

**PF56**

In vitro activity and chemical characterization of an apolar fraction of Morus alba leaf hot water extract

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White mulberry (Morus alba L.) leaf is a well known traditional medicine of type II diabetes, a progressive disease with a broad spectrum of complications which has increasing incidency worldwide. Most typically it is taken as tea, often in combination with other phytotherapies. Many constituents were found to contribute to the anti diabetic activity of mulberry leaf, including inosinosugars, flavonoids and related com-

**PF57**

Ethnobotany and ethnopharmacology of the genus Veratrum L. in veterinary medicine (Bosnia and Herzegovina, W. Balkan)

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Genus Veratrum L. (Liliaceae) in flora of Bosnia and Herzegovina (BiH) is represented with three taxa – white heliobele Veratrum album L., black heliobele Veratrum nigrum L. and green heliobele Veratrum lobelianum Bernh. White and green heliobele grows on mountain meadows, and black heliobele grows in strap of termofile oak-hornbeam forests. Dur-

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PF58

Phytochemical Study from Sonchus arvensis L. Leaves for Standardizing Traditional Medicine Extract

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Sonchus arvensis L. leaves are empirically used as a traditional medicine for asthma, cough, anti-inflammation and diuretic [1,2]. To ensure quality through identification and standardization of its extract, fingerprint/phytochemical study is needed. In this research, the phytochemical study was carried out by TLC (Thin Layer Chromatography) scanner and HPLC (High Performance Liquid Chromatography). From the results, n-hexane extract showed a better separation with toluen: ethyl acetate (93:7 v/v) and had specific retention factor 0.8; 1.3; 2.14 (254 nm) and 0.88; 1.29; 2.14 (366 nm). Chloroform extract showed specific retention factor 1.13; 1.30; 2.14 (254 nm) and 0.88; 1.29 (364 nm). Otherwise, clear separation of ethyl acetate extract was shown in chloroform: toluen: ethanol (4:4:1 v/v/v) with specific retention factor 0.98; 1.49; 1.9; 2.34; 2.49 (254 nm) and 0.98; 1.84; 1.95; 2.34; 2.50 (366 nm). From HPLC chromatogram at 254 nm, using acetonitrile-phosphoric acid mixture showed specific retention time at 2.46; 4.09; 4.83; 7.69; 10.02; 11.06; 11.73 minute for hexane extract and 3.66; 5.84; 9.68 minute for ethyl acetate extract. In conclusion, the specific retention time from both extracts can be used as fingerprint for standardization of traditional medicine extract of Sonchus arvensis leaves. References: 1. Foster S. & Duke JA (1995) A Field Guide to Medicinal Plants. Eastern and Central N. America. Houghton Mifflin Co., ISBN 0395467225. 2. Xu et al. (2008) Food Chemistry 111: 92 – 97.

PF59

HM-61, a Korean native plant extract, inhibits high glucose-induced ocular vessel alteration in zebrafish and prevents the development of diabetic ocular complications in diabetic db/db mouse

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Diabetes alters the structure and function of most cell types in the eye. The injury of ocular cell and retinal vasodilatation are the hallmark of diabetic ocular changes. HM-61 is an 80% ethanolic extract of Litsaea japonica (Thunb.) Jussieu, Korean native medicinal plant, with beneficial effects on diabetes. In this study, we investigated the preventive effects of HM-61 on diabetic ocular complications in zebrafish and diabetic db/db mouse. Tgflk1-1:EGFP zebrafish larvae which specifically express EGFP in all blood vessels were immersed in high glucose medium with or without HM-61 for 5 days post fertilization (dpf). HM-61 (100 and 250 mg/kg body weight) was also treated once a day orally for 12 weeks in db/db mouse. In zebrafish model of diabetes, HM-61 effectively inhibited high glucose-induced ocular vasodilatation (p < 0.01 vs. control). In diabetic db/db mouse, the treatment of HM-61 prevented the breakage of retinal-blood barrier and the injury of retinal vascular cells. In addition, diabetes-induced microvascular and neuronal cell apoptosis was significantly reduced in HM-61-treated db/db mouse (p < 0.01 vs. vehicle). Similarly, the administration of HM-61 also inhibited the development of diabetic cataract through the inhibition of sorbitol accumulation in lens fibers. These results indicate that HM-61 could provide a valuable therapeutic approach against diabetic ocular complications.

Acknowledgement: This research was supported by a grant (K10040) from the Korean Institute of Oriental Medicine (KIOM).

PF60

Effects of Angelicae Gigantis Radix (AGR) on Polycystic Ovary Induced by Estradiol Valerate in rats

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Angelicae Gigantis Radix (AGR) is the most frequently used medicinal plants for patients with gynecological problems, especially pregnancy (1,2). This study was designed to investigate the effects of AGR on Polycystic Ovary (POCO) induced by Estradiol valerate (EV) in female rats. We investigated the effects of AGR on Changes in body weights and food and water uptake for 5 weeks. In addition, we examined the effects on ovary weights. Finally, we also observed histopathological changes in POCO rats. In our results, AGR administration group showed ovary/body weight ratio to normal levels, which were lowered by induction of POCO (3). In histopathological observation, formation of cysts was suppressed in AGR group compared with non-treated POCO group. In conclusion, these results suggest that AGR can be used for patients with POCO to prevent formation of cysts or to delay and minimize the extent of ovary. References: 1. Yoo DL (2001)] Oriental Gynecology 14(1): 453 – 460. 2. Shin YW et al. (2003) Oriental Gynecology 16(4):180 – 188. 3. Farookhi R, Hemmins R, Braver J R (1985) Biology of Reproduction 32: 530 – 540.

PF61

Traditional Chinese medicines and their effective components for the treatment of rheumatoid arthritis: a systematic review

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Rheumatoid arthritis (RA) is a systemic, autoimmune, and one of refractory disease, which belongs to “arthromyodyina” in Chinese medicine. Traditional Chinese medicines (TCM) were utilized for treatment of RA in clinic for a long history. In recent years, a large number of experiments were carried out for anti-RA effects of TCM A and their effective components in China and many other countries. In this review, more than 20 Chinese medicines which are commonly used such as Tripterygium ignumlifolii Hook, Salvia miltiorrhiza Bunge, Paonia sterniana Fletcher, etc. and their effective components such as triptolide, sinomenine, total glucosides of paenoea for the treatment of RA are summarized. Furthermore, sinomenine injection, tripterygium glycosides tablet and total glucosides of paenoea as anti-RA drugs from TCM have been developed for therapy of RA and used popularly in clinic. Further investigations are required to elucidate the possible action mechanism of these medicines and components and determine their potential for clinical use needs to be demonstrated in clinical trials. Acknowledgement: This review was supported by the National Nature and Technology Commission Research Projects of Shanghai Municipality (100219172000).

PF62

Evaluation of antimicrobial effects of three traditional medicinal plants from Iran

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There is growing interest in use of plants as natural antimicrobial agents because they do not induce antibiotic resistance which is usually happened with the synthetic antibiotics. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infection diseases from various sources such as medicinal plants (1). The antimicrobial effects of different fractions of seed extract of Securigera securidaca L., fresh petal of Rosa damascena Mill. and aerial parts of Tripleurospernum discorfine Sch.Bip. extract were examined against four gram positive and four gram negative bacteria and 2 fungi, which were obtained.
from Department of Drug and Food Control, Faculty of Pharmacy, Tehran University of Medical Sciences. The seed of Securigera secundica, petals of Rosa damascena and top flowered of Tripleurospermum discerne were collected in September, May and July 2009 around the Fars, Gilan and Tehran Provinces of Iran, respectively. The antibacterial and anti-fungal activity were studied by cup plate diffusion method as described by Warnock DW (2) and Soybean Casein Digest Agar and Sabouraud Dextrose Agar were used as medium for the growth of bacterial and fungal strains, respectively. The petroleum ether and chloroform fractions of S. secundica showed antibacterial activities against Staphylococcus aureus and Pseudomonas aeruginosa, while methanol fraction had no antibacterial effects. R. damascena extract had antibacterial activities against Bacillus cereus, Staphylococcus aureus, Staphylococcus epidermidis and Pseudomonas aeruginosa. T. discerne extract demonstrated antibacterial effects against Staphylococcus aureus and Staphylococcus epidermidis. All the fractions of plants had no antifungal activities. References: 1-Berahou A, Auhmani A, Filé N, Benharfar A, Jana M, Gaddi CA (2007) Ethnopharmacol 112: 426 – 429 2-Warnock DW (1991) Methods with antifungal drugs In: Evans EG and Richardson MD. (Eds.) Medical Mycology: A Practical Approach. IRL Press, Oxford University Press. 179 – 200.

One of the Korean mistletoe species, Loranthus yadoriki Sieb. exhibited potent inhibitory activities against monoamine oxidases Hwang K1, Kim J1, Choi Y1, Choi K2, Park K2 1Plant Resources Research Institute, Duksun Women’s University, Seoul, Korea, 132 – 744 2Department of Agricultural and Food Science, Seoul National University.

It is well known that Viscum album L. is used in traditional medicine (TCM) and also contains small amounts of synephrine [1]. Although they have a wide usage in traditional medicine, there are only a few number of studies concerning the structure elucidation was achieved by 1D and 2D NMR spectroscopy (COSY, TOCSY, HSQC, HMBC) and FABMS.

The evaluation of biological activities of Loranthus yadoriki several bioassays were applied. Loranthus yadoriki showed potent inhibitory ability against both types of monoamine oxidases and dopamine beta hydroxylase. But Loranthus yadoriki did not inhibit NO production in the cell. We are isolating the bioactive compounds from this plant with MNO inhibitory activity as a guide assay. Acknowledgement: This work was supported by grants from Scientific research (KNA1 – 2-11,10 – 2) of Korea National Arboretum, Pocheon-si, Gyeonggi-do, Korea, 487 – 821.

One of the Korean mistletoe species, Loranthus yadoriki Sieb. exhibited potent inhibitory activities against monoamine oxidases Hwang K1, Kim J1, Choi Y1, Choi K2, Park K2 1Plant Resources Research Institute, Duksun Women’s University, Seoul, Korea, 132 – 744 2Department of Agricultural and Food Science, Seoul National University.


Antioxidant Activity of Eryngium kotschyi Boiss. Root Extracts

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Eryngium species, belonging to Apiaceae family are well known plants in ethnobotanical culture in the world and also in Turkey. They are used as antitussive, diuretic as well as for analgesic and antiinflammatory purposes. The herbal drugs for traditional Chinese medicines, In: Pharmeuropa 22.2 3. Bayer-(draft monograph), In: Pharmeuropa 22.4 2. EDQM (2010) Preparation of antifungal drugs In: Evans EG and Richardson MD. (Eds.) Medical Mycology: A Practical Approach. IRL Press, Oxford University Press. 179 – 200.


Antioxidant activity of Rhodomyrtus tomentosa (L.) Hassk. of Terengganu coastal area Afanani A, Abdul Manaf A Faculty of Agriculture and Biotechnology, Universiti Sultan Zainal Abidin, Terengganu, Malaysia

Rhodomyrtus tomentosa (L.) Hassk. has shown interesting capability to scavenge free radicals and hydrogen peroxide as well as play effective role inhibition of lipid peroxidation. Results of DPPH scavenging assay shows the IC50 of 30 μg/mL and the 80% maximum inhibition at the concentration of 100 μg/mL. The 50% inhibition against hydrogen peroxide is at the concentration of 0.17 μg/mL and the maximum inhibition of 98% at the concentration of 0.25 μg/mL. FTC and TBA assay shows the 77.11% and 95.88% inhibition, respectively. Acknowledgement: The authors are thankful to the Faculty of Agricultural and Biotechnology, University of Sultan Zainal Abidin for the funding and research facilities

Contents of ephedrine-like alkaloid synephrine in traditional Chinese decoctions Spriano D, Meier B 1Department of Pharmacognosy, Faculty of Pharmacy, University of Ankara, Ankara, Turkey; 2Department of Traditional Medicine, Faculty of Medicine, Ataturk University, Elazig, Turkey

The FDA’s ban on ephedra has led to an increase in the use of the ephedrine-like alkaloid synephrine, in dietary supplements for the purpose of body loss. Synephrine naturally occurs in bitter-orange (Citrus aurantium L.) and other Citrus species. Concerns have been raised about the safety of products containing synephrine. Tangerine peel (Citrus reticulata Blanco; Chenpi) is a herbal drug used in traditional Chinese medicine (TCM) and also contains small amounts of synephrine [1]. Traditional decoctions [2] of this drug are evaluated: i.e. the extraction yields for synephrine in dependence to extraction time. Thereof, an assumed daily intake is calculated for synephrine. Results showed a content of synephrine of 3.0 mg/g in the herbal drug (batch 1). Traditional decoction resulted in extraction yields of 63% synephrine, referred to dried drug. An extraction profile over time showed similar yields (about 67%) also after 3 hours of decoction. Maceration in cold water was about the same effective, yielding up to 71% of synephrine after 3 h. The analysis of a second herbal drug batch showed a synephrine content of 1.7 mg/g. Traditional decoction of it resulted in an extraction yield of 75% synephrine. A longer decoction lasting 2 hours lead to extraction yields up to 93%. It can be concluded that, assuming a daily dose of Chenpi of 3 – 9 g, there could result a daily intake of up to 19 mg synephrine. Such doses are below the levels exhibiting pharmacological effects, which are reported to be of 100 to 150 mg [3]. Acknowledgement: We thank Lian Chinaherb, Switzerland, for the supply of herbal drug material. References: 1. EDQM (2010) Mandarin episcarp and mesosarp (draft monograph), In: Pharmeuropa 22.4 2. EDQM (2010) Preparation of antifungal drugs In: Evans EG and Richardson MD. (Eds.) Medical Mycology: A Practical Approach. IRL Press, Oxford University Press. 179 – 200.
In African and Asian countries with low incomes up to 80% of the populations depend on traditional medicine for primary health care (WHO, 2008 World Health Report). In West-African countries and especially in the northern region of the Republic of Benin patients with pain-associated diseases used traditionally some plants like Entada africana Guill. & Perr., Ficus thornningii Blume, Entada collarium Freyrel., Fadogia agrestis Schwein. ex Hiern, Pilosilgisma thonningii (Schumach.) Milne-Redh. and Chasmanthera sp. Primary goal of this project was to test a potential analgesic effect of novel gel formulations containing a defined combination of ethanolic or aqueous extracts of these plants on pain associated with irritation provoked by arthritis or musculoskeletal trauma. Second objective was to compare effectiveness of the novel gel-formulations with diclofenac gel. The analgesic effect was assessed using criteria of evaluation within 10 days. 81.8% of the patients (n = 11) responded with reduced pain score after topical application twice daily of the gel formulation containing the ethanolic extracts, whereas the water-based formulation was less effective (57.1%; n = 7). Diclofenac gel (25 mg) reduced the pain by 58.3% (n = 12) of the patients within two weeks. The results demonstrated that the ethanolic extract was more effective than the aqueous extract and the well established diclofenac gel. Our study involving pain-associated patients emphasized the importance of an adequate formulation for extracts used in the traditional medicine and pointed out that a combination of plant extracts could an effective alternative to topically applied synthetic analogics. Further studies are necessary to examine the mechanisms contributing to the analgesic effect of the plant extracts.

Anti-inflammatory activity from Limonium brasiliense (Boiss.) Kunzte
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Limonium brasiliense Kunzte (Plumbaginaceae) is a medicinal plant, known as "Guaycuru" from southern Argentina. Infusion from the roots is popularly used in the treatment of hemorrhage, menstrual disorders, rheumatism and it is believed to have cardioprotective properties [1]. The aim of this work was to evaluate the anti-inflammatory activity of methanolic extract from roots of L. brasiliense and its major constituent, myricetin 3-O-rhamnoside, in vitro. [2, 3] This extract was partitioned with different solvents of increasing polarity to obtain sub-extracts that were fractionated by silica gel column chromatography, for isolation and purification of the active compounds. The fractions and isolated compounds have been tested in cultures cell lines. These were not cytotoxic against RAW 264.7 and HL60 cells lines. Thus, these fractions and the isolated compound have been tested in inhibition of Nitric Oxide (NO) overproduction on LPS-stimulated RAW 264.7 cells. The best anti-inflammatory potency (40 μg/mL = 63% inhibition) was provided by a fraction coming from the ethyl acetate sub-extract. This fraction contains myricetin 3-O-rhamnoside. (IC50 = 13.29 μM/mL) Also, we investigated the antioxidant effects of these fractions and the isolated compound on inhibition of intracellulare and extracellulare production of reactive oxygen species (ROS). These have inhibited both ROS production. The results presented demonstrated that myricetin 3-O-rhamnoside displayed a typical antioxidant activity; it markedly inhibited intracellular and extracellular ROS production. These results also support the claims of traditional medicine about the use of L. brasiliense roots in the treatment of inflammatory diseases. Therefore antioxidant research should also be extended to in vivo models. Acknowledgement: This work was supported financially by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, PIP N°0636), Universidad Nacional del Sur (UNS, PCIG
**PF71**

Evaluation of acute and sub chronic hepatotoxicity of hydroalcoholic extract of *Teucrium polium* L. in non-diabetic rats

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While it is well-known and widely used for its hypoglycemic and anti spasmodic properties in traditional medicine of many countries (1, 2), probable side effects of *Teucrium polium* L. (T.p), especially hepatotoxicity in diabetics, needs more investigation. The purpose of this study is to determine the acute and subchronic hepatotoxicity of T.p hydro alcoholic extract in non-diabetic rats. In acute phase, rats were given doses from 50 – 7000 mg/kg of the solution by gastric gavages. However, our paraclinic and histopathologic studies were focused on the dose of 3000 mg/kg. In sub chronic phase, 1000 mg/kg of the solution was given through drinking water once daily. On the day 45, liver damage was again evaluated through blood samples and biopsy. (3) There was no mortality seen, AST and ALT rose, more in females, but not to a significant level in either sex. Histopathologic examination revealed signs compatible with non specific reversible hepatic inflammation. The results were the same in both phases. Our study suggests that hydro alcoholic extract of *Teucrium polium* 1:1 is non-toxic in vivo and does not induce hepatotoxicity. However, the same result may not be seen in diabetic rats and that entails more investigation. Additionally, the growth place of T.p may have some effects on the results.

References:

**PF72**

Antioxidant and hepatoprotective activity of *Tragopogon porrifolius* methanolic extract

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*Tragopogon porrifolius* L. (Asteraceae), commonly known as purple salsify, is cultivated for its edible root and shoot. The present study investigates the in vivo and in vitro antioxidant activity of the methanolic extract of the aerial part of *T. porrifolius* as well as its protection against CCl4-induced hepatotoxicity in rats. Total phenolic and flavonoid contents, were assessed using the Folin-Ciocalteu and the aluminum chloride colorimetric methods and found to be 371 mg/g CAE and 16.6 mg/g QE respectively per gram dry weight of the extract. The FRAP assay is showed an antioxidant activity of 744 μmol Fe2+/g. In vivo, the extract (50, 100 and 250 mg/kg body weight) exhibited a dose dependent increase in the activity of liver antioxidant enzymes. The highest dose used increased the activity of catalase (CAT), superoxide dimutase (SOD) and glutathione-S-transferase (GST) by 222, 149 and 68% respectively. T. porrifolius extract also showed substantial hepatoprotective capacity against CCl4-induced hepatic injury by restoring the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) to normal levels at 250 mg/kg body weight dose. These findings suggest that *T. porrifolius* methanolic extract possesses antioxidant and hepatoprotective activity and can be used to prevent liver disorders. Acknowledgement: Mr. Jean Karam. References: 1. Tawaha K et al. (2007) Food Chem 104: 1372 – 1378. 2. Chandra T et al. (1987) Fitoterapia 58: 23 – 31.

**PF73**

In vitro schistosomicidal activity of triterpenoids from the African plant *Momordica balsamina* Ramalhete C1, Magalhães L2, Rodrigues V1, Mulhovo S3, Filho AS4, Ferreira MU5

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Schistosomiasis, also known as bilharzia, is a chronic liver and intestinal parasitic disease caused by trematode worms of the genus Schistosoma. Praziquantel is the only available drug against all forms of disease. The development of praziquantel resistance is a great concern and new drugs are urgently needed [1]. *Momordica balsamina* (Cucurbitaceae), commonly known as African pumpkin, is a vegetable widespread in tropical regions that has been used as food, mainly in sub-Saharan Africa. It has also been widely used in traditional medicine in Africa to treat various disease symptoms, mostly diabetes and malaria. In previous work, bioassay-guided fractionation of the methanol extract of the aerial parts of *M. balsamina* led to the isolation of several cucurbitane-type triterpenoids. Most of the isolated compounds as well as their acylated derivatives displayed antimalarial activity [2, 3]. Continuing our search for antiparasitic compounds, the aim of this work was to evaluate the in vitro schistosomicidal activity of several triterpenoids isolated from *M. balsamina* against Schistosoma mansoni adult worms [4, 5]. Praziquantel was used as positive control. A remarkable schistosomicidal activity was observed for two of the ten compounds tested (at 50 and 100 μM), which caused the death of all *S. mansoni* adult worms after 24 h of incubation. Both compounds, at 10 – 50 μM, induced significant reductions in the motor activity of the worms and significantly decreased the egg production. Furthermore, they were able (at 100 μM) to separate the adult worm pairs into male and female after 24 h. Acknowledgement: This study was supported by FCT, Portugal (SFRH/BD/22321/2005) as well as Fapesp (2006/60132 – 4) and CNPq, Brazil References: 1. WHO (2010). Fact sheet n/C30 115. 3. Ramalhete C et al. (2011) Bioorg Med Chem 19: 330 – 8. 4. Ramalhete C et al. (2011) Bioorg Med Chem 18: 5254 – 60. 4. Ramalhete C et al. (2011) Bioorg Med Chem 19: 330 – 8. 3. Magalhães LG et al. (2010) Parasitol Res 106: 395 – 401. 4. Magalhães LG et al. (2009) Parasitol Res 104: 1197 – 120

**PF74**

Phytochemical screening and the effects of aqueous extracts of *Phyllanthus amarus* leaves on the lipid profile and cardiac muscle cyclic guanosine monophosphate of male Guinea pigs

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Extracts of the leaves of *Phyllanthus amarus* Schumach. (Euphorbiaceae) are used as folk medicine for the treatment of jaundice and other diseases in Nigeria and other countries. Recently the extract is becoming popular for increasing or improving libido and reproductive functions in men. The effects of the aqueous extracts of the leaves of *Phyllanthus amarus* on lipid profile and the cardiac muscle cyclic guanosine monophosphate (cGMP) in male Guinea pigs was investigated and compared to the effects of sildenafil citrate on the same parameters. The phytochemical screening was also carried out. The results showed that the administration of aqueous extract of the *Phyllanthus amarus* leaves to the animals (100, 200 and 400 mg/kg body weight) caused a statistically non significant (p > 0.05) increase in cholesterol, triacylglycerol, low density lipoprotein and high density lipoprotein level while the administration of 100 mg/kg body weight of sildenafil citrate caused a non significant (p > 0.05) decrease in lipid profile levels but a non significant increase in the level of triacylglycerol. However the administration of aqueous extract of *Phyllanthus amarus* (100 and 200 mg/kg body weight) caused a non significant (p > 0.05) decrease in the level of cardiac cGMP, while the administration of 100 mg/kg body weight of sildenafil citrate and 400 mg/kg body of the aqueous extract cause a non significant increase p > 0.05 in the levels of cGMP. Furthermore, the phyto-
chemically screening of the leaves of *Phyllanthus amarus* revealed the presence of flavonoids, tannins, alkaloids, terpenoids, steroids, saponins and cardiac glycosides.

**PF75**
The effects of *Cucurbita pepo* seeds on testosterone induced benign prostatic hyperplasia

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The *Cucurbita pepo* L. (pumpkin) seeds are considered a snack food in most social gatherings in Lebanon and the Middle East. Many herbal combinations containing pumpkin seeds are used to treat symptoms of benign prostatic hyperplasia (BPH). Because the seeds are the most commonly consumed and not the seed oil, this study was carried out to examine the effects of pumpkin seeds on testosterone (3.57 mg/kg body weight) induced BPH in rats (1). After achieving hyperplasia (30 days), treatment with pumpkin seeds (10, 20, 30 and 60% w/w of chow) or finasteride (5 mg/kg body weight) was initiated for 12, 24, and 36 days. Results showed that pumpkin seeds exerted maximum inhibition (86.7, 98.0 and 98.4%) of hyperplasia at 30% w/w dose after 12, 24 and 36 days respectively. They were comparable to finasteride (78.1, 89.5 and 96.4%). There was no significant effect on weight gain in rats treated with testosterone and pumpkin seeds. Additionally, no significant effects were observed on levels of scOT-AST enzyme and ALP, while slight increase was observed on sGPT-AST. The findings on prostatic hyperplasia were confirmed by histopathological studies where tissue showed abundant stoma between glandular cells and lack papillary projections into the lumen of the glands. In conclusion, pumpkin seeds inhibit prostate hyperplasia induced by testosterone, and improve the histology of the prostate. Acknowledgement: Mr. Jean Karam. References: I. Gonzales G (2007) Asian J Androl 9(2): 245 – 251.

**PF76**
The most common medicinal plants of Sistan (Sistan & Balouchestan province, Iran) and some ethnobotanical aspects

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The uses of herbs to cure diseases have been common in folk medicine of Iran since the ancient times. In the present work, the most commonly medicinally important species used by local inhabitants of Sistan region in (south-east of Iran) were collected from various localities and identified by using related flora and comparison with herbaria specimens. This area is severely affected by arid and semi-arid climates with relatively poor vegetation. For each species botanical name, vernacular name, used part (s), popular medicinal usage, forms of preparation and application were also provided on the bases of ethnobotanical and folk beliefs in local culture. The essential oil content of some species is also determined by hydrodistillation method. Thirty species belonging to 22 families and 29 genera were recognized as the most common medicinal plants in the study area from which 2 species are cultivated (*Eucalyptus camaldulensis* Dehn., and *Aloe vera* L.), while the others grow as wild species. Also, only 2 species are monocotyledons and the others are dicotyledons. Some of the most important medicinal plants such as *Eucalyptus camaldulensis* (4%), *Cuminum cyminum* L. (3.5%), Trachyspermum ammicum Link (3%), Foeniculum vulgare L. (2%), *Nigella sativa* L. (1%), Mentha longifolia Huds. (1%) and *Citrus colocynthis* (L.) Schrad. (0.75%) showed high quantity of essential oils (Table 1), while *Peganum harmala* L. had the least amount (0.015%). The life forms of these plants were also determined. These medicinal plants are used in traditional medicine as diuretic, stomach improver, wound healing agent, antipyretic, expectorant, etc.

**PF77**
Prevention of carcinogen-induced mouse skin papilloma by *Daucus carota* (wild carrot) aqueous extract

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*Daucus carota* L. ssp. carota is a part of the folk medicine in Lebanon where it is used to enhance immunity and protect against many ailments among which are inflammation, gastric ulcer, and diabetes. Earlier we reported anti-inflammatory and anti-ulcer activities of the aqueous and methanolic extracts of the wild carrot umbels. Additionally, the aqueous extract of *D. carota* was found to possess anticancer activity against human promyelocytic leukemia HL-60 cells, while the oil extract exhibited chemopreventive effect against chemically induced skin cancer. The present study investigates the anticancer effects of the aqueous extract of *D. carota* umbels on DMBA-induced skin cancer in mice. The extract was administered to animals via either gavage or intraperitoneal routes for 20 weeks. Significant antitumor effects were observed with the intraperitoneal (250 mg/kg body weight) mode of treatment, where the percentage of papilloma incidence, yield and volume were reduced by 28, 23 and 86.4% respectively. Gavage treatment failed to inhibit tumor incidence, yield and volume. Intrapерitoneal treatment decreased hyperplasia and dermal infiltration with an increase in the level of hyperkeratosis. These findings demonstrate that *Daucus carota* aqueous extract possesses anti-tumor activity against DMBA-induced skin cancer. Acknowledgement: Mr. Jean Karam. References: 1. Diab-Assaf M et al. (2007) ACCR International Conference on Molecular Diagnostics in Cancer Therapeutic Development, Atalanta, USA. 2. Wehbe K et al. (2009) Journal of Complementary and Integrative Medicine(1): Article 7. 3. Abou Zeinab R et al, Pharm Biol in press.

**PF78**
Evaluation of cytostatic potential of *Helleborus purpurascens* extracts concentrated by membrane techniques

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*Helleborus* sp. is used in the adjuvant treatment of different tumors. Indications include various types of brain tumors in children, as well as prostate cancer, leukemia and lymphoma [1]. Our aim was to evaluate the cytostatic potential of *Helleborus purpurascens* Waldst. & Kit. concentrated extracts. Particular attention was devoted to investigate the possibility to purify and concentrate *Helleborus purpurascens* extracts by microfiltration, ultrafiltration and nanofiltration process, allowing the preservation of thermolabile compounds from the extracts and their antineoplastic properties examination. The bioactive compounds characterization has been effected by UV-Vis spectroscopy and HPLC. The concentrated Helleborus extracts “in vitro” testing, on Hela neoplastic cell cultures, has highlighted the cell proteinsynthesis alteration; protein dynamics modification; decrease of total cell number; cell viability diminution; inhibitory impact upon the cell cultures development. Concentrated Helleborus extracts induced an inhibiting effect upon cell cultures development of 90.7% at 72h, thus it can be considered a good source for further medicinal applications. Acknowledgement: This research was supported by the Romanian National Center for Program Management – P62076/2008 projects. References: 1. Jesse P et al. (2000) Pediatr Blood Cancer 32(4): 464 – 469.

**PF79**
NMR based Metabolomic Investigation of the Brazilian Medicinal Plant Carqueja: *Baccharis trimera* (Less.) DC

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In 2010 the ANVISA (the Brazilian Agency equivalent to the FDA in the USA) recognized 66 species of plants as medicinal plants and regulated its use [1]. Of most of these species only limited phytochemical information is available and doubts exist about the active components. On several species we initiated research with the objective to obtain the phyto-
tochemical fingerprints and relate them to the pharmacological activities. From *Baccharis trimera* (Less.) DC., a plant widely used in popular medicine, and known in Brazil under the name Carqueja, a series of samples was obtained from local markets and pharmacies. Using a protocol developed in our laboratory, the samples were directly extracted with deuterated solvents to provide two types of extracts: A polar aqueous extract and a palor chloroformic extract. These were subsequently analysed by NMR spectroscopy. In this comparison large differences were encountered in the chemical composition: e.g. the quantity of the flavonoid Epicatenin ranged between 0 and 10 mg/g of plant material. The differences in the phytochemical profiles generate large doubts about the efficient use of this medicinal plant. But, considering that the active components have not been identified, it also offers a possibility to find novel drug leads, by metabolomics, in which activity profiles of the individual samples are directly compared with the chemical profiles. With sufficient samples and efficient biological tests the active components will be revealed. Acknowledgement: CNPq, FAPERJ, CAPES.


**Effect of *Garcinia kola* Heckel seeds on bioavailability of two commonly used drugs in Nigeria (sulphamethazine and paracetamol)**

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*Garcinia kola* Heckel (Guttiferae), is a large forest tree found throughout Western and Central Africa. Widely known in commerce as ‘bitter kola’, its fruits are thus used locally as antidote to poisons and in cases of drug overdose. They reduce the effectiveness of drugs and toxic substances in general. They are thus used locally as antidote to poisons and in cases of drug overdose. The effects of concurrent administration of *G. kola* whole seed suspension were investigated on the bioavailability of two commonly used drugs in Nigeria, namely, sulphamethazine, a sulphonamide anti-biotic, and paracetamol, an analgesic and antipyretic compound. Five specimens of *G. kola* fruits were used. Two groups of rabbits (n=4 each) were treated by gavage with a concentration of 0.5 g/kg body weight of seed suspension given concurrent with 150 mg/kg body weight of sulphamethazine and paracetamol respectively. Control groups were given equivalent doses of either drug alone. Blood was withdrawn from the left ear at one hour intervals for five hours. Results showed that *G. kola* seeds decreased significantly (p<0.05) the bioavailability of the two drugs. Relative bioavailability was calculated to be 77.56% for sulphamethazine and 73.39% for paracetamol. The time of peak and peak concentrations were also reduced, while the concentration at one hour was only significantly different from that of paracetamol at p<0.05. These results suggest that *G. kola* seeds may reduce bioavailability by interfering with drug absorption across the gastrointestinal mucosa. Acknowledgement: The Management and Staff of Emmanuel Research Laboratory, Enugu Nigeria for allowing the use of their facilities.


**Purification of verbascone from plant extracts using column and countercurrent chromatography**

**Purification of verbascone from plant extracts using column and countercurrent chromatography**

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Verbascone, a phenylethanoid glycoside, displays diverse biological activities. The antifungal activity of *L. camara* extracts was tested by three methods to increase the verbascoside content could provide natural myco-biocides for postharvest control of pathogens on fruit. Verbascoside was estimated from dried *L. camara* leaves and flowers and leaves from *L. camara* could serve as a good source of verbascoside. Several stability tests were conducted to evaluate the stability of the compound under different conditions. The shelf life stability study proved that the compound is stable in a dry form when stored in the dark, but decomposes rapidly when exposed to light. Verbascoside also proved to be reasonably stable under steam distillation conditions.


**Comparison of mangiferin in mango leaf and honeybush infusions**

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Mangiferin reportedly exhibits antioxidant, anticancer, antimicrobial, antischlerotic, anti-allergic, anti-inflammatory, analgesic and immunomodulatory action in humans (1). Xanthone has demonstrated a potent anti-inflammatory activity on the proliferation of tumours and malformed cells. Mangiferin in leaves of mango tree cultivars to investigate the possibility of using mango leaves as a health beverage. Mangiferin was extracted by infusing the dried leaves in boiling water and the extract was subsequently quantified using UV-Vis (3), HPLC and HPTLC (4). Spectroscopic methods were used to analyse the powdered leaf materials. Chromometric analysis (O-PLS) was used to develop a predictive model for mangiferin. Extracts of the mango leaves were added to fruit juices and the stability of mangiferin determined regarding time, light and pH. The levels of mangiferin in mango were compared to those found in honeybush teas. Mangiferin levels found in a leaf infusion of mango indicated that mango leaves may have more health benefits than honeybush tea. References: 1 Mashibo M, He Q (2008) Compr Rev Food Sci Food Safety 7: 306 – 319 2 McKay DL, Bloomberg JB (2007) Phytother Res 21:1 – 16 3 Joubert E et al. (2008) Phytochem Anal 19:169 – 178 4 Rastogi R et al. (2007) Planar Chromat 20: 317 – 320.

**Inhibitory Effect of Crude Aqueous *Brucea amarissima* Extract on the Growth Profile of Oral Candida**

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The prevalence of oral Candida infections has increased, due to immunosuppressive effect of antifungal agents on resistant hosts (1,2). Growth rate is a key attribute of virulence among infectious microorganisms including *Candida* species. The aim of this study was to evaluate the growth inhibitory effect of *Brucea amarissima* (Lour.) Merr. leaves extract based on changes in the pattern of growth profile of *Candida* sp. *Candida albicans*, *Candida tropicalis* and *Candida dubliniensis* were used in this study. Crude extract of *B. amarissima* was prepared and the minimal inhibitory concentrations (MICs) towards *Candida* sp. were determined. The growth responses were recorded based on changes in the doubling time (g-values) and specific growth rates (r-values). The values in the presence of extract were computed as percentage in the optical density (OD) relative to the total cells suspension in the absence of extract. 0.12% w/v chlorhexidine (CHX)-containing mouth rinse and sterile distilled water were used as controls. *C. tropicalis* was found to have the highest growth rates indicating high bioactivities and reproducibilities. *C. dubliniensis* and *C. tropicalis* showed the highest reduction of r-values at a minimal concentration with 87.04% and 57.28%, respectively. At higher concentration (6 mg/mL), the extract exhibited significant reduction towards the growth (p<0.05). Also, was able to reduce the r-values of all *Candida* strains with more than 90% reduction. The extract

There are several species of medicinal plants known as “Node-to-dog”. The most used is the *Heteropterys aphrodisica* O. Mach. (Malpighiaceae). We conducted field and laboratory studies and also review on popular usage, occurrence and bioactivity of *H. aphrodisica* in southwestern Mato Grosso (MT-sw), Brazil from 2003 – 2009. The plant occurs in savannah and field areas. It is widely used as an aphrodisiac by local communities. Its beautiful flowers are ornamental, yellow, often visited by wild bees. The plant has long, expanded and nodulated roots, hence the name of “node-to-dog”. It spreads easily by seeds, adapts well in adverse environments and resists fires. Experiments on more appropriate systems of cultivation, developed in Cáceres (MT-sw), revealed that the species adapts equally well in mono and polyculture. The root of the adult plant is used in folk medicine to treat nervous disorders, sexual problems, high cholesterol, and is physical invigorating. The root is macerated in wine for consumption as an aphrodisiac. There are substances in the *H. aphrodisica* promising to treat fatigue, memory loss and Alzheimer’s disease. Phytochemical analysis revealed the presence, in root extracts, of polyphenols, tannins, alkaloids; cardiotonic, aromatic and flavonoid glycosides; and of saponins. In MT-sw it is a commonly used medicinal plant and the root is harvested in boxes. Acknowledgement: Fapemig – financial support, and for UNEMAT – institutional support; to the collaborators colleagues from the research group FLOBIO – (Plants carrying Bioactive substances)

Several plant species (> 10) known as “Leather hat” (*Echinodorus spp.* – Alismataceae) used in traditional medicine. We made field and laboratory studies and review on popular usage, occurrence and bioactivity of “Leather hat” in Mato Grosso (MT), Brazil, from 2003 – 2007. The following species have been known to occur in MT: *E. grandiflorus* Mitch., *E. lanceolatus* Ratj., *E. macrophthalmus* Kunth and *E. teretoscapus* Haynes. They prefer humid tropical conditions. The species *E. grandiflorus* and *E. macrophthalmus* are also used as tea. Studies of ethnic knowledge in southwestern MT revealed that leaves of “Leather hat” are used as anthophoric, antiseptic, diuretic, laxative, astringent, for gargling, washing sores, treating kidney, liver, arthritis, rheumatism, high blood pressure; roots – to treat atherosclerosis, hernia, boils; leaves and flowers – to treat syphilis, purgative; roots, leaves and flowers – for skin disorders. The healers collect the plant in the dry season, and indicate that *E. grandiflorus* and *E. macrophthalmus* are used as tea, poultice, and tincture. Some scientific studies show significant results with “Leather hat”: leaf infusion confirmed analgesic, anti-inflammatory and diuretic effect in experimental animals; tea showed diuretic activity; aqueous extract of dried leaves of the plant showed abortifacient activity in rats; prolonged use can lower blood pressure; and tincture, in excessive dose, can cause diarrhea. Use-potential: medical, industrial, ornamental, ecological. Acknowledgement: Fapemig team – for financial support; UNEMAT team – by institutional support; Colleagues of the research group FLOBIO – (Plants carrier of bioactive substances) who collaborated in this study.

In the present study we have investigated the antidiabetic effects of bulbs of Persian shallot *Allium ascalonicum* L., (Alliaceae) methanolic extract (AAE) at doses of 500 and 250 mg/kg bw. on Allsuan-induced diabetic male Wistar rats in comparison with Acarbose (as a reference drug), by measuring postprandial blood glucose (PBG), oral glucose tolerance test (OGTT), inhibition of rat intestinal α-glucosidases activities, changes in the levels of plasma lipid profiles antioxidant enzymes activities, including superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), pancreatic Insulin and cardiac Glut-4 mRNAs expression. In diabetic Wistar rats, in short term period, effects of AAE on PBG showed significant reduction after 24h of oral administration. After 3 weeks of treatment, significant chronic decrease in the PBG was observed. For OGTT, the increase in PBG levels reduced mildly in AAE treated diabetic rats, at 1hour. Intestinal sucrase and maltase activities were inhibited by AAE, 17.41% and 14.62% respectively. In diabetic rats, AAE also increased the activities of SOD by 65%, GPx by 43% and CAT by 55%, showing strong antioxidative effects. AAE demonstrated antihyperlipidemic properties by reducing serum TG, LDL, VLDL and TC. In addition, we have observed increased expression of Ins and Glut-4 genes in diabetic rats treated with methanolic extract of *Allium ascalonicum*, compared to control group. Acknowledgement: The authors would like to thank the Cellular and Molecular Department of University of Tehran for financial support. References: Vincent AM, Russell JW, Low P, Feldman EL (2004) Endocr Rev 25: 612 – 28. Marcus AO (2001) Postgrad Med 110: 113 – 23. Day C (1998) Br J Nutr 80: 5 – 6. Augusti KT & Sheela CG (1996) Experientia 52: 115 – 120. Feshani AM, Kouhsari SM, Mohammadi S (2010) J Ethnopharmacol 133: 67 – 74.
Molecular and biochemical effects of the methanolic extracts of the leaves of Salvia officinalis on diabetic male wistar rats

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Common sage (Salvia officinalis L.) is among the plants that are claimed to be beneficial to diabetic patients and previous studies have suggested that this plant has hypoglycaemic effects in normal and diabetic rats. In the current study, we have investigated the effects of methanolic extract of S.officinalis leaves on blood glucose, plasma biochemical parameters, pancreatic insulin and cardiac Glut-4 mRNAs expression, inhibition of rat intestinal α-glucosidase activities and erythrocyte antioxidant enzymes. Treatment of Alloxan monohydrate -induced diabetic rats with oral administration of sage leaves methanolic extract for 3 weeks, resulted in a significant reduction in blood glucose (Glucose oxidase assay); Total cholesterol (TC), triglycerides (TG), LDL/HDL ratio and VLDL were mildly decreased after treatment by the extract of the plant. We have also observed significant enhancement in activity of blood antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), we have observed increased expression of ins and Glut-4 genes in diabetic rats treated with methanolic extract of S. officinalis, compared to control group. The extract showed strong inhibition effect on intestinal α-glucosidase enzymes activities. Acknowledgement: The authors would like to thank the Cellular and Molecular Department of University of Tehran for financial support. References: Lin YF, Tsai HL, Lee YC, Chang SL (2005) Journal of Nutrition 135: 2457 – 61. Lu YR and Foo LY (2001) Tetrahedron Lett 42: 8223 – 8225. Wang M et al. (1998) J Agric Food Chem 46: 4869 – 4873.

New Approaches in Characterisation of Herbal Preparations

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New techniques have recently been established in instrumental analytics and molecular biology, which are also applicable to medicinal plants could demonstrate the suitability of such methods for the identification of herbal preparations by PCR-based methods and for characterisation of herbal preparations by NMR-fingerprinting in combination with principal component analysis [1, 2]. The next step is characterisation of biological activities and correlation with fingerprint profiling. Extracts with solvents of different polarity (Ethanol, Ethanol/Water, Dichloromethane) were obtained from herbal substance of Cheilosium majus L. and characterised by means of HPLC and NMR-fingerprinting. Extracts were applied to human liver cells (HePG2) and cell proliferation was monitored in a real time cellular monitoring system, xCELLigence (Roche). Growth of HepG2 cells was drastically inhibited when administered orofratically to diabetic rats of the plant extract. We have also observed significant enhancement in activity of blood antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), we have observed increased expression of ins and Glut-4 genes in diabetic rats treated with methanolic extract of S. officinalis, compared to control group. The extract showed strong inhibition effect on intestinal α-glucosidase enzymes activities. Acknowledgement: The authors would like to thank the Cellular and Molecular Department of University of Tehran for financial support. References: Lin YF, Tsai HL, Lee YC, Chang SL (2005) Journal of Nutrition 135: 2457 – 61. Lu YR and Foo LY (2001) Tetrahedron Lett 42: 8223 – 8225. Wang M et al. (1998) J Agric Food Chem 46: 4869 – 4873.

Assessing the in vitro gastric stability and intestinal transport of selected natural molecules

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Natural antioxidants in foods and plants play a major role in helping the body’s defense system to fight the destruction caused by reactive oxygen species. They may act by decreasing oxygen concentration and as metal inactivators, hydroperoxide decomposers, oxygen scavengers and synthases (Shahidi, 1997). Several have had the possible beneficial effects of natural antioxidants in the human body, without considering the influence the gastrointestinal tract may have on the composition, activity and absorption of these compounds (Cao et al., 1998; Serrano et al., 2007). This study aimed at assessing the in vitro gastrointestinal stability and intestinal transport of orally ingested anti-oxidants in food and plants. Curcumin, epicatechin, ferulic acid, gallic acid, quercetin, resveratrol, rosmarinic acid, and rutin were selected. Compounds were incubated in simulated gastric fluid (SGF, pH 1.2) for 1 hour and in simulated intestinal fluid (SIF, pH 6.8) for 3 hours. Concentrations were detected before and after incubation. The results indicated that all the compounds were stable in SGF, only epicatechin and rutin were unstable in SIF (with 6.27% and 5.16% degradation, respectively). The in vitro transport experiment was conducted across porcine intestinal tissue in the apical-to-basolateral direction in a Sweetana-Grass.
Antihyperlipidemic and antidiabetic effects of Pinus koraiensis leaf oil

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Metabolic disease is a complex syndrome to develop cardiometabolic risk factors, including central obesity, insulin resistance, glucose intolerance, dyslipidemia and hypertension. In the present study, anti-diabetic and hyperlipidemic activities of essential oil from leaves of Pinus koraiensis Sieb. & Zucc. (EOPK) were evaluated. EOPK significantly reduced the blood glucose concentration in streptozotocin (STZ)-induced diabetic mice without weight loss, while significant weight loss was observed in STZ treated mice. Furthermore, EOPK significantly suppressed the production of α-amylase, an enzyme that catalyzes the breakdown of carbohydrate into glucose, in a dose-dependent manner and also prevented the STZ-induced cytotoxicity and nitric oxide (NO) production in HIT-T15 pancreatic β-cells. In addition, EOPK significantly inhibited the level of human cholesterol acyltransferase (hCAT)-1 and -2 and reduced low-density lipoprotein (LDL) oxidation in a dose-dependant manner with an IC50 value of 40 μg/mL. Also, Gas chromatography-mass spectrometry (GC-MS) revealed that EOPK contains alpha-pinene (21.3%), alpha-terpineol (11.0%), 8-3-carene (10.2%), terpinolene (7.2%), camphene (6.2%) and limonene (5.2%). Taken together, our findings suggest EOPK can be a potent pharmaceutical agent for prevention and treatment of metabolic syndrome including diabetes and hyperlipidemia. Acknowledgement: This work was supported by a grant from the Next-Generation BioGreen 21 Program (PJ007998), Rural Development Administration, Republic of Korea. References: 1. Yang X et al. (2008) Fito- terapia 79(3):179 – 81. 2. Lee JH et al. (2008) Microbiol Biotechnol 18(3):497 – 502. 3. Nyenwe EA et al. (2011) Minerva Endocrinol 36(2):129 – 45.

Antioxidant activity, total phenolic and flavonoid content of Ficus deltoidea Jack varieties

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Six varieties of Ficus deltoidea Jack namely F. deltoidea Jack var. deltoidea, F. deltoidea Jack var. angustifolia (Miq.) Corner, F. deltoidea Jack var. intermedia Corner, F. deltoidea Jack var bilobata Corner, F. deltoidea Jack var. trenggannuensis Corner and F. deltoidea Jack var. kunstleri (King) Corner were collected from various locations in Peninsular Malaysia. The total phenolic content in Ficus deltoidea var. intermedia was remarkably high, followed by varieties kunstleri, trenggannuensis, deltoidea and bilobata. The lowest flavonoid content was observed in the angustifolia variety. Antioxidant activity of aqueous extracts was determined by measuring DPPH and H2O2 scavenging activities. Very strong antioxidant activity was observed for the extract of intermedia variety with IC50 of 40 μg/mL, whereas moderate activities were recorded in the extracts of bilobata and kunstleri varieties with IC50 of 150 and 200 μg/mL, respectively. Lower DPPH scavenging activities were observed in the extracts of trenggannuensis, deltoidea and angustifolia varieties; with IC50 of 325, 380 and more than 500 μg/mL, respectively. Higher H2O2 scavenging activity was observed in all varieties studied, when measured at 500 μg/mL as compared to vitamin C.

An ethnobotanical survey of medicinal plants in Valopei district of Savadkouh (Mazandaran-Iran)

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This study was aimed to identify wild plants collected for medical purposes by the local people of Valopei County, located in the Northern of Iran, and to establish the uses and local names of these plants. Field study was carried out over a period of two years (2009 – 2011). Valopei district with over thousand years oldness, 52 villages and high rich knowledge about useful plants is situated in central Elburz region of Iran. A total of 66 plant species belonging to 61 genera and 39 families have been reported from the study area [1]. The most encountered medicinal plant family was Lamiaceae with seven species. The plants are used as medicinal, food and vegetable, provender, treatment of animal diseases, hunting, building construction, manure, weaving and dyeing, respectively based on significance. The use of several species such as Ligularia persica Boiss., Albizia julibrissin Durazz., Ruscus hyrcanus Woronow, Gedchitsia caspica Desf., Tamus communis L., Hyoscyamus niger L., Quercus castaneifolia C.A.Mey., Punica granatum L., Calystegia sepium (L.) R.Br. and Potentilla reptans L. are reported for the first time. Mode of preparation and administration are discussed along with the family and local names of plants and plant parts used [2]. References: 1. Rechercher KH (1963 – 1999) Flora Iranica, Vol. 1 – 174. Akademische Druck und Verlaganstalt Graz. Austria. 2. Martin GJ (1995) Ethnobotany. A methods manual. London: Chapman & Hall.

Ethnobotanical and ethnopharmacological study in the practice of midwifery in pastoral Iran

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In this study the ethnobotanical and ethnopharmacological features of twenty medicinal plants native to Iran were investigated. Midwives in pastoral communities across the world are very important as main health care providers, but few researches have recognized the therapeutic plants engaged in this age-old practice. Semi-structured interviews were carried out with 5 midwives in 12 pastoral communities near Kermanshah, Iran, concerning the plants they utilize during child delivery as well as pregnancy. Twenty different plant species used to treat 5 conditions happening during the pregnancy, birth and postpartum stages were recorded. Most plants and uses were reported by only one or two midwives. It is interesting to mention that most midwives in this area had emigrated from different parts of the country. Approximately all the midwives used or knew of plant remedies for the treatment of miscarriages, postpartum abdominal pain and hemorrhages, retained placenta, and for speeding up contractions during labor. The most commonly cited plants as well as those for which there was greatest consensus tended to be widespread cultivated or wild species. Although use of medicinal plants by midwives has been reduced as a result of retraining programs by government health centers, midwives’ knowledge of medicinal plants may provide an important resource for improving maternal-infant health in Iran and elsewhere. References: 1. Tahraoui A, El-Hilaly J, Israli ZH, Lyoussi B (2007) Ethnopharmacol 110: 105 – 117. 2. WHO (2000), www.who.int/diabetes/facts/world-figures (World Health Organization, Switzerland).

Myoporum laetum G.Forst. (Myoporaceae) is an evergreen ornamental shrub and it flowers from May to June [1]. Fractionation and isolation of the butanol extract of *Myoporum laetum* yielded five major flavonoids, luteolin 4”-O-"l-rhamnoside, 5-methoxy-luteolin 7-"o-"l-rhamnoside, 5’-hydroxy-luteolin 7-"o-"l-D-glucoside (tricetin 7-"o-"l-D-glucoside), luteolin and apigenin. Their structures were determined by chromatographic and spectroscopic methods. The hepatoprotective and antioxidant activities of the butanol extract against liver injury induced by repeated doses of the profibrotic hepatotoxicant dimethylsulfoxide (DMSO). Oral supplementation of butanol extract to progenofos treated animals successfully modulated the hepatotoxicant induces deviation in the liver function markers (liver oxidative and antioxidant markers) indicating its potential hepatoprotective and antioxidant abilities. References: 1. Blackburne et al. (1972) Aust J Chem 25: 1787 – 96.

In previous studies *Apodytes dimidiata* E.Mey. ex Arn. (Icacaceae) displayed activity against leishmaniasis [1]. Therefore, a bioassay guided isolation was performed in order to isolate the active constituents. Six saponins, never isolated from nature before, were identified by LC-MS/ MS, GC-MS and 1D and 2D NMR: apodytine A (compound 1), apodytine B (compound 2), apodytine C (compound 3), apodytine D (compound 4), apodytine E (compound 5), apodytine F (compound 6). Compounds 1, 2 and 3, having an acetyl group at the same position in the aglycon part of the molecule, were more active against *Leishmania infantum* (IC50 values < 1 µM) and cytotoxic against MRC-5 cells (CC50 values < 100 µM) than compounds 4, 5 and 6 (IC50 < 10 and CC50 < 20µM respectively), which contain a hydroxyl functionality at the same position. These compounds, responsible at least in part for the antileishmanial activity of the plant, also showed a haemolytic activity, and antiamoebic activity in the rat aorta ring assay. The latter might be due to a non-selective toxicity. Since saponins are known for their molluscicidal activity, the presence of these compounds might explain the use of *Apodytes dimidiata* against the snails *Bulinus africanus* and *Biomphalaria pfeifferi*, intermediate host snails of Schistosoma spp. [2].
Figure 1: Compound 1 – 6 (Apodyteine A – F)


Previous phytochemical investigation of Ormosiaceae kirkii S.Moore (Pa-pilinaceae) has led to the isolation and identification of (3-β-D)-β-hilflavonoids, some of them with antimalarial activity (Dhooghe et al., 2010). The major compound of the total extract (80% MeOH) was (+)-chamaequasmin. However, a series of minor constituents remained unidentified. The aim of this work was to further characterise the 80% MeOH extract from O. kirkii and to identify the minor constituents using an integrated platform of LC-MS and LC-SPE-NMR. The crude 80% MeOH extract of O. kirkii was partitioned into four fractions by means of liquid-liquid extraction: n-hexane, chloroform, ethyl acetate and water. The chloroform and ethyl acetate fractions were used for the LC-SPE-NMR study. By using the multiple trapping technique several components were enriched on the SPE cartridges. After drying and eluting with deuterated methanol into 3 mm NMR tubes, high-resolution NMR spectra were recorded. The structure elucidation of these compounds was based on 1D and 2D NMR, and MS data. A total of sixteen compounds were identified and assigned in the HPLC chromatogram: sikkianin B (1), sikkianin C (2), galbrosiflavonane A (3), diphyllolone (4), (+)-chamaejasmin (5), isoschamaejasmin (6), apigeninyl-(1-3,2-3)-naringenin (7), liquiritigeniyl-(1-3,2-3)-naringenin (8), (-)-3,3'-biquitinogen (9), 3,5'-dimethyldihydropyridine (10), 4'-hydroxydihydropyridine (11), 7-O-glucosylchamamejjasmin (12), dihydropyrdin (13), oromoracin (14), 7-O-glucosyl-dihydrodihydropyridine (15), and isovertxin (16). In addition to the twelve constituents (5-16) that had been isolated before using semi-preparative HPLC, four more compounds were obtained (1-4).


Dryobalanops aromatica is one of the Dipterocarpaceae families which contain oligostilbenos that show various biological activities. The oligostilbene in Dryobalanops genera is very unique, as some compounds such as cis- and trans-diptoindonesin B and malayanol A have different oxidative pattern compared to other oligostilbenos in Dipterocarpaceae [2, 3]. The aim of this study is to isolate the oligostilbene constituents in Malaysian D. aromatica C.F.Gaertn. and to determine their cytotoxic activity. The dried powder of the stem bark of D. aromatica was macerated with acetone and evaporated under reduced pressure. The crude of acetone extract was subjected to vacuum liquid chromatography (VLC) to give 10 major fractions. Purification of the sixth fraction with radial chromatography and am (13) gave laevifonol (1) (93 mg) and amegolosin E (2) (397 mg) while the fifth fraction afforded α-viniferin (3) (91 mg) and ε-viniferin (4) (20 mg) and the tenth fraction yielded diptoindonesin A (5) (30 mg). The effect of the isolated compounds (1 – 3) against HL-60, MCF-7, HepG2, A-549 and WRL-68 cell lines were evaluated by using


Figure 1: Compound 1 – 6 (Apodyteine A – F)
In our continued efforts to identify the immunoactive constituents of a local mistletoe species in Eastern Nigeria, a novel sesquiterpenoidal acid and an alkaloid from leaves of the Eastern Nigerian mistletoe with potent immunostimulatory activity on C57BL/6 spleenocytes

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A novel sesquiterpene acid and an alkaloid from leaves of the Eastern Nigerian mistletoe with potent immunostimulatory activity on C57BL/6 spleenocytes

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Flavonoids from Centaurea gloriosa and antioxidant activity of its extract

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In the early nineties the presence of flavonoids in herbal began to attract the attention of a number of researchers, as a result of their biological and physiological importance [1]. The present study deals with the isolation and identification of flavonoids from Centaurea gloriosa Vahl. and evaluation of antioxidant activity of the extract. The aqueous alcoholic extract (MeOH:H2O 7:3) of Centaurea gloriosa aerial parts was subjected to extensive repeated column chromatography on polyamide, and Sephadex LH-20 resulted in two new flavonoids named querectin 6-methoxy –7-O-galactoside (1) and querectin 6,4- dimethoxy –7-O-galactoside (2) as well as apigenin 8-C-glucoside, apigenin 6-C-glucoside, quercetin 6-methoxy, quercetin and apigenin. Structures of the isolated compounds were established by chromatography, UV, HRESI-MS and 1D/2D 1H/13C NMR spectroscopy. The radical scavenging activity of the extract was quantified spectrophotometrically, using DPPH radical. The effective dose 50 (ED50) of the extract was compared with that of standard antioxidants as vitamin C.

radial chromatography (CHCl₃-MeOH system) to yield compound 1. Fraction 5 was subjected to VLC (Hex: EtAc: Ac system) and purified by radial chromatography repeatedly (CHCl₃:EtAc:MeOH system) to give compound 2, 3 and 4. The structures of compounds 1–4 were determined based on spectroscopic data including UV, IR, 1D and 2D NMR and comparison with those previously reported. The similarity to these published data from Tanaka et al. [1, 2] and Ito et al. [3] suggested compound 1 as davidiol A, a trimer resveratrol and compound 2, 3 and 4 as tetramer resveratrols namely hemisephenol, isohopeaphenol and hemsleyanol D, respectively. Acknowledgement: Scholarship of one of the authors was financed by the National Science Fellowship (NSF) from Ministry of Science, Technology and Innovation Malaysia (MOSTI). References: [1] Tanaka T et al. (2000) Phytochemistry 53: 1009 – 1014. [2] Tuikinan AS et al. (2005) Biochemical Systematics & Ecology 33: 631 – 634. [3] Tanaka T et al. (2001) Heterocycles 55:729 – 740.

Isolation of Trimer stilbenoids from the bark of Shorea maxwelliana

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The stem bark of Shorea maxwelliana King (2.85 kg) was collected from reserved forest Jengka, Pahang, Malaysia. Three major trimer stilbenoids have been isolated from acetone extract of the stem bark of Shorea maxwelliana. Sample of S. maxwelliana was macerated in acetone for five times, and the solvent evaporated using rotary evaporator to produce crude acetone extract. The acetone extract (178.90 g) was fractionated by a series of solvent partitions into an EtOH soluble phenolic fraction (25.78 g). This fraction was repeatedly chromatographed on silica gel columns with Hex-EtOH (1:1) and EtOH, and then with a CHCl₃-MeOH gradient of increasing MeOH to provide four fractions (A–D). Fraction A was the n-hexane soluble fraction (126 g). Fraction B (226 g) was subjected to silica gel chromatography eluted with CHCl₃-MeOH (95:10 to 80:20) to give compound 1 (500 mg). Fraction C (9.8 g) was subjected to silica gel chromatography eluted with CHCl₃-EtOH-MeOH (85:1:0.5) to afford compound 2 (250 mg). Compound 3 (185 mg) was obtained from fraction D (11.2 g) by silica gel chromatography eluted with CHCl₃-MeOH (8:2) [2]. The molecular structures of 1, 2 and 3 were determined based on spectroscopic data, including UV, IR, 1H NMR, 13C NMR, 2D NMR and comparison with that reported in the literature. Spectral data of compounds 1, 2 and 3 showed very close similarity to α-viniferin [1], vaticanol A [1] and copaliferol A [2]. Acknowledgement: The authors would like to thank the Ministry of Science, Technology and Innovation, Malaysia (MOSTI) for funding the research grant, Fundamental Research Grant Scheme (FRGS), 600-BMI/FRGS 5/3 (Ps 19/2009) and National Science Fellowship (NSF) for financing the study of one of the authors. References: [1] Sahidin EEH et al. (2007) Journal Matematika dan Sains. 12(3): 113 – 118. [2] Sotheeswaran S., Sultanbawa M U S and Surendrakumar S (1983) Journal of Chemical Society Perkin Transactions (1): 659.

A study on exudates and micromorphology of Primula

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The genus Primula L. comprises more than 400 species, grouped in 6 subgenera and 37 sections [1]. Especially the production of oily or farinaceous exudates on aerial surfaces of leaves, stems, calyces and flowers is a conspicuous character of this genus. These exudates consist primarily of un-substituted flavones and other flavones with unusual substitution patterns, which are probably derived from a still unidentified biogenetic pathway [2]. Exudate profiles were monitored by HPLC and TLC, and known structures were identified by comparison of their UV-spectra and retention times with those of reference compounds of our spectral library. New structures were elucidated additionally by NMR spectroscopy. The auto-fluorescent property of some of these flavonoids was used for studying their accumulation in glandular hairs in vivo by using an epifluorescence microscope. Different colors of fluorescence were observed within a single leaf of P. vulgaris Huds., while leaves of P. vialii Delavy ex Franch. showed uniform fluorescence. The significance of our findings in relation to chemodiversity, morphology, and micromorphological character differentiation will be discussed. Acknowledgement: Hochschulfreibildungsstiftung der Stadt Wien, Gesellschaft zur Förderung der Pflanzenwissenschaften. References: [1] Richards J (2002) Primula, B.T. Batsford Ltd. London. 2. Valant-Vetschera KM et al. (2009) NPC 4: 365 – 370.

New Lanostane Triterpenoids from Antrodia camphorata

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Many polyoپes are used for medicinal purposes in traditional Chinese medicine. Antrodia camphorata (M. Zang et C.H. Su) Sheng H. Wu, Rynward et T.T. Chang, known as “niu-chang-chih”, is restricted to the endemic tree, Cinnamomum kanehira Hayata. Traditionally the fungus has been used for the treatment of food and drug intoxication, diarrhea, abdominal pain, hypertension, skin itching, and liver cancer. The components of this fungi have shown activities such as anti-inflammatory, immune-modulation, anti-Helicobacter pylori, and neuroprotection from Aβ damage. Here, we present our chemical studies on the mixture of fruiting body and mycelia of solid cultures of A. camphorata. As a result, eight new lanostane triterpenoids, 3,7,11-triexo-5α-lanosta-8,24-(E)-diene-26-ol (1), methyl 11α-hydroxy-3,7-dioxo-5α-lanosta-8,24(14S)-diene-26-oate (2), methyl 3,7,11,12,15,23-hexaexo-5α-27-cis-lanost-8a,16-ene-26-oate (3), ethyl lucidante A (5), ethyl linidicenolate F (6), acetyl ganolucidic acid A (7), 3,11,15,23-tetraexo-5α-27α,lanosta-8,16-dienoic acid (8), were isolated and elucidated. These compounds were evaluated for their cytotoxicity against several human tumor cell lines.

New type of polyacetylene sesquiterpenoid conjugates from Notopterygium incisum

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Qiang Huo, the dried rhizome and root of both Notopterygium incisum Ting ex H. T. Chang and N. forbesii Boiss. (Umbelliferae) are used widely in China for treating cold, and inflammatory diseases. Peroxisome Pro-liferator Activated Receptor gamma (PPAR-gamma) is involved in inflammatory processes, and has become an important pharmacological target. From the dichloromethane extracts of the underground parts of N. incisum, we have obtained through bio-guided isolation, a series of falcarindiol derivatives with significant PPAR-gamma agonistic activity (2–8), which were identified by NMR as unique polyacetylenes (1–8), which were identified by NMR as unique polyacetylenes fused with sesquiterpenoids. They are a second type of polyacetylene adducts connected through an ether bond, besides previously reported coumarin adducts. The sesquiterpenoid moiety of 5 and 6 is reported for the first time.
New Pyrones from the Mangrove Endophytic Fungus Pestalotiopsis sp
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Pestalotiopsis is generally found as endophytes of tropical plants and as prolific producers of structurally unusual natural products [1]. Our previous chemical investigations on fungus species Pestalotiopsis JCM2484 isolated from the Chinese Mangrove plant Rhizophora mucronata Lam. have led to the isolation of a series of natural products and yielded over twenty different compounds with seventeen of them being new natural products including chromones, cytosporones and coumarins [2–3]. Following cultivation of the fungus now yielded eight new pyrene derivatives (1) as well as a known compound. The structures of all compounds were unambiguously established from their spectroscopic data, that included HR-ESIMS and 1- and 2-dimensional NMR spectroscopy, and by comparison with the literature [4]. Our findings proved endophyte genus Pestalotiopsis to be particularly productive.

Figure 1: Structure of pyrone

Acknowledgement: We gratefully acknowledge the funding provided by the Austrian Science Fund (FWF) within project NFN S 10705-B13.

Isolation of three oligostilbenes from the bark of Shorea bracteolata
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Shorea species is the largest subfamily of Dipterocarpaceae and are the source of resveratrol oligomers (oligostilbene), sesquiterpenes and triterpenes (Aminah et al., 2002). Shorea bracteolata Dyer – also called white meranti is locally known as “Meranti Pa’ang” and is widely distributed in Sumatera, Peninsular Malaysia, Indonesia and Singapura. The
tree is up to 50 m in height and the timber is light hardwood. The study was undertaken to extract and isolate the chemical constituents from the stem bark of *Shorea* species namely *Shorea bracteolata* and to elucidate the structures of the chemical constituents isolated by using modern spectroscopic methods. The stem bark of *S. bracteolata* (4 kg) was dried and cut into small pieces and ground to powder about 1 mm mesh size using grinder. The sample was extracted with acetone, filtered and evaporated in vacuo at 40°C to yield crude extract (320 g). Diethyl ether was added to crude extract to remove the tannin. The tannin free crude extract was fractionated using vacuum liquid chromatography (VLC) to give six fractions. Fraction 2 was subjected to radial chromatography [EtOAc:MeOH (9:1)] and [CHCl3:Acetone:MeOH (7:2:1)] afforded hopeaphenol (Figure 1). Double purification of fraction 5 using radial chromatography [EtOAc:MeOH (9:1)] and [CHCl3:Acetone:MeOH (7:2:1)] afforded hopeaphenol (3) (Guebailia et al., 2006). The structure of the isolated compounds was determined based on analysis of spectroscopic data, including NMR, UV, IR and comparison with previous reported studies.

Linalool is a monoterpenol that is widely used in fragrance industry and cosmetics. The effect of linalool on cultured cells (HepG2, MCF7, HeK293, Caco2 and NIH3T3) was investigated. We have recently reported a significant decrease of 50% and 100% in the viability of HepG2 by 0.4 μM and 2 μM linalool, respectively (1). Other studies reported the cytotoxicity of linalool on hematopoietic malignancies but not on normal human cells (2). These findings identify linalool as a potential anticancerogenic molecule. The aim of this study was to investigate the importance of the structural features of linalool in exerting the effect on HepG2 cells. Eleven chemicals with were tested. HepG2 cells were treated with various chemicals at 2 – 500 μM for 24 hours and the viability was estimated using MTT. None of the screened compound had the same potent effect of linalool (2 μM). No effect was demonstrated at concentrations lower than 50 μM. We obtained cell death in HepG2 74% with myrcene & nerolidol (100 μM); 55% with trans-2-nonenal, Decanal (100 μM); 20% with Nonyl aldehyde, citronellal, citral, Perillaldehyde, trans-2-octen-1-ol, and 1-octen-3-ol at (500 μM). Our findings suggest that the effect of linalool is specific to 1-ene 3-ol moiety. The hydroxyl group needs to be tertiary. Hydration of myrcene and L-perillaldehyde did not have any significant effect on HepG2 viability. This may be attributed to: favorability of the hydration reaction and to possible steric effect. Alternative metabolism of linalool into other products may not be ruled out.

Acknowledgement: The authors would like to thank Faculty of Applied Sciences, Universiti Teknologi MARA, Malaysia for financing this research project. The scholarship of one of the authors was financed by UBTM Fellowship.

References:

Figure 1: Structure of compound 1 – 3

**PG21**

Synthesis and Evaluation of Gamogonic Acid Analogs as Cytotoxic Agents


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Gamboic acid (1, GA) was isolated from the resin of *Garcinia hanburyi* Hook. f. (Clusiaceae), a tree growing in Southeast Asia. The resin is used in folk medicine and as coloring agent in China (1,2). The chemical structure of GA shows a unique 4-oxatricyclic[4.3.1.0^3,7]decan-2-one ring system attached to a xanthone backbone. In order to analyze structure-activity relationships (SAR) of GA, we converted it into xanthone derivatives (Figure 1) and performed the synthesis of some structurally related prenylated derivatives, using as building blocks 1,3,6-trihydroxyxanthene (2) and tested them for cytotoxic activity. All newly synthesized compounds were assessed for in vitro cytotoxicity against 4 human cancer cell lines: KB (nasopharyngeal), KBvin (multidrug-resistant nasopharyngeal over-expressing P-gp), A549 (lung) and DU-145 (prostate). Among them, compounds 9 and 10 showed remarkable IC50 values of 0.91 and 0.82 μg/mL, respectively, against KBvin cells.


**PG22**

Linalool Effect on HepG2 cells: Structure Function Relation

**Structural Analogs**

**Linalool Effect on HepG2 cells:** The effect of linalool on human HepG2 cell viability was evaluated, and the results were compared with those for other compounds with similar structural features. Linalool was found to be significantly cytotoxic at concentrations of 0.4 μM and 2 μM, with 50% and 100% cell death, respectively. Other compounds such as myrcene and l-perillaldehyde were less effective, demonstrating the importance of the 1-ene 3-ol moiety in exerting a cytotoxic effect.

**Conclusion**

Linalool is a potential anticancerogenic molecule, and its effect on HepG2 cells is specific to the 1-ene 3-ol moiety. Future studies could focus on the development of new structural analogs to further explore the potential of linalool in cancer therapy.

**Acknowledgment**

The authors would like to thank the Faculty of Applied Sciences at Universiti Teknologi MARA, Malaysia for their support in this research project. Additionally, gratitude is extended to the scholarship of one of the authors, financed by UBTM Fellowship.

**References**


**Figure 1:** The structures of compounds 9 and 10

**PG23**

New Alkaloids, Sessilifoliamides K and L from the Roots of Stereona sessilifolia

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Plants belonging to the genus *Stereona* (family Stereoneaceae) are noted for producing a series of alkaloids with unique structures, most of which are characterized by incorporating a pyrrolo[1,2-a]azepine core. Of the genus *Stereona* plants, *Stereona japonica* (Blume) Miq., *S. tuberosa* Loure, and *S. sessilifolia* both contain interesting natural products.

Plants belonging to the genus *Stereona* (family Stereoneaceae) are noted for producing a series of alkaloids with unique structures, most of which are characterized by incorporating a pyrrolo[1,2-a]azepine core. Of the genus *Stereona* plants, *Stereona japonica* (Blume) Miq., *S. tuberosa* Loure, and *S. sessilifolia* both contain interesting natural products.
and S. sessilifolia Franch. & Sav. have been used in China and Japan as an insecticide and also as a remedy for cough, and their biological activities are considered to be related to their alkaloid components. In our studies on the chemical constituents of S. sessilifolia, we isolated eleven new alkaloids, sessilifloramines A–I and sessilifloraline A, with novel alkaloid skeletons. In this meeting, the isolation and structure determination of further new Stemona alkaloids, sessilifloramines K and L are represented.

**Reference:**

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**Chemodiversity of Pentadesma grandifolia (Clusiaceae) from Cameroon**

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Within the family Clusiaceae, the genus Pentadesma is represented by three species only, which are distributed in the tropical regions of Africa and America. Pentadesma grandifolia Baker f. is used in African folk medicine, and the roots and stem bark are applied to treating fever and malaria in the western part of Cameroon [1]. Xanthones, biflavonoids and triterpenoids are the major secondary metabolites of this genus as reported recently [1]. A new glycosylated biflavonoid and a further xanthone derivative were identified now from the same accession originating from Cameroon [1]. Their structures were elucidated by NMR spectroscopy and classical chromatographic techniques. The significance of bioactivity results and reported bioactivities of Pentadesma compounds are shortly discussed. Acknowledgement: The authors are thank to the financial support from the National Science Council of Taiwan (NSC-99–2320-B-007–Q01). References: 1. Liao JC (1996) Moraceae in Flora of Taiwan, 2nd edition, Vol. 2: 136 – 137. Editorial Committee of the Flora of Taiwan. Taipei 2. Hakim EH et al. (2006) Nat Med 60: 161 – 184. 3. Jagtap UB, Bapat VA (2010) J Ethnopharmacol 129: 142 – 166.

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**Triterpenoids and Flavonoids from Pavetta corymbosa**

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The genus Pavetta have long being used in ethnomedicine as anti-malarial, remedy for tuberculosis and for relieve of stomach pain [1]. The literature does not report any phytochemical studies on Pavetta corymbosa (DC.) EN. Williams. The dichloromethane and the ethyl acetate extracts were investigated for phytochemical constituents. Fractionation of the dichloromethane extract by Flash column chromatography, sephadex LH-20 and Preparative TLC afforded the triterpenes: α-amyrin, lupenol, ursoic acid and a mixture (1:1) of β-sitosterol and stigmasterol, while the ethylacetate extract was fractionated over sephadex LH-20 to give the known flavonoids: quercetin, quercitin 7-O-rhamnoside and kaempferol. The structures were elucidated by NMR spectroscopy and compared with literature (2, 3, 4) and are reported here for the first time. References: 1. Dalziel JM and Hutchinson J (1955) Useful plants of West Africa. Crown Agents for Oversea Publication, London 2. Reynold WF et al. (1999) Braz Chem Soc 10(3): 237 – 240 3. Ahmad SH and Nordin HL (1998) ARBEC II: 1 – 6 4. Mabry T.J et al. (1970) The Systematic Identification of Flavonoids Springer-Verlag Publication, New York.

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**Chemical constituents of Artocarpus xanthocarpus and their inhibitory effects on melanin biosynthesis**

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Artocarpus xanthocarpus Merr. is an evergreen monoeious tree with milky juice, distributed in Philippines, Borneo and Taiwan [only on Lanyu] [1]. Various phenolic compounds, including isoprenylated flavonoids, stilbenoids, and 2-arylbenzofurans are widely distributed in plants of the genus Artocarpus. Many of these compounds exhibit cytotoxic, anti-inflammatory, antibiotic, antimarial, antitubercular, antiviral and antioxidant activities [2, 3]. In continuation of our research on natural whiteners, the present study aims to characterize the chemical constituents of A. xanthocarpus and demonstrate their potential whitening potency. Three new compounds, artocanthocarpuones A-C (1–3), and twelve known compounds (4–15) have been isolated from the root of A. xanthocarpus. Their structures were determined on the basis of spectroscopic evidence. These compounds were evaluated for their antioxidative property, tyrosinase inhibitory potential and cytotoxicity. In B16F10 melanoma cells, compound 1, nor-artocarpetin (4), oxyresveratrol (5), albinan (4) and steppogenin (9) reduced tyrosinase activity and also inhibited the α-MSH induced melanin production. We may conclude that isolates of A. xanthocarpus with antioxidant and tyrosinase reducing activities may be considered as depigmenting agents. Acknowledgement: The authors are thank to the financial support from the National Science Council of Taiwan (NSC-99–2320-B-007–Q01). References: 1. Liao JC (1996) Moraceae in Flora of Taiwan, 2nd edition, Vol. 2: 136 – 137. Editorial Committee of the Flora of Taiwan. Taipei 2. Hakim EH et al. (2006) Nat Med 60: 161 – 184. 3. Jagtap UB, Bapat VA (2010) J Ethnopharmacol 129: 142 – 166.
Evaluation of effects of climate condition on the quality of sugar beet production in Iran

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This research has been carried out during the 9 years (1998 – 2008) and in some provinces (Chehar Mahal Bakhthiari, Isfahan, Qazvin, Zanjan, Azarbayejan Gharbi) in 5 sugar factories. Our aim was the evaluation of qualitative specifications of sugar beet produced in different climates and their effects on the amounts of sugar produced. Conclusions of this research were based on the analysis of 13811 samples of sugar beet consignments received during 9 years. The results have shown that amounts of sugar changed from min. 15.38% to max. 62.2%. K+ contents varied between min. 5.06 and max. 8.1; while Na+ varied from min. 1.8 meq to max 5.49 meq., and harmful nitrogen ranged between 1.96 to 4.37. These results showed the annual variations in the quality of sugar produced in Iran with respect to geographical variations.

Structural analysis of arabinogalactan-proteins from suspension cultures of Pelargonium sidoides DC.

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Pelargonium sidoides DC. is a traditional medicinal plant from South Africa. An aqueous-ethanolic formulation of the roots is approved for the treatment of acute bronchitis. The main effects could be related to antibacterial activities and the stimulation of the non-specific immune system by the main components of Pelargonium sidoides: coumarins, phenols and tannins [1]. Due to wild harvesting, Pelargonium sidoides is an endangered species. Therefore the propagation of the plant materi-

al by cell cultures and the extraction of ingredients are interesting tasks. From suspension cultures of Pelargonium sidoides high amounts of pure Arabinogalactan-proteins (AGPs) could be isolated by precipitation with β-glucosyl Yariv reagent. These AGPs have been investigated with regard to their structure. Quantification of neutral sugars by acetylation pointed out arabinose (Ara) and galactose (Gal) as dominating mono-
saccharide residues in a ratio of 1:2. Colourimetric determination of uronic acids revealed an amount of 6 – 8%. Linkage type analysis in combination with the reduction of the uronic acids showed that the main components are 1,3,6-Gal(p), 1,3-Gal(p) and 1-Ara(f) as well as minor amounts of 1,6-Gal(p), 1-GlcA(p), 1,4-Gal(p), 1-Gal(p), 1,5-Ara(f) and 1.2-Ara(f). Molecular weight of AGPs has been determined by size exclusion chromatography with laser light scattering detection and found to range between 80 and 85 kDa. The characterisation of the AGP-protein moiety pointed out an untypical low protein content for AGPs with 1%. According to the amino acid analysis the protein moiety consists of high amounts of Hyp (42.8 – 51.1%) as well as Pro, Gly, Glx, Asp, Ser, Ala, Leu and Thr. References: 1. Kolodziej H (2008) Planta Med 74: 661 – 666

Figure 1: Structures of new compounds 1 – 5

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Thymol, benzofuranoid, and phenylpropanoid derivatives: anti-inflammatory constituents from Eupatorium cannabinum subsp. asiaticum

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Eupatorium cannabinum L. subsp. asiaticum Kitam. (Compositae) [1] is a perennial herb distributed in Himalaya mountain range, China, and Tai-
wan. E. cannabinum, locally called ‘Taiwan ze-pan’ or ‘liu-yue-see-pan’, has been used as a folk medicine to treat hepatitis, headache, diarrhea, hypertension, and Diabetes mellitus in Taiwan. Sesquiterpene lactones, diterpenoids, flavonoids, pyrrolizidine alkaloids, thymols, benzofurans, and their derivatives are widely distributed in plants of the genus Eu-
patorium. Many of these compounds were found to exhibit cytotoxic, antimicrobial, and anti-inflammatory activities. In our studies on the anti-inflammatory constituents of Formosan plants, many species have been screened for in vitro inhibitory activity on neutrophil pro-inflam-
matory responses, and E. cannabinum has been found to be an active

Species. Five new compounds, 9-0-angeloyl-8,10-dehydrothymol (1), 9-
(3-methylbutanoyl)-8,10-dehydrothymrol (2), eupatobenzofuran (3), 2-
hydroxy-2,6-dimethylenobenzofuran-3(2H)-one (4), and 1-(2-hydroxy-4-
methylphenyl)propan-1,2-dione (5) and 16 known compounds have been isolated and identified from the aerial part of E. cannabinum subsp. asiaticum. Compounds 6 – 8, 11, 13, and 15 exhibited inhibition (IC50 values < 18.4 μM) of superoxide anion generation by human neutrophils in response to formyl-L-methionyl-L-leucyl-L-phenylalanine/cytochala-
sin B (FMLP/CB). Compounds 2, 5, 10, 13, and 15 inhibited FMLP/CB-
induced elastase release with IC50 values < 18.3 μM.

Value-added products from Pinus banksiana wood

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Pinus banksiana Lambert (Pinaceae) is a boreal conifer species, with a sparse, variable crown and spreading branches at maturity. The species is widely available in Canada, and an important timber species for pulp and lumen [1]. In this work, the chemical composition, such as ash, lignin, cellulose and hemicellulose contents, of the P. banksiana wood chips from Eastern Canada, was determined. In addition to timber and pulp, P. banksiana wood may be a rich source of unexploited potentially novel bioactive compounds. A previous study showed that P. banksiana wood extracts possessed strong antioxidant and anti-tumor activities [2]. In this work, the chemical constituents of the extracts from P. banksiana wood were further investigated. The GC-MS results of essential oils from P. banksiana wood showed that there were 76 volatile compounds presented, including phenolic acids, phenylpropanoids, al-
kaloids and terpenoids. Among those determined, 1-Naphthalenecar-
boxylic acid was the most abundant (29.06%). Based on the successive Sephadex LH-20 column chromatographic separation of P. banksiana wood aided by Thin Layer Chromatography, 5 yellowish low-molecu-
lar eight natural compounds, including 2 flavan-3-ols ([–]-Catechin (I) and [–]-Epicatechin (II)), a phenolic acid [Caffeic acid (III)], a phenyl-
propanoid [Isoconiferin (IV)] as well as a lignan [Cedrusin (V)], were isolated. Structure elucidation of the isolates was based on their physio-
chemical and spectroscopic data. To the best of our knowledge, this was
the first time of isolation low-molecular-weight natural compounds from *P. banksiana* wood. The results in the study might lead to the further development of high value-added products from this pine species.


**PG32**

**Activity-guided supercritical CO$_2$ isolation of antioxidative constituents from *Eucommia ulmoides***

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Increasing evidence exhibited that antioxidants played very important role in protecting against various diseases like cancer, atherosclerosis, diabetes, cataracts, neurodegenerative disorders [1]. *Eucommia ulmoides* Oliv., the sole species in Eucommia genus and Eucommiaceae family, is a large medicinal hardwood native to China and widely cultured in Eastern Asia and has widely been used as a tonic to strengthen the kidney and liver, against diabetics, strong bones, ache knees, treat lower back pain, prevent fatigue and miscarriage [2, 3]. In present study, crude extracts of *E. ulmoides* wood were initially obtained by supercritical CO$_2$ isolation. And DPPH free radical scavenging assay, a standard in vitro model, was employed for the activity-guided purification to identify the antioxidative constituents of *E. ulmoides*. The crude extracts were suspended in water, and then successively partitioned to a serious of polar solvents to yield fractions soluble in n-hexane, dichloromethane, ethyl acetate, n-buthanol and water. Ethyl acetate fraction exhibited most significant capacity to scavenge DPPH radical, and thus was further subjected to repeated sephadex LH-20 open column to separate the individual antioxidative compounds. Guided by DPPH assay, the fraction having superior activity was identified as containing five major yellow amorphous compounds, including gallic acid, ellagic acid, caffeic acid, (+)-catechin and kaempferol and were obtained. The structures of the isolated antioxidants were mainly elucidated and established by spectroscopic analysis, such as NMR and MS, as well as cellulose TLC. To the best of our knowledge, this was the first time of phytochemical investigation of *E. ulmoides* wood. Acknowledgement: This work was financially supported by Program for New Century Excellent Talents in University (NCET 2010). Foundation for the Development of Science and Technology in Tianjin Universities (No. 20080616), National Natural Science Foundation of China (NSFC, No. 31000279), Program for New Century Excellent Talents in University (NCET 2010) and Natural Science Foundation of Tianjin City (No. 09JCYBJC15800). References: 1. Si CL et al. (2009) Planta Med 75: 1165 – 1167. 2. Chang H., Yan SZ (1979) Flora Republicae Popularis Sinicae. Science Press. Beijing. 3. Takamura C et al. (2007) J Nat Prod. 70: 1312 – 1316.

**PG33**

**Phytochemical investigation of *Galanthus transcaucasicus* Fomin, as a source of isoquinoline alkaloids**

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*Galanthus transcaucasicus* Fomin (Amaryllidaceae) is an endemic species of the Caucasus region and the Alborz mountains in Iran and is used in folk medicine to recover paralysis and nerve pain (1, 2). All species of *Galanthus* are famous for their bioactive alkaloids such as galanthamine, an acetylcholinesterase inhibitor, which is used for the treatment of Alzheimer’s disease (2). This study is designed to identify major constituents of the alkaloid fraction of *G. transcaucasicus* Fomin. The plant was collected from Alborz mountain area (Rostnam abad) in February 2008.

The bulbs of the plant were percolated with 96% ethanol and alkaloid fraction of the plant was prepared. Major constituents of alkaloid fraction were purified using different chromatographic methods. Finally, five isoquinoline type alkaloids involving galanthamine, narwedine, lycorine, caranine and tazettine were identified with spectroscopic methods. The results showed that this species can be considered as a source of isoquinoline type alkaloids especially galanthamine which is a long acting, competitive and reversible acetylcholinesterase inhibitor (2). References: 1. Bastida J et al. (2000) The alkaloids. Elsevier Scientific Publishing. Amsterdam 63: 87 – 179. 2. Heinrich M et al. (2004) J Ethnopharmacol 92: 147 – 162.

**PG34**

**New skeleton polyacetylene ferulic acid conjugates from *Notopterygium incisum***

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In our search for natural products activating PPAR-gamma we have isolated three polyacetylene ferulic acid conjugates (1-3) from the underground parts of *Notopterygium incisum* Ting ex H. T. Chang (Qiang Huo), in addition to previously isolated polyene-yne derived compounds. Their structures were elucidated by NMR and MS. Compound 1 is formed by an ester bond, while compound 2 and 3 represent two new skeletons. Pharmacological evaluation of the isolated compounds is in progress.

**Figure 1:** structures of polyacetylene ferulic acid conjugates from *N. incisum.*

**PG35**

Isolation of anthocyanins with identified qualitative-quantitative properties

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Technology of natural substances isolation is substantial element affecting on the quality of natural preparations. The isolation of the plant substances from a raw material is carried out by distillation and extractions with different solvents. The methods used for anthocyanin separation due to high temperatures, etc. destroy their structures and decrease their therapeutic effects. The present study is aimed to establish optimal procedures for anthocyanins after their extraction to secure the stability of substances by lyophilisation. The freeze drying technology seems to be an appropriate method to keep the structure and qualitative-quantitative properties of these natural components. The focus of lyophilisation technology is vacuum sublimation of ice crystals, i.e. phase transition from solid to gas state, which is created during freezing the water solution. The process is carried out in three phases: freezing, primary drying and secondary drying. LC/MS/IT/TOF equipment is used for the determination of anthocyanin qualitative-quantitative characteristics before and after lyophilisation. The biological and antimicrobial properties of these natural components are tested as well. The freeze drying technology is used to establish a new lyophilisation technology called Mediproduct Company in Lipany, the East Slovakia. Keywords: Hydrolyzation, lyophilisation, plant material, secondary metabolites Acknowledgement: The participation is supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic, the project: 00162 – 0001 (MS SR-3634/2010 – 11).

**PG36**

Secondary metabolites from the Root of **Rhaphiolepis indica** var. tashiroi and Their Anti-inflammatory Activity

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**Rhaphiolepis indica** (L.) Lindl. ex Ker var. tashiroi Hay, ex Matsum (Rosaceae) is an evergreen shrub or small tree which distributes from India to southern China, the Ryukyus, Japan, Korea, and Taiwan at low altitudes. The methanolic extract of the root of this species showed anti-inflammatory activity using N-formylmethionylleucylphenylalanine (fMLP)-induced production of superoxide anion, an inflammatory mediator produced by neutrophils in vitro. Previously, we reported seven compounds including four new dibenzofuran derivatives, rhapinofuran A – D (1–4), two new biphenyl derivatives, rhaphinol and rhaphinol A – B (5–6) along with one new natural product, ferreirin from the active EtOAc-soluble layer of this plant’s root. Continuing investigation of the active EtOAc-soluble layer of this plant’s root led to the isolation of three new triterpenoids, namely 2a,3β-dihydroxyolean-11,13(18)-en-28,19-olide (7), from a new naturally occurring triterpenoid from the root of this species. The compounds are elucidated by spectral analyses. Among the isolates, 3, 6, and 7 exhibited potent inhibition against fMLP-induced superoxide production with IC50 values less than 8.36 μM. Acknowledgement: National Science Council of the Republic of China (NSC 97 – 2320-B-037 – 010-MY3).

**PG37**

Secondary Metabolites from the Root of **Neolitsea daibuensis** and Their Anti-inflammatory Activity

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**Neolitsea daibuensis** Kamikoti (Lauraceae) is a small semideciduous trees, endemic to Taiwan, confined to broad-leaved forests from 800 to 1000 m in the southern part. Recently, approximately 40 species of Morosan Lauraceous plants have been screened for anti-inflammatory activity using an inducible nitric oxide synthase (iNOS) assay, and the methanolic extract of the root of this species was shown with potent inhibition of NO production without any cytotoxicity on RAW 264.7 cells. Besides, the chemical constituents of its root have not extensively been studied yet. Bioassay-guided fractionation of the ethyl acetate soluble layer of the root of this species led to the isolation of three new alkaloids: daibucarbolines A – C (1–3), three new sesquiterpenoids: daibulactones A – B (4–5), and daibuoxide (6), together with twenty known compounds. The structures of these compounds were determined by spectroscopic analysis. Among the isolates, daibucarbone (A), hiranlactone B, isolinderalactone, 7-O-methylinderalactone, and prunetin showed iNOS inhibitory activity with IC50 values as 18.41 ± 0.47, 29.30 ± 0.92, 0.30 ± 0.01, 19.55 ± 0.71, and 10.20 ± 0.33 μM, respectively. Acknowledgement: National Science Council of the Republic of China (NSC 99 – 2300-B-037 – 009).

**PG38**

Phytochemical Investigation of **Himatanthus sucuba** bark leading to the identification of novel and anti-inflammatory compounds

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**Himatanthus sucuba** (Spruce ex Müll. Arg.) Woodson (Apocynaceae) is used in the Amazonian region for the treatment of inflammatory diseases [1]. The bark of this medicinal plant was phytochemically investigated guided by an LPS/TNF stimulated assay measuring E-selectin and IL-8. Out of bioactive fractions 11 constituents were isolated and identified by MS and 1D and 2D NMR experiments as iridoids (plumericin, plumericin, allamandin, and ferreirin), and five new iridoid glucosides: 18.41 ± 0.47, 29.30 ± 0.92, 0.30 ± 0.01, 19.55 ± 0.71, and 10.20 ± 0.33 μM, respectively. Acknowledgement: National Science Council of the Republic of China (NSC 97 – 2320-B-037 – 010-MY3).

**PG39**

New isoflavones and bioactive constituents from the fruits of *Psoralea corylifolia* Chen T1, Chen C1, Lai R1, Chen H2, Kuo W2, Liao T1
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**Psoralea corylifolia** (Chinese name Buguzhi), dry fruits of leguminous plant *P. corylifolia* L., is one of the most popular traditional Chinese medicines. This crude drug has used for the treatment of pollakiuria, enuresis, osteoporosis, depression, and various kidney problems. It is reported to contain coumarins, flavonoids, alkaloids, essential oil, and terpenoids. Many of these compounds were found to exhibit anti-allergic, antioxidant, antitumor, insecticidal, and antimicrobial activities. Investigation on *n*-hexane- and EtOAc-soluble fractions of the fruits of *P. corylifolia* has led to the isolation of two new isoflavones, 7-O-methylflavone A (1) and 7-O-isoprenylflavone B (2), together with eight...
known compounds, including angelicin (3), psoralen (4), bavachalcone (5), bakanuchiol (6), 12,13-dihydro-12,13-epoxybakanuchiol (7), p-hydroxybenzaldehyde (8), b-sitosterol (9), and stigmasterol (10). The structure of new compounds 1 and 2 was determined through spectroscopic and MS analyses. Among the isolated compounds, psoralen (4) exhibited inhibition (IC₅₀ value = 110.0±0.60 μg/ml) of superoxide anion generation by human neutrophils in response to formyl-L-leucyl-L-phenylalanine/cytochalasin B (fMLP/CB).


A new ferulic acid ester, a new ellagic acid derivative, and other constituents from Pachycentria formosana: Effects on neutrophil pro-inflammatory responses

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A new benzophenol glucogluconol derivative with an adamantyl skeleton and other constituents from Garcinia multiflora: Effects on neutrophil pro-inflammatory responses

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Garcinia multiflora Champ. is a small evergreen tree, distributed in South China, Hong Kong, and Taiwan [1]. The genus Garcinia (Guttiferae) comprises about 400 species with pantropical distribution. In Taiwan, the genus Garcinia is represented by three species, viz., G. linii, G. multiflora, and G. subelliptica. Plants of this genus contain a variety of secondary metabolites including xanthones, benzophenones, phloroglucinols, terpenoids, biflavonoids, and their derivatives. Many of these compounds exhibit antioxidant, trypanocidal, cytotoxic, antitubercular, anti-inflammatory, and anti-HIV activities. As part of our studies on the anti-inflammatory constituents of Formosan plants, many species have been screened for in vitro inhibitory activity on neutrophil pro-inflammatory responses. In the course of this screening, an AcOEt-soluble fraction of the fruit of G. multiflora exhibited inhibitory activities with IC₅₀ values of 7.21±1.07 and 6.01±0.37 μg/ml, respectively, against fMLP/CB-induced superoxide anion generation and elastase release. Investigation of the active fraction afforded a novel benzophenol glucogluconol derivative, garcinmultiflorone D (1), with an unusual adamantyl caged skeleton and four known compounds. The structure of 1 was determined through extensive 1D/2D NMR and mass-spectrometric analyses. Garcimultiflorone D (1) exhibited inhibitory activities with IC₅₀ values of 7.21±1.07 and 6.01±0.37 μg/ml, respectively against fMLP/CB-induced superoxide anion generation and elastase release.


New biphenyl derivatives and anti-inflammatory constituents from the stem bark of Magnolia officinalis

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The stem bark of Magnolia officinalis Rehd. et Wils. (Magnoliaceae) has been used as a traditional medicine for the treatment of gastrointestinal disorders, bronchitis, and emphysema, in China, Taiwan, Japan, and Korea [1]. Chemical studies have revealed a variety of neo-lignans and
alkaloids as constituents of this plant. Many of these compounds exhibit central depressant effect, muscle relaxation, and antigastric ulcer, anti-

bacterial, antiallergic, vasorelaxant, and neurotrophic activities. Investi-
gation on EtOAc-soluble fraction of the stem bark of M. officinalis has led to the isolation of three new biphenyls, 5,5'-diallyl-2,2'-hydroxybenzophenyl-2,2'-dialyl-2'-allyloxybiphenyl-2-ol (2), and 5,5'-diallyl-2,2'-hydroxybenzophenyl-2-ol (3), together with 12 known compounds, including four neolignans, magnolol (4), honokiol (5), (+)-monoterpenylmagnolol (6), and randalain (7), two norlignans, 

methylmagnolol (8) and randalain (9), and six steroids, β-sitosterone (10), stigmasta-4,22-dien-3-one (11), β-sitosterol (12), stigmasteral (13), 3β-hydroxystigmast-5-en-7-one (14), and 3β-hydroxystigmast-5,22-dien-7-one (15). The structure of new compounds (1-3) were determined through spectroscopic and MS analyses. Among the isolates, magnolol (4) and honokiol (5) exhibited potent inhibition against FMLP-

induced superoxide production with IC50 values of 4.42 ± 0.24 and 0.68 ± 0.20 μg/mL, respectively. In addition, magnolol (4) inhibited fMLP/CB-induced elastase release with an IC50 values of 1.45 ± 0.20 μg/mL.

Figure 1: Structures of new compounds 1 – 3

Acknowledgement: This research was supported by grants from the Na-
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MYS and NSC 98 – 2326-B-127 – 001-MYS), awarded to Prof. J.-J. Chen.

New Coumarin Derivative and Bioactive Constituents from the fruits of Cnidium monnieri

Cnidium monnieri (L) Cusson (Apocaceae)1 is a crude drug ‘Fructus Cni-
dii’ (Chinese name Shechaungzi) used in traditional Chinese to treat impotence, frigidity, and skin-related disease. Coumarins, chromones, and their derivatives were isolated from this plant in previous studies. Many of these compounds were found to exhibit in vitro, antifungal, and antibacterial activities. Investigation on EtOAc-solu-
ble fraction of the fruits of C. monnieri has led to the isolation of a new coumarin, 3-O-demethylmuurral (1), together with 12 known compounds, including 11 coumarins, meranzin hydrate (2), peroxycoumarin (3), auraptenol (4), demethylauraptenol (5), peroxymuraftol (6), murafzel (7), osthol (8), bergapten (9), isopimpinellin (10), xanthotoxin (11), and xanthotoxol (12), and a chromone, cniidinol A (13). The structure of new compound 1 was determined through spectroscopic and MS analyses. Among the isolates, osthol (8) completely inhibited ADP-induced platelet aggregation at 100 μg/mL. Xanthotoxin (11) showed complete inhibitory activity on platelet aggregation at 100 μg/mL induced by arachidonic acid.

Figure 1: Structure of new compound 1

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tional Science Council of the Republic of China (NSC 95 – 2326-B-127 – 001-
MYS and NSC 98 – 2326-B-127 – 001-MYS), awarded to Prof. J.-J. Chen.

Helicia regetiensis Masamune (Proteaceae) is an endemic evergreen trees, growing in thickets at lower elevations in the central and southern parts of Taiwan. Flavonol glycosides, phenolic glycosides, benzenoid gly-
cosides, and their derivatives distributed in plant of genus Helicia. In our studies on the antitubercular constituents of Formosan plants, 1,200 species have been screened for in vitro antitubercular activity, and the H. regetiensis has been found to be an active species. However, the chemical constituents and biological activities of this plant have never been studied. Bioassay-guided fractionation of the active EtOAc-soluble fraction of the root of this species has led to the isolation of three new compounds, including helicinol A (1) helicinol B (2) and helicinone (3), together with eleven known compounds, including one cyclopane, ker-
madecin H (4), three flavones, 5-hydroxy-3,7,4′-trimethoxyflavone (5), 5-hydroxy-3,6,7,4′-tetramethoxyflavone (6), and 5-hydroxy-3,6,7,8,4′-pentamethoxyflavone (7), one fatty acid, stearic acid (8), one benzoqui-
none, α-tocopherol quinone (9), and five steroids, β-sitosterone (10), a mixture of β-sitosterol (11) and stigmasterol (12), and a mixture of 3-O-
β-D-glucopyranosyl β-sitosterol (13) and 3-O-β-D-glucopyranosyl stig-
masterol (14). The structures of these new compounds were determined through spectroscopic analyses including 2D-NMR data. The successive isolation and antitubercular assay are still in progress. Acknowledge-
ment: National Science Council of the Republic of China (NSC 99 – 2326-B-
057 – 010).

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MYS and NSC 98 – 2326-B-127 – 001-MYS), awarded to Prof. J.-J. Chen.
**PG46**

**Chemical Constituents and Bioactivities from the Root of *Piper taiwannense***

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*Piper taiwannense* Lin & Lu (Pipercaceae) is a woody climber, endemic to Taiwan, distributed in forests at low to medium altitudes throughout the island. Previously, four new compounds and 25 known compounds, along with anti-platelet aggregation activity were reported from the stem of this species. The methanolic extract of the root of this plant showed potent antibacterial activity against *Mycobacterium tuberculosis* H37Rv and inhibitory activities against platelet aggregation induced by collagen. The aim of this study is the isolation of chemical constituents and their bioactivities of the root of this species. The methanolic extract of root was partitioned into EtOAc and H2O-soluble layers. Biosay-guided fractionation of the active EtOAc-soluble layer led to the isolation of eight new compounds, *taiwanenosols A, B* (1, 2), *twadienol A, B* (3, 4), *neo-taiwanenosol A, B* (5, 6), *1,2-diacetoxy-6'-methoxy-4'-allylbenzene* (7), and *1-acetoxy-2-hydroxy-4'-allylbenzene* (8), and the last two were first isolated from nature, along with 16 known compounds. The structures of these isolates were elucidated by spectral analysis. Among the isolates, the mixture of 8 and 2-acetoxy-1-hydroxy-4'-allylbenzene (9), 1,2-dihydroxy-4'-allylbenzene (10), and 1,2-diacetoxy-4'-allylbenzene (11) showed potent inhibitory activities against platelet aggregation induced by collagen, with IC50 values as 1.7, 0.8, and 0.5 µg/mL, respectively. The successive isolation and bioactivity assay of the isolates are under progress.

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**Bioactivity-guided isolation of leishmanicidal chalcones from *Piper delineatum***

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Leishmaniasis affects approximately 12 million people worldwide, primarily in developing regions [1]. Conventional chemotherapy with pentavalent antimonials is considerably toxic and prone to induce resistance. Second-line drugs, such as amphotericin B and its lipid formulations, are either toxic or expensive for routine use in developing countries. At the same time, the efficacy of miltefosine against cutaneous leishmaniasis remains to be ascertained [2,3]. Therefore, there is an urgent need for a novel, effective and safe drug for the treatment of these diseases [4]. In our ongoing study of potential leishmanicidal agents from *Piper species* [5], we have carried out a bioguided fractionation of the chalconic extract of the leaves of *Piper delineatum* Trel. This extract was subsequently partitioned between water and organic solvents of increasing polarity, giving CH2Cl2 and EtOAc fractions. These extracts and the remaining aqueous layer were tested for their leishmanicidal activity against promastigote forms of two strains of Leishmania (*L. amazonensis* and *L. braziliensis*). The bioactive CH2Cl2 fraction was subjected to column chromatography, yielding thirteen fractions. The most active fraction (IC50 1.1 and 1.3 µg/mL against *L. amazonensis* and *L. braziliensis*, respectively) was further subjected to repeat chromatography, affording two new bioactive trans-chalcones: 2',3',4'-trihydroxy-6'-methoxy-chalcone and 2',3',4'-trihydroxy-5,6'-dimethoxy-chalcone, whose structures were elucidated by means of spectrometric and spectroscopic techniques. These results support the use of the *Piper* species as a traditional remedy in the treatment of parasitic diseases. Acknowledgement: We are indebted to the Agenzia Canaria de Investigación, Innovación y Sociedad de la Información (C200801000040) and RED RAPMA/OPS projects for financial support. TJC is grateful to MAEC-AECID for a fellowship.


**Figure 1:** Structures of the new naturally occurring compounds

**PG47**

**Flavonoid trglycosides from *Astragalus armatus***

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*Astragalus armatus* Willd. is an endemic shrub of the Northern Africa (Algeria, Morocco, Tunisia), is distributed in the pre-Saharan zone and is associated with the desertification in arid areas due to overgrazing [1]. In Tunisia it is used as tonic, stimulant and in cases of anaemia [2]. From the roots of *Astragalus armatus* Stapf, [1] the only phytochemical work on *S. dichroantha* around 900 species worldwide. There are 90 species of *Astragalus* genus in Turkey half of which are endemic including *S. dichroantha* Stapf [1]. *Salvia dichroantha* Kirmizibekmez H, Bardakçı H, Yeşilada E, Hökmann F
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The genus *Salvia*, being the largest genus of Lamiaceae family, contains around 900 species worldwide. There are 90 *Salvia* species growing wild in Turkey half of which are endemic including *S. dichroantha* Stapf [1]. Some *Salvia* species are used as carminative, spasmylic, diuretic, antiseptic as well as against cold and cough in Anatolian folk medicine [2]. The only phytochemical work on *S. dichroantha* which was conducted on the roots revealed the presence of abietane-type diterpenes [3]. In the continuation of our work on the secondary metabolites of Lamiaceae, we herein report the isolation and structure elucidation of diverse compounds from the aerial part of title plant. Chromatographic studies on the H2O and CHCl3 subextracts of the *MeOH* extract led to the isolation...
of two megastigmage hormone, premaionone (1), and salvinolside B (2), an aliphatic alcohoholic alcohol, (3R)-1-octen-3-ol-3-O-β-D-xyllypyronosyl-(1→6)-D-β-D-glucopyranosyl (3), a flavonoid, 5-hydroxy-3,7,4′-trimethoxyflavone, two hydroxybenzoin acid derivatives, rosmarinic acid and 3-O-methyl-rosmarinic acid, and sucrose. The structures of the compounds were established by means of 1D- and 2D-NMR experiments and MS. To the best of our knowledge, compound 1 is being reported for the first time from Lamiaeae, while compounds 1 and 3 are new for the genus Salvia. This work also constitutes the first phytochemical study on the aerial parts of *S. dichroantha*. References: 1. Hodge IC (1982) Salvia L. In: Davis PH (ed) Flora of Turkey and East Aegean Islands. Edinburgh University Press. Edinburgh. 2. Baytop T (1999) Therapy with Medicinal Plants in Turkey, Nobel Tıp Kitapevleri, Istanbul. 3. Kawazoe K et al. (1999) Phytochemistry 50: 493 – 497.

Iridoid, phenylethanoid and flavonoid glycosides from *Sideritis trojana* Bornm. (Lamiaceae) is utilized as an herbal tea for various purposes. Previously, several diterpenes were reported from *S. trojana* [1]. However, there is no report on its iridoid and phenolic constituents. In the continuation of our work on the bioactive secondary metabolites from Lamiaceae family, we now describe the isolation and structure elucidation of the secondary metabolites from the roots of *S. trojana* as well as their antioxidant activity. From the H₂O-soluble portion of the MeOH extract, a new iridoid glycoside, 10-O-(E)-feruloylmelittoside (1) was obtained in addition to four known iridoid glycosides (melittoside, 10-O-(E)-p-coumaroylmelittoside, stachysosides E and G). Moreover, five phenylethanoid glycosides (verbascoside, isoasactoside, lamalboside, leonose A, isovaladinufolioside), three flavone glycosides (isoscutterellatin 107-O-6′-O-acetyl-β-D-glucopyranosyl-(1→2)−β-D-glucopyranoside, 4′-O-methylisocutellarin 7-O-6′-O-acetyl-β-D-glucopyranosyl-(1→2)−β-D-glucopyranoside, 3′-hydroxy-4′-O-methylisocutellarin 7-O-6′-O-β-D-glucopyranosyl-(1→2)−β-D-glucopyranoside) and a benzyl alcohol derivative (di-O-methylcereatin) were obtained and identified. Characterization of the isolates was carried out by using NMR experiments and HR-MS. All compounds were tested for their antioxidant activity by in vitro TEAC assay and some of them exhibited moderate activity (0.97 – 1.44 mM) if compared with the reference compound (quercetin, 1.86 mM). References: 1. Aytac Z and Aksoy N (2000) Flora Mediterranea 10: 181 – 184. 2. Topcu G et al. (2001) Nat Prod Let 16: 33 – 37.

Isolation and identification of rotenoids in *Pachyrhizus tuberosus* seeds

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Amazonian yam bean (*Pachyrhizus tuberosus* Spreng., Leguminosae) is a perennial twining plant indigenous to South America used for food and as a fodder. Large tuberous roots, which are eaten fresh or roasted, contain high amount of protein and easily digestible carbohydrates. Leaves, stems, roots, ripe pods, and seeds can be toxic to humans due to the presence of isocryptic and piscicidal compounds of isoflavonoid type called rotenoids [1]. Aqueous/methanol extract obtained from dried seeds (collected in Peruvian Amazon) was pretreated on an immunoaffinity column (IAC) [2] and subsequently analyzed by reverse phase HPLC with diode array detector. Immunosorbents for IAC are characterized by high molecular selectivity so that single group of structurally related compounds can be targeted. Certain levels of compounds with immunoreactivity similar to isoflavonoids were identified. The extract was subsequently separated by flash chromatography into fractions and semipreparative HPLC column into individual compounds. The structures were identified by ¹H and ¹³C NMR spectroscopy and confirmed by HR-MS. A study of chemical constituents of the seeds of *P. tuberosus* led to the isolation and identification of one new rotenoid of chemical formula C₁₇H₁₄O₅, along with five other known rotenoids (rottenone, pachyrhizine, neutenone, erosine and 12α-hydroxydolucene) [3]. The new rotenoid was assigned structure of 12α-hydroxyrotenone based on the detailed analysis of ¹H and ¹³C NMR spectra. According to previous literature, various biological activities of rotenoids, we suppose that future research on this new rotenoid may lead to new findings in the phytochemistry of these bioactive compounds.

**Figure 1:** 12α-hydroxyrotenone


Searching for iridoids from tropical plants: detection, isolation and antibacterial activity

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Iridoid glycosides, which form an important group of cyclopentane monoterpenoids, are biosynthesized by a large number of plant species belonging to approximately twenty important botanical families. Although they possess a wide range of pharmacological and biological properties, such as anti-inflammatory, anti-microbial, anti-fungal, antiviral, anti-oxidative, immunomodulatory, neuroprotective, etc. [1,2], no molecule is currently used as a drug. However, iridosides, which possess a highly functionalized aglycon, may be regarded as starting material for the synthesis of a new generation of chiral molecules. In this context, we have launched a research project aimed at searching for new chiral scaffold of iridoid-type from higher plants of the tropical biodiversity. This project, part of an ANR program called IRNA-CHIR, focuses on species, which contain high iridoid content. For this study, approximately 500 species were selected from iridoid-containing families, and were subjected to a methanolic extraction followed by di-chloromethane recovery. Then a methodology, based on the combination of different analytical and spectroscopic techniques such as TLC, LC/UV/DELD, LC/MS and NMR, was developed in order to select the plants of interest. Parallel to this study, we conducted an evaluation of the antimicrobial potential of extracts and isolated compounds. Our initial results show that no new iridoid was discovered to date, despite the fact that the species selected have never been studied, and few containing-iridoid extracts exhibit potent antibacterial activity. We will present and discuss the methodology used to detect and isolate iridoids, and the antibacterial activity of the extracts selected and pure compounds. Acknowledgement: This 4-years project is funded by the French National Research Agency. We are grateful to our foreign partners: Dr K. Awang (University Malaya, Malaysia), Dr. P. Rasoanaivo (IMRA, Madagascar), Dr. V.H. Nguyen (VAST, Vietnam), Dr. B. Kiremire (University of Makerere, Uganda) enabling us, through official agreements between CNRS and var-
Phenylated acetophenone derivatives constitute a characteristic chemical group of constituents of *Acronychia pedunculata* (L.) Miq. [1]. In continuation of a previous study, seven acetophenone dimers were isolated among them five structural isomers [2,3]. Such acetophenones exhibit particular structural characteristics as fully substituted and polyhydroxylated aromatic rings. The presence of inter- and intra-molecular hydrogen bonds and their conformational behavior due to the occurrence of rotamers complicates their structure elucidation. In the present study, NMR spectroscopy was used in order to determine the structures and the different rotamers of all isolated acetophenones. The developed methodology included variation of different solvents (DMF–DMSO, CDCl 3, CpD 6) as well as acquisition of NMR spectra in a broad range of temperatures (0 to 47°C) where acetophenone was used as a model compound. Two principal rotamers of acrovestone are the most populated in CDCl 3, solution at 0°C, while at 47°C their representative NMR signals are not resolved due to fast inter-conversion between the rotamers. According to our study, *Acronychia* acetophenone rotamers’ determination can be accomplished with NMR spectroscopy, by changing the polarity of solvent used as well as by altering temperature conditions of measurements. In parallel, an LC-APCI(+)-HRMS and MS/MS method was developed for the analysis of acetophenone derivatives using a LTQ-Orbitrap mass analyzer. A characteristic ion corresponding to the major fragment at m/z 319 was defined and used as diagnostic peak of the isolated phenylated acetophenone dimers. This novel developed LC-MS/MS method could be applied for the detection and identification of acetophenone-type prenylated acetophenone dimers in other substrates.

**References:**


**Figure 1:** Structure of new compounds 1 and 2

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**PG54**

Alkaloids from *Meiogyne virgata* (Annonaceae)  
Alias A 1, Awang K 2, Li A 3, Bihud N 1, Kasim N 1, Alias A 1, Awang K 2, Li A 3, Bihud N 1, Kasim N 1

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The genus *Meiogyne* (family Annonaceae) consists of about 24 species, widely distributed in Indo-China, Thailand, Peninsular Malaysia, Sumatra, Java, Borneo and the Philippines. Several plants of the genus *Meiogyne* has been used as a folk medicine in Malaysia. Genus *Meiogyne* has been reported to contain various types of alkaloids. In this work, we investigated the chemical constituents of the bark of *Meiogyne virgata*. MeOH and the different rotamers of all isolated acetophenones. The developed study included variation of different solvents (DMF–DMSO, CDCl, CpD) as well as acquisition of NMR spectra in a broad range of temperatures (0 to 47°C) where acetophenone was used as a model compound. Two principal rotamers of acrovestone are the most populated in CDCl, solution at 0°C, while at 47°C their representative NMR signals are not resolved due to fast inter-conversion between the rotamers. According to our study, *Acronychia* acetophenone rotamers’ determination can be accomplished with NMR spectroscopy, by changing the polarity of solvent used as well as by altering temperature conditions of measurements. In parallel, an LC-APCI(+)-HRMS and MS/MS method was developed for the analysis of acetophenone derivatives using a LTQ-Orbitrap mass analyzer. A characteristic ion corresponding to the major fragment at m/z 319 was defined and used as diagnostic peak of the isolated phenylated acetophenone dimers. This novel developed LC-MS/MS method could be applied for the detection and identification of acetophenonetype prenylated acetophenone dimers in other substrates.


**Figure 1:** Structure of new compounds 1 and 2

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**PG55**

New labdane-type diterpenoids and anti-inflammatory constituents from the rhizome of *Hedychium coronarium*  
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**Hedychium coronarium** is a terrestrial epiphytic herb, which is distribution in India, Malaysia, Vietnam, southern China and Taiwan [1]. Two new labdane-type diterpenoids, hedychicoromin (1) and peroxycoronarin D (2), together with eleven known compounds, including calcaratarin (3), (+)-coronarin A (4), coronarin D (5), coronarin D methyl ether (6), (E)-labda-8(17),12-diene-15,16-dial (7), ergosta-4,6,8(14),22-tetraen-3-one (8), β-sitosterol (9), stigmasterol (10), oleic acid (11), stearic acid (12), and palmitic acid (13) have been isolated from the rhizomes of *Hedychium coronarium*. The structure of new compounds 1 and 2 was determined through spectral analyses including extensive 2D NMR data. Among the isolates, calcaratarin (3), coronarin A (4), (E)-labda-8(17),12-diene-15,16-dial (7), and oleic acid (11) exhibited potent inhibition (IC50 < 6.17 μg/mL) against FMLP-induced superoxide production and elastase release.


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**PG56**

Sesquiterpenoids from the root of *Solanium erianthum*  
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**Solanium erianthum** D. Don (Solanaeae) is an evergreen shrub or small tree which is native of South America, widespread in tropical Asia and Oceania [1]. It is a traditional folk medicine used for the treatment of rheumatism, edema, cough, carbuncles, eczema, toothache and dermatitis [2]. In a screening program of Formosan plants, the active constituent was purified and characterized through spectroscopic methods such as ID-NMR (1H, 13C, DEPT), 2D-NMR (COSY, HMQC, HMBC), UV, IR and MS and comparison with published data. Acknowledgement: Universiti Teknologi MARA, Universiti of Malaya, Ministry of Higher Education for research grants and Ministry of Science, Technology and Innovation Malaysia for scholarship awarded to Alias. A. References: 1. Tadic D, Cassels BK, Leboeuf M and Cave A (1987) Phytochemistry 26(2): 537–541 2. Alias A, Hazni H, Mohd Jaafar F, Awang K and Ismail NH (2010) Molecules 15: 4583–4588

**References:**
The fruits and seeds (star anise) of *Illicium* plants are used traditionally as spices and folk medicines in Southern China. Those are one of the most important natural resources of shikimic acid (SA). The raw material of the antiviral drug Tamiflu. China is the largest star anise supplier in the world and 80% of raw resources are from Guangxi province. A simple and rapid HPLC method was established to analyze the content of SA in the fruits and leaves of 22 samples from different species and habitats, and SA with high content was found in the fruits of three species (>8%) and leaves of eight species (>5%). Thus these materials can be used as the raw resources of SA. Researches found that even the trace amount of anisatin and its analogs could arouse toxic effects. The mechanism studies revealed they are non-competitive antagonists of GABA receptor. Chemical investigations on three *Illicium* plants resulted in 14 sesquiterpenoids including anisatin and its analogs. The structures of new compounds 1 and 2 were determined through spectroscopic and MS analyses. Among the isolates, 5-hydroxyauranetin (3) and isohemigossylic acid lactone-7-methyl ether (10) exhibited potent inhibition against N-formyl-L-methionyl-L-leucyl-L-phenylalanine-induced superoxide production with IC_{50} values of 28.84 ± 2.26 and 12.77 ± 2.48 µM, respectively.

**Figure 1:** Structure of new compounds 1 and 2

Acknowledgement: This research was supported by grants from the National Science Council of the Republic of China (NSC 95 – 2320-B-127 – 001-MY3 and NSC 98 – 2320-B-127 – 001-MY3), awarded to Prof. J.-J. Chen.

Isolation and structural elucidation of coumarins from *Micromelum falcatum* (Rutaceae)

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*Micromelum falcatum* Tanaka (Rutaceae) is a small tree growing in Southeastern Asia [1] showing protective and therapeutic effects against cold and rheumatoid arthritis according to the traditional medicine of China [2]. The leaves of the plant, collected in Cambodia, after drying and pulverization (20g) were extracted successively with CH₂Cl₂ (200 ml × 3), MeOH (200 ml × 3) and H₂O (200 ml × 3). In parallel, a protocol using NH₃ and extraction (150g) with EtOAc and MeOH was followed for focused isolation of alkaloids [3]. The isolated compounds was performed by HRMS and NMR (¹ & ²D) spectroscopy. The identification of the isolated compounds was performed by HRMS and NMR (¹ & ²D) spectroscopy.

Figure 1: Microcoumarin

Figure 2: Microfalcin

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Anti-oxidative and anti-inflammatory activities of caffeoyl derivatives from the barks of *Ilex rotunda*


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The barks of *Ilex rotunda* Thunb. (IR) have been used for the treatment of common cold, tonsillitis and intestinal ulcer. As part of our continuous search for new anti-oxidative and anti-inflammatory agents from natural sources, we tried to isolate caffeoyl derivatives from the bark of IR. Activity guided isolations of the 80% MeOH extract yielded two new caffeoyl hemiterpenes (1 and 2), tentatively named rotundarpene and rotundarpenoside B, together with 6 caffeoyl derivatives which were methyl caffeic acid (3), 5-caffeoylquinic acid (4), 3,5-dicaffeoylquinic acid (5), 1,4-dicaffeoylquinic acid (6), 3,4,5-tricaffeoylquinic acid (7) and 3,5-dicaffeoyl shikimic acid (8), respectively. In order to evaluate anti-oxidative and anti-inflammatory activities of 1-8, DPPH radical scavenging activity and inhibitory activity on nitric oxide production in LPS-stimulated RAW 264.7 cells were measured in vitro. All caffeoyl derivatives (1-8) showed potent anti-oxidative and anti-inflammatory activities, and these results suggested that caffeoyl derivatives from IR might be developed as anti-oxidative and anti-inflammatory agents.

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References: 

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Four new kaempferol glycosides from the leaves of *Brugmansia suaveolens*

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*Brugmansia suaveolens* (Humb. & Bonpl, ex Willd.) Bercht. & C. Presl (Syn. *Datura suaveolens*; Common name: Angel’s trumpet) is a flowering shrub of Solanaceae family and it is native from coastal regions of the rainforest in Southeast Brazil. This plant has been investigated due to its anti-inflammatory and wound healing activities [1] and mainly due to the presence of alkaloids [2]. Nevertheless, only few studies related the characterization of flavonoid glycosides [3]. This prompted us to investigate the ethanolic extract prepared from its leaves. In order to have pure compounds, the plant material was submitted to successive chromatographic separations using open column chromatography and HPLC on RP-18. Up to now, four new flavonol glycosides, namely, kaempferol 3-O-β-D-glucopyranosyl-(1′′→2′′)-Oα-L-arabinopyranosyl-7-O-β-D-glucopyranoside (1), kaempferol 3-O-β-D-glucopyranosyl-(1′→2′)-Oα-L-arabinopyranosyl-7-O-β-D-glucopyranoside (2), kaempferol 3-O-β-D-[2′′→3′′,(4′,3′,4′-dihydroxy-cinnamoyl)-β-D-glucopyranosyl-(1′→2′)-Oα-L-arabinopyranosyl-7-O-β-D-glucopyranoside (3), and kaempferol 3-O-β-D-glucopyranosyl-(1′→2′)-Oα-L-arabinopyranosyl-7-O-β-D-glucopyranoside (4) were isolated and identified by means of extensive spectroscopic methods including 1D-(1H and 13C) and 2D NMR experiments (COSY, HSQC and HMBC) as well as ESI-MS.

References:
Phytochemical investigations of Alyssum corsicum

The genus Alyssum belonging to family of Cruciferae is represented by 89 species in Turkey, 52 of them being endemic [1]. Although there are no reports of the medicinal uses of Alyssum corsicum Duby, the aerial flowered part, flowered stems and inflorescences of A. maritimum (L.) Lam. are employed as renal lithotripter in infusion and decoction in the Iberian Peninsula. It is also claimed to be a hepatic lithotripter, and to have other benefits associated to the hepatic function (hepatoprotective, antiicteric, the last use in veterinary) [2]. In Iran, seeds of A. minutum Patrin ex DC. are used as a treatment for fevers and other ailments. Glucosinolates, hydrocarbons, fatty acids and flavonol 7-glucuronides were isolated from the genus Alyssum previously [3]. Glucosinolate profiles of the seeds of the various Alyssum species were also screened by Ion-Pair LC-MS method [4]. This is the first phytochemical report on Alyssum corsicum. In this study three known compounds (Tamarixin 3,7-diglucoside, Tamarixin 3-O-D- glucopyranoside-7-O-a-rhamnopyranoside, Tamarixin 3-O-B-D-glucoside) were isolated from the MeOH extract of of Alyssum corsicum by using preparative chromatographic methods. The structure elucidation of the isolated compounds was based on analyses of their spectroscopic data (1D and 2D NMR). References: 1. Davis PH (1965) Flora of Turkey and East Aegean Islands. University Press. Edinburgh. 2. Parada et al. (2009) J Ethnopharmacol 124: 609 – 618. 3. Afsharypuor S, Lockwood GB (1986) J Nat Prod 49: 944 – 945. 4. Bennett RN et al. (2004) Agric Food Chem 52: 428 – 438.

Constituents of Verbascum reeseanum

The genus Verbascum (Scrophulariaceae) is represented by 228 species, of which 185 are endemic in the flora of Turkey and East Aegean Islands [1]. Verbascum species contain biologically active compounds, such as flavonoids, phenylethanoid and neolignan glycosides, saponins, and iridoids [2]. The leaves and flowers of Verbascum are reported to have expectorant, mucolytic and demulcent properties, and are used to treat respiratory disorders such as bronchitis, dry coughs, tuberculosis and asthma in traditional Turkish medicine [3]. In this study, dried and grinded plant material (Verbascum reeseanum Hub.-Mor. – whole plant) was extracted with MeOH, and then treated with n-hexane. The n-butanol fraction from the methanol extract was submitted to several preparative chromatographic methods such as VLC, normal phase silica gel CC and reversed phase RP-18. The structures of the known compounds were determined as ajugol, lawsoniaside B, ajugoside, coniferin and sinatol, by a combination of one- and two-dimen- sional NMR techniques, and mass spectrometry. References: 1. Davis PH (1978) Flora of Turkey and East Aegean Islands. University Press. Edinburgh 2. Tatlı II, Akdemir ZS (2004) Fabad J Pharm Sci 29: 93 – 107. 3. Tatlı II, Akdemir ZS (2006) Fabad J Pharm Sci 31: 85 – 96.

Efficient isolation of bioactive constituents from Greek Fabaceae species through elaboration of counter-current chromatography (CCC) approaches

Fabaceae family contains plants that are characterized by significant biological activities. The main edible plants of this family constitute an important part of the Mediterranean diet and contain secondary metabolites with considerable estrogenic, antioxidant and chemopreventive activity.1 Recent years CCC has become a method of choice in separation and purification of natural products. The advantage of this method is the ability to separate substances from large volume of crude and complex extracts which is crucial in further analysis as far as identification and biological control activity (2,3). In the present study the application of CCC is demonstrated as a main separation technique in the phytochemical analysis of seven Fabaceae species growing in Greece. The analysis of active extracts of Vicia faba L., Lotus siculus L., Tetragonolobus purpur-eus Moench and Genista h assaultana (Bald.) Buchegger took place by use of line coupling of CCC technique with sephadex column or preparative HPLC. In the case of Lotus edulis L., Lathyrus la florus (Desf.) Kuntze, and Genista halocyli Heldr. the direct isolation of active compounds (flavonoids, isoflavones and phenolic acids) was achieved from the complex extracts using dual mode or gradient mode CCC. The purity and identity of isolated compounds was confirmed by NMR spectroscopy. It is worth noting that the phytochemical analysis of L. siculus, T. purpureus and G. h assaultana is presented for the first time. In conclusion, it is clearly indicated that counter-current chromatography is a valuable technique and can be successfully employed for rapid and effective separation of natural compounds from crude active extracts of Fabaceae species. References: 1. Spanou C et al (2008) J Agric Food Chem 56: 6967 – 6976. 2. Bertoth A et al. (2009) Pure Appl Chem 81(2): 355 – 360. 3. Sutherland IA et al. (2009) Chromatogr A 1216: 740 – 753.
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A New Guianolide from the aerial parts of Centaurea pannonica (Heufel) Simonik.
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Centaurea is a complex genus of about 500 species belonging to the Asteraceae family [1]. Sesquiterpene lactones are the main chemical taxonomic markers of the genus [2,3]. Some members of this genus are used in folk medicine [4]. In the present study, we report the main compounds isolated from Centaurea pannonica (Heufel) Simonik, a taxon belonging to the section Jacea. The plant was collected in Smedjia region-Serbia, on September 2008. The aerial parts were extracted according to the Bohlmann isolation method, slightly modified [5]. One new naturally occurring sesquiterpene lactone (Fig.1), six known guaianolides, namely babylin A, chlorohyssopifolin C, chlororepdiolide, retusin, kaemferol trimethyl ether, pheophytin A, were isolated by repeated CC and RP18-HPLC. The structure of the isolated compounds were elucidated by spectroscopic methods, particularly high-field NMR spectroscopy (1 H-NMR, 1 H-1 H COSY, HSQC, HMBC). So far, the presence of guaianolides is characteristic for the taxa of the section Jacea [6, 7].

Figure 1


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Enrichment of bioactive phenolic compounds from aqueous solution by foam separation

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Olive mill wastewater (OMWW) is an abundant source of polyphenols [1]. Due to their different bioactivities, OMWW would be a worthwhile source of highly valuable compounds for pharmaceutical and food industry. However, a simple and cost-effective extraction technique has still to be found. The present study aimed to evaluate foam separation for the isolation of phenolic compounds from OMWW. Thereby, surface active substances can be enriched in an up-rising foam produced by introducing gas in an aqueous solution [2]. Running the process with inert gas at room temperature provides a mild technique for heat- and oxygen-sensitive substances. Aqueous solutions of phenolic acids (vanillic acid, 4-hydroxybenzoic acid, 4-hydroxyphenylacetic acid, 4-hydroxybenzoic acid, protocatechuic acid), all found in OMWW, were used as simple models of OMWW. The added cetyltrimethylammonium bromide (CTAB) acted as a foaming agent and as an anion-collector for the deprotonated reference substances. Enrichment factors (concentration in the collected foam relative to the concentration in the feed solution verified via HPLC analysis) for the phenolic acids were optimized by varying important process parameters like pH, CTAB concentration, biophenol concentration, and gas flow rate. As a result, enriched extracts were obtained for all tested substances. Acknowledgement: This work was funded by the Bundesministerium für Bildung und Forschung, BioDisc. References: 1. Obied HK et al. (2005) Agric Food Chem 53: 823 – 937. 2. Lemlich R (1986) Ind Eng Chem Res 50: 16 – 29.

PG70

Microgram-scale, in vivo natural product discovery using zebrafish bioassays, UHPLC-TOF-MS and microflow NMR:
Identification of anticonvulsants in the Philippine medicinal plant Solanum torvum Crawford AD1, Challet S1, Buenafe OE2, Harvey AL1, Esquerra CV1, De Witte PA1, Wolfenden J2

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The rapid acquisition of structural and bioactivity information on natural products at the sub-milligram scale is key for performing efficient bioactivity-guided isolations. We have recently established zebrafish as an ideal in vivo system for natural product discovery [1,2]. Zebrafish offer...
the possibility of rapid in vivo bioactivity analysis at the microgram scale, an attractive feature when combined with high-resolution fractionation technologies and microgram-scale analytical methods such as UHPLC-TOF-MS and microflow NMR. Using this platform, we have performed high-resolution in vivo bioactivity profiling of Solanum torvum Schult., one of several Solanaceae species used as medicinal plants for the treatment of epilepsy [1–6]. The crude methanolic extract of S. torvum stem bark exhibited strong anti-convulsant activity in a microtiter plate-based zebrafish seizure assay. UHPLC-TOF-MS profiling revealed the presence of numerous steroid glycosides. An initial enrichment step localized the bioactivity to the steroid fraction. High resolution fractionation by semi-preparative LC-MS enabled the resolution of most constituents directly in a single 96-well microtiter plate. The analysis of these wells by microflow NMR in combination with LC-MS profiling allowed dereplication of the active compounds, identifying them as spirostanol glycoside derivatives and estimating their microquantities for quantitative assessment of anticonvulsant activity in zebrafish. These results indicate the potential of zebrafish bioassay-guided microfractionation in combination with UHPLC-TOF-MS and microflow NMR to rapidly identify bioactive natural products. References: 1. Crawford AD et al. (2008) Planta Med 6: 624. 2. Crawford AD et al. (2011) PLoS ONE 6:e14694. 3. Glauser G et al. (2009) J Agric Food Chem 57: 1127. 4. Guimberteau E (1951) Medicinal Plants of the Philippines, Bureau of Printing, Manila. 5. Barber CF (1895) JAMA 25: 1023, 6. Trusch D (1904) Journal de Médecine de Paris 8: 74.

Several species of the large and taxonomically complex genus Alpinia are of ethnomedicinal importance in (sub)-tropical regions of Asia and Oceania. Different parts of the plants (rhizomes, leaves, fruits) are used mainly for medicinal purposes, as food additives and spices (e.g. A. officinarum Hance, A. galanga Willd., and A. oxyphylla Miq.). Current comparative studies on the chemical profiles of rhizomes of 25 Alpinia species revealed the presence of labdane diterpenes, acetylated phenylpropenes, kavalactones, specific flavonoids, and diarylheptanoids, each being derived from distinct genetically defined pathways. Phytochemical methods applied include various chromatographic techniques for profiling (HPLC-UV/Vis, TLC) and isolation (CC, MPLC, pTLC), and spec-troscopic methods applied include various chromatographic techniques for profiling (HPLC-UV/Vis, TLC) and isolation (CC, MPLC, pTLC), and spectroscopic methods (NMR, MS) for structure elucidation of obtained compounds. Most of the extracts and purified compounds were routinely checked for bioactivity, against the phytopathogenic fungus Cladosporium sphaerospermum and the pest insect Spodoptera littoralis. In addition, some of the pure compounds isolated were studied for their anti-angiogenic properties in a zebrafish model system. The labdane-diterpene zerumin A dose-dependently inhibited vessel formation on both wild type and Tg(fli1a:EGFP)y1 zebrafish embryos through effects on multiple molecular targets related to angiogenesis. The occurrence of different secondary metabolites within rhizomes of Alpinia is presented, and bioactivity results are discussed.


Euphorbia species, commonly named spurge, have been widely used in traditional medicine to treat several diseases, like tumors and warts [1]. From these species, a wide range of structurally unique polyoxygenated macrocyclic diterpenes as jatrophanes, lathyranes and their polycyclic derivatives, have been isolated. These compounds have shown several important biological activities including anti-cancer properties, modulation of multidrug resistance in cancer cells and apoptosis induction [2–4]. Euphorbia boetica Boiss. is an herb endemic to Europe, commonly found in Southwest of Iberic Peninsula (Algarve and Alentejo). In our continuing investigation for biologically active molecules, the methanol extract of E. boetica aerial parts has been studied. The crude methanolic residue was suspended on a methanol-water mixture and extracted with ethyl acetate. Repeated column chromatographic fractionation and further purification by HPLC of the ethyl acetate soluble part afforded six diterpenes with the lathryhane skeleton that have a new acylation pattern. In addition, a cycloartane triterpene was also isolated and identified. The chemical structures of the isolated compounds, including stereochemical features were deduced from their physical and spectroscopic data, which include Infrared Spectroscopy, low and high resolution Mass Spectrometry (MS), and extensive one- and two-dimensional Nuclear Magnetic Resonance studies (1D and 2D-NMR). Acknowledgement: This work was supported by Fundação para a Ciência e Tecnologia (FCT) (Project PTDC/QUI-QUI/099815/2008) References: 1. Hartwell J (1965) Lloydia 32: 153 – 205. 2. Lage H et al. (2010) Phytomedicine 17: 441 – 448. 3. Duarte N et al. (2008) Bioorg Med Chem 16: 9321 – 9330. 4. Duarte N. et al. (2007) Bioorg Med Chem 15: 546 – 554.

Multidrug-resistance phenomenon (MDR) to anti-cancer drugs is one of the most serious obstacles in the success of a chemotherapeutic treatment. P-glycoprotein (P-gp) is often implied in the efflux of drugs as anti-cancer drugs, vinca alkaloids, and other non-related drugs, lowering the effective concentration of such drugs in the cytoplasmatic compartment [1]. One of the most promising approaches to overcome MDR is the development of molecules that can effectively modulate the activity of P-gp, thus inhibiting the drug efflux. In the past decades, several natural and synthetic compounds have been reported as MDR modulators, but none is currently available for the clinical practice. In previous works, we have isolated, from Euphorbia species (Euphorbiaeae), several macrocyclic jatrophane [2,3] and lathyrane-type [3,4,5] diterpenes with strong P-gp modulation activity. In order to obtain a library of bioactive lathyrane and jatrophane diterpenes, required for QSAR studies and further refinement of an in-house P-gp modulators pharmacophore model, the phytochemical study of Euphorbia piscatoria Ait., an endemic species from Madeira island traditionally used in fishing activities, has been carried out. Fractionation by chromatographic methods of the crude methanolic extract of the aerial parts of Euphorbia piscatoria yielded a large amount of a lathyrane-type diterpenoid that was acylated, using different alkanol and acyl chlorides/anhydrides. Several new esters were obtained whose structures were assigned based on spectroscopic methods namely 1D NMR (1H, 13C), DEPT and 2D NMR (COSY, HMBC, HMQC) data. Acknowledgement: This work was supported by Fundação para a Ciência e Tecnologia (FCT) (Project PTDC/QUI-QUI/099815/2008 and grant SFRH/BD/72915/2010). References: 1. Teodori E et al. (2002) II Farmaco 57: 385 – 415. 2. Valente C et al. (2004) Planta Med 70: 81 – 84. 3. Duarte N et al. (2006) Planta Med 72: 162 – 168. 4. Duarte N et al. (2008) Bioorg Med Chem 16: 9323 – 9330.

Unusual Cycloartane Saponins with Cytotoxic Activity from Astragalus stereocolex Bornm. Yağcın FN1, Piacente S2, Perrone A2, Capasso A2, Duman H3, Cals T1

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The genus Astragalus L., which is included 10 subgenera and approximately 150 sections, today it contains about 2500 – 3000 species and subspecies according to some sources (1 – 2). In Turkish folk medicine, the aqueous proctics extracts of some Astragalus species (declared by the healer) are used to treat leukaemia as well as for wound healing (3). Six new cycloartane-type triterpene glycosides (1 – 6) were isolated from Astragalus stereocolex Bornm. along with six known cycloartane-type glycosides (Figure). Their structures were established by the extensive use of

All the isolated compounds were evaluated for their in vitro biological activities, such as antiviral, antimicrobial, and anti-inflammatory activities. The structures of the compounds were elucidated by comparing their spectral data with those reported in the literature. The isolated compounds were evaluated for their in vitro antibacterial activity against Gram-positive and Gram-negative bacteria. Some compounds showed inhibitory activity mainly against Gram-positive and Gram-negative bacteria. Some compounds showed inhibitory activity mainly against Staphylococcus aureus ATCC 6538 with MIC values ranging from 12.5 to 50 μg/mL. These compounds might be promising leads for the development of new antiviral agents. Acknowledgement: This study was supported by a fellowship from FCT, Portugal (reference number SFRH/BPD/37179/2007).

References:

**Antimicrobial constituents from the African medicinal plant Zanthoxylum capense**

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The genus Zanthoxylum, comprising approximately 250 species, is well known for its ethnobotanical uses among the Rutaceae family [1]. Previous studies have demonstrated that plants belonging to this genus are rich sources of biologically active compounds, such as alkaloids, aliphatic and aromatic amides, coumarins, as well as lignans [2]. Zanthoxylum capense (Thunb.) Harv. is a medicinal plant indigenous to Zimbabwe, South Africa, and Mozambique. Traditional healers use the decoction of its roots for snakebites, and the decoction of its root barks to treat tuberculosis, paralysis, and relief of toothache. However, until date there have been relatively few phytochemical studies on this species [3, 4]. During our search for bioactive compounds from the methanolic extract of Z. capense roots, we have isolated a new benzophenanthridine alkaloid and two new 2-arylbenzofuran neolignans. In addition, several known compounds were also isolated, including six alkaloids with the known compounds were also isolated, including six alkaloids with the molecular formula of [M+Na]+ adduct ions yielded the highest content of structural information. Reference: 1. Choi HJ et al. (2001) Dyes Pigment 49: 15 – 20.

**Anti-Influenza Viral Flavonoids from Persicaria filiforme**

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Persicaria filiforme Nakai, which belongs to the family Polygonaceae, is a perennial herb growing in mountains area of Korea [1]. It has been used as a traditional medicine for the treatment of various bleeding, diarrhea, dysentery, and stomachache [2]. In the course of a search for anti-influenza viral compounds from natural products, we have found that the methanol extract of P. filiforme has anti-viral activity against influenza A virus. To date, there are no reports on the phytochemical constituents and biological activities of P. filiforme. A new flavonol glycoside and eight known flavonoids were isolated from the EtoAc and n-ButOH fractions of P. filiforme. The known compounds, quercitrin (2), juglansin (3), avicularin (4), afzelin (5), quercitin (6), kaempferol (7), quercitin 2’-O-gallate (8) and quercitin 3’-O-gallate (9) were identified by and comparison of their spectral data with literature values. The structure of a new flavonol glycoside (1), named persicarsioside A, was determined as quercitin 3-0-b-D-apiofuranosyl-(1-2)-6-l-rhamnopyranoside from spectral data and chemical evidence. The anti-influenza viral activity of the isolates 1-9 were evaluated using cytopathic effect (CPE) inhibition and antiproliferative assay against influenza A (H1N1) virus [3]. Compounds 1, 3, and 7 showed antiviral activity. The anti-viral compounds were subjected to inhibition activity against neuraminidase in a virus. The effects of compounds was evaluated using neuraminidase inhibition assay in influenza A (H1N1) virus [5]. The activity of neuraminidase decreased significantly by tested compounds, however half reduction of enzymatic activity was shown at relatively high concentration. Reference: 1. Lee TB (1999) Illustrated flora of Korea. Haengmoona. Seoul. 2. ZhonghuaBencao Compilation Committee (1999) ZhonghuaBencao (2). Shanghai Science and Technology Publisher. Shanghai. 3. Player MR et al. (1998) Proc Natl Acad Sci USA 95: 8874 – 8879. 4. Jeong HJ et al. (2009) Bioorganic & Medicinal Chemistry 17: 6816 – 6823. 5. Song J-M et al. (2005) Antiviral Research 68: 66 – 74.

**Isolation and Tandem Mass Spectrometric Characterization of Selected Crocus sativus L. (Saffron) Bioactive Compounds**

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Saffron, the dried stigmas of Crocus sativus L., is an expensive spice that is used mainly as a medicine or food coloring and flavoring agent in different parts of the world. The major biologically active ingredients of saffron are crocin analogues, including crocins 1 – 4, which are all glucosides of trans-crocetin, a carotenoid derivative. cis-Crocetin and its glycosides are also present, although they make up the minor components in saffron [1, 2]. In addition, saffron also contains the flavonoid derivatives, picrocrocin and its aglycone safranal, in lower quantities. In this study, extraction of saffron was carried out using 50% methanol and was evaluated qualitatively using HPLC-DAD profiling. According to the Rt and UV spectra, crocin analogues (cis and trans), kaempferol glycosides, picrocrocin and safranal were detected. The analytical method that was used for the profiling was transferred to preparative MPLC and HPLC scale and was used for the fractionation and purification of the aforementioned constituents, respectively. The complete identification of the purified compounds was performed using NMR (1 & 2D) and HRMS/spectrometric methods. Fingerprinting of crocetin derivatives was performed by LC-electrospray (ESI) mass spectrometry (MS) on Orbitrap and QqRTof systems. Moreover, selected saffron compounds (e.g., trans-crocin, cis-crocin and picrocrocin) were further characterized by various tandem mass spectrometric techniques such as vacuum MALDI-TOF/TOF-MS in combination with collisional induced dissociation, intermediate pressure MALDI-QqRTof-MS, ESI-QqRTof-MS/MS, ESI-ITQ-orbitrap-MS/MS and ESI-IT-MS/MS. Furthermore, high-energy CID on the stable [M+Na]+ adduct ions yielded the highest content of structural information.

References:

**Isolation, Purification and Structure Elucidation of Diterpenoids from the Roots of Salvia chorassanica Bunge**

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Salvia contains around 900 species worldwide, mainly in central Asia. The genus has 58 species and one hybrid in Iran, in which 17 of them are...
endemic to the country [1]. *Salvia chassarranica* Bunge is one of the Iranian endemic species of *Salvia* that only grows in Iran that belongs to the Lamiaceae family. There is not any reported literature on *S. chassarranica* so the present project set to start search for finding various diterpenoids from this plant. Air- dried and powdered roots of *S. chassarranica* were extracted with EtOAc (3 x 3L), for about 24h at ambient temperature. After filtration, the combined extracts were concentrated yielding 20g of total extract. Two diterpenoids, Taxodione (1) [2] and Ferruginol (2) [3], were isolated by means of chromatographic methods mainly column chromatography checked by TLC, and purified by preparative RP-HPLC. The structures of compounds 1 – 2 were determined on the basis of spectroscopic data [4,5] using 1H NMR, 13C NMR, DEPT-135, HMBC, HSQC and NOESY experiments. In conclusion, based on the result obtained from our study *S. chassarranica* can be considered a rich source of different abietane diterpenoids. This is the first report of compounds isolated from this plant. References: 1. Emami A et al. (2008) de l‘Universite d‘Iran des Sciences Medicales pp. 362 – 391. 2. Kuchpan SM et al. (1968) Journal of the American Chemical Society 90: 5923 – 5924. 3. Son Kh et al. (2005) Bioorganic & Medicinal Chemistry Letters 15: 2019 – 21. 4. Marcos IS et al. (2010) Tetrahedron 66: 7773 – 7780. 5. Tezuka Y et al. (1998) Pharmaceutical Society of Japan 46: 107 – 112.

**Figure 1**

A new cycloartane-type glycoside from *Astragalus schottianus* Boiss
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*Astragalus* L., the largest genus in the family Leguminosae, is represented by 445 species, of which 224 are endemic. They can be attributed to 62 sections in the flora of Turkey [1,2,3]. The roots of *Astragalus* species represent a very old and well-known drug in traditional medicine for its usage as an antispasmodic, diuretic and tonic drug [4]. In the district of Anatolia, located in South Eastern Turkey, an aqueous extract of the roots of *Astragalus* is traditionally used against leukemia and for its wound-healing properties. Known biologically active constituents of *Astragalus* roots represent two major classes of chemical compounds, polysaccharides and saponins [4]. In our continuing search on Turkish *Astragalus* species, we have isolated a new cycloartane-type triterpene glycoside from methanolic extract of *A. schottianus* by combined chromatography on reverse phase C-18 and silica gel. The structure of the new compound was determined as 3-O-β-D-xylopyranosyl-3b,6β,16β-20S,24S,25-hexahydroxy-cycloartane by the extensive use of 1D and 2D-NMR techniques and mass spectrometry. This compound represents the first entry of the series of cycloartane-type compound possessing a 20-OH functional group in *Astragalus* genus.

**Figure 1**

Flavonol glycosides and a saponin from *Chenopodium foliosum* Asch.

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Three new flavonol glycosides and a new saponin, namely 6-methoxykaempferol-3-O-β-gentiobioside, gomphrenol-3-O-β-gentiobioside, gomphrenol-3-O-α-L-rhamnopyranosyl-1→2-O-β-D-glucopyranosyl-1→6-O-β-D-glucopyranoside and 3-O-β-D-glucopyranosylo-30-normedicagenic acid-38-O-β-D-glucopyranosylester as well as the known flavonol glycosides patuletin-3-O-β-gentiobioside and spinacetin-3-O-β-gentiobioside were isolated from the aerial parts of *Chenopodium foliosum* Asch. The structures of the compounds were established by means of spectroscopic methods (1D and 2D NMR, UV, IR, and HRMS). DPPH free radical scavenging activity and cytotoxicity (MTT-test) of the new compounds were assessed as well. Acknowledgement: This study was supported by Medical Science Council at the Medical University of Sofia (Project 36/2011)
Terpenoids from the Root of Salvia hypoleuca Benth  

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The genus Salvia comprises nearly 900 species and is one of the largest members of the lamiaceae family. Fifty eight species of this genus are found in Iran, 17 species of them are endemic. In this study, the roots of Salvia hypoleuca Benth. were collected at flowering stage from Tehran province (Iran) and dried at room temperature, in shade. Dried plant materials were cut into small pieces and extracted with ethyl acetate by percolation method. Three sterols, stigmasterol, sitosterol and stigmasterol, two diterpenes, manool and 7α-acetoxy royleanone and five triterpenes, ursoic acid, oleoanolic acid, 3-epicorosolic acid, 3-epimaslinic acid and coeloneolic acid, were isolated and purified by column chromatography (silicagel normal and reverse phases, Sephadex LH20).

The structures of these compounds were identified by spectroscopic methods including 1H-NMR, 13C-NMR, DEPT, HSQC, HMBC and H-H COSY. These compounds have been reported for the first time from Salvia hypoleuca in which coeloneolic acid has not been previously reported from the genus Salvia. Keywords: Lagochilus cabulicus, flavonoid, chromatography, spectroscopy Acknowledgement: This research was supported by the Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences.

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Phytochemical study of Lagochilus cabulicus Benth  

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The genus, Lagochilus, belongs to Lamiaceae family and consists of 44 species all over the world, 33 of which grow in central Asia. Five species of this genus have been found in Flora Iranica and 4 species exclusively grow in Iran. Chemical studies on some Lagochilus species have studied [3–12]. One of these species, Lagochilus cabulicus Benth., was collected during flowering stage, dried at ambient temperature and vacuum dried and cut into small pieces. Plant material was successively extracted with ethyl acetate and methanol solvents using percolation method. Main compounds were separated and isolated by column and thin layer chromatography. The isolated compounds were identified by spectroscopic methods, including 1H-NMR and 13C-NMR. In conclusion, four flavonoids, Tricetin 3'-methyl ether (1), Quercetin (2), Quercetin 3-O-D-l-rhamnopyranosyl (193 Da), the 6''-O-D-pentosyl-8-O-[(3-hydroxy-3-methylglutaroyl)-2''-O-pentosyl-C-hexosyl-apigenin (MW 724 Da) and the 6'-O-D-pentosyl-3'-methylglutaryl-2''-O-pentosyl-C-hexosyl-apigenin (MW 708 Da). Attending that half of these compounds were herein described for first time in Fabaceae, overall, the present work is a valuable contribution for the phenolic elucidation of all Cytisus genus as well as of Fabaceae family.

Figure 1: Structures for flavones identified in Cytisus multiflorus

Polymeric biophenols in olive mill wastewaters

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Olive mill wastewater (OMW), the effluents generated in the olive (Olea europea L.) oil extraction industry operating in three-phase mode, are phytotoxic mainly due to its high phenolic content [1]. On the other hand, attending to the potential health-benefits of some of their phenolic compounds, OMW are now regarded as a potent source of biophenols for food and pharmaceutical industries. An important portion of the OMW biophenols include the secoiridoids found in olive pulp and their derivatives formed along the olive oil extraction process [2]. Still, due to the complex composition of OMW, many phenolic compounds remain unknown. Their structural identification can encourage the search of new bioactive compounds in OMW and contribute to further valorize this waste. In the present work, electrospray ionization-mass spectrometry analysis in the negative mode with direct infusion of OMW aqueous acetone purified extracts allowed to identify new major polymeric compounds, detected as [M-H]- ions at m/z 909, 1071, 1457, 1075 and 1613. These compounds could be classified into two groups: I- derivatives of a lignostigoid glucoside isomer and II- oleuropein oligomeric compounds. Attending that the scavenging ability of a polyphenolic compound is increased by its degree of polymerization [3], bioactivities related to that capacity are expected at least for some of these compounds.
The constituents from the stem of Clausena lansium and their bioactivities

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Two new glycosides, clausenosides A (1) and B (2) and three new carbozol alkaloids, clausenamine A (3), clauamine A (4) and clauamine B (5), together with fifty known compounds were isolated from the stem of Clausena lansium Skeels. Their structures were determined by spectroscopic methods, including CD spectroscopy and 1D and 2D NMR spectra. Compound 4 has an 1-oxygenated carbozole framework with a rare 2,3-lactone ring. Compound 5 represents the first acetal carbozole alkaloid, of which the absolute configuration was determined by Mosher’s method. The cytotoxicity of compounds 3–6 against a limited panel of cancer cell lines and the anti-inflammatory activity of 8–27 were evaluated.

Figure 1: Structures of compounds 1 – 5 from the stem of Clausena lansium

Acknowledgement: The authors are grateful for financial support from the National Science council of Republic of China awarded to T-S. Wu. Thanks are also given to Associate Prof. Vu Xuan Phuong (Institute of Ecology and Biological Resources, Vietnamese Academy of Science and Technology). This study was supported in part by Taiwan Department of Health Clinical Trial and Research Center of Excellence (DOH 100-TD-B-111 – 004).

Bioactivity guided isolation of iridoid and flavonoid glycosides from four Veronica species

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The genus Veronica L. (Plantaginaceae) has been used traditionally for the treatment of a number of diseases. The use of Veronica species for influenza, coughs, inflammation, rheumatic pains and cancer was reported in many countries [1,2]. Earlier investigations performed on Veronica species resulted in the isolation of mainly iridoid glucosides, especially benzoic and cinnamic acid esters of catalpol, some phenylethanoid and flavonoid glycosides [3,4]. It is represented by 79 species in Turkish flora, 26 of which are endemic [5]. In this study, the antioxidative activity of four different Turkish Veronica species were investigated and bioactivity guided isolation was carried out to examine the chemical composition of V. serpyllifolia L. Further, 2,2-diphenyl-1-picrylhydrazyl (DPPH), nitric oxide (NO) and superoxide (SO) radical scavenging assays

Figure 1: Bioactivity guided isolation of iridoid and flavonoid glycosides from four Veronica species
were performed for guiding of the bioactivity. *V. chamaedrys* L. was found to be the most bioactive species, followed by *V. serpyllifolia*. *V. fuhsi I Freyn & Sint.* was found to be the least active species. Thin layer chromatographies of their water extracts showed *V. chamaedrys* to contain a large proportion of phenylethanoid glycosides, the remaining species showed the presence of a large proportion of flavonoid glycosides. Their structures were determined as iridoid glucosides verposide, catapavoside, veronicoside and flavonoid glycosides 4"-methylapigenin-7-O-ramnosopyranosyl-acetylglucopyranoside, 3'-O-methylutelitin-7-O-ramnosopyranosyl-acetylglucopyranoside using different 1D and 2D NMR techniques. Isolation and structure determination studies on bioactive compounds of genus Veronica are still continuing. Acknowledgement: This study was supported by the Scientific and Technical Research Council of Turkey (TUBITAK) Project No: 108T158. References: 1. Baytop T (1994) Therapy with Medicinal Plants in Turkey (Past and Present). Publications of Istanbul University, Istanbul. 2. Fujita T et al. (1995) Econ Bot 49: 406 – 422, 3. Harpur US et al. (2003) 2 Naturasforsch 58c; 481 – 484. 4. Saracoglu I et al. (2004) Phytochemistry 63: 2379 – 2385. 5. Davis P (1978) Flora of Turkey and the East Aegean Islands. Vol. 6. University Press. Edinburgh.

**PG91**

High-speed countercurrent chromatography of *Harpagophyllum procumbens* constituents and their identification by TLC-MS

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Harpagophyllum procumbens DC. (Pedaliaceae), known as Devil’s claw, is native to the arid regions of Southern Africa including the Kalahari desert. The dried secondary root tubers have been used to reduce pain and inflammation especially in rheumatism and arthritis [1]. Iridoid glycosides are considered to be the main pharmacologically active constituents with other constituents such as phenylethanoid glycosides and flavonoids contributing to the effect [2]. Rapid isolation and identification of the constituents was necessary in order to acquire sufficient quantities of the reference compounds for use in further biological studies as well as to develop quantitative calibration models. To achieve these goals, a methanol extract of the secondary root tubers was rapidly filtered over silica gel to remove sugars and other polar compounds. The resulting fraction, which consisted mainly of iridoid and phenylpropa-

doid glycosides, was subjected to high-speed countercurrent chromatography (HSCCC). This allowed a one-step separation of the major constituents. The minor constituents were obtained either by a second HSCCC operation or by a final column chromatographic step. In order to distinguish close-running compounds in the absence of reference standards, TLC-MS [3] was performed on the extract and the isolated constituents. This method could be used, for example, to distinguish the closeluting pair 8-8-p coumaroylharpagide and 8-8-p-feruloylharpagide [2]. Acknowledgement: The authors thank the National Research Foundation of South Africa for financing this study. References: 1. Qi J et al. (2006) Phytochemistry 67: 1372 – 1377. 2. Karioti A et al. (2011) J Pharm Biomed Anal 55: 479 – 486. 3. Reich E, Widmer V (2009) Plant Med 75: 711 – 718.

**PG92**

Antiplasmodial and antitrypanosomal triterpenoids from *Salvia hydrangea* with rare carbon skeletons

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Salvia hydrangea DC. ex Bentham, endemic to Iran, has been widely used in traditional Iranian medicine. Fractionation of the hexane extract of the aerial parts of this plant led to the isolation of hydrangdione-A (1) and hydrangdione-B (2), two new triterpenoids with rare carbon skeletons. Their structures were established on the basis of an extensive spectroscopic analysis, including 1D and 2D NMR, and by comparison of their NMR data with those of the related compounds. The IC50 of the compound 1 and 2 were determined against two parasites and rat myo-

**PG93**

Flavonoid constituents from *Morettia philaeana* and their antimicrobial activity

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A successfull petroleum ether, diethyl ether, ethyl acetate, butanol and methanol extracts of *Morettia philaeana* (Delile) DC. (Cruciferae) flower-

ing aerial parts were tested for their antimicrobial activity. The ethyl acetate and methanol extracts were found to be most effective against most of the tested organisms. The chemical investigation of these extracts afforded nine flavonoids using chromatographic techniques. These are kaempferol, kaempferol 3-O-glucoside, kaempferol 3,7-di-O-glucose, kaempferol 3-O-sophoroside-7-O-glucoside, quercitin, quercitin 3-O-j-glucoside, quercitin 3-O-j-gentibioside, orientin and isoorientin. Their structures were established through chemical and spectral analysis. All flavonoids were evaluated to show a broad antimicrobial spectrum of activity on microorganisms including seven bacterial and two fungal species. Among them, the isolated aglycones had stronger bioactivity than their glycosides. Acknowledgement: This work was financially supported by the Phytochemistry and Plant Systematic Department, National Research Centre, Giza, Egypt.
1,3,5-Trihydroxy-2,6,7-trimethoxyxanthone (Holt et al. 1997) Compound from dichloromethane extract was assayed according to the method of References:


**MAO Inhibitory Activity of Xanthones from Dichloromethane Extract of Polygala supina**

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Eight xanthones as inhibitors of monoamine oxidase have been isolated from dichloromethane extract of aerial part and roots of Polygala supina Schreb. The isolated xanthones were characterized by spectroscopic methods such as 1D, 2D NMR, and MS data analyses as 6,8-Dihydroxy-2,3,4-trimethoxyxanthone (1), 2,4,6,8-Tetrahydroxy-3,7-dimethoxynanthone (2), 2,3,7-Trimethoxyxanthone (3), 3,7-Dihydroxy-1,2-dimethoxynanthone (4), 1,3,6-Trihydroxy-2,7-dimethoxyxanthone (5), 1,3,5-Trihydroxy-2,6,7-trimethoxyxanthone (6), 3,6,8-Trihydroxy-1,2-dimethoxynanthone (7), 1,3,7-Trihydroxy-2,6-dimethoxynanthone (8). MAO-A (monoamine oxidase-A) activity of isolated xanthones (1 – 8) from dichloromethane extract was assayed according to the method of Holt et al.1 Compound 5 showed the best activity with IC50 value of 0.24 mM, also 1 and 4 showed good activity with IC50 values of 2.12 mM and 3.64 mM, respectively. The compounds 2, 6, and 7 showed mild activity with IC50 values of 12.21, 43.80, and 23.98 mM. However, compounds 3 and 8 were not active. References: 1. Holt A et al. (1997) Analytical Biochemistry 244: 382 – 392.

**New N-alkylamides from Anacysys pyrethrum**

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The roots of Anacysys pyrethrum DC (Asteraceae) are frequently used in traditional medicine e.g. as aphrodisiac1. Depending on the extraction method and solvent, different yields of N-alkylamide constituents can be found, possibly resulting in alterations in biological effects and toxicity. Therefore, analytical profiling of the bio-active N-alkylamides in these plant preparations is an inevitable quality parameter, with liquid chromatography-electrospray mass spectrometry (HPLC/ESI-MS) as recommended technique for comprehensive analysis of alkylamides in plant extracts [2 – 4]. An N-alkylamide profiling from an ethanolic extract of the genus Salvia species has never been identified in Nature. The genus Salvia is a rich source of terpenoids with structural diversity. Apart from sesquiterpenoids as unusual constituents of Salvia species [12], it is a source of di- and triterpenoids with unprecedented carbon skeletons [3]. Aiming at identifying structurally interesting and bioactive metabolites from the Salvia species, we examined the extract of Salvia hydrangea DC. ex Benth. In our search for new bioactive natural products, a novel triterpenoid (hydrangdione C, 1) was isolated from the hexane extract of this plant. The skeletal type displayed by hydrangdione C was noticeable for its unusual carbon ring skeleton with a unique five membered ring D substituted by an acetyl group. This is the first report of a natural triterpenoid with a five-membered ring D. The structure of 1 was established by comprehensive 1D NMR, 2D NMR, and HRMS spectroscopic analysis and subsequently confirmed by a single-crystal X-ray diffraction study.

**Sesquiterpene lactones and other constituents from Hedyosmum brasiliense**

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Hedyosmum brasiliense Miq. is an endemic, aromatic arborescent shrub, which is the only representative of Chlorantaceae in Brazil. There have been few studies that seek to determine its chemical composition and/or pharmacological effects. This work describes five new compounds: two guaianolides, two elemnolides and a dimeric lindenanolide, which were tested against Mycobacterium tuberculosis, together with some widespread phenolics. All the structures of the isolated compounds were elucidated based on extensive analysis of 1D and 2D NMR and MS data, and also in comparison with the published data. The new compounds found were: 1,2-eq-10α-hydroxy-podaodionin (3), 1,3,7,10-tetrahydro-podaodionin (4), 15-acetyl-isogermacrene (5), 15-hydroxy-isogermaenolide (6) and brasiliensolide (7) – which is the first dimeric sesquiterpenoid identified in the Hedyosmum genus. The phenolic compounds isolated were scopoletin, vanillin, vanillic acid, protocatechuic aldehyde and ethyl caffeate. The isolated sesquiterpene lactones, at concentrations in the range of 1 – 30 μM, did not show in vitro anti-mycobacterial activity, since M. tuberculosis cultures exposed...
to the Hedysosyum brasileense-derived substances kept growing but were sensitive to isoniazid, an antibacterial agent.

medication usage. 1,2 Our previous work has led to the isolation of 90-plus alkaloids from different Stemona species. In our effort to identify alkaloidal constituents from the extract of a Vietnamese species, an LC-MS method was established for a rapid and sensitive screening of the specific compounds. 3 On the basis of more than 90 alkaloids, we established a MS database of all these reference compounds by UPLC/ESI-LTQ. By picking the specific peak in the LC chromatogram, extracting its MS, MS2 and MS3 spectra, and then comparing with those of the standard samples, we can do a rapid identification of main structures in the extract. Such technology was successfully applied for the chemical diversity investigation of three Stemona species – S. sessilifolia Franch. & Sav, S. japonica Franch. & Sav, and S. tuberosa Franch. The results revealed that the alkaloids varied greatly with species and habitats, but not with collecting seasons. S. tuberosa is the commonly-used species while having the most complicated metabolites which structural types were influenced extremely by ecological environment. S. sessilifolia growing in Tsuchu, Anhui province with the highest amount of the most active stemosipine, was determined to the best species for medicinal usage. All these data, combined with the pharmaceutical experiments, supplied the scientific evidence for guiding the usage of TCM baibu. Specific compounds 3. On the basis of more than 90 alkaloids, we established a MS database of all these reference compounds by UPLC/ESI-LTQ. By picking the specific peak in the LC chromatogram, extracting its MS, MS2 and MS3 spectra, and then comparing with those of the standard samples, we can do a rapid identification of main structures in the extract. Such technology was successfully applied for the chemical diversity investigation of three Stemona species – S. sessilifolia Franch. & Sav, S. japonica Franch. & Sav, and S. tuberosa Franch. The results revealed that the alkaloids varied greatly with species and habitats, but not with collecting seasons. S. tuberosa is the commonly-used species while having the most complicated metabolites which structural types were influenced extremely by ecological environment. S. sessilifolia growing in Tsuchu, Anhui province with the highest amount of the most active stemosipine, was determined to the best species for medicinal usage. All these data, combined with the pharmaceutical experiments, supplied the scientific evidence for guiding the usage of TCM baibu.

Keywords: 

Stemona species (Stemonaceae) are plant resources of traditional Chinese medicine ‘baibu’, which had long been used as antitussive and insecticidal agents. Stemona alkaloids, featuring a pyrrolo[1,2-c]azepine or pyridin[1,2-c]azepine nucleus, are believed to be responsible for their antibacterial activity.

Figure 1: Isolated compounds of Hedysosyum brasileense

Figure 2: Key NOE correlations observed for compounds 4 and 5

Acknowledgement: The authors are grateful to CNPq and CAPES for financial support, and Norberto P. Lopes and José C. Tomaz (FCFRP-USP) for the antimicrobial assay.

The fungal isolate *Penicillium brevicaespunctum* which isolated from the associated marine alga *Pelorocladia* sp. in autumn season was able to produce 11 clear and active compounds, separated by the best solvent system dichloromethane: methanol (95:5 v/v). Compounds 4 and 9 were considered as antibacterial compounds, active against Gram positive (*B. subtilis*) and Gram negative (*E. coli*) bacteria. Malt extract broth medium with initial pH 4 when incubated at 28°C in an incubator shaker at 200 rpm for 12 days were the most favorable conditions for compound 4 production (19.87 mg/L). The suitable conditions for compound 9 production (121.15 mg/L) were potato carrot broth medium, initial pH 4, incubation temperature 26°C at 180 rpm after incubation period for 10 days. Structural elucidation of the pure compounds suggested that compound 4 may be [D(+)-2-ethyl hexyl] phthalate], and compound 9 may be fungisterol or one of its isomers. Pure compounds were evaluated for cytotoxicity towards 6 different types of tumor cell lines performed in Cancer Biology Department, National Cancer Institute, Cairo, Egypt. The results revealed that, the maximum concentration of compound 4 (100 μg/mL) kills about 30% of lung cells. The maximum concentration of compound 9 (100 μg/mL) kills approximately 40% of the viable infected liver cells and also kills approximately 50% of the viable infected lung cells at concentration equal to 91.6 μg/mL. It can be concluded that compound 9 can be recommended as an anticancer compound.

Multiple resistance and environmental pollution to chemical pesticides with increasing world population led to the development and the improvement of new trends to pesticides from natural product. The application of natural product as well as the biological control specific agents especially nucleopolyhedrovirus (NPV) are considered very important tool to avoid the contradiction between pest control and clean environment. Furthermore, the biological control specific agents have many advantages such as their low mammalian toxicity and no adverse effect on plant growth and seed viability. The brown alga Sargassum species (Family: Sargassaceae) were widely distributed in worldwide. They have been used as food, as well as in industry and medicine for various purpose. Pharmacopeial constants, phytochemical screening, determination of minerals and trace elements of *Sargassum asperifolium* were performed using Kjeldal method and amino acid analyzer, respectively. The alcoholic extracts as well as amino acid composition of three algae were performed using in addition, they have high Ca, K, Mg & Fe contents. The protein content have high ash content and gave positive for sublimenable matter, volatilization of minerals and trace elements of

**Abstracts | 59th International Congress of the GA | 4th-9th September 2011, Antalya, Turkey**

**PH3**

**Effect of dieckol from Ecklonia cava on glucose transport in L6 muscle cells**

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Ecklonia cava, a brown sea algae that is grown in the coast of Jeju island in Korea, has a variety of biological activities. We investigated the effect of dieckol (DEK) isolated from Ecklonia cava on glucose transport and its related signaling pathways in L6 muscle cells. DEK increased the basal glucose transport independent of insulin. Glut4 was also translocated from cytoplasm to plasma membrane in response to DEK. PKB/Akt as well as ERK was phosphorylated by DEK in a dose-dependent manner. DEK also stimulated phosphorylation of AMPK, an insulin-independent stimulator of glucose uptake. DEK-stimulated increase of Glut4 translocation and of glucose transport were sensitive to both inhibition of PI3 kinase (by wortmannin) as well as AMPK (by compound-c). These results suggest that DEK from Ecklonia cava can be applied to ameliorate abnormalities in glucose metabolism like as insulin resistance or diabetes mellitus.

**Figure 1:** DEK stimulate glucose consumption independent of insulin in L6 muscle cells.

**Figure 2:** DEK-induced Glut4 translocation to plasma membrane is sensitive to inhibition of PI3-kinase as well as AMPK in L6 muscle cells.

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**PH4**

Composition of fatty oil of sea urchin eggs from Barents Sea

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The regular sea urchin Strongylocentrotus pallidus is a widespread species in high-Arctic waters. However, little is known about lipid composition of sea urchin eggs from northern Barents Sea. Sea urchin was harvested by divers in Barents Sea close to Murmansk (Russia) in November 2010. The eggs have been (or were) found to contain 20% (dry matter) total lipid fraction. Lipids were extracted from eggs with chloroform/methanol (2/1) by sonification in 30 min. Bound fatty acids were esterified by sodium methoxide, and the methyl esters were analysed by GC using a DANI 3865 GC equipped with SP 2340, 60m; 0.25mm i.d. (Supelco, Switzerland) column. The oven temperature was programmed from 70°C to 130°C at a rate of 10°C/min, to 235°C at a rate of 2.5°C/min. Programmed temperature vaporizer (PTV) increased from 70°C to 235°C, and FID temperature was set at 235°C. Peak identification was...
Marine dinoflagellates of the genus Amphidinium are well-known as a producer of unique cytotoxic metabolites. We have isolated a new 12-membered macrolide, iriomoteolide-12a, from the benthic dinoflagellate *Amphidinium* species collected off Iriomote Island, Japan. In this symposium, we will discuss the isolation and structural elucidation of this new macrolide. The dinoflagellate *Amphidinium* sp. (strain KC09055) was cultivated in 50 L of 1% Prowazek’s enriched seawater (PES) medium, 16 h light and 8 h dark. The algal cells obtained from 50 L of the medium were extracted with MeOH/toluene (3:1). The toluene-soluble materials were subjected to a SiO2 column, C18 column and one of a macrolide fractions containing compounds were separated by C18 HPLC to afford a new compound, iriomoteolide-12a. Iriomoteolide-12a was obtained as colorless amorphous solid, and the molecular formula of C_{21}H_{24}O_{8} was revealed by HRESIMS data. ^13C NMR data disclosed the presence of a total of 25 carbon atoms due to five quaternary carbons, one of which is a carbonyl carbons, eight methine carbons, five methylene carbons, and seven methyl carbons. The skeleton is related to that of amphidinolide Q [1, 2].

References:

**PH7**

**Antimicrobial active compounds of green alga Ulva rigida collected from Ghar El Melh lagoon (North of Tunisia)**

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The green alga *Ulva rigida* is wellspread within Tunisian coast mainly in the northern region of Ghar El Melh lagoon with important blooms particularly in warm seasons [1]. The aim of this study is to evaluate its antimicrobial potential against pathogens bacteria and fungi. Thus, polar and non polar organic crude extracts of dried *Ulva rigida* collected from Ghar El Melh lagoon (37° 10.8’ N, 10° 16.8’ E), were tested against eighteen pathogenic species of bacteria and the yeast *Candida albicans*. The dichloromethane/methanol extract which exhibited the most significant activity was therefore subjected to column gradient chromatography on silica gel and led to one hundred thirty seven fractions. Eluates were subsequently grouped into twenty nine sub-fractions based on their similarity in Thin Layer Chromatography (TLC). According to TLC visualisation by phosphomolybdic acid and Libermann’s spraying, and UV examination, we retained sub-fractions considered of further interest, to isolate antibacterial compounds through bioassay-guided fractionations. Purification processes (column chromatography with Silica gel and Sephadex LH-20 and preparative TLC) of the most active fractions led to purified and semi purified compounds with strong antibacterial activity especially against *Staphylococcus aureus* and *Micrococcus sp.* which are recognized amongst most common human pathogens. Nevertheless, active compounds showed weak value of CMI. H NMR and 13C NMR provided structural information about active compounds. References: 1- Shili A et al. (2000) Journal of Costal Conservation 8: 127 – 134.

**PH8**

**Antimicrobial potentialities of Ulva rigida epiphytic bacteria**

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Marine epiphytic bacteria (72 strains) were isolated from green alga *Ulva rigida* collected in two different biotypes (Cap Zebib, rocky shore: 30 strains and Ghar El Melh lagoon: 34 strains) and alga surrounding water (eight strains). All isolates were identified based on their 16S rDNA sequences and tested for antimicrobial effect against several human and fish pathogens (*Vibrio tapetis*, *Staphylococcus aureus*, *Pseudoalteromonas sp* and *Salmonella typhimurium*) were mostly inhibited by the isolates tested, while, *Vibrio species* (V. anguillarum, V. tapetis and V. alginolyticus) were resistant. *Candida albicans* was inhibited only by the two *Stappia* species isolated from *Ulva rigida* of Cap Zebib locality. Further investigations continue on the inhibition effect of *Ulva rigida* organic crude extracts against epiphytic bacteria isolated, in order to assess degree of affinity of epiphytes to their proper host. Keywords: *Ulva rigida*, *Epiphytic bacteria*, *Antimicrobial activities* Acknowledgement: The authors thank Ms. Veronique Confiurus-Gans, Department of Marine Microbiology, Netherlands Institute of Ecology, NIOO-KNAW, Yerseke, The Netherlands, for her assistance and help with PCR and DNA sequencing.
Effects of Nitrogen Fertilizer on Growth, Seed Yield and Oil Seed Content of Nakedseed Pumpkins (Cucurbita pepo subsp. pepo var. styriaca)

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The red seaweed Delesseria sanguinea dominantly populates a large artificial reef at Nienhagen in the Baltic Sea. It contains substantial amounts of sulfated polysaccharides (D.s.-SP), which consist of a homogenous fraction of branched sulfated xylolgalactans (gal-xyl -5.4) and exhibit a pharmacological profile indicating anti-inflammatory and anti-skin aging potencies [1 – 3]. Compared with heparin, D.s.-SP revealed stronger inhibitory effects on the enzymes elastase, hyaluronidase, heparanase, collagenase as well as on complement activation, cell adhesion to P-selectin and cytokine release from LPS-activated monocytes, but have only moderate anticoagulant activity. Their hyaluronidase and complement inhibitory activities proved even superior than those of the anti-inflammatory β-1,3-glucan sulfate PS 3. Crucial for an economic use is the availability of adequate amounts of D.s.-SP with reproducible high quality. For evaluation and optimization, 30 D.s.-SP batches were harvested and extracted since 2005 resulting in almost 200 D.s.-SP batches. By a standardized procedure (extraction (EX) with water for 8 h at 85°C), the D.s.-SP can be isolated in reproducible high quality. However, as found by a second 8 h-EX, the first 8 h-EX is incomplete. Subsequently modified EX-procedures led to following yields: 8.8% (1 x 8 h-EX), 13.3% (2 x 4 h-EX), 15.0% (2 x 2 h-EX) and 17.9% (4 x 2 h-EX). Consequently, a 2 x 2 h-EX (15.0%) seems to be a rational compromise. Moreover, the D.s.-SP obtained by shorter EX contained less glucose, which partly represents co-extracted starch: 14.4% (1 x 8 h-EX), 10.92% (2 x 4 h-EX), 9.0% (2 x 2 h-EX) and 11.74% (4 x 2 h-EX). The glucose content was further reduced by precipitating the extracted D.s.-SP with 70% instead of 90% ethanol. In conclusion, after stepwise optimization of the isolation procedure, the D.s.-SP from Nienhagen are ready for an economic use. Acknowledgement: This project is financed by the EU (FIAF/EFF) and the LFALF Mecklenburg-Vorpommern.

References:

Figure 1: The reaction catalyzed by daurichromenic acid synthase

References:

Development of new genomic and genic SSR primer pairs for carrot

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Carrot (Daucus carota L.) is one of the most economically important and the most popular vegetables cultivated worldwide among the members of the family Apiaceae. The carrot and its relatives are extensively grown worldwide for both fresh market and processing purposes. Despite the economic importance of carrot, there is still a need for the development of more effective tools for genetic improvement.

PI1 Molecular characterization of daurichromenic acid synthase from Rhododendron dauricum

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Rhododendron dauricum L. (Ericaceae) produces daurichromenic acid (DCA), an anti-HIV component [1]. DCA is a terpenophenol, and would be biosynthesized from grifolinic acid via oxidative cyclization of the far-nesyl group, the reaction analogous to those reported for cannabinoid biosynthesis [2]. We attempted to amplify cDNA fragments encoding DCA synthase by homology-based RT-PCR with degenerate primers designed from conserved sequences in cannabinoid synthases and related plant oxidasises. Then, the 3’ and 5’-ends of cDNA were obtained by rapid amplifications of cDNA ends. Consequently, three cDNA clones, that encode polypeptides named RdOx 1–3, were cloned. RdOx 1–3 consisted of 533, 533 and 534 amino acids containing a FAD binding motif. In addition, these polypeptides had > 50% identities with cannabinoid synthases. The heterologous expression system for RdOx1 was established using Pichia pastoris as a host. The recombinant RdOx1 and 2 could produce DCA from grifolinic acid, whereas RdOx3 showed no DCA-producing activity, suggesting that RdOx 1 and 2 are active DCA synthase in R. dauricum. DCA synthase would be applied for biotechnological production of DCA because the substrate grifolinic acid has been isolated from a mushroom Albatrellus disensus in a large amount [3].
of family Apiaceae. Despite its importance for human nutrition, health, and development of new drugs, genomic resources in carrot are relatively underdeveloped and the use of molecular markers in carrot has limited to a few reports of several researches [1]. Among the molecular markers microsatellite or simple sequence repeat (SSR) has much superiority in genetic studies since they are co-dominant, highly polymorphic, and reliable PCR procedure [2,3,4]. But, the number of microsatellite primer pairs flanking the microsatellites in ESTs and genomic DNA library limited in carrot gene use to utilize microsatellites in carrot genetic studies, new microsatellite primer pairs are required. To date at the NCBI, 3845 nucleotide sequences and 93 expressed sequence tag (EST) sequences are available for all Daucus species [March 2011]. We developed 14 microsatellite primer pairs using ESTs and genomic DNA library data bases in the NCBI databases. Microsatellites were determined using Exact-Tandem Repeat Analysis program and primer pairs flanking the microsatellites were designed using Primer3 software [5,6]. Microsatellite primer pairs developed in the present study (Table 1) will enhance generic studies in carrot. Besides, transferability of these microsatellite primer pairs from carrot to other members of the Apiaceae family is important for future genetic studies in the Apiaceae family. Acknowledgement: This research is supported by the Scientific Research Projects Coordination Unit of Akdeniz University. References: 1. Cavagnona PF et al. (2009) Mol Genet Genomics 281: 273 – 288. 2. Karaca M, Ince AG (2008) J Genet Genomic 87: 83 – 86. 3. Ince AG et al. (2010) Mol Breeding 25: 645 – 658. 4. Ince AG et al. (2010) Mol Breeding 25: 491 – 499. 5. Ince AG et al. (2008) Plant Cell Tissue Organ Cult. 94: 281 – 290. 6. Ince AG et al. (2010) Plant Mol Biol Rep 28: 285 – 291.

Transferability of EST-Microsatellite Markers to some Labiatae Genera

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Enhancing effect of Methyl Jasmonate on antioxidative capacity of Bunium persicum under Cadmium stress

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Methyljasmonate (MeJA) is a compound that used as plant growth regulation and is currently being used in cancer research. Bunium persicum

The effect of silicon on membrane integrity and antioxidative pigments on Echium amoenum that exposed to cadmium stress

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Some researchers reported that silicon (Si) increase tolerance in some higher plants against biotic and abiotic stress. The beneficial effects of Si are mainly associated with its high deposition in plant tissue and enhancing their strength and rigidity. We investigated the role of Si against cadmium stress in (Echium amoenum Fisch. & C.A.Mey.) in greenhouse condition. When the seventh leaf appeared, plants were pretreated with five levels of Si: 0.0, 0.5, 0.7 and 1.5 mM Si (as sodium trisilicate, Na2SiO3) and then the plants were treated with two levels of Cd (30 and 90 mM). The effects of Silicon and Cd were investigated on some physiological and biochemical parameters such as: lipid peroxidation (malondialdehyde (MDA) and other aldehydes), anthocyanin and flavonoid content. Our results showed that Cd significantly increased MDA, other aldehydes, anthocyanin and flavonoids content in Echium and silicon offset the negative effect and increased tolerance of Echium against Cd stress. From these results we concluded that Si increase membrane integrity and antioxidative ability in this plant against cadm...
Diterpenes including the phytalexins and phytohormone gibberellic acid are one of biologically important pharmaceutical sources from natural origin. *Scoparia dulcis* L. (*Scrophulariaceae*), a tropical medicinal plant, produce tetracyclic diterpenes such as scopaducic acid B (SDB) and scopadulciol (SDC). SDB exhibits various pharmacological activities including inhibitory effects on replication of herpes simplex virus type 1 and antitumor. Furthermore, SDB formation in *S. dulcis* leaf tissue is rapidly and transiently stimulated by addition of methyl jasmonate (MeJ) as an elicitor. In order to gain insight into the molecular mechanisms underlying diterpene biosynthesis, we have focused on cytochrome P450 (P450) enzymes often appear to form key regulatory steps in plant secondary metabolism. As a first step, we performed differential display analysis of P450 genes induced during elicitation of SDB biosynthesis after Mj addition to *S. dulcis* leaf tissues. As a result, nine genes were found to be up-regulated and were highly homologous to the corresponding region of P450 CDNA. We further examined the gene expression in the course of induction of SDB biosynthesis by MeJ or Yeast extract. In addition, we also isolated a gene encoding ent-kaurene synthase (KS) which catalyzes the cyclization of copalyl diphosphate to ent-kaurene from *S. dulcis* and analyzed its function. The KS gene has been duplicated in the *S. dulcis* genome and is highly expressed in mature leaves. Here, we discuss the physiological roles of these isolated P450s and KS in diterpene biosynthesis in *S. dulcis*.
PI11 Insights from P-Glycoprotein in-silico modelling
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P-glycoprotein (P-gp) is the most representative member of the ABC transmembranar transporter superfamily, often implicated in the multidrugs resistance phenomenon (MDR) [1]. Several models have been proposed for the efflux mechanism, namely the hydrophobic pore, the flip-flop model and, more recently, the hydrophobic vacuum-cleaner model. Using the bacterial transporters Sav1866, BtuCD or MsbA, several homology models have been constructed. However, the majority proved to be inaccurate due to errors introduced during the homology process, originating misleading results. The recently published crystallographic structure of the murine P-glycoprotein [2] constitutes a more suitable working model. However, a linker known to regulate substrate’s specificity and to be involved in the conformational changes that accompany ATP hydrolysis was not mapped [3]. Starting with the murine P-gp crystallographic structure, we built a system comprising a correctly protonated P-gp structure inserted in a lipid bilayer inside a molecular dynamics simulation box with respective counter-ions and waters to solvate all the system. Variations on this system were studied that allowed examining the influence of the linker and lipid type on the P-gp structure stability. The lipid environment and bilayer rigidity was also tested by studying systems with and without cholesterol. Different force field parameterizations were used for quality assessment. The molecular dynamics systems were simulated for tens of nanoseconds using the GROMACS simulation package and the new insights gathered from the simulations namely dynamic and static properties both from P-gp and lipids will be presented and discussed. Acknowledgement: This study was supported by FCT, Portugal (project PIDDQ/UI-QUI/099815/2008) References: 1. Juliano R et al. (1976) Biochimica et Biophysica Acta 455: 152 – 162 2. Allen J et al. (2000) Science 292: 1718 – 1722 3. Sato T et al. (2009) FEBS 276: 3504 – 3516

PI12 DNA-based molecular screening and identification of Veronica sp
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Southeastern Europe represents an important center of genetic diversity for many groups of Veronica. It was estimated that about 80 species of Veronica, representing 10 subgenera, are found in Europe [1]; out of these, about 40 have been reported in literature as being present on the Romanian territory [2]. Data about the chemical composition have been found for ten Veronica species from the Romanian flora; these species have a complex and variable biochemical composition, with many secondary metabolites used in pharmacognosy [3]. We aimed to apply molecular techniques to different Veronica species present in Romania, in order to obtain reliable means of authentication of the raw plant material and medicinal products which contains V. officinalis and other species of the genus. Nuclear ribosomal internal transcribed spacer region (ITS) and plastid DNA (cpDNA) intron sequences have been used for PCR amplification. The rpsb-trnC spacer region, one of the most variable plant markers of the plastid genome [4] and the psbA-trnf spacer, a highly variable cpDNA region [5] were amplified from different veronica species. The length both DNA fragments taken into evaluation for their putative usefulness as markers for plant authentication were highly variable among the Veronica species tested; the length variability in coherent with the molecular data reported from phylogenetic studies [6]. These two spacers could be successfully used as potential DNA barcode marker and as an alternative way to rapidly authenticate the plant species. Acknowledgement: This study was supported by UEFISCDC/project 32151/2008. References: 1. Albach DC et al. (2004) Taxon 53: 429 – 452. 2. Ichim MC et al. (2010) Bulletin UASVM Agriculture 67(2): 482. 3. Crisan G et al. (2008) Rev Med Chir Soc Med Nat 113(2): 81 – 85. 4. Shaw J et al. (2005) Am J Bot 92: 142 – 166. 5. Kress WJ et al. (2005) PNAS 102(23): 8369 – 837. 6. Albach DC, Meudt HM (2010) Mo. Phyl Evol 54: 457 – 471

PI13 Evaluation of the Effect of Licorice Extract on Proliferation and Differentiation of Human Mesenchymal Stem Cells into Osteoblasts
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Estrogen deficiency caused osteoporosis during the first decade after Menopause. Estrogen replacement therapy is effective in osteoporosis caused by menopause, but it has some effects, such as carcinogenesis and uterine bleeding. Recent studies have focused on replacement natural compound that contain phytoestrogens. phytoestrogen is natural compound derived from plant, which exist estrogen-like activities. Licorice is one of the medicinal plant that have phytoestrogen and its extract indicate activity as Estradiol in some parameters. To evaluate the effect of licorice extract on the proliferation and osteogenesis of human mesenchymal stem cells were determined by MTT method. In real-time PCR. Our results show that licorice extract were increased the proliferation and differentiation of hMSC in a dose dependent manner (significant at 10,25,50,100 ug/ml). Real-time pcr analysis shown that licorice extract treatment induced an increase in the expression of BMP-2, Runx-2, Alp, osteocalcin and sp-1 in day 6 and 12, hence ICI 182780, an specific estrogen receptor antagonist inhibit the effect of licorice extract on differentiation, we found that licorice extract stimulates osteoblastogenesis via estrogenic activity and can be used as alternative natural medicine for bone disease such as osteoporosis

PI14 Methyl Jasmonate-induced biosynthesis of taxol and expression of certain related genes by Hazelnut (Corylus avellana L.) cells
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Taxol (Paclitaxel), a diterpene alkaloid against cancer, was originally isolated from Taxus sp. and recently was shown to be produced by hazel nut as well. To develop an optimal bioprocess for pacitaxel supply, taxol biosynthetic pathway regulation must be better understood. In the present study, the effects of methyl jasmonate (MJ) on taxol production and phenyl alanine ammonia-lyase (PAL), deoxyxylulose phosphate reductoisomerase (DXR) and 3-hydroxy-3-methylglutaryl CoA reductase (HMGR) genes expression were investigated in suspension-cultured hazelnut cells. The cultures were treated with MJ (0, 25, 50 and 100 μM) 8 days after subculture. According to the results, cell growth and viability decreased but lipid peroxidation rate, phenolics and taxol production increased by these treatments. In those cells treated with 100 μM MJ, extracellular and intracellular taxol were respectively 168 μg/L and 20.5 μg/g DW (dry weight) for, 3 and 2.3 times higher than those of the control cells. The expression of the PAL and DXR genes were respectively 20.5 and 15 times of those of control cells. The expression of MJ and DEXR genes were respectively maximum after 48 h and 72 h of the MJ treatment, but the expression of HMGR gene was suppressed by MJ suggesting that the terpenoid part of taxol is more derived from non-mevalonate route and is originated from plastidic terpenoid pathway rather than cytosolic route of terpenoids production. References: Bestoso F et al. (2006) BMC Biotechnol 6: 45. Hoffman A et al. (1998) Spectroscopy 13: 22 – 32. Ottaggio L et al. (2008) J Nat Prod 71: 58 – 60. Rezaei A, Ghanati F, Behmanesh M (2010) 6th International Workshop on Biological Effects of Electromagnetic Fields pp 70 – 71. Wu J, Lin L (2003) Appl Microbiol Biotechnol 62: 151 – 55.

Topic J: Nutraceuticals and Dietary Supplements

PI15 Quantitation of Undervitamined Omega-3 and Omega-6 Fatty Acids in Foods by HPLC and Charged Aerosol Detection
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The omega fatty acids are a group of compounds that include essential n-3 and n-6, and nonessential n-9 analytes. The omega-3 fatty acids,
which also include eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA], are required for normal growth and health. Although both omega-3 and -6 fatty acids can give rise to eicosanoid-signaling molecules (prostaglandins, prostanoylthromboxanes and leukotrienes), the omega-6 eicosanoids are generally pro-inflammatory and may play a role in disease. It appears that the amounts and balance of omega fatty acids in a person’s diet affect their eicosanoid-controlled functions. A proper balance of omega fatty acids in the diet is important. Traditionally, omega-3 fatty acids are measured using gas chromatography (GC). For foods, analytes are extracted from the samples prior to hydrolysis to release the fatty acids from their triglycerides, and then converted to their volatile methyl-esters prior to analysis by GC. Regardless, this approach is tedious, time-consuming, and the high temperatures can cause polyunsaturated fatty acid oxidation. Charged aerosol detection (CAD), a universal mass-based approach, is sensitive, has a wide dynamic range, and has a major advantage in that all nonvolatile analytes give similar response independent of chemical structure. No derivatization is required, and unlike UV detection, the analyte does not need to contain a chromophore. Presented here is a simple and direct HPLC-CAD method that can be used to measure omega-3, -6, and -9 fatty acids in traditional and commercially produced meat, fish, and oils, as well as over-the-counter supplements.

Potential preventive and therapeutic effects of date palm (Phoenix dactylifera) pollen grain on cadmium – induced testicular injury in rats

This study was investigated the possible preventive and therapeutic effects of date palm (Phoenix dactylifera L.) pollen grain (DPP) on cadmium (Cd) -induced testicular damage using quantitative biochemical and histopathological approaches. A total of 25 adult male rats were randomly divided into five groups. control; DPP treated group received a 15 days of DPP suspension before CdCl2 injection. While, DPP pretreatment as preventive intervention failed to testicular histology and reclaimed sperm abnormalities, lipid peroxidation were significantly elevated. Reduced glutathione, serum testosterone and Johnsen's score. Meanwhile, DPP treated group received a 56-day of DPP suspension folowing CdCl2 injection. CdCl2 (1.2 mg/kg bwt) was intraperitoneally injected while post-treated group received a 15 days of DPP suspension before CdCl2 injection. CdCl2 (1.2 mg/kg bwt) was intraperitoneally injected as a single dose and DPP (120 mg/kg bwt) was given by gavage suspended in distilled water. Cd treated group showed significantly decrease reproductive organs index weight, sperm count and motility, reduced glutathione, serum testosterone and Johnsen's score. Meanwhile, sperm abnormalities, lipid peroxidation were significantly elevated. Necrotic changes with poor spermatogenesis to complete spermatogenic arrest were the key histopathological finding. Although the mechanism is not clear, improved sperm quality and antioxidant status, elevated testosterone level, restored testicular spermatogenesis were noticed in DPP post-treated group as therapeutic intervention. While, DPP pretreatment as preventive intervention failed to attenuate the adverse effects of cadmium.

Anti-metalloproteinase-9 Activities of Selected Indonesian Zingiberaceae Rhizome Extracts in Lipopolysaccharide-induced Human Vascular Endothelial Cells

Atherosclerosis arises from chronic inflammation triggered by bacterial infection that activates degradation process by matrix metalloproteinases (MMPs). Zingiberaceae, a group of tropical food crops grown in Indonesia and other Southeast Asia regions, has been traditionally used for food coloring, seasoning, culinary, and medicinal purposes. However, its efficacy as natural vascular protection has not been explored. Our previous studies demonstrated that Kaempferia pandurata Roxb. possessed MMP-2 and MMP-9 inhibitory effects in human gingival and oral epithelial cells induced by Porphyromonas gingivalis, suggesting its potential therapeutic for natural periodontal therapy. Here, we examined the effects of 10 Indonesian Zingiberaceae rhizome extracts on inhibition of MMP-9 expression in human vascular endothelial cells treated with lipopolysaccharide (LPS) in vitro by conducting gelatin zymogram, Western blotting, and RT-PCR assays. LPS (2 μg/ml) significantly elevated the expression of MMP-9 secretion, protein, and mRNA in vascular endothelial cells. Selected Zingiberaceae extracts (1 and 5 μg/ml), i.e. Curcuma xanthorrhiza, C. aeruginosa, C. mangga, C. longa, Kaempferia galanga, Alpinia galanga, and Zingiberaceae officinale, effectively attenuated the expression of MMP-9 secretion, protein, and mRNA in LPS-induced vascular endothelial cells. Furthermore, MMP-9 expression was specifically blocked by MAPK inhibitors, i.e. PD 98059 (ERK1/2 inhibitor), SB203580 (p38 inhibitor), SP600125 (JNK inhibitor), and PI3K inhibitor (LY294002), indicating that MAPK and PI3K signaling pathways are involved in regulation of MMP-9 gene expression in LPS-induced vascular endothelial cells. These results suggest that selected Indonesian Zingiberaceae rhizomes with potent MMP-9 inhibitory activity may have potentials on prevention and protection of vascular diseases particularly atherosclerosis.

Formation of 5-HMF in making aged garlic (Allium sativum L.) under different condition

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5-Hydroxymethylfurfural (HMF) is derived from dehydration of sugars and has been identified in processed foods [1]. The biological function of HMF have revealed as antiglycating and thiosinase inhibitor [2,3]. This study was performed to find out the amount of HMF and free sugars from the aged garlic when it is treated by temperature at 60 and 75°C and different incubation period from 7 to 35 days. HMF and free sugars from the hot-water extracts of aged garlics were analyzed with GC/MS, LC/MS, and HPLC. The amount of HMF was high at 75°C and 35 days incubation. Among free sugars, the only fructose except glucose and sucrose was formed and converted to HMF at high temperature and long incubation period. However, fructose formed in low temperature made aged garlic was rarely converted to HMF. This result indicates that formation of HMF can be dependent on the temperature and incubation period for making aged garlic. References: 1. Chen S et al. (2009) Food Chem 114: 582 – 588. 2.Abdulmalik O et al. (2005) British J Haematol 128: 552 – 561. 3. Sharma V et al. (2004) Phytotherapy Res 18: 841 – 844.

Sesamin and sesamol contents in various commercial sesame oils

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Sesame (Sesamum indicum L.) seed and oil have been categorized as one of the representative health food and widely used for good flavor and taste in China, Japan, and other East Asian countries for a long times. Sesame seed and oil contain abundant lignans [1] such as sesamin, sesamolin, and others. Sesamin and sesamolin are major lignans in sesame, sesame oil, and its biological effects have been extensively studied. These components are believed to play an important role in the oxidative stability of sesame oil [2]. It is important to understand the variation in the contents of these physiologically active constituents in sesame oil. This knowledge of their levels and forms in sesame seed, sesame oil, and functional food products of sesame is beneficial to control the quality of the sesame seed and sesame oil, and to develop the sesame oil manufacturing technique. The aim of this study was to establish the methods of sample pretreatment and simultaneous determining the sesamin and sesamolin in the sample of sesame oils by RP-HPLC. The methods developed in this work were used to determine the sesame and sesamolin in ten different brands of sesame oils collected in the Chinese markets. The mean contents of total lignans, sesamin and sesamolin were 8.16, 5.14, and 3.02 mg/g respectively. The results shown that can be used to control the quality of sesame oils, and to estimate the dietary intake of sesame lignans. And also it will be beneficial to improve the processing technique in industry. Acknowledgement: This research work was supported by the Research Council of Zhejiang University and Skyherb Ingredients. References: [1] Daisuke N et al. (2006) J Pharm Exp Ther 318: 328 – 335 [2] Nakai M et al (2006) Biosci Biotechnol Biochem 70: 1273 – 1276.
Studies on the stability of secoisolariciresinol diglucoside in flaxseed powder
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Secoisolariciresinol diglucoside (SDG) (Fig.1) is an essential component (11.9 to 25.9 g/kg [1]) of lignans in flaxseeds. Recently, SDG has drawn more and more attention because of its health benefits. A number of animal studies have shown that SDG may help fight many diseases in the modern society, including breast cancer [2], cardiovascular malfunction [3], diabetes [4] and prostatic hyperplasia [5]. With a novel perspective, we focused our study on the effect of food processing methods, including steamed, boiled, fried and deep fried, and processing times on the stability of SDG in flaxseed powder. In this paper, the samples of four processing times were prepared for each processing method. The concentrations of SDG in different samples were determined by RP-HPLC [6]. The chromatographic analysis was performed on a Diamonsil C18 column (150×4.6 mm, 5 μm). Acetonitrile and 1% aqueous acetic acid was selected as the mobile phase. The detection wavelength was 280 nm. A comprehensive study on the effects of different processing methods was made in Fig.2. For steaming process, little effect on SDG content was observed. While for the boiled dishes, i.e. the medicated soups, although little impact was exerted on the content of SDG in flaxseed powder remnant, small amount of SDG was released from flaxseed into the filtrate (the soup). As for fried and deep fried dishes, the loss of SDG was inevitable, but shorter processing time lead to less decrease in SDG, flaxseed could also be employed if cooked properly.

Figure 1: Chemical structure of SDG

Figure 2: Comprehensive comparison of SDG content in different processing methods. a. Steaming; b. Boiling; c. Frying; d. Deep Frying.


Lectins from Vigna radiata - A potential health supplement
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There has been growing interest in use of nutraceuticals of plant origin, because of their impact on the status of human health and disease prevention. Plant lectins, a unique group of proteins and glycoproteins with potent biological activity, occur in commonly consumed foods such as legumes. The lectins can be used as dietary supplements due to their potential benefits of enzyme inhibition. The present research work discusses lectins from Vigna radiata (L.) R.Wilczek as an effective and economical source of natural antioxidants and α-amylase inhibitors which can prove to be a potential dietary and health supplement. Lectins from Vigna radiata were extracted by macerating the seed meal in 50 mM phosphate buffer saline (pH 5.2) containing D-(-)-Galactose at 40 °C. The lectin rich extract was prepared by salt precipitation. The precipitate was dialysed against distilled water for 48 hours. The lectins were purified by Size exclusion Chromatography by using Sephadex G75. The presence of lectins was confirmed by hemagglutination assay. The fractions showing agglutination were pooled together and lyophilized. The yield of relatively purified lectins was found to be 0.04±4%/w/w. Lectin rich extract was evaluated for anti-oxidant property by TBARS assay. The fractions showing agglutination were pooled together and lyophilized. The yield of relatively purified lectins was found to be 0.04±4%/w/w. Lectin rich extract was evaluated for anti-oxidant property by TBARS assay. The IC50 value of the lectin rich extract was found to be 598.32 ± 3.2 μg/ml. The extract was also subjected to the evaluation of α-amylase inhibitory activity. The IC50 value was found to be 552 ± 6.09 μg/ml. This study provides evidence on the potential health benefits of lectins from Vigna radiata, thus confirming the traditional claim. References: 1. Heller VG (1927) JBC 435 – 442.

Protective effects of saffron and trans-crocetin on glutamate mediated excitotoxicity in rat neuroblastoma cells
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Neuronal dysfunctions or even cell death are often accompanied by an exceeding release of glutamate. Excessive glutamate level induces unregulated stimulation of NMDA receptors. In previous studies we found an antagonistic effect of hydro-ethanolic saffron extract (CSE) and trans-crocetin, a carotenoid from saffron, on NMDA receptors (Berger et al., Neuroscience, 180: 238 – 247, 2011). In this study we evaluated the protective effects of CSE and trans-crocetin on glutamate mediated excitotoxicity on rat B104 neuroblastoma cells using cell-based cytotoxicity tests and a fluorescent stain with 4’,6-diamidino-2-phenylindole (DAPI-staining). Glutamate applied for 24h decreased concentration dependently (0.1 – 20 mM) cell viability (MTT-test) and increased LDH activity (LDH-test). The number of annexin-V and 7-AAD positive cells was also augmented and DAPI staining showed an enhanced number of pycnotic nuclei. The glutamate effect was partially inhibited by kynurenic acid (10 mM), an antagonist on excitatory amino acid receptors. After pre-incubation with CSE or trans-crocetin for 2 h followed by 24 h co-incubation with 10 mM glutamate a reduced excitotoxicity could be found. CSE 500 μg/ml significantly increased cell viability from 27 ± 4 % to 63 ± 5 % and decreased LDH activity from 207 ± 5 % to 125 ± 18 %. DAPI-staining showed no differences between CSE treated and control cells. Trans-crocetin 50 μM fully abolished the glutamate effects on cell viability, LDH activity, and DAPI-staining. We conclude that the neuroprotective effects of saffron and trans-crocetin which was previously demonstrated on cortical neurons are partially mediated by attenuation of glutamate mediated excitotoxicity.
Red clover (Trifolium pratense L., Fabaceae) is important source of isoflavones, among which the most present are: daidzein, genistein, formononetin and biochanin A [1]. These substances are considered to be beneficial for reduction of menopausal symptoms, prevention of osteoporosis, cancer and cardiovascular diseases. Since red clover extracts are used for production of dietary supplements, it is important to evaluate profile of these active compounds in plant parts. The aim of this study was to determine content of daidzein, genistein, formononetin and biochanin A in different plant parts of red clover, and to investigate which isoflavone is present in highest concentration. Stems, leaves and flowers of five red clover cultivars were grounded and mixed with water 30 minutes on 37°C. After that, 3 M HCl and 96% ethanol were added and mixture was heated to boiling. Extracts were then filtered by gravity. Total isoflavone content was on average 1.50 mg/g, and the lowest in stems of red clover cultivars (0.27 mg/g). The highest individual concentrations of all investigated isoflavones were found in leaves of different cultivars. On average, in all analyzed samples formononetin was the most present isoflavone. References: 1. Sivesind E, Seguin P (2005) J Agric Food Chem 53: 6397 – 6402. 2. Krenn L et al. (2002) J Chromatogr B 777: 123 – 128.

Fatty acid profile from samples of hemp seeds of dioecious and monoecious hemp varieties approved in Romania

Cannabis sativa L. hemp seeds can be a complete and balanced source of fatty acids, with an optimal omega 6/omega 3 ratio of 3:1 [1], but with limited use in Romania because of the stigma of drug. Whole hemp seeds have oil content of about 25 – 35% [2], but in Russia there is a cultivated variety called “oliferica” which contains 40% oil [3]. About 30 – 35% of the weight of hempseed is an edible oil that contains about 80% as essential fatty acids (EFAs), linoleic acid, omega-6 (LA, 55%), alpha-linolenic acid, omega-3 (ALA, 22%), in addition to gamma-linolenic acid, omega-6 (GLA, 1 – 4%) and stearidonic acid, omega-3 (SDA, 0 – 2%). This study aims to investigate the impact of the extraction technology of oil content and fatty acid profile from samples of hemp seeds of dioecious and monoecious hemp varieties. Hemp oil has been extracted from the seeds by cold pressing using a press and by Soxhlet method with a Velp block of mineralization. Investigation of fatty acid profile and oil content was performed by gas chromatography GC-MS. Hemp seeds not only contain essential fatty acids, but come with substantial contribution of 20 – 25% protein, essential amino acids, which make hemp seed an ideal food for vegetarians, successfully replacing the lack of animal protein, with a high nutritional value for human consumption in salads, bread and even chocolate. References: [1] Callaway JC. (2004) Euphytica 140: 65 – 72 [2] Şandru ID, Paraszchiviu R, Găuţă C (1996) Cultura câinei, Helicon, Timişoara [3] Defeure JL and Pate DW (1996) Journal of the International Hemp Association 3(1): 4 – 7.

The contents of heavy metal (Pb, Cd and Zn) in plant Taraxacum officinale Weber

The species of Taraxacum officinale Weber (Asteraceae) is a very popular medicinal and edible herb. Since time immemorial have been used in traditional phytotherapy in Bosnia and Herzegovina [B-H] [1]. The young shoots and inflorescences are used as health food [2]. Dandelion is widespread. Most often inhabit different anthropogenic habitats that are loaded with different pollutants, including heavy metals [3]. This is a serious limitation for safe and sustainable use of this plant in medicine and dietetics. Investigation the content of heavy metals in roots and aerial part of the dandelion included 30 localities of B-H which are under different anthropogenic influence. Samples have been prepared using standard methods. Measurement of concentrations of heavy metals was carried out by atomic absorption spectrophotometry. The content of heavy metals cadmium, zinc and lead in Taraxacum officinale varied depending on the vegetative part of plants, season, location, then the type of soil, the intensity of anthropogenic influences, soil pH, the interaction of the tested elements, climate conditions and other environmental factors. The concentration of cadmium ranged from 0.02 – 0.8 mg/kg, the concentration of zinc was 30 mg/kg to 100 mg/kg. The concentration of lead varied from 0.1 – 10 mg/kg. There are significant differences in the concentration of metals between the sites under severe anthropogenic pressure and a larger site outside the pollution. In many localities have been established concentrations that are not allowed. References: 1. Redzic SS (2007) Coll Antropol 31: 869 – 890. 2. Redzic SJ (2006) Ecol Food & Nutr 45(3): 189 – 232. 3. Redzic S et al. (2009) Planta Med 75: 902 – 902.
We have recently shown that Ecd prevents osteoporosis and decreases visceral fat mass in ovx rats. This animal model is known to develop a metabolic syndrome which includes hypercholesterolemia, hypertriglyceridemia and an impaired oral glucose tolerance test. These impaired metabolic parameters are due to increased cytokine secretion by the increased number of adipocytes. Estradiol (E2) and beta-ecdysone (Ecd) are known to reduce visceral and subcutaneous fat loads and E2 normalizes the deranged metabolic parameters. Whether this can also be achieved by a treatment with Ecd was not studied hitherto. Ovx rats were orally treated with E2 (0.108 mg/animal/day) or Ecd (18.56 mg/animal/day) and following necropsy 4 weeks later serum cholesterol, LDL, HDL and triglycerides were determined. In addition an oral glucose tolerance test (OGTT) was performed. Serum cholesterol, LDL and HDL were reduced by E2 whereas triglycerides were increased. Ecd decreased cholesterol, LDL and also triglycerides but increased HDL. Clearance of glucose following an OGTT lasted longer in the ovx controls than in the E2 and Ecd treated animals. It is concluded that Ecd shares the positive effects of E2 on cholesterol and glucose clearance but prevents the adverse acting stimulation of triglycerides by E2. Hence, Ecd may be a novel non-estrogenic alternative for prevention and treatment of the metabolic syndrome. Acknowledgement: This work was in part funded by VerdeVital GmbH

Soybean [Glycine max (L.) Merr.] is an annual plants species and is locally known as "Soya Fasulyesi". Soybean is consumed as a food and vegetable plant and its recorded folklore uses for the medicinal purposes. In this present study macro (N, P, K, Ca, Mg, Na,) and trace elements (Fe, Mn, Zn, Cu) of soybean cultivars cultivated under the controlled conditions in Turkey have been studied. Macro and trace elements were determined by using various techniques. N was determined by the dry combustion method using elemental analyser, P was measured by a colorimetric method, whereas K and Na by flame photometry. Finally Ca, Mg, Fe, Cu, Zn and Mn was detected and quantified by atomic absorption spectroscopy (AAS). All experiments were performed qualitatively and quantitatively with comparison to a certified reference plant material statistically. The results of elemental analyses showed that N ranged 5.41 – 5.82%, P ranged 5250 – 6100 ppm and K ranged 15915 – 19645 ppm. To the best of our knowledge, this is the first report on macro and micro elements of cultivated Turkish soybean cultivars. As a conclusion, the elemental composition and the nutritional value soybean cultivars are worthwhile to investigate with comparison to other Glycine sp. used medicinally.

Elemental Compositions of Soybean Cultivars Cultivated in Turkey

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Soybean (Glycine max (L.) Merr.) is an annual plants species and is locally known as “Soya Fasulyesi”. Soybean is consumed as a food and vegetable plant and its recorded folklore uses for the medicinal purposes. In this present study macro (N, P, K, Ca, Mg, Na,) and trace elements (Fe, Mn, Zn, Cu) of soybean cultivars cultivated under the controlled conditions in Turkey have been studied. Macro and trace elements were determined by using various techniques. N was determined by the dry combustion method using elemental analyser, P was measured by a colorimetric method, whereas K and Na by flame photometry. Finally Ca, Mg, Fe, Cu, Zn and Mn was detected and quantified by atomic absorption spectroscopy (AAS). All experiments were performed qualitatively and quantitatively with comparison to a certified reference plant material statistically. The results of elemental analyses showed that N ranged 5.41 – 5.82%, P ranged 5250 – 6100 ppm and K ranged 15915 – 19645 ppm. To the best of our knowledge, this is the first report on macro and micro elements of cultivated Turkish soybean cultivars. As a conclusion, the elemental composition and the nutritional value soybean cultivars are worthwhile to investigate with comparison to other Glycine sp. used medicinally.

Elemental Compositions of Soybean Cultivars Cultivated in Turkey

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Histopathological and immunohistochemical study of the effect of Punica granatum extract on Azoxymethane induced colon cancer in Rats

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Chemoprevention has become an important area in cancer research due to the failure of current therapeutic modalities. Polyphenol-rich dietary foodstuffs have attracted attention due to their cancer chemopreventive and chemotherapeutic properties. The modulating effects of aqueous methanol of Punica granatum L. peels at doses (200 and 400 mg/kg) on colon carcinogenesis initiated with azoxymethane (AOM), were investigated in male rats by weekly s.c. injections of 15 mg/kg body wt for 12 weeks. Histopathological studies on AOM-treated rats revealed dysplasia of the colon histochiarchitecture, which showed signs of improvement following P. granatum administration, was found to significantly and dose dependently decrease the total number of aberrant crypt foci (ACF) per rat. Cell proliferation in the colon, as shown by proliferating cells nuclear antigen (PCNA), was also reduced in those treatments. AOM-treated rats exhibited alterations in cancer tumour markers gamma glutamyl transpeptidase (gamma-GT), carcinoembryonic antigen (CEA), pathophysiological markers (alkaline phosphatase (ALP) and lactate dehydrogenase (LDH)) and oral administration of P. granatum restored the levels of these marker enzymes. Also, pro-inflammatory proteins (inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) and pro-inflammatory cytokines tumour necrosis factor (TNF)-alpha and interleukin (IL-6) in AOM group exhibited elevated expression of all these inflammatory proteins. P. granatum administration reduced COX-2, iNOS, TNF-alpha and IL-6 as confirmed by immunohistological analysis during AOM-induced colon carcinogenesis. Our results suggest that P. granatum could exert a significant chemopreventive effect on AOM induced colon carcinogenesis is probably due to combined effect of polyphenolic compounds.

Investigation on Compositions of Seed Oil and Yield of Silylin of Seeds from Silibum marianum (L.) Gaertn. Cultivated in Konya Ecological Conditions

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Milk thistle (Silybum marianum (L.) Gaertn.) is an herbal supplement used to treat liver and biliary disorders. The active constituent of milk thistle is silymarin, a mixture of flavonolignans. It is also contains important fatty oil acid anasturated. In this study, researches have been conducted in Medicinal – Aromatic Plants laboratory and Medicinal and Aromatic Plants Experimental Farm of Field Crops Department, Agriculture Faculty, Selcuk University. The aim was to determine the effect on yield and quality some characters of organic fertilizers applied at the different doses on milk thistle (S. marianum) cultivated under Konya (Turkey) ecological conditions. It were applied at the three different doses sheep manure as organic fertilizer. In this study; plant height, plant seed yield, yield of crude oil, composition and yield of oil and flavonoid (silymarin) were examined. According to results of this research; plant height, seed yield, yield of crude and composition and yield of oil and flavonoid (silymarin) varied between 73’–118 cm, 720 – 1480 kg/ha, 20 – 28% and 1.1 – 3.1, respectively. In this research; high silymarine yield, crude oil yield, fatty acid composition and drug of milk thistle (S. marianum) cultivated in Konya ecological conditions were obtained from 15000 kg/ha from applied organic fertilizer.

Antioxidant properties of wild Solanum nigrum ripe fruit

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This work was to examine hepatoprotective properties of Solanum nigrum, commonly known as Black Nightshade, a medicinal herb grown in Egypt used traditionally in oriental medicines and believed to have various biological properties. A crude ripe fruit ethanol extract was made, lyophilized to give 2.86 g/100 g. Two sets of experiments were done, in vitro antioxidative experiments and in vivo biological assays. The results revealed that crude ethanol extracts of Black Nightshade ripe fruit had strong antioxidant activity, for example in a DPPH assay at 500 ppm of the extract, a 68% reduction of DPPH radicals was detected, with total antioxidant capacity at 8.45 ± 0.031 as ascorbic acid equivalents. In vivo, the extract; 100, 200 or 300 mg/Kg; showed valuable activity as a hepatoprotective agent on oral one dose CCl 4-treated experimental rats shown by an increase in total serum soluble protein, albumin, and a remarkable reduction in the serum activity of AST, ALT and ALP as well as bilirubin and uric acid. For example, the serum total albumin level was reduced from 6.29 ± 0.12 g/dl in the healthy normal control animals to 3.35 ± 0.17 g/dl (53% at normal control) for CCl 4 intoxicated control rats, but recovered to 5.62 ± 0.39 g/dl by 300 mg per kg body weight for rats on daily oral post-treatment for 5 days. Collectively, Black Nightshade ripe fruit ethanol extracts were shown to be an effective hepatoprotective agent in vivo due to their high content of antioxidant and bioactive plant secondary metabolites. Key words: Solanum nigrum L, Black Nightshades, Hepato-protective, Antioxidant, CCL 4, Administration: The authors thanks Dr. Enam Abdel-Mobdee, Biochemistry Department, Faculty of Agriculture, Cairo University for his great support. Thanks to Kamela Alegre, American PhD student at Universitat Autonoma de Barcelona, for Language revision. References: Lin H et al. (2008) Chemico-Biological Interactions 171: 283 – 293 Loganayaki N, Sidduraju P and Manian S (2010) Food Science and Biotechnology 19(1): 121 – 127

Hypcholesterolemic Effects of Glyphaea brevis (Spreng.) Monach. in Normal And Streptozotocin-Induced Diabetic Rats

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Hypercholesterolemia, sometimes linked to diabetes, is a public health concern since it paves the way to severe complications such as hypertension and stroke. The search for innovative, natural and safe treatments to reverse the condition remains imperative. This study was aimed at evaluating the hypcholesterolemic potential of Glyphaea brevis (Spreng.) Monach. aqueous extract (AE) in both normal and streptozotocin-induced diabetic rats. AE was given on a daily basis to rats by gastric intubation at 500 mg/kg for 30 days in a controlled study. At the end of experiment, administration of AE significantly reduced the levels of fasting blood total cholesterol (normal: -38.54%, p < 0.01; diabetic: -22.08%, p < 0.01); LDL-cholesterol (normal: -72.85%, p < 0.01; diabetic: -38.15% p < 0.01) while no significant change was observed in triglycerides. Moreover, atherogenicity indices total cholesterol/HDL-cholesterol (TC/HDL-c) and LDL-cholesterol/HDL-cholesterol (LDL-c/HDL-c) were significantly reduced at the end of study (TC/HDL-c: normal: -43.88%, p < 0.01; diabetic: -44.11%, p < 0.01) while no significant change was observed in atherogenic index. The results suggest the hypcholesterolemic effect of G. brevis. Such effect would be accountable of the presence of flavonoids (revealed by phytochemical screening) that may inhibit enzymes such as hydroxymethyl-CoA (HMGCoA) reductase that are involved in cholesterol biosynthesis. The outcome of our study could find applications in the development of alternative means of treatment or prevention of hypercholesterolemia and its associated complications.

The pericarp of Pismum sativum L.(Fabaceae) as a biologically active waste product

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Food industries generate large amounts of wastes and byproducts which contain biologically active compounds. The recycling of these wastes could be of economic benefits. Pericarp of Pismum sativum L pods is separated from the seeds which are processed as frozen foods. Most of the phytochemical studies on Pismum sativum dealt mainly with the seeds. With the aim of utilization of waste products as biologically ac-
Auricularia auricula-judae (Bull.) J.Schr/C246t. and nutritional food and because of their special flavour and texture [1]. Mushrooms have been used for many years in oriental culture as tea and been carried out by high-performance liquid chromatography coupled to nolic composition, protective and cytotoxic effects of these mushrooms.

Qu/C216l. are two edible mushrooms and they have been reported to have oxidant activity as measured in various in vitro atiators and cytokines in the blood. The extract also showed good anti-inflammation (carrageenan paw edema) and chronic inflammation (adjuvant-arthritis) in rats. It also reduced the level of inflammatory mediators and the anti-diabetic activity of the 80% aqueous – acetone extracts, antimicrobial screening of the saponifiable fraction and the anti-inflammatory activity of the unsaponifiable matter were studied, together with the antioxidant activity of the 80% aqueous – acetone extract. Results showed that the two extracts were relatively safe. The saponifiable fraction showed significant antibacterial activity, but no effects against fungi or yeast. The unsaponifiable matter displayed significant anti-inflammatory activity. The 80% aqueous – acetone extract showed potential anti-hyperglycaemic activity.

PK1

High-performance liquid chromatography (HPLC) analysis of phenolic compounds in two edible mushrooms extracts and their protective effect against oxidative damage in BHK-21 cell line Oke Attanatas F, Ashim B

Mushrooms have been used for many years in oriental culture as tea and nutritional food and because of their special flavour and texture [1]. Auricularia auricula-judae (Bull.) J.Schr, and Pleurotus eryngii (DC.) Quël, are two edible mushrooms and they have been reported to have many biological activities [2,3,4]. In this study we investigated the phenolic composition, protective and cytotoxic effects of these mushrooms. Analysis of phenolic compounds in these edible mushrooms species has been carried out by high-performance liquid chromatography coupled to photodiode array detector (HPLC-DAD). Twelve of the 14 phenolic compounds were identified and quantified by comparing their chromatographic characteristics and absorption spectra with that of the standard compounds. The analysis showed that p-hydroxybenzoic acid, catechin, gallic acid and caffeeic acid were the major phenolic components in the extract. Protective effect of these mushroom on H2O2 induced oxidative cell damage was determined by using MTT (3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyltetrazoliumbromide, a yellow tetrazole) assay. All the extracts exhibited protective effect against H2O2 induced oxidative cell damage but the highest activity was observed for A. auricula-judae aqueous extract (89.5 ± 1.8% cell viability at 0.1 mg/ml) (Fig. 1). A. auricula-judae extracts (at concentration of 0.025 – 0.100 mg/ml) were not toxic to baby hamster kidney fibroblast cell line (BHK 21) (Fig. 2.). The results of this study indicated that the extracts exhibited interesting protective effect against H2O2-induced baby hamster fibroblast cell line and they may be used as natural sources in pharmaceutical industry for the prevention of conditions that occur due to oxidative damage.

Figure 1: Protective effect of the mushrooms extracts in BHK 21 cells pretreated with the extracts at concentrations (0.100 mg/ml) for 24 h and exposed to 1 mM H2O2-induced oxidative stress. Each value represents the mean ± SD of five wells.AAE: A. auricula-judae aqueous extract-AME: A. auricula-judae methanol extract; PAE: P. eryngii aqueous extract; PME: P. eryngii methanol extract

Figure 2: Cytotoxic effect of the mushrooms extracts in BHK 21 cells pretreated with the extracts at concentrations (0.025, 0.050 and 0.100 mg/ml) for 24 h. Each value represents the mean ± SD of five wells.

Artemisinin, a sesquiterpene lactone endoperoxide isolated from the herb Artemisia annua L. (Asteraceae), is a highly potent antimalarial compound, which is efficient against multidrug-resistant strains of Plasmodium falciparum. The promotion of artemisinin-based combination therapies (ACTs) by the WHO during the past years lead to a strong pressure on the world market of artemisinin. The artemisinin world therapies (ACTs) by the WHO during the past years lead to a strong pressure on the world market of artemisinin. The artemisinin world market is volatile and therefore efforts to improve performance of this culture are often limited. The use of varieties with high artemisinin content is a key factor for the development of such cultures. This should secure the supply of artemisinin, lower its cost of production and improve the competitiveness of this new culture with other commercial crops. After the variety Artemis, Mediplant introduces a new variety called Apollon with about 20% yield increase, Performances of this new hybrid, with artemisinin content nearing 1.5%, are being presented.

Use of paclitaxel on balloon catheters against restenosis

PK3

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Besides the established use as chemotherapeutic agent against breast or ovarian cancer, paclitaxel coated on medical devices such as stents and balloon catheters has recently been applied in local prophylaxis and therapy of arterial stenosis/restenosis [1]. Paclitaxel is particularly suitable to inhibit injury-induced excessive intravascular scar formation following balloon angioplasty because of its strong and persistent anti-proliferative properties [2]. The aim of this work was to optimize the balloon coating for clinical application. The challenge is to guarantee firm adherence of coated paclitaxel on its way through a hemostatic valve and atherosclerotic arteries to the target, and to allow optimal release at the lesion site. Paclitaxel was coated on the surface of angioplasty balloon catheters by a semiautomatic Hamilton microsyringe. HPLC showed a coating of ca. 3.0 μg/mm2 of paclitaxel. When introducing the catheter into the artery, the loss of paclitaxel was shown to be in the range of 15%. Upon the inflation of a balloon catheter in the stenotic segment of the artery, the surface gets in contact with the vessel wall for a few seconds up to one minute only [3]. After removal of the device from the artery ca. 10% of the drug were retrieved on the balloon, indicating the release of ca. 90%. The proportion of the vessel was ca. 10% as assessed in a porcine model. The pharmacological effect measured by angiography using the diameter stenosis as a parameter supported the benefits of the processed coating for clinical practice. References: 1. Rowinsky EK and Donehower RC (1995) N Engl J Med 332: 1369 – 1377. 2. De Labriolle A et al. (2009) Catheter Cardiovasc Interv 73: 643 – 652. 3. Waksman R and Pakala R (2009) Circ Cardiovasc Med 332: 1004 – 1014. 2. De Labriolle A et al. (2009) Catheter Cardiovasc Interv 73: 643 – 652. 3. Waksman R and Pakala R (2009) Circ Cardiovasc Med 332: 1004 – 1014. 2. De Labriolle A et al. (2009) Catheter Cardiovasc Interv 73: 643 – 652. 3. Waksman R and Pakala R (2009) Circ Cardiovasc Med 332: 1004 – 1014.

Improvement of high-fat-diet-induced metabolic syndrome by ethanol extract of Polygonatum falcatum (ID1215B) in mice

PK5

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Sirtuin, a NAD+-dependent class III histone deacetylase, is closely related to calorie restriction (CR) – mediated life span extension in yeast and rodents. Recently, it has been also reported that sirtuins improve various chronic diseases associated with metabolic dysfunction such as diabetes and obesity. In this study, we screened to find Sirt1 (a mammalian homolog of sirtuin) modulating herbal extracts. We identified the ethanol extracts of Polygonatum falcatum A.Gray (ID1215B) increased the expression of Sirt1 protein in HEK293 cells and further investigated the effects of ID1215B on metabolic syndrome in mice. The metabolic effect was evaluated in male C57Bl/6j mice administered in high fat (HF) diet that were orally given a dose of 250 or 750 mg/kg/day of ID1215B significantly decreased body weight gain, lipid accumulation in adipose tissue, serum triglyceride and free fatty acid levels and improved insulin resistance. In addition, ID1215B increased the expression of genes related to mitochondrial biogenesis and fatty acid oxidation in the HF-diet mice. Taken together, our results indicate that ID1215B may be a promising anti-obesity therapeutic agent that could improve the metabolic syndrome including the insulin resistance and hyperlipidemia as well as body weight gain. References: 1. Rodgers JT et al. (2005) Nature 434: 113 – 8. 2. Lagouge M et al. (2006) Cell 127: 1109 – 22 3. Milne JC et al. (2007) Nature 450: 712 – 6

Researchers regarding skin anti-photoaging effect of Linum usitatissimum L. oil by using in vivo skin imaging dermatologic evaluation

PK6

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Due to its content of unsaturated fatty acids and lignans (acting like phytoestrogens) Linum usitatissimum L. oil has been proved several pharmaceutical properties in dermatologic and cosmetic field. The current stage of knowledge concerning the dermatological uses of this oil is: - photoprotection effect, an well-known propriety, already used by a lot of pharmaceutical laboratories; - sebum-regulating and non-comedogenic effect of linum seeds lignans. The main lignan in flaxseed is secoisolariciresinol diglucoside (SDG) which plays a major role in the sebum rate decrease, by being a 5 alpha-reductase inhibitor. - unsaturated fatty acids are essentials for maintain the epidermal physiology. LA is the most abundant fatty acid in the epidermis. Importantly, it is the precursor to ceramides, a major component of the extracellular lipid matrix that forms the stratum corneum permeability barrier (SCPB). [2,3] Additionally, omega 3 fatty acids are involved in prevention of skin cancers. Our study is advocated the skin anti-photoaging effect of Linum usitatissimum oil. This effect was performed in vivo, on healthy volunteers, by registering the wrinkles involution with ProDerm Skin Analyser. We admitted in study 22 females volunteers, ages between 33 an 56 years. The imagistic evaluations were registered after 14 days, 21 days and respectively 28 days of daily application. The oil content of the...
Falcarnel is a natural C17-polyacetylenic pesticide (phytoalexin) present in Apiaceae vegetables such as carrot (*Daucus carota* L.). Recently it has attracted a lot of attention due to its interesting biological activities such as cytotoxic, antibacterial, antimycobacterial [1]. However, falcarnel suffers from photo- and thermal degradation, due to the presence of unstable triple bonds in its structure, which limits its possible applications. In the present work the thermal and photo-stability of falcarnel alone and in complex with β-cyclodextrin was studied. Falcarnel was isolated from the endemic Sardinian plant *Sezeli praecox* (Camisians) Camisians (*Apiaceae*) [2]. Falcarnel/β-cyclodextrin complexes were prepared and the inclusion complex was initially characterised by NMR (ROESY) spectroscopy. Accelerated thermostability testing proved to be an extremely aggressive method for this type of constituent resulting in the complete degradation of both, the compound and its inclusion complex. On the other hand, photostability studies were carried out successfully as the β-cyclodextrin complex provided protection to the substance which kept its macroscopically properties and protected the substance from degradation. In comparison the photostability assay generated a loss of 15% in uncomplexed falcarnel. Therefore, inclusion in β-cyclodextrin was proved to be a good method for the photoprotection of falcarnel.

**Figure 1:** Falcarnel/β-cyclodextrin inclusion complex


Curcumal is a natural polyphenolic constituent of *Curcuma longa* L. It has been generally associated with a large number of biological activities, including anti-oxidant, anti-inflammatory [1] and anti-cancer [2] properties. Although curcumal is a safe molecule even at high doses, its therapeutic use is limited by its low hydro-solubility in acid or physiological pH [3] and, consequently, by its poor bioavailability. Another drawback for clinical application of curcumal is its rapid hydrolysis under alkaline conditions and its photochemical degradation. The overall aim of this project is to increase solubility and stability of curcumal by microinclusion in cyclodextrins. Solubility studies of curcumal in presence of different concentrations of natural (α, β, γ) and semi-synthetic cyclodextrins (HEβ, DMβ, TMβ, RAMEB, HPI, HPβ) were carried at different temperatures (25 – 37 °C). Thermodynamic parameters related to complex formation (∆G, ∆H and ∆S) were also evaluated. Stoichiometry of curcumal inclusion and apparent equilibrium constants (K1, K2) were evaluated by Job’s plot method using UV detection. Inclusion of curcumal into selected cyclodextrins was obtained by co-fusion, co-lyophilization, co-evaporation and physical mixture. Complex characterization was achieved by DSC, UV, NMR and HPLC/DAD analysis. Between the cyclodextrins tested the most efficient in order to maximise curcumal solubilisation was DMβ and the most effective complexation technique was the co-lyophilization. This latter was then employed, after curcumal complexation, for the realization of a topical formulation, useful as local anti-inflammatory medicament, and pharmacokinetic was evaluated by in vitro test using Franz cells apparatus. References: 1. Dong-Oh M (2008) Bioch Bioph Res Comm 375: 275 – 279. 2. Preetha A et al. (2008) Cancer Lett 267: 133 – 164. 3. Tonnesen HH et al. (2002) Int J Pharm 244: 127 – 135.

**Figure 1:** Falcarnel/β-cyclodextrin inclusion complex

**References:**


**Development and stability of semisolid preparations based on Gardenia jasminoides Ellis extract**

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The fruit of *Gardenia jasminoides* Ellis has been traditionally used as a Chinese medicine for centuries in China, as well as in other Asian countries [1]. The major and characteristic constituents of Gardenia fruit are iridoid glycosides such as geniposide, gardenoside, genipin-1-β-gentibioside, geniposidic acid, acetylgensioside, scandioside methyl ester, shanzhiside and gardoside. Modern clinical and pharmacological research has revealed that Gardenia fruit has anti-inflammatory properties, cytotoxic effects, as well as protective against oxidative damage [1,2]. In the Chinese Pharmacopoeia a topical application of the fruit of gardenia, powdered and mixed with water is reported [8]. Gardenia fruit also represents the principal effective ingredient of a preparation with antiinflammatory and analgesic properties, “Zhongtong Caiji”, a kind of liniment for external use, formulated with more than ten different herbal drugs [3]. The development of three semisolid preparations (anphyl cream, sepgel and natrosol gel) based on *Gardenia jasminoides* Ellis extract is reported. Aqueous methanol (50% v/v) was selected in order to exhaustively extract the active constituents, iridoids (20% w/w). Stability of the developed preparations was investigated according to ICH guidelines. In vivo permeation of three selected formulations is investigated using the “skin stripping” test, according to the FDA, in healthy subjects. Analysis of iridoids both in the extract and in the stratum corneum were performed by HPLC-DAD-MS method. The sepgel gel showed the best stability and release profile in the in vivo tests. References: 1. Wang Y et al. (2010) Int J Pharm 352: 72 – 77. 2. Park E-H et al. (2003) Phytotom Res 17: 961 – 962. 3. Pharmacopoeia of the People’s Republic of China (2005) vol. 1, 95 – 96.

**Effects of different irrigation intervals on yield and yield components of black cumin (Nigella sativa)**

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Black cumin (*Nigella sativa* L.) is a medicinal plant with economic influence, especially in medicine production. A randomized complete block (RCB) experiment with three replications was conducted in 2010, to evaluate yield and yield components of black cumin (*Nigella sativa*) under no irrigation and three different irrigation intervals (7, 14 and 21 days) at research station of Islamic Azad university of Maragheh. Plants were sown in plots 20 cm plant to plant distance and 50 cm apart rows. Three irrigation intervals had significant effects on all studied characteristics. Results showed that increasing irrigation intervals to 14 days, increased number of capsules per plant, number of seeds per capsules and grain yield per plant, but produced smaller seeds. The lowest numbers of capsules and grain yield per plant were obtained in no irrigation treatment. Lowest number of seeds per capsules and the largest grains produced in 21 days intervals. Increasing yield per plant in 14 irrigation interval was mainly attributed to the highest number of capsules per plant and number of seeds per capsules. References: 1. Ghomarina H, Khosravy H, and Sepehri S (2010) J Medicinal Plants Research 4(16): 1612 – 1616. 2. Mohhebi M and Maleki H (2010) Advances in Environmental Biolog 4(1): 10 – 13. 3. Pourouzpour Gh and Moghadam P (2007) Agronomy and Horticulture 19: 43 – 47.
Pharmacoeconomic evaluation of peppermint tea-bag products using graph theory

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Peppermint (Mentha x piperita L.) has very long tradition of medicinal use due to its essential oil content. It is often also used as tea and food flavoring. The content of essential oil of peppermint leaf is crucial for its medicinal, but also flavoring effects. There are plethora of tea products on the market for the consumers to choose from, usually based on the product’s price and the external package appearance. The consumers commonly don’t have the insight into the tea-bag’s content pharmaco- nystic and chemical quality. We have used pharmacoeconomic framework to make the surveillance of ten peppermint tea-bag products present on Bosnian and Herzegovinian market. Consumers were asked to give grades 1 – 10 for the products external packaging. The unit price was determined for each product. Pharmacists were asked to rate organoleptic appearance of herbal content of tea-bags. Modified methods of GC and GC-MS analysis described by Kowalski and Wawrzykowski where used for the essential oil determination. The graph theory was chosen to sum up all results of different parameters for each product and give a quantitative estimate of the pharmacoeconomic acceptability. Relationship between tested parameters is presented in Figure 1. The obtained results reflect balance between external value on one side (> 1) and pharmacognostic quality (> 1), while value of 1 represents perfect balance of tested opposites. Of ten tested products, in 8 predominated external value (0.19 to 0.70), while in 2 products predominated pharmacognostic quality (1.67 and 1.77). The graph theory was useful in assessment of herbal products.

Figure 1: Relationship between tested quality parameters


Phytochemical and hypoglycemic effect investigation of methanolic flower extract from Piper claussenianum

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Species of the genus Piper, the most important genus of Piperaceae Family, are widely used in traditional medicine for the treat many conditions. Chemical investigations of Piper species revealed many bioactive metabolites as amides, lignans, alkaloids, terpenes, flavonoids among others. In order to investigate native Piper species, phytochemical analysis of Piper claussenianum flowers was tested for hypoglycemic effect on rats with type 1 diabetes. Seven days after the induction, rats with glucose levels above 200 mg/dL began to be treated with vehicle or extract. Treatment lasted for 14 days and rats had glucose levels measured on days 0, 5 and 14. Glucose levels of both groups were also measured after 7 days of treatment interruption. Glucose levels of vehicle and extract groups were: day 0 (346.14 ± 41.67 and 275.29 ± 27.13, p < 0.05, n = 7), day 5 (290.14 ± 32.65 and 122.71 ± 7.19, p < 0.05, n = 7), day 14 (370.75 ± 77.89 and 137.50 ± 17.70, p < 0.05, n = 4). Seven days after treatment interruption, glucose levels were 304.14 ± 71.16 (vehicle, n = 7) and 255.50 ± 114.86 (extract, n = 4). Thus, the results suggest the remarkable presence of 2',6'-Dihydroxy-4'-Methoxychalcone on methanolic flower extract of Piper claussenianum has a noteworthy role to reduce blood glucose levels in rats with type 1 diabetes. Acknowledgement: The authors thank to CNPq.

Stability and staining property of gel from roselle calyx extract and butterfly pea flower

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Roselle calyx and Butterfly pea flower are among plants which have been used for coloring of food and beverage. The combination of the water extraction of the two plants present tanning color and was found interesting to study the property of an herbal staining gel. The herbs were extracted with boiling water for 2 hours, filtered and lyophilized until dried. The gel base was prepared by using combination of sodium carboxy methyl cellulose gel base and hydroxyethylcellulose 4000 gel base in 1:1 ratio. The herbal extracts were varied amount to add to the gel base. The color number in an expanded color chart were use to evaluate the usable color. The staining gel was tested for cracking, precipitation, changing of color, changing of pH before and after heating cycle. The gel was tested for staining property by applied 0.5 g of gel on the pig skin and left for 1 hr then determine the color by using a color scale. The Mexameter MX 18 was used for determined of color uniformity of skin. The permanent of staining was tested by stirring the pig skin after staining for 24 hrs with 500 millilitres of water for 20 minutes and compare the color change. The gel with 1.35% of roselle extracted and 0.15% of butterfly pea extract was selected to be a staining gel. The color pH and physical property of gel are not changing after freeze and thaw for 3 cycles. The gel present consistency and permanent of staining. Acknowledgement: The faculty of pharmaceutical science Khon Kaen university for supportive of grant. References: 1. Kazuma K et al (2003) Phytochemistry 64: 1133 – 1139. 2.Therkildsen P et al (1998) Skin Res Technol 4: 174 – 179.

Stem gum of Moringa oleifera as pharmaceutical excipient

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The herbal gum exudates from the stem of Moringa oleifera Lam. is sparingly soluble in water but swells in contact with water, giving a highly viscous solution. It consists of arabinose, galactose, and glucoronic acid. In the present study the formulation of paracetamol tablets by
using Moringa oleifera gum as a binder was performed. The four different tablet formulations loaded drug as paracetamol were prepared by wet granulation method. The binder concentrations used in the formulation were 2, 4, 6 & 8% w/w of Moringa oleifera gum. Tablets were subjected for evaluation of hardness, friability, drug content uniformity. The preliminary evaluation for granules was done by measuring the granule size, angle of repose and percentage fines. The percent friability was in the range and tablet showed 98% to 99% of labeled amount of paracetamol indicating uniformity in drug content with 6 to 20 mm disintegration time and more than 90% dissolution at 80 to 90 min. Tablets at 6% w/w binder concentration showed more optimum results as tablet binder. Paracetamol tablets were prepared using newly developed herbal gums as binder for the controlled release. The results indicated that tablets were successfully formed and displayed good binding properties which can come as the new source of binder. The herbal gums obtained from Moringa oleifera stem gum could be utilized in the development new pharmaceutical formulations.

Development and evaluation of conventional and PEGylated curcumin liposomes, absorption and tissue distribution studies in mice

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Curcumin is the main biological active polyphenolic compound present in the rhiromes of turmeric (Curcuma longa Linn.), has a wide biological and pharmacological profile. It has been reported to possess anti-oxidative, anti-inflammatory and anti-carcinogenic properties [1 – 3]. Many recent clinical study reports have revealed that curcumin has many beneficial properties in the treatment of various diseases in man such as pancreatic cancer and inflammatory bowel disease [4,5]. Despite these promising effects a poor oral absorption due to its extremely low aqueous solubility and rapid metabolism result in low oral systemic bioavailability, thus limiting its clinical use. In order to overcome bioavailability drawbacks, this work proposes inclusion of curcumin into liposomal carriers. Liposomes were prepared by thin layer evaporation technique using phospholipon 90G, cholesterol and PEG-GSPE. Temperature of rehydration of thin film and curcumaminizations were optimized in order to maximise efficiency of entrapment of drug inside the vesicles. Vesicles were characterized by dynamic light scattering and HPLC/DAD. The pharmacokinetic profile was tested in mice after i.p administration using stealth and conventional vesicles and an alcoholic solution of the drug. After 48 h, organs (liver, spleen, intestine, mesentry and lung) were removed and curcumin dosed by HPLC/DAD/MS analysis. Liposomal inclusion increases bioavailability of curcumin in plasma, in particular, stealth formulation. A further accumulation was found among the different tested formulations. A further advantage of PEGylated liposomes is the high EEC of curcumin in the vesicles (70% compared to 47% of conventional liposomes). References: [1] Ruby et al. (1995) Cancer Lett 94: 79 – 83. [2] Lukita et al. (2007) Cancer Lett 255: 170 – 181. [3] Johnson et al. (2007) Cancer Lett 255: 170 – 181. [4] Dhillon et al. (2006) J Clin Oncol 24: 14151. [5] Holt et al. (2005) Dig Dis Sci 50: 2191 – 2193.

Effect of Urtica dioica on proliferation of HCT-116 colon cancer cell line

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Urtica dioica L. (stinging nettle), a member of the Urticaceae family is a plant which has been used as a remedy for diabetes mellitus [1], benign prostatic hyperplasia (2), arthritis (3), allergic rhinitis (4), hypertension and cardiovascular disease (5). In recent studies it was shown that extract of Urtica dioica exhibited significant growth reduction in human prostatic epithelial cells (6) and inhibition on adenosine deaminase activity in prostate tissue. In this study we aimed to investigate the effect of Urtica dioica on proliferation of HCT-116 colon cancer cell line. Herbal preparation of Urtica dioica was made by ethanol extraction which was followed by evaporation. HCT-116 cells were incubated with different doses of Urtica dioica ranging from 3.33 mg/ml to 42.8 mg/ml for 24 hours. Cell viability was measured with MIT test. Results showed that Urtica dioica (33.3 mg/ml, 42.8 mg/ml) inhibited HCT-116 colon cancer cell proliferation significantly (p < 0.001). Further studies are needed to reveal the effectiveness of Urtica dioica as an alternative therapy for colon cancer. References: 1) Rasal VP, Shetty BB, Srinathambi A, Yesmhana S, Ashok P (2006) Int J Pharmacol 4(2): 22. 2) Krzeski Tet al.
In recent years, the design of multifunctional polymeric materials in the submicrometre size has been considerably improved due to their wide applications in the fields of biomedicine. Particularly, hollow polymeric nanoparticles and micelles have attracted a great deal of attention due to their wide range of applications. These structures have potential utility in encapsulation and controlled release of various biomolecules such as drugs, peptides and genes. A variety of multi-stimuli-responsive nanoparticles have been synthesized that are capable of conformational and chemical changes on receiving an external signal. These changes are accompanied by variations in the physical properties of the polymer. The signal is derived from changes in the materials’ environment, such as a change in temperature or in pH. On the one hand, we have synthesized, characterized and studied micro- and nano-spheres for magnetic and non magnetic properties. Specifically, pH and thermal responsive hollow microspheres were prepared using the distillation precipitation polymerization method with magnetic nanoparticles encapsulated either in the shell or in the core. These novel hybrid microstructures were characterized with transmission electron microscopy, scanning electron microscopy, dynamic light scattering, vibrating sample magnetometry, X-ray diffraction and FT-IR spectra. On the other hand, polymeric micelles seem to be one of the best carriers for delivering hydrophobic drugs. They are formed by the self-assembly of amphiphilic block copolymer in aqueous solutions and have a spherical shape and a size in nano-range. Anticancer drugs that are incorporated into and a size in nano-range. Anticancer drugs that are incorporated into micelles were shown to improve their stability and efficiency. Acknowledgement: This work was supported by scientific programme “IDEAS”, ERC Advanced Grand Nanotherapy. Project Reference: 232959. References: [1] Kataoka K. et al. (2003) Angew Chem Int Ed. 42: 4640 – 4643. [2] Minoko S et al. (2010) Nature Materials 9:101 – 113. [3] Piskin E et al. (2010) Micr. Meso. Chem. 32: 534 – 595. [4] Sukhorukov GB et al. (2004) Langmuir 20: 7265.
Inter-population variation in phenolic content of *Teucrium chamaedrys* L. from the localities in the Balkan Peninsula

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Total phenolic content and flavonoid concentrations in methanolic extracts obtained from *Teucrium chamaedrys* L. in five natural populations of the Balkan Peninsula and a garden population were investigated and compared. The above-ground parts of plants were collected during the flowering phase and the methanolic extracts were prepared. The total phenolic content of the extracts was determined using Folin–Ciocalteu reagent and expressed as gallic acid equivalent. The obtained values varied between 142.04 mg GAE/g and 265.91 mg GAE/g. The concentration of flavonoids was determined using AlCl₃ and expressed as rutin equivalent. The obtained values ranged between 55.66 mg Ru/g and 90.48 mg Ru/g. The highest phenolic content was found in the plants collected from the mountain areas (Bulgaria, Serbia, Bosnia and Herzegovina) and somewhat lower content was found in plants from Mediterranean localities (Montenegro, Croatia). The lowest level was found in the extract obtained from the cultivated plant (Greece). The highest concentration of flavonoids was found in the plants from Mediterranean localities (Croata, Montenegro), while the levels were lower in the other samples and ranged between 50 and 70 mg Ru/ml. On the basis of comparative analysis, the plants collected at higher altitude localities were found to be richer in total phenolics, while higher concentration of flavonoids was found in *T. chamaedrys* from Mediterranean localities. A cultivar of *T. chamaedrys* had lower concentration of phenolics in comparison with natural populations. The results obtained in the analysis point out that the concentration of phenolics depend on the ecological properties of the plant habitats. Acknowledgement: Ministry of Science and Education, Republic of Serbia (III41010)

Phytochemical and pharmacological studies of *Ficus auriculata* Lour. (Family Moraceae) cultivated in Egypt

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This study scientifically examined the phytochemistry, antibacterial and anti-inflammatory potencies of two extracts of *Ficus auriculata* Lour. Eight known compounds, including: betulinic acid, lupeol, stigmasterol, bergapten, scopoletin, 7α-sitosterol-3-O-β-D-glucopyranoside, myrcegen and quercetin-3-O-β-D-glucopyranoside were isolated from the petro-methanolic extract. The structures of these compounds were elucidated on the basis of various spectroscopic methods. This is the first report on compounds separation from *Ficus auriculata* (Moraceae). Concerning the biological studies, the results revealed that both extracts were effective against *gram + ve* bacteria (*Staphylococcus aureus* and *gram – ve* bacteria *Esherichia coli*) by agar well diffusion method. However, ethanolic extract of leaves exhibited greater antibacterial activity than the ethanolic extract of fruits. Meanwhile, the ethanolic extract of leaves at dose of 500 mg/kg exhibited significant anti-inflammatory effect using carrageenin-induced rat hind paw oedema model. Keywords: *Ficus auriculata*, Moraceae, antibacterial activity, anti-inflammatory

Moringa oleifera-treated dry season-turbid Well-water in Enugu Metropolis, Nigeria: A comparative evaluation

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1Fellowship award of Nigerian Institute of Science Laboratory Technology Ministry of Science and Education, Republic of Serbia (III41010)

Water and sanitation services provide a cost-effective solution for alleviating the impact of water-borne diseases. Polluted water is gateway to infectious pathogens leading to a both acute and chronic-diseases worldwide. With the ultimate objective of contributing to the improvement of the quality control of drinking water, we report here, the main application of *Moringa oleifera* Lam. seed extract in the treatment of 25 natural underground well-water samples randomly collected from the three most populous cities in Enugu Metropolis, in southeastern Nigeria. The assessed parameters were salinity, pH, conductivity, total dissolved solids (TDS), total solids (TS), total suspended solids (TSS), turbidity and microbial load before and post-treatment with both alum (as a standard agent) and *M. oleifera* aqueous and ethanolic extracts at equal concentrations of 60 mg/L. The result of the finding showed the ability of *M. oleifera* seed extract to remove organic matter (natural humic substances and micropollutants) thereby avoiding water degradation (mainly bad odours and taste, formation of disinfection by-products such as trihalomethanes) and in addition to having a potent antimicrobial activity which alum naturally lacked. The ethanolic extract of *M. oleifera* had broader spectrum of antibacterial activity than aqueous extract. The alum-treated water samples showed increased salinity and pH in addition to other by-products. From the foregoing, the use of *M. oleifera* aqueous and ethanolic seed extracts as alternative biocompatible flocculants in water treatment in Enugu Metropolis could be recommended. Acknowledgement: *This work is a product of research for a Fellowship award of Nigerian Institute of Science Laboratory Technology (NISL)*

The effect of *Salvia virgata* on GSH-Px Activities of HepG2 cells

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*Turkey is an important country for *Salvia* species. The flora of Turkey includes 88 species of the genus *Salvia*. *Salvia virgata* Jacq. which has shown to be extremely rich with the phenolic compounds that allows this species to be a important member of antioxidative plants. This study was performed to investigate the effect of different *Salvia* extracts on GSH-Px activities of HepG2 hepatocarcinoma cells. The 70% methanol and water extracts were prepared from the aerial parts of *S. virgata* collected from Bursa, Turkey. Gallic acid and rosmarinic acid were used as positive controls. The cells at a number of 2 x 10⁶ cells per well were incubated for 24 h with the extracts and the positive controls under %5 CO₂ at 37 °C. The GSH-Px activities of the cells were then analysed spectrophotometrically via a multifunctional microplate reader. Phenolics rich extract of *S. virgata* has enhanced the GSH-Px activity more than water extract where their effect was just in between the rosmarinic acid and gallic acid positive controls. These results reveal that both extracts mostly the phenolics rich extract of *S. virgata* methanol supports the antioxidant activity in the hepatocarcinoma cell line and these results confirm that it can further effect the glutathione reserves of these cells. This preliminary results needs to be further investigated over the GSSG, GSH and total glutathione and selenium levels. Keywords: *Salvia Virgata*, HepG2, GSH-Px, antioxidant References: 1. Koser M, Goger F, Baser KHC (2008) J Agric Food Chem 56(7):2369 – 74 2. Tosun M, Erçisli S, Şen alguém M, Özer H, Polat T, Ozturk E (2009) Biol Res 42(2):175 – 81 3. Tepe B (2008) Bioresearch Technol 99(6):1584 – 8*
Anti-inflammatory effect and lipoidal content of *Acrocarpus fraxinifolius* Wight & Arn leaves

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*Acrocarpus fraxinifolius* Wight et Arn commonly known as mundane and shingle tree, it is a stately deciduous tree, attaining heights of 30 – 60 m; stem cylindrical, free of branches for up to 75% of its total height. It can achieve a diameter of over 200 cm. The dried powdered leaves of *Acrocarpus fraxinifolius* was successively extracted with solvents of increasing polarities (petroleum ether, chloroform, ethyl acetate and methanol) as well as the total ethanol extract of the powder was prepared. The petroleum ether extract was fractionated into unsaponifiable matter (USM) and fatty acids (FA). The FA fraction was methylated to give FAME fraction. GC/MS analysis of the USM revealed the identification of twenty five compounds represented 74.2% of the total USM, with phytol (43.73%) as the major compound followed by butylated hydroxy toluene (9.96%), n-hentriacontane (6.02%) and squalene (3.56%). Oxygenated compounds represented 59.3% of the total USM. GC/MS analysis of FAME fraction revealed the identification of twenty two compounds represented 85.49% of the total fraction, with methyl 9,12,15-octadecatrienoate (21.05%) as the major compound followed by methyl hexadecanoate (20.09%), methyl 9,12-octadecadienoate (19.58%) and methyl octadecanoate (8.96%). The unsaturated FA represented 41.61% of the total fraction. The acute anti-inflammatory effect of the total ethanol and successive extracts was evaluated by measuring the percentage of reduction of rat hind paw oedema induced by carrageenan [1] which revealed good effects exhibited by all extracts. Ethyl acetate extract was found to be the most potent (93.11% potency) in comparison with indo- methacin (100% potency). References: 1. Winter GA et al. (1962) Proc Soc Exp Biol Med III: 1544 – 1547

PL10

Bioactive Constituents from *Gleditsia triacanthos* L. leaves

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**Gleditsia triacanthos** L. is a deciduous tree belonging to family Fabaceae. It flowers in July, and the seeds ripen from October to November. Different extracts of *Gleditsia* posses important pharmacological activities in treating rheumatoid arthritis [1], as anti-mutagenic [2], anticancer [3] and have significant cytotoxic activity against different cell lines [4]. The dried powdered leaves of *G. triacanthos* was successively extracted with solvents of increasing polarities (petroleum ether, chloroform, ethyl acetate and methanol) as well as the total ethanol extract of the powder was prepared. The petroleum ether extract was fractionated into unsaponifiable matter (USM) and fatty acids (FA). The FA fraction was methylated to give FAME fraction. GC/MS analysis of the USM revealed the identification of twenty four compounds represented 76.86% of the total USM, with squalene (22.68%) as the major compound followed by nonacosenoic acid (21.03%) and isophytol (14.70%). The oxygenated compounds represented 25.38% of the total fraction. GC/MS analysis of FAME fraction revealed the identification of twenty two compounds represented 85.87% of the total fraction, with methyl 9,12,15-octadecatrienoate (31.37%) as the major compound followed by methyl hexadecanoate (19.35%), methyl 9,12-octadecadienoate (13.52%) and methyl octadecanoate (8.95%). The unsaturated FA represented 45.64% of the total fraction. The acute anti-inflammatory effect of the total ethanol and successive extracts was evaluated by the carrageenan induced rat hind paw oedema test Winter, et al. [5], which revealed a moderate effect of all extracts. The most potent effect was exhibited by 100 mg/kg b.wt. of the total ethanol extract (74.60% potency) in comparison with indomethacin (100% potency). References: 1. DaiY, Ye W, Fu L (2002) Patent Appl. 2,002,160,095,31. 2. Lim J et al. (2005) Chem Pharm Bull 53(S): 561 – 564. 3. Klyosove A, Platt D (2001) Patent Appl. W0 276, 474 4. Zhong L et al. (2004) Planta Med 70(9): 797 – 802. 5. Winter GA et al. (1962) Proc Soc Exp Biol Med III: 1544 – 1547

PL9

The first antibacterial activity report of three selected Malaysian rainforest medicinal plants *Nematoloha* A, *Aminimoghadamfarouj* N, Rajagopal M, Khojf T, Wiart C

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Despite increasing resistance among clinically important gram-negative and gram-positive pathogens to many common antibacterial agents, many large pharmaceutical companies are showing decreased interest in this product area that is so critical to public health. This downturn in antibacterial discovery and development, in turn, is leaving us vulnerable to emerging resistance, particularly to recently arrived vancomycin-resistant Staphylococcus aureus and multiply resistant, gram-negative *Pseudomonas aeruginosa*, which we do not have adequate antimicrobial therapy in the future. Rainforest plants possess therapeutic potential, including anti-microbial activity [1,2]. Therefore, this study is a screening program of several extracts from three endemic medicinal plants in Malaysian rainforest which have not been fully discovered and investigated for their antimicrobial properties against several *Gram* negative and *Gram* positive bacterial strains. These plants included families namely Annonaceae, Ebenaceae and Burseraceae. The antibacterial activity of hexane, chloroform and ethanol fractions of some parts of *Uvaria grandiflora* Roxb. (Annonaceae), *Dirosyos wallichii* King & Gamble (Ebenaceae) and *Ceratium pateninnervium* Miq. (Burseraceae) was determined against Gram-positive bacteria *Bacillus cereus* ATCC 10876, *Staphylococcus aureus* ATCC11632, *Methicillin resistant Staphylococcus aureus* ATCC43300 and Gram-negative bacteria *Pseudomonas aeruginosa* ATCC 10145 and *Escherichia coli* ATCC 10536 using the disk diffusion method. Results showed that the bark ethanol fraction of *U. grandiflora*, the fruit hexane fraction of *D. wallichii* and the leaf ethanol fraction of *C. pateninnervium* are active (Table 1). The results indicate that these medicinal herbs can be used as active and potent ingredients in the formulation of natural antibacterial products. References: 1 - Wiart C (2006) Medicinal Plants in Europe. *Origanum vulgare* L. *Thymus capitatus* (L) Hoffmanns and *Satureja montana* L., are endangered species and are included in Albanian National Red Data Book. All of these three plants produce essential oils which are rich in phenolic compounds (Carvacrol or thymol are the dominant phenols in their essential oils) and are used as oregano spices. Using R and Mathematica7 to study the variability of these medicinal plants, provide a very interesting example for further essential oils research on less known MAP resources of the European flora and protect biodiversity. The combinations of modern statistical analyses provide the clear method to analysis the variability of essential oils in MAPs. This will lead to better statistical understanding of the data and higher economic gains.

PL11

Statistical software R and Wolfram Mathematica7 in studying variability of *Satureja montana* in Albania

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Albania is one of the most important exporters of medicinal and aromatic plants in Europe. *Origanum vulgare* L., *Thymus capitatus* (L.) Hoffmanns and *Satureja montana* L., are endangered species and are included in Albanian National Red Data Book. All of these three plants produce essential oils which are rich in phenolic compounds (Carvacrol or thymol are the dominant phenols in their essential oils) and are used as oregano spices. Using R and Mathematica7 to study the variability of these medicinal plants, provide a very interesting example for further essential oils research on less known MAP resources of the European flora and protect biodiversity. The combinations of modern statistical analyses provide the clear method to analysis the variability of essential oils in MAPs. This will lead to better statistical understanding of the data and higher economic gains.

PL12

Effect of biostress on accumulation of secondary metabolites in *Hypericum* species

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In the presence of a pathogen attack, plants developed a vast array of metabolic defense responses sequentially activating the production of bioactive secondary metabolites [1]. The present study aimed to detect how fungal and bacterial biostress alter the phytochemical profile of *Hypericum perforatum* L. and *H. triquetrifolium* Turra known for their pharmacological activities. The greenhouse grown plants were inocu-
lating with four different doses of the fungal pathogen *Seimatosporium hypericum* and the soil bacterium *Pseudomonas putida*. Secondary metabolites were analysed by HPLC-DAD. An analysis of covariance was used to measure the overall effect of different inoculation doses of microorganisms on concentrations of metabolites. According to the results, inoculation of *H. perforatum* with both microorganisms had a significant effect on positive changes of hypericins, hyperforin, rutin, hyperoside, isoorceuticin and total phenolics. In *H. triquetrifolium* the amounts of hypericins and phenolic compounds did not vary significantly under the higher doses of inoculums with exception of a positive accumulation of hyperforin. The increased accumulation of hyperforin can be described to be very important in plant defense mechanism triggered by some of the components excreted by the microorganisms. The increased accumulation of hyperforin can be described as a most important compound to be very important in plant defense mechanism triggered by some of the components excreted by the microorganisms. The comparison of the microorganisms’ effect on the biosynthesis of secondary metabolites showed that pathogenic fungi seem to have more influence than bacteria. The two species of *Hypericum* showed differences in the accumulation of secondary metabolites induced by biostress. Biological stimuli of microorganisms may allow a specific modulation of the biosynthesis of some desirable metabolites in plants. Acknowledgement: The research was supported by Research Council of Lithuania; project number MIP-57/2010. References: 1. Conceição et al. (2006) Florcultura, Ornamental and Plant Biotechnology Vol. 3: 487.

**PL13**

The content of fagopyrin and polyphenols in common buckwheat (*Fagopyrum esculentum* Moench) sprouts depends on growing conditions and the phase of development

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Dried buckwheat herb (*Fagopyrum esculentum* Moench) is used in medicinal products and the fresh green plant parts, especially sprouts, are consumed as a vegetable [1,2]. The herb contains fagopyrins, which cause sensitivity to light after the ingestion of large amounts of the green parts of buckwheat [3]. The aim of this study was to investigate the impact of different growing conditions and development phase on the content of fagopyrin and phenolic compounds in buckwheat sprouts. Total flavonoid and total phenol contents, fagopyrin content and antioxidative activity were determined spectrophotometrically. Fagopyrin and flavonoids were located almost exclusively in cotyledons. It was found that the content of fagopyrin in 14-days-old buckwheat sprouts grown in a sprouter was nearly the same as reported for mature plants, but the content of polyphenols was only at approximately 20 to 30%. The safe intake of buckwheat sprouts was then estimated to be at least 40 g per day. References: 1. Hinneburg I, Neubert Reinhard HH (2005) J Agric Food Chem 53: 3 – 7. 2. Kreft J et al. (2006) Food Chem 98: 508 – 513. 3. Chick H, Ellinger P (1941) Physiol 100: 212 – 230.

**PL14**

Comparative phytochemical study on Veronica officinalis L. and Veronica chamaedrys L

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Criteria to avoid the substitution of *Veronica officinalis* L. (common speedwell) with other species of the genus *Veronica* (*Plantaginaceae* sensu APG 2003, formerly *Scrophulariaceae*) are urgently needed [1], especially *Veronica chamaedrys* L. (germander speedwell), widely spread and without therapeutic action. We have studied the differential phytochemical characters, for the two species regarding the iridoids and polyphenolic compounds content. In these species we have determined the aucubin and catalpol content by using a HPLC analysis with mass spectrometry detection. The content of aucubin is 107.4 µg/g for *V. officinalis* and 328.6 µg/g for *V. chamaedrys* The content of catalpol is 232.2 µg/g for *V. officinalis* and 144.4 µg/g for *V. chamaedrys* [2]. The polyphenolic compounds were determined in the two species before and after acid hydrolysis. The identification of these compounds was achieved through a HPLC analysis with mass spectrometric detection, by comparison with 18 polyphenol standards. The quantitative analysis of the polyphenols, based on UV detection, was performed using an external standard method [3]. The most significant difference between the two species is in the qualitative and quantitative content of polyphenolic compounds and it can be a criteria to phytochemical differentiation of *V. officinalis* and *V. chamaedrys* Acknowledgement: This work was supported by the grant PN II 32515(2008) financed by MECI Romania References: [1] APG (Angiosperm Phylogeny Group). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II (2003) Bot J Linn Soc. 141: 390 – 430 [2] Cisar G et al. (2010) Farmacia 58(2): 237 – 242 [3] Crisan G, Vlase L, Balica G, Crisan O (2009), Rev Med Chiir Soc Med Nat Iaşi 113(2): Supplement nr. 4, 81 – 85

**PL15**

Pharmacognostic studies and establishment of quality parameters of Albizia altissima (Hook.f) Hutch et Dandy

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Albizia altissima (Hook.f) Hutch et Dandy is a deciduous tree that grows up to 15 m in height and 25 cm in diameter and is found in various parts of Africa from Sierra Leone to West Cameroon, Sudan and up to Angola. It is used as a fish poison and in traditional medicine for the treatment of mental illness, snake bites, stomachache and toothache. The use in ethnomedicine for the treatment of mental disorders has been scientifically investigated and the results validated the ethno medicinal use Pharmacognostic studies of the leaves were carried out following the World Health Organization guidelines on the establishment of quality standards for medicinal plants. Other physiochemical parameters were also determined. Pharmacognostic investigations include macro and microscopic studies on fresh green leaves, powdered leaves, physiochemical constants like total ash, extractable (water and alcohol) material and chromatographic fingerprint analysis. The results showed the presence of paracytic stomata in the leaves exclusively on the lower surface, free prisms of calcium oxalate and clusters of calcium oxalate crystals embedded in the epidermal cells. The amount of water extractable matter was 130 mg/g, that of alcohol extractable matter 150 mg/g and the ash value was 30 mg/g. The results of this study are helpful for the preparation of a monograph for the *Albizia altissima*: Acknowledgement: The authors acknowledge Ajibesin K K and Raji R References: World Health Organization (1998) Quality control methods for medicinal plants, Geneva

**PL16**

Development of a novel botanical drug (DA-9701), as a new prokinetic agent


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Functional dyspepsia (FD) is a highly prevalent chronic gastrointestinal disorder that causes a considerable burden to both the patient and society. In the past ten years, several herbal extracts were reported from natural sources in our laboratory. This research was also carried out as a continuous work on bioactive extracts and for the development of prokinetic drugs from natural sources. Based on our prokinetic prescreening data, *Corydalis tuber* (*Corydalis remora* Nakai) and *Pharbitis nil* (Pharbitis nil Choisy) were selected for this research. A prokinetic agent, DA-9701 has potential as a safe and effective prokinetic agent capable of lessening gastrointestinal symptoms and increasing quality of life in FD patients with abnormalities in GI motor function. At the present time, product development is in progress for complement of phase...
Diabetic neuropathy is one of the most common causes of chronic neuropathic pain. In our search for bioactive constituents from plant sources, we found a diabetic neuropathy agent, DA-9801. Ethanol extract of two herbal mixture (the rhizome of Dioscorea japonica Thunberg and Dioscorea nipponica Makino. DA-9801 induces increases in endogenous Nerve growth factor (NGF) levels, and thereby has a protective effect against diabetic neuropathy. NGF plays an important role in the survival and maintenance of neurons in the nervous system and in nerve injury repair. The rhizome of D. japonica has been used in traditional medicine in East Asia to strengthen stomach functions and to dilute sputum in TCM. The rhizome of D. nipponica has been used in traditional medicine in East Asia for treatment of rheumatoid arthritis and diabetes. After phytochemical investigation we found 2 new furanostanol saponins besides 13 known compounds in DA-9801. We evaluated the anti-diabetic neuropathic effect of DA-9801 in a streptozotocin (STZ)-induced animal model. After treatment with DA-9801, NGF levels increased significantly in STZ-induced diabetic rats. Results from a nociceptive test (thermal & mechanical hyperalgesia) showed an increased latency time in groups treated with DA-9801 when compared with control and reference drug groups. The results suggest that DA-9801 may improve the damage produced by diabetic neuropathy via increasing the level of NGF in target tissue, shows improvement on nerve conduction velocity (NCV) and recovery from nerve degeneration. Therefore DA-9801 might have a potential therapeutic effect in patients with diabetic neuropathy. Acknowledgement: This study was supported by grant from the Korea Healthcare Technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (2011-A11082).

It was found that long-term ingestion of cocoa flavonols was able to prevent a variety of dermal disorders associated to UV exposure, like decrease of skin thickness and skin density (1), possibly by means of production of glycosaminoglycans and collagen (2). The antidepressant activity seems to be the underlying mechanism (3). These findings led to the development of a polyphenol enriched cocoa bean (Theobroma cacao L.) extract for further use as a cosmetic ingredient. The proposed method of extraction of polyphenols was able to avoid the formation of insoluble tannins that normally occurs in the production of chocolate (4). The extract was evaluated by three different and complementary DPPH, Lipoperoxidation and Plasmidial DNA Protection Assay. In The DDPH assay, the extract showed an IC_{50} of 7 x 10^{-1} mg/ml, the same protection achieved by the control BHT at 0.01 %. In the lipoperoxidation assay the lowest concentration tested, 25 ug/ml, showed a reduction of oxidation of 33.8% of the liposomes. The maximum level of protection was achieved by 50ug/ml and was not surpassed by greater concentrations. In the Plasmidial DNA Protection Assay, the damnification of the supercoiled (SC) DNA is done by UVA (4.7 J/cm²) and riboflavin (phototoxic under UVA radiation) to form the dammified open circle (OC) DNA. The greater concentration tested i.e 183.4 ug/ml exhibited the same level of protection of quercetin at 1mM. The results showed support indirect evidence for the use of the present extract as an anti-aging ingredient. References: 1. Heinrich U et al. (2006) J. Nutr. 136: 1556 – 1569 2. Casser P et al. (2008) International Journal of Cosmetic Science 30: 339 – 345 3. Han B & Nimmi ME (2005) Connective Tissue Research 46: 251 – 257 4. Wollgast J, Ankla, E (2000) Food Research International 33: 423 – 447

This study scientifically examined the phytochemistry, antibacterial and anti-inflammatory potencies of two extracts of Ficus auriculata Lour. (Moraceae). Eight known compounds, including; betulinic acid, lupeol, stigmastanol, bergapten, scopoletin, β-sitosterol-3-O-β-D-glucopyronoside, myricetin and quercetin-3-O-β-D-glucopyranoside were isolated from the petroleum ether, chloroform and ethyl acetate fractions of alcoholic extracts of the leaves and fruits of Ficus auriculata. The structures of these compounds were elucidated on the basis of various spectroscopic methods. This is the first report on compounds separation from Ficus auriculata. Concerning the biological studies, the results revealed that both extracts were effective against gram +ve bacteria (Staphylococcus aureus) and gram –ve bacteria (Escherichia coli) by agar well diffusion method. However, ethanolic extract of leaves exhibited greater antibacterial activity than the ethanolic extract of fruits. Meanwhile, the ethanolic extract of leaves at dose of 500 mg/kg exhibited significant anti-inflammatory effect using carrageenan-induced rat hind paw oedema model. Keywords: Ficus auriculata, Moraceae, antibacterial activity, anti-inflammatory

Lavandula luisieri (Rozeira) Rivas-Martinez is a Lamiaceae endemic in Iberian Peninsula (1). The morphology and histochemistry of vegetative and reproductive structures of specimens collected in SW Portugal, during 2007 – 2010, were investigated by LM and SEM. Non-glandular multicellular branched stellate hairs and peltate and capitiate I and II, glandular hairs were identified on those structures. Glandular hairs exhibit different secretory modes and almost all showed mixed secretions, hydrophilic and lipophilic, in their nature, except peltate hairs, where lipophilic secretions prevail. A preliminary phytochemical screening through TLC on silica gel plates on ascending polarity plant extracts confirmed the results of the histochemical tests: phenolics, flavonoids and terpenes were present and alkaloids were absent. The antibacterial activity of L. luisieri extracts was determined against different bacteria responsible for infectious diseases in human: Gram-positive bacteria (Staphylococcus aureus ATCC 6538, S. epidermidis ATCC 12228, Enterococcus faecalis ATCC 51299); Gram-negative bacteria (Salmonella typhimurium ATCC 13311, Klebsiella pneumoniae ATCC 5997, Pseudomonas aeruginosa ATCC 9027) and the alcohol-acid resistant bacillus Mycobacterium smegmatis ATCC 19016. Appropriate antibiotics were used as positive controls. All extracts exhibited low activity against Gram-negative bacteria (MICs > 125 µg/mL) and were active against Staphylococcus aureus. The n-hexane extract inhibited S. typhimurium at a concentration of 62 µg/mL, while the dichloromethane and methanol extracts showed the same MIC value of 62 µg/mL for Gram-positive bacteria. Dichloromethane, methanol and water extracts inhibited the Mycobacterium smegmatis bacillus growth with MICs of 32, 15 and 32 µg/mL, respectively. These results are statistically significant and are worthy of further studies. Acknowledgement: Elmo Nunes, Paula Pees. Refer- ences: 1. Morales R (2000) Portugalae Acta Biol 19: 31 – 48

PL17

PL18

PL19

PL20
Sanguisorba l. is a Rosaceae distributed throughout the northern hemisphere. Some species are known to show hypoglycemic and hemostatic properties [1], antimicrobial (2) and antiviral activities (3). Sanguisorba hybrida (L.) Nordborg is endemic in Portugal (4) and was selected for pharmacognostic studies including a preliminary phytochemical survey and an evaluation of its potential against human pathogens. Samples were collected in SW Portugal (38° 8’ N – 8° 33’ W) during 2009 – 10 and identified at LUSU. Under microscopy techniques non glandular and glandular multicellular trichomes were seen on both leaf surfaces. With histochemical tests the terpenoids and phenols were the most relevant compounds detected. Powdered plant material was extracted with n-hexane, dichloromethane, ethyl acetate, methanol and water. Their phytochemical analysis established the presence of flavonoids, saponins and saponin-rich plant-extracts from other plant-families were time-consuming to purify/to create substances [5]. In our work we focused on Cistaceae and we demonstrated important antiviral activity on PI-3 with a range of 64 – 16 μg mL⁻¹ [16 – 0.0625 μg mL⁻¹] showed a significant antiviral activity on HSV-1 with the MTIC of 16 μg mL⁻¹ [1]. Only the BuOH extract of S. wiedemannii demonstrated important antiviral activity on PI-3 with a range of 64 – 16 μg mL⁻¹ of inhibitory concentration for CPE, which was close to the anti PI-3 activity of oseltamivir. This study has shown that S. wiedemannii extracts have important antiviral activities and can be used as a source for drug development. References: 1. Rebecca CB et al. (2004) Antiviral Res 61: 73 – 81. 2. Hall CB (2001) N Engl J Med 344: 1917 – 28. 3. Davis PH. (1982) Flora of Turkey and the Aegean Islands. Edinburgh.

In vitro antiviral activity and cytotoxicity of the extracts of Salvia wiedemannii Boiss. Ustun O1, Ozcelik B2, Baykal T1

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Herpes simplex type 1 (HSV-1) and Parainfluenza-3 (PI-3) viruses are important pathogens for humans. Although antiviral drugs are available, resistance to these antiviral medications has been increasing [1,2]. S. L. (Lamiaceae) is widely distributed in Turkey by 94 taxa belonging to 89 species, with a 50% ratio of endemicism (4). Some parts of Salvia species have been used in Turkish folk medicine for the treatment of various disorders and symptoms, including catarrh, cold, wounds, stomachache, flatulence, constipation, rheumatic pain, wards, sunstroke, and hemorrhage. In addition, there are also some reports on the antiviral effects of Salvia species. In this study, we evaluated the antiviral efficacy of S. wiedemannii Boiss. extracts on HSV-1 and PI-3 viruses by using Vero cell lines. Antiviral efficacy of these extracts, obtained from aerial parts of S. wiedemannii, was compared to that of acyclovir and oseltamivir. The H₂O, CHCl₃, and EtOH extracts of S. wiedemannii (16 – 0.0625 μg mL⁻¹) showed a significant antiviral activity on HSV-1 with the MTIC of 16 μg mL⁻¹. Only the BuOH extract of S. wiedemannii demonstrated important antiviral activity on PI-3 with a range of 64 – 16 μg mL⁻¹ of inhibitory concentration for CPE, which was close to the anti PI-3 activity of oseltamivir. This study has shown that S. wiedemannii extracts have important antiviral activities and can be used as a source for drug development. References: 1. Rebecca CB et al. (2004) Antiviral Res 61: 73 – 81. 2. Hall CB (2001) N Engl J Med 344: 1917 – 28. 3. Davis PH. (1982) Flora of Turkey and the Aegean Islands. Edinburgh.
Biologically active flavonoid glycosides from *Horwoodia dicksoniae* Turrill

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Three flavonoid glycosides and one aglycone have been isolated from the ethanolic extract of *Horwoodia dicksoniae* Turrill for the first time, and their structures were assigned from 1H- and 13C-NMR spectra (DEPT) and from negative ESI-MS, as luteolin-7-O-β-D-glucopyranoside (1), api-genin-8-C-β-D-galactopyranoside (2), luteolin-6-C-β-D-glucopyranoside (3) and luteolin (4). The SRY cytotoxicity assay was used to investigate the antitumor activities of the ethanolic extract, compounds 1, 3 and 4. Compound 1 showed the highest cytotoxic activity against the three human cell lines; HEPG2, HCT 116 and MCF 7 (IC50 10.7, 9.3 and 9.9#g/ml, respectively). Compared to thestandard anticancer drug doxorubicin, Compound 4 showed selective antitumor activity against the colon cell line (IC50 9.5 #g/ml). The present investigation also demonstrates the protective effect of compounds 1, 3 and 4 with antioxidant potential, in glycerol-induced myocardial function failure in rats. Treatment with each of these compounds attenuated renal dysfunction, and restored the oxidant balance by decreasing renal MDA levels, increasing the activity of the depleted renal antioxidant enzymes, and the non enzymatic antioxidant GSH. They also, decreased the elevated serum inflammatory marker (TNF-α), and ameliorated apoptosis and kidney damage by reduction in caspase-3 activity. Compound 1 showed the highest biological activity.

Biodiversity assessment of Veronica sp. in Romania for their characterization, preservation and sustainable use in pharmacognosy

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Veronica is the most species-rich genus of Plantaginaceae family with about 800 species. The majority of the species are herbs, forbs, subshrubs or perennials and they are very diverse from an ecological point of view [1]. Based on morphological and molecular analyses it was estimated that about 80 species of Veronica are found in Europe; about 40 species found in nine different subgenera are endemic to Europe and about 40 species have been reported in literature as being present in Romania [1, 2]. The genetic diversity and the chemical composition, useful for pharmacognosy [3] (V. officinalis, especially), of the Veronica species have encouraged different types of research to be carried out. Our aim is to assess the biodiversity of the species from Veronica genus on the Romanian territory in order to contribute to the protection of their biodiversity. These studies will subsequently result in offering new nian territory in order to contribute to the protection of their biodiversity. These studies will subsequently result in offering new

Centipeda cunninghamii, an australian traditional medicinal plant

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Centipeda cunninghamii (DC.) A.Braun & Asch. is an endemic Australian Asteraceae with a long history of traditional use as a medicinal plant for treating wounds, infections and inflammation. Whilst its essential oil composition, principally chrysanthenyl and sabiny acetates, has been known for some time, there was little scientific information regarding its phytochemistry and biological activity. Investigations on aqueous ethanolic extracts confirmed its anti-inflammatory and antioxidant (ORAC) activity. Detailed investigations suggest the extract acts against a range of inflammatory markers including COX-1, COX-2, NO and TNF-α, but not through the lipoxigenase pathway. Seventeen compounds were isolated and subsequent bioassays indicated that the anti-inflammatory activity was linked to flavonoids, whilst the antioxidant activity was attributed to both flavonoids and a group of novel heptaneoic acid cinnamoyl esters. The latter compounds are ring-opened quinic acid derivatives and appear to be unique to this species. Optimisation of growing, post-harvest and extraction conditions based on quality markers have been developed for future production and product development.

Biodiversity of carrot genetic resources – variation in secondary metabolites

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Daucus genetic resources comprises a few thousand accessions collected in gene banks mainly in USA, Europe and Russia. In 2009 a sub collection of 94 accessions representing edible wild carrots was established that should represent available biodiversity. The choice of the accession was done mainly based on their passport data supplemented with data obtained during European programme on carrot characterization during which morphological characters were assessed. The aim of the presented work was to assess variation of the chosen accessions with regard to their composition of secondary metabolites. The analytical investigation was focused on carotenoids, including alpha-, beta-carotene, lutein, lycopene and their precursor phytoene, reducing and non-reducing sugars, phenolics, including anthocyanins, flavonols and phenylpropanoids and tocopherol. The results obtained revealed considerable variation of secondary metabolites content depending on genetic background. Edible carrots possessed higher carotenoid content, while phenolics dominated in wild relatives. Several accessions with high level of these compounds with importance for human health were identified. These materials may be prioritized in genetic and breeding programs for the development of high nutritional carrot cultivars. Acknowledgement: Research was supported by Polish Ministry of Agriculture and Rural Development (grant No. HON 078 dec-1/10).

Quantitative Determination of Lycorine in *Galanthus* x*vulcaninae* nothosubsp. *subplicatus*

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*Galanthus* x*vulcaninae* (J. Allen) Beck nothosubsp. *subplicatus* (N. Zeybek) A. P. Davis (Amaryllidaceae) is a hybrid between *G. nivalis* L. and *G. plicatus* M. Bieb. subsp. *hyazantius* (Baker) D. A. Webb. This *Galanthus* L. hybrid is endemic and it occurs naturally in northwest Turkey [12]. Lycorine, a common alkaloid found in Amaryllidaceae plants, has been shown to possess important biological activities including antiviral [3], cytotoxic [4] and antimarial activities [5]. In the present study, a reversed-phase high-performance liquid chromatographic method has
been used for the quantitative determination of lycorine in the aerial parts and bulbs of G. xalvenetini noothsubsp. subplicatus [6]. A simple method for the extraction of lycorine in low-mass plant samples was employed utilizing pre-packed columns with diatomaceous earth (Ex- trel-.) [7]. The chromatographic separation was carried out using an isocratic system with a mobile phase of trifluoroacetic acid-water-acetonitrile (0.01: 95: 5) applied at a flow rate of 1ml min⁻¹ using diode array detector. The linearity of the method was studied by injecting five known concentrations of lycorine in the range of 0.5 – 8 μg ml⁻¹. The calibration curve for lycorine was determined as Y = 14.9668622X + 0.7717199. The content of lycorine in the bulbs of G. xalvenetini noothsubsp. subplicatus was found to be 0.0028%. Lycorine was not detected in the aerial parts of this plant. Acknowledgement: This study was financially supported by Ege University Research Fund (09/ECZ/037) and partially supported by TUBITAK (TBAG-104T272) and EBİTEM (2007-BIL-007). References: 1. Davis AP et al. (2001) Kew Bull 56: 639 – 647. 2. Davis A (2006) The Genus Galanthus-Snowdrops in the Wild, in Bishop M., Davis A.P., Grimshaw J. (Eds.), Snowdrops, A Monograph of Cultivated Galanthus. Griffin Press Publishing Ltd. Cheltenham. 3. Szlávik L et al. (2004) Planta Med 61: 77 – 79. 5. Sener B et al. (2003) Phytotherapy Res 17: 1220 – 1223. 6. Mustafa NR et al. (2003)) Liq Chromatogr RT 26:3217 – 3233. 7. Berkov S et al. (2008) Phytochem Anal 19: 285 – 293.

PL31 From structural studies of natural products to the discovery of a selective antimalarial derivative: a serendipity story
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In our continuing effort directed to the search for new antimalarial natural compounds from plants of the tropical biodiversity, the phytochemical study of Canthium majus showed that this plant was a source of new chemical entities with potential antiparasitic activity. One of these entities was tested against the amastigote and promastigote forms of Trypanosoma brucei and Plasmodium falciparum and showed weak antiplasmodial activity due to a possible stomatocytogenic[1] effect. The structures of the diarylheptanoid glucosides were similar to those isolated from the rhizomes of Taccia chantrieri André by Yokosuka and co-workers[2]. After chemical modifications of the natural glucosides, including hydrolytic cleavage, methylation and esterification, the determination of their absolute configuration using the CD excitation chirality method [3] applied to acyclic 1,3 dibenzoates [4] systems has been successfully achieved. Naturally-occurring diarylheptanoid glucosides and their derivatives were evaluated in vitro, for their antiparasitic activity against Plasmodium falciparum (FC8), Leishmania donovani (amastigote and promastigote forms) and Trypanosoma brucei as well as for their cytotoxic activity against HL-60, KB and MRC5 cell lines. Among 17 compounds investigated, one diarylheptanoid exhibited a selective antimalarial activity and no cytotoxicity. Acknowledgement: This work was supported by an ICN-CNRS grant to one of us (MAA B.). We express our thanks to G. Aubert for the cytotoxicity assay, Pr. P. Loiseau and Pr. C. Bories for the antileishmanial and antitypansomosal activity. References: 1. Ziegler H L et al. (2002) Antimicrob Agents Chemother 46: 1441. 2. Yokosuka A et al. (2002) Nat Prod 65: 283. 3. Harada N et al. (1972) Acc Chem Res 5: 257. 4. Harada N et al. (1991) Am Chem Soc 113: 3842.

PL32 Antiprotozoal and cytotoxic activities of some mushrooms from Turkey
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Protozoal infections still constitute a major health problem worldwide. Due to emerging resistance to common antiprotozoal agents, new drugs are urgently needed. Mushrooms are simple, non-photosynthetic organisms widespread in the world flora. Some are inedible or toxic, but many of them are used medicinally or in cooking. Some biological effects, e.g. antioxidant, immunomodulatory, antitumor, antimicrobial and antiprotozoal of mushrooms have been shown [1 – 4]. In this study, we evaluated the in vitro antiparasitic and cytotoxic potential of Ethanolic extracts of some mushrooms growing in Turkey, namely Polyphorpus gigu, P. sulphureus, P. anamoss, P. rhedes, P. pinicola, P. volvatus, P. bodius, Cantharellus cibarius, Ganoderma applanatum, Fomes fomentarius, Clavulinina cerin, Cortinaris orellanus and Trametes versicolor. The test organisms used were Trypanosoma brucei rhodesiensis, T. cruzi, Leishmania donovani and Plasmodium falciparum. Cytotoxic effects of the extracts were also assessed towards primary mammalian L 6 cells. All mushroom extracts were active against T. brucei rhodesiensis with P. rhedes being the most potent (IC50 0.59 μg/ml). The most potent extracts against L. donovani and P. falciparum were those of P. gigu, P. sulphureus, P. anamoss, P. pinicola, F. fomentarius, C. cerin and T. versicolor (IC50 values 1.39 – 2.73 μg/ml). The extracts did not show any cytotoxicity against L 6 cells. To our knowledge, this is the first biological activity and antiprotozoal screening study carried out on Turkish mushrooms. The activity-guided isolation of the most active extracts is in progress. References: 1. Ribiero B et al. (2008) Food Chem 110: 47 – 56. 2. Guerra DCM et al. (2007) Int Immunopharmacol 7: 1160 – 1169. 3. Kaneno R et al. (2004) Int Immunopharmacol 7: 1160 – 1169. 3. Kaneno R et al. (2004) Int Immunopharmacol 7: 1160 – 1169.

PL33 Climate change impact on conservation status of wild Melissa officinalis L. (Lamiaceae) populations in Armenia
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Climate change and temperature may lead to long-term irregularities in inter-specific interaction and may alter plant populations’ dynamics, its structure and ecosystem functioning in the region [1,3]. Studies on possible effects of climate change on medicinal plants biodiversity and conservation status are particularly significant due to their value within traditional systems of medicine and as economically useful plants. Currently, only limited knowledge on conservation status under the impact of global climate change of these species is available in Armenia [2]. Anthropic threats to biodiversity (overpopulation, deforestation and urbanization) have simultaneously hindered research and increased the need for it. From 2006 – 2009, field studies were conducted to find
out changes the growth, phenological and habitat characteristics of Melissa officinalis L., population size and location (GPS mapping). In 2010, we have implicated these research data to carry out future assessment of the risk analyze and impact of global climate change on its population distribution and conservation status. Neural network and genetic algo-
rithms have been identified as stochastic self-learning methods to in-
vestigate hidden regularities between different data. Certain factors, such as biological characteristic of plants, habitat of the populations, anthropogenic threats and climate change have been identified as the key elements. In fact, vulnerability of plant population, particularly will increase central and northern part of the country, as they identified to be comparatively stressful environment under global climate change and anthropogenic threats, which included: poor land management, increasing population pressure, and excessive collection of plants. References: 1. Hughes L (2000) Trends Ecol Ecol 15: 56 – 61. 2. IUCN-WHO-WWF (1993) Guidelines on the Conservation of Medicinal Plants, IUCN, Gland, Switzerland, 50 p. 3. Bishop JC, Schemske DW (1998) Ecol 79: 534 – 546

under Shirvan conditions. This research was conducted at Research Farm of the Faculty of Shirvan Agriculture, Ferdowsi University of Mashhad, Iran in 2009 – 2010. Five safflower cultivars (Cinaa, CW-440, Sahuripa-88, Chochlan local and Isfahan local) were used in this study. The re-
search was randomized complete block, split plot design with three replications. Different sowing time significantly affected yield, oil con-
tent and fatty acid composition of the genotypes used in this study. There was interaction between genotypes and sowing times. According to the results of this research: China had highest yield in autumn and spring sowing (2989 and 2120 kg/ha respectively). Also Sahuripa-88 has showed the highest oil content in autumn and spring sowing (32 and 28.9% respectively). Yield and oil content in autumn sowing was highest (847 kg/ha and 2.1%). The palmitic, stearic and oleic acid increased but linoleic, and linolenic acid decreased in autumn sowing. According to the results of the study it was found that autumn sowing was suitable than spring sown and Cina genotype have desirable potential for plant-
ing in Shirvan region.

Cheemosystematics, or chemotaxonomy, is the attempt to classify and identify plants, according to demonstrable differences and similarities in their secondary metabolites. Thus, chemotaxonomic markers are powerful tools for the identification of a wide variety of plants [1]. Chinese forest scientists have been making significant efforts to develop fast-growing trees due to the extreme shortage of wood resources. Tri-
plold Populus tomentosa Carr. (Salicaceae), the cloned hardware poplar species from Populus tomentosa, has been receiving the most attention [2]. However, secondary metabolites of triploid P. tomentosa have never been studied to date, though poplars have been widely used in folk medicines for the treatment of various diseases [3]. This work was car-
rried out to investigate the secondary metabolites and the chemosyste-
matic markers from triploid P. tomentosa. Column chromatographic pur-
ification of triploid P. tomentosa extracts resulted in the isolation of twelve phenolics: grandidentatin, isograndidentatin A, isograndidentatin B, grandidentatin, here in triploid P. tomentosa extracts, namely arctigenin, trachelogenin and matairesinol. Arctigenin isograndidentatin A, isograndidentatin B, grandidentatin, here in triploid P. tomentosa was interesting and glucosides of 1,2-dihydroxycholhex-
ane acylated by p-coumaric acid (or p-coumaric acid derivatives) could be considered as useful chemosystematic marks within the Salicaceae family, which was also well in accord with the our previous conclusion [4]. Acknowledgement: This work was financially supported by Program for New Century Excellent Talents in University (NCET 2010), Foundation for the Development of Science and Technology in Tianjin Universities (No. 200808616), the National Natural Science Foundation of China (NSFC, No. 31000278) and Nature Science Foundation of Tianjin Municipal Government (No. 09JCYBJC1800). References: 1. Bohm BA (1987) The Bot Rev 53: 197 – 279. 2. IFL TN et al. (2011) Bioreseourses 6: 232 – 242. 3. SI CL et al. (2009) Chem Nat Compd 45: 634 – 636. 4. SI CL et al. (2009) Biochem Syst Ecol 37: 221 – 224.

PL34

Effects of autumn and spring sowing on yield, oil content and fatty acid composition of safflower (Carthamus tinctorius L.) cultivars in Shirvan region

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Yield, oil content and fatty acid sythesis of crop are influenced by a lot of factors such as genotype, ecology, morphology and management (plant-
ing date, plant density, fertilization etc.). The aim of this study was to determine effect of Autumn and spring sowing on yield, oil content and fatty acid composition of safflower (Carthamus tinctorius L.) cultivars

PL35

PL36

PL37

Discoveries of new indoleamine-2,3-dioxygenase inhibitors from Carthamus tinctorius

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Indoleamine-2,3-dioxygenase (IDO) is the rate limiting step of tryptophan catabolism. As its expression is induced by Type II interferone (INF-γ), it is involved in inflammatory diseases [1]. In neurological disorders, de-
gradation of tryptophan can reduce serotonin synthesis, which is related to major depression [2]. Furthermore, quinolinic acid originating from tryptophan catabolism has neurotoxic effects. In cancer cells, the expres-
sion of IDO leads to a local suppression of T-cell responses and promotes immune tolerance. Therefore, IDO is an interesting target for therapeutic intervention in these conditions [1]. As Carthamus tinctorius L. was used in ethnopharmacology against inflammatory diseases and also against cancer [3] and depression [4], this plant was investigated for IDO inhibi-

Effects of chemical and organic fertilizers on number of corm and stigma yield of saffron (Crocus sativus)

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Saffron (Crocus sativus L.) is the world’s most expensive spice and 95% of the production is coming from Iran [1]. The aim of this study was to better understanding the effects of different organic and chemical fertilizers on number of corm and stigma yield of saffron. This experiment was conducted in Organic Farm of Ferdowsi University of Mashhad, Iran, based on CRBD with three replications. The experimental treatments were four different fertilizers including chemical (50 – 250, 100 – 250 and 300 – 250 kg/ha N-P2O5), cow manure (20, 40 and 60t/ha), sheep manure (20, 30 and 40t/ha) and hen manure (5, 10 and 15t/ha). The results which is reporting here, came from fifth year of the experiment. Results showed that the highest fresh flower and dry stigma yield were

Effects of autumn and spring sowing on yield, oil content and fatty acid composition of safflower (Carthamus tinctorius L.) cultivars in Shirvan region

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Yield, oil content and fatty acid sythesis of crop are influenced by a lot of factors such as genotype, ecology, morphology and management (plant-
ing date, plant density, fertilization etc.). The aim of this study was to determine effect of Autumn and spring sowing on yield, oil content and fatty acid composition of safflower (Carthamus tinctorius L.) cultivars

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ing date, plant density, fertilization etc.). The aim of this study was to determine effect of Autumn and spring sowing on yield, oil content and fatty acid composition of safflower (Carthamus tinctorius L.) cultivars
obtained from chemical fertilizer (300 – 250 kg/ha N-P2O5) and then from cow manure (20t/ha) treatments. Fresh flower and dry stigma yield were increased by increasing the nitrogen level in chemical fertilizer and increasing sheep manure levels. The same results has been reported by Behnia et al [1]. Behzad et al [2] showed that application of 200 kg ammonium phosphate plus 30 tons of cow manure produced the highest stigma yield. Rezvani moghdam et al. [4] reported that cow and chemical fertilizers produced more flower and stigma yield than hen manure. Sheep manure at 40t/ha produced the highest mother corn and replacement corn per clump. Saffron is a low nutrient demand plant and requires a modest amount of nutrients [3]. References: 1- Behnia MR et al. (1999) Agron Crop Sci 182: 9 – 15. 2- Behzad S et al. (1992) Acta Hortic 306: 337 – 339. 3- Housini M (1998) Iranian Scientific and Industrial Research Organization, Press -Khorasan Center. 4- Rezvani Moghdam M et al. (2006) 2nd International Symposium on Saffron Biology and Technology. Iran.

In the order Asterales only two species are known to have proteolytic activity in their latices, first Taraxalis from dandelion Taraxacum officinale Webb s.l. and second Parthenin from Guayule Parthenium argentatum L... Both are characterized as serine endopeptidases [1]. Proteolytic enzymes isolated from plant latex have received special attention in the pharmaceutical industry and biotechnology due to their property of being active over wide range of temperature and pH. Nearly half of the commercially available enzymes are proteases, frequently used in food processing, tendonization of meat, brewing, cheese elaboration, bread manufacturing, leather and textile industries [1]. In this investigation the latex of 40 species of the Asteraeaceae family and 8 species of the Campanulaceae, which are not biochemical characterized before, were collected in the Botanical Garden Berlin. To determine proteolytic activity we used the fluorogenic substrate BODIPY FL casein (Molecular Probes, Inc., USA) [2]. To investigate the type of endopeptidases, the latex samples were pre-incubated with specific inhibitors for serine endopeptidases. The latex was hydrolysed with different concentrations of pepsin, trypsin, pronase and elastase from Bacillus subtilis. The enzyme solution was added to the latex and the reaction was allowed to proceed for 24 h at 37°C in a shaking water bath. The enzyme solution was added to the latex and the reaction was allowed to proceed for 24 h at 37°C in a shaking water bath. The enzyme activity was determined using a UV spectrophotometer (Model: 1260) on a 1cm cell. The absorbance at 500nm was measured. The results were expressed as the amount of proteinase activity in U/ml of latex. Each experiment was performed in triplicate and the results were expressed as the mean ± SD. The normality of the data was checked using the SPSS program. Statistical analysis was performed using one-way ANOVA test followed by Duncan multiple range test. A significance level of P<0.05 was considered.

PL42

Screening of Zambian Ficus species for antibacterial and antymycobacterial activity

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Members of the genus Ficus (Moraceae) are traditionally used in Zambia against many infectious diseases, including bacterial (oral, chest and diarrhoeal), mycobacterial and fungal (ringworms) infections [1]. Based on this information, we collected different plant parts (leaves, stem and root barks) of eight Zambian Ficus species: F. ovata Vahl, F. wakefieldii Hutch, F. natalensis Hochst. F. sanisbarica Warb. subsp. macroaspera, F. Iutea Vahl, F. ingens (Miq.) Miq., F. sycomorus L. subsp. gnaphalocarpa (Miq.) F. sycomorus L. subsp. sycomorus. The dried plant materials were extracted with methanol (CR-MeOH) and further partitioned with methanol (K-MeOH) subextracts. We recently investigated the antifungal activity of the extracts were also analyzed. BHT, BHA, ascorbic acid, gallic acid and rosmarinic acid were used as positive controls. Phenolics rich extract of aq. methanol showed more scavenging activity on DPPH/C30 than water extract whereas water extract more scavenged the ABTS+/C30 radical. The aq. methanol extract more reduced the ferric(III) to ferro(II) in a certain proportion than water extract. All extracts were cytotoxic against MRSA 1199B, Enterococcus faecalis NCTC 10418 and S. virgata strains. The dried plant materials were extracted with methanol (CR-Me) and further partitioned to obtain n-hexane (K-Hex), chloroform (K-CHCl3) and aqueous methanol (K-MeOH) subextracts. We recently investigated the antifungal effect of CR-Me extracts against Trichophyton species, the causative agents of ringworm infections [3]. Herein we screened the CR-Me extracts and the subextracts for antibacterial and antymycobacterial activity using agar disc diffusion and MTT assays, respectively. Test organisms were Gram-positive [Staphylococcus aureus NCTC 12695, methicillin-resistant Staphylococcus aureus MRSA 1199B, Enterococcus faecalis 13379] and Gram-negative [Escherichia coli NCTC 10418] bacteria, plus Mycobacterium tuberculosis (H37Rv strain). The CR-Me extracts of the stem barks were active against all Gram-positive microorganisms. Of the subextracts, K-MeOH-solubles exhibited the best activity with inhibition zones of 11 mm at 100 µg/disc concentration against all three Gram-positive bacteria. Moderate antibacterial activity was observed in some K-Hex and K-CHCl3 subextracts, with K-CHCl3-solubles of F. ovata stem bark exhibiting the highest activity (MIC 128 µg/ml). These results provide a scientific basis supporting the use of species in traditional herbal preparations in Zambia. Acknowledgement: UK Commonwealth Scholarship Commission and the Rick-Cannell Travel Fund of the School of Pharmacy are acknowledged for funding. References: 1. Kuete V et al. (2008) Ethnopharmacol 124: 556 – 561. 2. Fowler DG (2007) Zimbabwe Med J 17: 1220 – 1223. 3. Rantamarianpignonna D et al. (2007) Fittotera 78: 482 – 489. 4. Sritongkul J et al. (2008) Acta Hort 804: 367 – 372.

PL43

Impact of nitrogen nutrition on growth and plant quality of Centella asiatica

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Due to its bioactive triterpene saponins, Centella asiatica (L.) Urb. has been used as a medicinal herb since ancient times and its economical importance is still rising [1,2,3]. Up to now plants are collected spontaneously which implicates a large variation in plant and product quality depending on the origin, genotype and time of harvest of the plants [1 – 4]. To assure high plant quality, it will be necessary to encourage cultivation of Centella. So far, there is scarce information on cultivation techniques, especially on mineral nutrition of Centella plants. The aim of this study was to investigate the effects of nitrogen on growth and saponin biosynthesis of Centella asiatica and to find appropriate parameters to evaluate quality of plant material by non-destructive measurements in situ. Plants were grown for eight weeks in rock wool cubes in a greenhouse and fed with five nutrient solutions differing in their nitrogen concentrations. Number of leaves and stolons, length of stolons, assimilation rate and leaf green intensity were monitored weekly. Non-destructive measurements were conducted with a portable optical sensor (Multiplex® Research, Force-A, France) recording the fluorescence signature which is associated with plant constituents. Fresh and dry weight of leaves and stalks, leaf area and specific leaf weight were determined. Leaf chlorophyll and leaf nutrient content was carried out once at the end of the study. Leaf samples for determination of triterpenoid content were harvested four times. Analyses of asiaticoside and asiatic acid by HPLC are in progress and will be discussed. Acknowledgements: Regionale 2010, State of North Rhine-Westphalia (Germany); Institute of Systematic Botany, The New York Botanical Garden (USA), A.N. Nicolas; Institute Malacée de Recherches Appliquées (Madagascar), D. Randriamampionona; National Center for Natural Products Research, University of Mississippi (USA), B. Avula; Unité d’Analyse Chimique et Physique-Chimique des Médicaments et Pharmacognosie, Université Catholique de Louvain (Belgium), M.H. Rafamantarisa; Institute of Nutritional Sciences, University of Bonn (Germany), B.F. Zimmermann. References: 1. Thomas MT et al. (2010) Ind Crop Prod 32: 545 – 550. 2. Devkota A et al. (2010) Biochem Syst Ecol 38: 12 – 22. 3. Randriamampionona D et al. (2007) Fittotera 78: 482 – 489. 4. Sritongkul J et al. (2008) Acta Hort 804: 367 – 372.

PL44

Antioxidant Properties and Phenolic Composition of Salvia virgata from Turkey

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Several biochemical reactions generate reactive oxygen species and these are crucial of damaging crucial bio-molecules [1]. Free radicals are very important in food products, because oxidative degradation of lipids is one of the main factors limiting their shelf-life [2]. In recent years, national antioxidants have been focused on because of the harmful effects of synthetic antioxidants [3]. S. officinalis L. (Lamiaceae), is an important source of antioxidant compounds and have wider implications for the dietary intake of natural antioxidants [3]. Turkey is an important country for Salvia species. The flora of Turkey includes 88 species of the genus Salvia. The 70% methanol and water extracts were prepared from the aerial parts of S. virginica Jacq. collected from Bursa, Turkey. All the extracts were analyzed by HPLC and in vitro antioxidant assays. The 1,1-diphenyl-2-picrylhydrazin (DPPH®), 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS®) radical scavenging activity and β-carotene bleaching methods were used. Total phenolic compounds and reductive activity of the extracts were also analyzed. BHT, BHA, ascorbic acid, gallic acid and rosmarinic acid were used as positive controls. Phenolics rich extract of aq. methanol showed more scavenging activity on DPPH® than water extract whereas water extract more scavenged the ABTS® radical. The aq. methanol extract more reduced the ferric(III) to ferro(II) in a certain proportion than water extract. All extracts were inhibited linoleic acid peroxidation in β-carotene bleaching test and shown more activity than rosmarinic acid. Rosmarinic acid was found as the main component and caffeic acid, ferulic acid and uroline-7-O-glycoside were identified in the extracts. References: 1. Kumaran A, Joel Karunakaran R (2006) Food Chem 97: 109 – 114. 2. Pizzalle L et al. (2002) J Sci Food Agric 82: 1645 – 1651. 3. Kintzios SE (2000) Sage The Science of Food Systems, London, 27 – 53 and 185 – 192.
The plants are one of the attractive sources of novel antitumor compounds. Isolation of pharmaceuticals from plants is difficult due to their extremely low concentrations. To extend the research to human clinical studies, we needed to find a reliable supply of plant material, produced target compounds. As part of our ongoing program on the investigation of Linum species, cell cultures of L. leonii F.W.Schultz, were examined. We have established several callus and suspension cultures and checked for the occurrence of lignans. The main component in cell cultures of L. leonii was isolated and analyzed by means of GC-MS and NMR. The EI-MS of the isolated compound showed an ion at m/z 364 and mass fragmentation, which is consistent with the data for an aryl naphthalene lignan, the 1H NMR spectrum showed that the isolated compound is justicidin B. Justicidin B produced by in vitro cultures of L. leonii was isolated and analyzed by means of GC-MS and NMR. The EI-MS of the isolated compound showed an ion at m/z 364 and mass fragmentation, which is consistent with the data for an aryl naphthalene lignan, the 1H NMR spectrum showed that the isolated compound is justicidin B. Justicidin B produced by in vitro cultures of L. leonii was tested for cytotoxic activity and induction of apoptosis in MDA-MB-231 and MCF-7 breast cancer derived cell lines. The tested lignan evoked strong, concentration-dependent cytotoxicity in both cell lines, whereby MCF-7 proved to be more sensitive. The 24h treatment of both cell lines increased the level of apoptotic DNA fragmentation; however the proapoptotic activity is completely inhibited if the cells are co-incubated with the non-selective pan-caspase inhibitor Boc-Asp(OMe)-fluoromethyl ketone (PC1), which implies that justicidin B, activates PCD via caspase-dependent mechanisms. Exposure of MDA-MB-231 cells with justicidin B leads to concentration-dependent decrease in the NFKB expression; strong NFKB expression is observed in MCF-7 cells. Acknowledgement: Financial supports from Ministry of Educaton and Science, Sofia, Bulgaria (grant D002 – 128/08 I. Ionkova) is acknowledged.

Investigation on the antimicrobial activity of Acroptilon repens (L.) DC. Noroozi M*, Rajabi A1, Eivazi S1, Sadeghinikoo A2, Amin G2
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Acroptilon repens (L.) DC. (Russian knapweed) is a perennial herbaceous plant belonging to the family Asteraceae. This plant is native to Mongolia, western Turkistan, Iran, Turkish Armenia and Asia Minor [1]. The objective of this study was to evaluate antimicrobial activity of aerial parts extract of A. repens against 4 pathogenic bacteria. The samples were collected from Takestan, Ghaevin province in June 2007. The CH3Cl, EtOAc and MeOH extracts of the aerial parts of the plant obtained by percolation method were investigated for antimicrobial activity against Staphylococcus aureus, Escherichia coli, Salmonella typhi and Bacillus subtilis. The present study showed that all the extracts of A. repens showed significant antibacterial activity against gram positive bacteria at dilutions of 1/10, whereas no effect was observed against gram negative bacteria. In comparison, the MeOH extract showed the most potent effect on B. subtilis. A. Repens, with potential activity on S. aureus and B. subtilis can be considered as a prominent source for obtaining new natural antibiotics. So the isolation of the active compounds and understanding the mechanism of inhibition would be of interest. Acknowledgement: This research has been supported financially by Student’s Scientific Research Center, Tehran University of Medical Sciences grant number: 7132 – 61 – 02 – 87. References: 1. Maddox DM, Mayfield A, Poritz NH (1985) Weed Sci 33: 315 – 327.

Curcumin As Anti-Oxidative Stress In Iron Toxicity
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Iron is an important element for normal cellular physiology, but an excess might induce the formation of oxygen free radicals (1). Thus, in the present study the oxidative stress induced by chronic iron intoxication was investigated by analysis of some enzymatic and non-enzymatic antioxidants: superoxide dismutase (SOD), catalase (CAT), ceruloplasmin (Cp), glutathione (GSH) and ascorbic acid (AsA) in liver and spleen homogenates of treated and control rats. The average values of TBARS, NO and OH radicals were significantly (p = 0.001) elevated in iron-overloaded rat groups compared with the corresponding control group. The average amount of iron was significantly (p < 0.001) elevated whereas that of copper was significantly (p < 0.001) reduced in iron-overloaded rat groups. Inversely, the administration of curcumin as iron-chelating agent with SOD- and CAT-like activities before setting the iron overload improved the above biochemical parameters (2). The administration of curcumin as iron-chelating therapy ameliorated the oxidative stress of excess iron either by decreasing iron level or by scavenging reactive oxygen intermediates, which could be clinically useful. References: 1. Luccisoli F, Caliguri M, Roberti MF, Perazzo JC, Fraga CG (1999) Arch Biochem Biophys 372(1): 37 – 43. Z. Abou-Seif M, Badria F, and Houssein W (2004) Arab J Lab Med 30(2): 191 – 206.

Screening methods to determine potential bioactivity of endophytic fungi from Vitis vinifera L. plant Vetschera KM, Zahradnik C
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Fungal endophytes are widespread in plants and colonize living internal tissue of their hosts symptomlessly [1]. They are well known for their beneficial effects for their hosts, providing increased tolerance against abiatic and biotic stresses, enhancing inter alia resistance to insect pests and fungal or microbial infection. This is largely due to their production of bioactive secondary metabolites [2]. Recently, Zingiberaceae species were studied and several bioassays successfully developed to test for antifungal activities [3]. These tests were now applied to the analysis of Vitis plants and their endophytic fungi, including routinely: a) competition tests against Cladosporium sphaeromosum, to investigate the dominance of the endophyte compared to its competitor in vitro [4]; b) thin layer-bio-autography with the crude endophytic extract and subsequent determination of inhibition halos, caused by separated compounds after spraying with conidiospores of C. sphaeromosum; c) establishing species-specificity by cultivation of the endophytic fungus on media containing the crude extract of the respective plant; d) performing a modified ELISA-test with the fungal crude extract to determine the median effective concentration (EC50) for inhibition of the growth of C. sphaeromosum. Several fungal endophytes have so far been isolated from Vitis vinifera L. cultivars, and respective results will be presented. The methods described provide tools not only for testing for antifungal activities, but also for subsequent isolation of bioactive compounds, and eventually for their practical applications in pest control. Acknowledgement: The financial support of “Society for the Advancement of Plant Sciences” is gratefully acknowledged. References: 1. Petrine O (1991) Microbial Ecology of Leaves, Springer Verlag, New York. 2. Gao F et al. (2010) Afr J Microbiol Res 4(13): 1346 – 1351. 3. Zahradnik C (2010) Pilzendophyten aus Alpinia malaccensis und Curcuma sp.: Kultur, Se-kundärstoffprofil und Bioaktivität. Diploma thesis, Univ. of Vienna. 4. Yuen TK (1999) Micro Ecol 37: 257 – 262.
PL50

Quantitative Determination of Gallic acid and Cyanidin-3-O-Glucoside within Sumac Extracts by HPLC-MS/MS
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Rhus coriaria L., commonly known as sumac (also spelled sumach), grows wild in the region extending from the Canary Island over the Mediterranean coastline to Iran and Afghanistan. It is native to the Mediterranean and southeast Anatolian region of Turkey [1]. The fruits are red colored and contain one seed. It’s dried and ground leaves have been used as a tanning agent due to their high tannin content. Previous phytochemical studies of this plant reported that it contained flavones, tannins, anthocyanins, and organic acids [2]. In this study gallic acid and cyanidin-3-O-glucoside contents of Water and MeOH 3/70 extracts of sumac were investigated using with HPLC ESI/MSMS MRM method. The assay performed in different concentrations of gallic acid and cyanidin-3-O-glucoside chloride as standard solutions. The diagnostic fragmentations of gallic acid and cyanidin-3-O-glucoside were used 168.7/125 – 79 and 448.7/287 – 150 respectively for MRM quantitative determination. Cyanidin-3-O-glucoside contents of 100 g each of aq. Methanol and water extracts of sumac were found as 0.007 ± 0.001 g and 0.566 ± 0.005 g in 100 g each of aq. Methanol and aqueous extracts, resp. References: 1. Dogan M and Akgul A (2005) J. Med. Food 8: 154–163; 2. Jahaniani F et al. (2005) Phytochemistry 66: 1581 – 1592.

PL52

Screening of Dracocephalum kotschyi accession for surface flavonoids and rosmaryc acid
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Dracocephalum kotschyi Boiss, of the Labiatae family, is an endemic and native herbaceous plant of Iran, where it is known as Badrandjboie-Denniae and Zarrin-Gah[r]. It has been traditionally used as a folk medicine and an additive to improve the taste and scent of tea and yogurt. D. kotschyi is an important source of essential oils [2] and flavonoids such as xanthomicrol[3], calycopterin and cirsimaritin with antioxidant properties. In this study we collected plant samples in 13 natural habitats to locate valuable accessions for domestication and breeding. Methanolic (80%) extract of leaves was used to quantify xanthomicrol, cirsimaritin, calycopterin, apigenin and rosmaryc acid, which were also identified and quantified by ESI-MS and HPLC-DAD methods. Plants collected from the central regions of Iran showed the highest levels of methylated flavonoids whereas the plants from the north of the country contained the most rosmaryc acid. Taken as a whole, our results show that flavonoid contents in D. kotschyi depend on environmental conditions; a semi-arid climate and high exposure of the plants to UV radiation leads to an accumulation of methoxy derivatives, while wet and cold conditions increase rosmaryc acid accumulation. References: 1. Mozaffarian V. (1998) A Dictionary of Iranian Plant Names. Farhang Moaser Publication. Tehran. 2. Fattahi M et al. (2010) Planta Med 76: 1271. 3. Jahaniani F et al. (2005) Phytochemistry 66: 1581 – 1592.

PL51

Free Radical Scavenging Activities of Flavonoids from Cistus salviifolius L.
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The genus Cistus (Cistaceae) is represented by 21 species on worldwide and 5 species in Turkish flora. The Cistus genus has widespread utilization in Turkish folk medicine such as against rheumatism, for hemorrhoids, to cure sterility, kidney and urinary inflammations, as a hemostatic, antipyretic, expectorant, sedative, and for peptic ulcer, as well as diabetes mellitus. Anti-inflammatory, anti-helicobacter pylori, anti-inflammatory, anti-microbial activities and cytotoxic effects of Cistus species were also reported. In this study, pharmacognostical investigations have been carried out on Cistus salviifolius L. The aerial parts of the plant were extracted with n-hexane and MeOH respectively. The methanolic extract was partitioned with petroleum ether and n-BuOH. Isolation of secondary metabolites from n-BuOH extract were performed with various chromatographic methods. The n-BuOH extract yielded three flavonoid aglycones; CS-1: Kaempferol, CS-2: Quercetin, CS-3: Myricetin, four flavonoid glycosides; CS-4: Kaempferol 3-O-(6''-O-trans-p-coumaroyl)-galactopyranoside, CS-5: Quercetin 3-O-a-arabinopyranoside, CS-6: Quercetin 3-O-b-galactopyranoside and CS-7: Myricetin 3-O-b-galactopyranoside. The structures of these compounds were elucidated using spectroscopic methods (UV, IR, ¹H-NMR, ¹³C-NMR, 2D-NMR and MS). The free radical scavenging activity of these compounds was measured by DPPH method and found as Quercetin (IC₅₀: 4.23) > Kaempferol (IC₅₀: 6.24) > Quercetin 3-O-b-galactopyranoside (IC₅₀: 8.26) > Myricetin (IC₅₀: 9.76) > Myricetin 3-O-b-galactopyranoside (IC₅₀: 10.05), respectively. According to these results quercetin is more active than ascorbic acid and has similar activity with the caffeic acid used as standards.

PL53

Anti-obesity effects of Geranium thunbergii extract via improvement of lipid metabolism in high-fat diet-induced obese mice
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Geranium thunbergii Siebold & Zucc. (Geraniaceae) is a traditional herb with anti-diarrheal, anti-inflammatory, and anti-oxidative effects. This study investigated the anti-obesity properties of an extract of Geranium thunbergii (GTE) in high-fat diet-induced obese mice. GTE treatment significantly reduced body weight, adipose tissue mass, adipocyte size, and serum triglyceride, total cholesterol, and low density lipoprotein-cholesterol levels in obese mice relative to the high-fat diet-fed mice. It also decreased serum leptin levels and increased adiponectin levels. The serum levels of aspartate transaminase, alanine transaminase, blood urea nitrogen, and creatinine were not significantly changed in GTE-treated mice compared to their levels in normal diet and high-fat diet-fed mice. Furthermore, GTE suppressed the mRNA levels of sterol regulatory-element-binding protein 1c, peroxisome proliferator-activated receptor γ, adipocyte fatty acid-binding protein, and fatty acid synthase in the adipose tissues of obese mice. These results suggest that GTE ameliorated high-fat diet-induced obesity by altering adipokines levels, and downregulating the expression of transcription factors and lipogenic enzymes involved in lipid metabolism.
Antimicrobial, antioxidant and phytochemical investigations of sea buckthorn (Hippophae rhamnoides L.) organs

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Hippophae rhamnoides L. (Elaeagnaceae), commonly known as sea buckthorn, is a thorny bush with orange berries naturally distributed in Asia and Europe. Fruits of H. rhamnoides have been used by Chinese, Mongolian and Tibetan medicines for decades, and possessed considerable medicinal and nutritional values like antimicrobial, antitumoral, antioxidant and dermological effects [1,2]. However the therapeutic potential and phytochemical diversity of the other H. rhamnoides parts remain unexplored. In this work we present the phytochemical and bio-activities screening of seed, leaf, stem and root of H. rhamnoides. The crude extracts were obtained by Pressurised Liquid Extraction (PLE) using ethanol. Each extract was then partitioned by liquid-liquid extraction using three solvents of different polarities: aqueous, ethyl acetate and ethanol. From the ethyl acetate soluble fraction of the Phyllanthus atropurpureus Bojer cultivated in Egypt, six compounds were isolated and identified based on spectral data (IR, UV, Mass (FAB, EI), 1H-NMR and 13C-NMR). Four compounds are vobasinyl-iboga bisindole alkaloids as active constituents. The structures were established from 1D and 2D NMR spectra at low temperature. A hypothesis of biogenesis is proposed. The antiparasitic on Plasmodium falciparum, Trypanosoma brucei brucei and Leishmania donovani and cytotoxic activities were evaluated on all obtained molecules. The selectivity towards parasites was determined. The attempt for the access to the target of active molecules on Plasmodium falciparum is discussed.

PL55

New vobasinyl-iboga bisindole alkaloids with antiparasitic activities from Montafara sessilifolia
girard de l'OrlÉans-

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Muntafara sessilifolia (Baker) Pichon or Tabernaemontana sessilifolia is an endemic plant of Madagascar which belongs to the Apocynaceae family.
The tubers of Corydalis cava Schwigg. & Kort. were extracted with ethanol and the summary alkaloid extract was fractionated in silica gel chromatography column using step gradient elution with hexane, chloroform and ethanol. Repeated column chromatography, preparative TLC and crystallization led to the isolation of fifteen isosuloline alkaloids. The chemical structures of isolated compounds were determined on the basis of spectroscopic techniques and by comparison of literature data. Isolated alkaloids were tested on ability to inhibit human erythrocyte acetylcholinesterase, serum butyrylcholinesterase (IC50) and for its free-radical scavenging activities (EC50). Cholinesterase inhibitory activities were determined in vitro by modified spectrophotometric Ellman’s method [1]. (+)-canadaline inhibited acetylcholinesterase as well as butyrylcholinesterase in a dose-dependent manner with IC50 values 20.1±1.1 μM and 85.2±3.2 μM, respectively. (+)-canadine with an IC50 value 12.4±0.9 μM was the most potent inhibitor of acetylcholinesterase, whereas (+)-(p)-corydalinine and (+)-(p)-bulbocapnine were effective inhibitors of butyrylcholinesterase with IC50 values 46.2±2.4 μM and 67.0±2.1 μM. Other isolated alkaloids were considered inactive (IC50 >100 μM). Free-radical scavenging activities of isolated alkaloids were tested in vitro by means of the DPPH test [2]. The highest activities exhibited (-)-scoulerine, (-)-sinocatine and (+)-bulbocapnine with EC50 values 102±6.2 μM, 209±8.1 μM and 279±16.7 μM, respectively. Other isolated alkaloids were considered inactive (EC50 >1000 μM). Acknowledgement: The study was supported by grants of GA UK No. 122309 and SVV-2010 – 263 – 002. References: 1. Eillman L, Courtney D, Andres A, Featherstone R (1961) Biochim Pharm 7: 88 – 95. 2. Polšček M, Skála P, Opletal L, Jahodár L (2004) Anal Bioanal Chem 379: 754 – 758.

Biodiversity of high mountain flora as a source of new medicines – Dinaric Alps (W. Balkan) Redzic S Dep.of Botany, Fac. of Science University of Sarajevo

The biodiversity of high mountain flora is very rich [1]. It still pharmacologically poorly investigated. This is especially true in areas that are rich in endemic species like this Dinarides (Western Balkans). This is an important resource in getting new drugs [2]. The aim is to make identification of potentially endemic medicinal plants and their biochemical background. In order to achieve objective of the research methodology was applied: field research on different profiles, including ethnobotanical interviews, followed at the end by comparative taxonomic-biochemical method. In the mountainous zone of the western Balkans was found 2500 species [3]. Very small number used in the official pharmacy and medicine. As potentially are 1500 species of medicinal plants. On the basis of their taxonomic similarity is expected and biochemical similarity, the pharmacological activity, as well. As a real or potential sources of alkaloids are the species of the genera: Onosma, Moltkaea, Colchicum, Senecio, Cynanchum, Astragalus, Oxytropis, Vicia, Papaver, Euphorbia, Edraianthus, Campanula; heterodiers are species of genera: Arctous, Ferulago, Atamantha, Pancica, Bupleurum, Seseli, Genista, Gentianella, Gentiana, Frangula, Rhamnus; saponosides are: Verbacsum, Scrophularia, Primula, Solidanella, Dianthus, Silene, Arenaria, Minuartia, Knautia, Scabiosa, Viol, etc.; tannins are: Geum, Potentilla, Sibirea, Cra- teagus, Dryas, Saxifraga, Geranium, Asplenium, etc.; terpenoids are species of genera: Centaurea, Hieracium, Hypochaeris, Aphricarpos, Petasites, Homogyne, Stachys, Satureja, Scutellaria, Euphrasia, Pedicularis, Veronica, Iris, Pinus, etc.; carbohydrates are: Orchis, Gymnadenia, Dactylorhiza, etc. and lipids are species of genus Linum. As a precondition for its use, we planned to conduct basic botanical and pharmacognostic research. The material was sampled 2007 at the mountains of Sarajevo (800 to 1500 m). All studies were carried out in accordance with the European Pharmacopoeia IV monograph. The leaves have trans-servital histological structure. Stomata are anomocytic. Type of stoma is anomocytic. Root has the primary and later secondary structure. Use of spectrophotometric analysis and paper chromatography fortified the ratio between chlorophyll a and b 2:1. Chlorophyll a is 0.6 mg/g; chlorophyll b is 0.383 mg/g and carotenoids 0.285 mg/g. The proportion of plant pigments indicates the high antioxidant activity of this plant. The roots of this species has been prepared for chemical analyses. For the separation of metabolites was used thin layer chromatography method. Standard analysis showed the presence of sucrose and aramargin. The method of micro-sublimation proved the presence of genistin. Preliminary and basic results suggest that the roots and aerial parts of Gentiana cruciata could be a useful replacement for the very popular and highly endangered species Gentiana lutea. Gentiana cruciata L. (Gentianaceae) is a widely distributed species in the area of Dinarides. [1]. It is used in traditional medicine in some mountainous areas of Bosnia [2]. As related species of Gentiana lutea is endangered, [3] similar uses of the other species of Gentiana in modern phytotherapy are investigated. Gentiana cruciata has such capabilities. As a precondition for its use, we planned to conduct basic botanical and pharmacognostic research. The material was sampled 2007 at the mountains of Sarajevo (800 to 1500 m). All studies were carried out in accordance with the European Pharmacopoeia IV monograph. The leaves have trans-servital histological structure. Stomata are anomocytic. Type of stoma is anomocytic. Root has the primary and lower secondary structure. Use of spectrophotometric analysis and paper chromatography fortified the ratio between chlorophyll a and b 2:1. Chlorophyll a is 0.6 mg/g; chlorophyll b is 0.383 mg/g and carotenoids 0.285 mg/g. The proportion of plant pigments indicates the high antioxidant activity of this plant. The roots of this species has been prepared for chemical analyses. For the separation of metabolites was used thin layer chromatography method. Standard analysis showed the presence of sucrose and aramargin. The method of micro-sublimation proved the presence of genistin. Preliminary and basic results suggest that the roots and aerial parts of Gentiana cruciata could be a useful replacement for the very popular and highly endangered species Gentiana lutea. Gentiana cruciata L. subsp. symphandra (Murb.) Hayek. References: 1. Redzic S (2006) Proc. 1st IFOM Intern. Conf. Organic Wild Production 117 – 141. 2. Redzic SS (2007) Coll Antropol 31: 869 – 890. 3. Redzic S et al. (2009) Planta Med 75: 902 – 902.
Elemental compositions of Echinacea purpurea, E. pallida radix and herb cultivated in Turkey

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Echinacea sp. (Asteraceae) are one of the most popular medicinal plants used in phytotherapy. In this present study, E. purpurea (L.) Moench and E. pallida (Nutt.) Nutt. were successfully cultivated under controlled conditions in experimental fields in Konya. Dried aerial parts and the roots of 36 cultivation samples were investigated for their macro (N, P, K) and micro (Ca, Mg, Na, Fe, Mn, Zn, Cu) trace elemental compositions using various techniques. N was determined by the dry combustion method using elemental analyses, P was measured by a colorimetric method, whereas K and N by flame photometry. Finally Ca, Mg, Fe, Cu, Zn and Mn was detected quantitatively for (AA) and (ICP-AES). All experiments were performed qualitatively and quantitatively with statistical data comparison to a certified reference plant material, respectively. The total dry matter content, including those of the areal parts of the crops ranged 25 – 30%. The results of elemental analyses showed that N ranged 0.54 – 1.69%, P ranged 1100 – 2600 ppm and K ranged 9990 – 29585 ppm. To the best of our knowledge, this is the first report on micro and macro elements of cultivated Turkish Echinacea sp. As a conclusion, the elemental composition and the nutritional value E. purpurea and E. pallida are worthwhile to investigate with comparison to other Echinacea sp. used medicinally.

Bioassay-guided fractionation and cytotoxic activity of flavonoids from Echinochloa crus-galli L. (Barnyard Grass)

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Echinochloa crus-galli L. (Family Poaceae) is a problematic summer weed found in rice fields and moistened soil worldwide and known as Barnyard Grass [1]. Bioassay-guided fractionation of the seeds of Echinochloa crus-galli L. isolated to isolation of two cytotoxic flavonoids after screening against four human cancer cell lines: MCF-7 (breast cells), HCT-116 (colon cells), HELA (cervical cells) and HEPG-2 (liver cells) using the sulfur-hodamine B (SBH) colorimetric assay [2]. Different extracts showed a dose dependent inhibition in a range of 5 – 50 μg/ml. The ethanolic extract (95%) proved to be the most active extract against HELA cell line (IC50 = 12 μg/ml). On the other hand, the hexane and chloroform fractions exhibited moderate activities against HEPG-2 (IC50 = 15 μg/ml) and HCT-116 (IC50 = 171 μg/ml) cell lines, respectively. Two flavonoids were isolated from the chloroform fraction and identified as 5,7-dihydroxy-3’,4’,5’-trimethoxy flavone (1) and quercetin (2) [3]. Compound 1 exhibited potent cytotoxic activities against HELA cell line (IC50 = 4.5 μg/ml) and HEPG-2 cell line (IC50 = 4.5 μg/ml), which were comparable to doxorubicin (IC50 = 4.3 μg/ml). Compound 2 showed moderate cytotoxic effects against MCF-7, HCT-116, HELA and HEPG-2 cell lines with IC50 values of 12.7, 20.4, 13.9 and 11.3 μg/ml, respectively. Acknowledgement: The authors are grateful to Prof. Dr. Osama El Koptay, Professor of Botany, Faculty of Agriculture, Cairo University for authentication of the plant and Prof. Dr. Samia Shouman, Professor of Clinical Biochemistry, National Cancer Institute, Cairo, for her help in carrying out the cytotoxic assays. References: 1. Apfelbaum S, Sams C (1987) Natural Journal 7: 68 – 74. 2. Skelan P, Stroeng R (1990) N G Natl Cancer Inst 82: 1107 – 1112. 3. Mabry TJ, Markham KR, Thomas MB (1996) The Systematic Identification of Flavonoids, 2nd ed., Springer-Verlag, Berlin.

Plant pigments in some medical plants of family Lamiaceae (Bosnia and Herzegovina, W. Balkans)

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Plant pigments chlorophyll and carotenoids are very important group of primary and secondary metabolites, resp. Besides their role in process of photosynthesis and plant protection from extensive radiation, they have huge appliance in pharmaceutical industry, cosmetology and dietetic. Plant pigments are also given significant role in anti-oxidant activity [1, 2]. Goal of these studies has been qualitative – quantitative analysis of main and side pigments in selected medicinal species of wild flora in BiH, including endemic species. Plant materials were gathered during different seasons. They were transported fresh to the laboratory where qualitative (paper and thin layer chromatography) and quantitative (spectrophotometric) analyses took place. Results (Table 1) showed significant presence of chlorophyll a, chlorophyll b and carotenoids. Ratio between chlorophyll a and chlorophyll b was rarely 3:1, as stated in classical literature but rather close to 3:2 and more, which makes these species even more medicinal and gives them higher potential for anti-oxidant capacity in [3].

Table 1: Contents of plant pigments in selected plants of Lamiaceae family

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Locality (100 – 2,000 m altitude)</th>
<th>Chlorophyll a (mg in g fresh leaves)</th>
<th>Chlorophyll b (mg in g fresh leaves)</th>
<th>Carotenoids (mg in g fresh leaves)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Origanum vulgare L.</td>
<td>Igman Mt.</td>
<td>4,592</td>
<td>4,114</td>
<td>1,619</td>
</tr>
<tr>
<td>Origanum heracleeticum L.</td>
<td>Mostar</td>
<td>5,210</td>
<td>4,252</td>
<td>1,731</td>
</tr>
<tr>
<td>Satureja subspicata Bartl. ex Vis.</td>
<td>Velez</td>
<td>4,750</td>
<td>3,870</td>
<td>1,670</td>
</tr>
<tr>
<td>Satureja montana L.</td>
<td>BH coast</td>
<td>6,452</td>
<td>4,445</td>
<td>1,756</td>
</tr>
<tr>
<td>Microtoma thyrsiflora (Koehne) Fisch.</td>
<td>Sarajevo</td>
<td>5,780</td>
<td>2,100</td>
<td>0,890</td>
</tr>
<tr>
<td>Thymus aureopectus (Bieb) K.Maly</td>
<td>Konjic</td>
<td>5,723</td>
<td>1,124</td>
<td>0,679</td>
</tr>
<tr>
<td>Thymus bulbosus Borbas</td>
<td>Bjelavica Mt.</td>
<td>4,678</td>
<td>1,670</td>
<td>1,620</td>
</tr>
<tr>
<td>Thymus brascoceus V. ex Beth</td>
<td>Trebinje</td>
<td>5,120</td>
<td>2,020</td>
<td>1,230</td>
</tr>
<tr>
<td>Acinos ocrantus (K. Malo) Silik</td>
<td>Konjic</td>
<td>4,345</td>
<td>1,970</td>
<td>0,890</td>
</tr>
<tr>
<td>Nepeta pannonica L.</td>
<td>Visoko</td>
<td>5,670</td>
<td>2,230</td>
<td>1,120</td>
</tr>
<tr>
<td>Salvia officinalis L.</td>
<td>Jajshiki</td>
<td>5,100</td>
<td>1,800</td>
<td>1,234</td>
</tr>
</tbody>
</table>


Protective effects of Sedum caespitosum and its polyphenolic compounds against to H2O2 induced cytotoxicity

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There are 33 Sedum species growing in Turkey and some Sedum species have been employed in folk medicine for their anti-inflammatory, wound healing, and diuretic properties as well as treatment of chronic viral hepatitis. The crude MeOH extract prepared from the aerial parts of Sedum caespitosum (Carv.) DC. and its EtOAc n-BuOH, H2O subextracts were screened for their protective effect against to H2O2 induced cytotoxicity at different concentrations in human red blood cell. The EtOAc subextract were found to be the most protective one and its chemical composition was further analysed. Five polyphenolic secondary metabolites including gallic acid (1), kaempferol 3-o-rhamnopyranoside (2), quercetin 3-O-β-glucopyranoside (3), quercetin 3-O-α-rhamnopyranoside (4), myricetin 3-O-α-rhamnopyranoside (5) were isolated from the EtOAc extract by successive chromatographic methods. The structures of the isolated compounds were elucidated by 1D- and 2D-NMR techniques. This is the first phytochemical work on S. caespitosum. The protective effect of the isolates against to H2O2 induced cytotoxicity in human red blood cells also evaluated at 5 and 10μg/ml and kaempferol 3-o-rhamnopyranoside was shown most protective effect. Acknowledge-
Fagopyrin and its derivatives in buckwheat (Fagopyrum sp.)

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Fagopyrin and its derivatives are dimerized anthraquinone polyphenolic substances from buckwheat. They act as photosensitizers upon excitation with visible light (540–610 nm), which causes phototoxic effect after ingestion of large amounts of buckwheat called fagopyrism (1). According to known structures and transformations of hypericin derivatives from St. John’s wart and their similarity to fagopyrin, several forms of fagopyrin derivatives have been postulated as pseudofagopyrin and protofagopyrin (2). It was shown that pre-forms of fagopyrin transform to fagopyrin in plant extract under daylight exposure (3). We optimized the extraction method of fagopyrin from plant material. We developed a HPLC method coupled with fluorescence detector (excitation wavelength 330 nm, emission 590 nm) for separation and detection of those compounds. The HPLC method yielded several chromatogram peaks with close retention times presenting different forms of fagopyrin. Various buckwheat products available on the market were analyzed using this method. The highest amount of fagopyrins was found in buckwheat herb samples and less in fruit samples, which contain most of the compounds in peels. Buckwheat herb was used for further observation of the nature of fagopyrins in plant extract. As described previously (4), we observed that the content of different forms of fagopyrin varied due to different extraction conditions (time, temperature, solvent). Since the differences were observed even if the conditions changed after the end of the extraction (removal of the herbal substance), we assume that this changes were due to transformations of fagopyrins and not only due to extraction efficacy. The transformations were at least partly reversible. References: 1. Chick H, Ellinger P (1941) Physiol 212 – 230. 2. Brockmann H, Lackner H (1979) Tetrahedron Letters 18: 1575 – 1578. 3. Habermann B (2000) Arch Farm, Farm Med Chem 333, Suppl, 2. 4. Hinnebusch, Neubert Reinhard HH (2005) Agric Food Chem 53: 3 – 7.

Seasonal variation of lipophilic constituents in roots of Echinacea purpurea and E. pallida

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Echinacea purpurea (L.) Moench and E. pallida (Nutt.) Nutt. are widely used for the unspecific enhancement of the immune system. The plants originate from North America and are grown all over the world as garden flowers or as medicinal plants. Lipophilic constituents such as alkaloids and ketokalenes/ketoalynes are believed to be among the active metabolites in E. purpurea and E. pallida, respectively, with the highest concentrations being found in the roots. Most investigations on roots have been conducted on plants younger than one or two seasons and few have investigated the seasonal variation of lipophilic constituents in roots of Echinacea species. From early winter 2009 to fall 2010 five 4 – 5 year old E. pallida and seven 3 – 4 year old E. purpurea roots from the same population of plants were collected throughout one year (i.e. before and after soil freeze in winter, mid spring, at high soil temperature in the summer and mid fall). Lipophilic constituents were extracted from milled freeze dried roots with EtOH-H2O (70:30) and analyzed by HPLC-PDA and LC-MS/MS. The highest concentration of alkaloids in E. purpurea roots was found when soil temperature was just above 0°C after winter and during summer, when the soil temperature was high. In the first case dodeca-2E,4Z-diene-8,10-diylnic acid isobutilamide was the major alkalide and in the latter case dodeca-2E,4,8Z,10E/Z-tetraenoic acid isobutilamides were the major constituents. For E. pallida roots the highest concentration of 2-ketokalenes and -alkynes were found when the soil temperature was just above 0°C after winter and here the major constituent was pentadeca-8Z,13Z-dien-11-yn-2-one.


Study of content and composition of anthocyanins in selected plants species

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Anthocyanins are heteroglycosides composed of aglycone – anthocyanide and sugar moiety. They are the final product of flavonoid production in secondary metabolism of plant cells. They are characteristic with antioxidant effects, through which they have positive effect on human organism. There is a large number of anthocyanids, out of which only six are of the greatest importance, those with hydroxylic group at C-3 location. They are cyanidine, pelargonidine,peonidin, delphinidine, petunidine, malvidin. These are present in large amounts in plant species Vitis vinifera L., Vaccinium corymbosum L. and Sambucus nigra L. In the berries of Vitis vinifera they are accumulated in hypodermal cell layer of peel, or in some cultivars. Except for pelargonidine anthocyanins contain all important anthocyanidines, with predomination of malvidine. Total content of anthocyanins in fresh berries ranged from 0,50 to 4,99 g.kg-1 and in peels from 20,7 to 66,6 mg.g-1 of peels dry matter. In the anthocyanins of Vaccinium corymbosum there were identified cyanidin, delphinidin, malvidin and peonidin. Their total content varies significantly depending on variety. Total determined content of anthocyanins ranged from 290,16 to 1343,08 mg.dm-3. Sambucus nigra contains five important anthocyanidines: cyanidine 3-sambubiosid-5-glucoside, cyanidine 3-5-digluco side, cyanidine 3-sambubioside, cyanidine 3-glucoside and cyanidine 3-rutinoside. The content of identified anthocyanins in fruits of this species ranges from 602,9 to 1265,3 mg.100 g -1. The amount of accumulated anthocyanins pigments depends on variety, ecological conditions standard of agricultural technology, and particularly on the temperature and solar radiation. Acknowledgement: The participation is supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic, the project: 00162 – 0001 (MS SR-3634/ 2010 – 11).
PL69

Genetic and Environmental Variations in Bacilain, Bacilein and Wogonin Contents in Scutellaria baicalensis
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Scutellaria baicalensis has long been used to treat fever, cough, diarrhoea and infection in Chinese medicine, and flavonoids such as baicalin (BA), baicalein (BE), and wogonin (WO) have been identified. To understand genetic and environment-dependent variations, 15 Scutellaria baicalensis landraces were collected from Korea and China, and cultivated under different nitrogen fertilizer application and planting density conditions, and resultant changes in BA, BE, and WO were evaluated. Tested 15 landraces exhibited BA, BE, and WO contents ranging 4.56 to 13.59%, 0.28 to 5.54%, and 0.50 to 1.63% with an average of 9.66%, 2.09%, and 0.52%, respectively. Among tested 4 levels (0 to 500 kg/ha) of nitrogen fertilizer application 300 kg/ha resulted in highest BA (10.3%) and BE (1.3%), as well as WO (0.4%) contents, corresponding to about 10% increase compared to 0 kg/ha. Six different levels of planting density (2 and 3 Rows x 1, 5, 10 cm distance), however, showed no difference in flavonoid contents. Similarly no difference in flavonoid contents could be observed between Scutellaria baicalensis harvested from early June 6th to Aug. 3rd. When flavonoid contents in different plant parts were compared, leaf and stem of Scutellaria baicalensis showed no BE and WO under our experimental conditions, while relatively low BA (0.67% in leaf and 1.56% in stem) could be found in leaf. Higher composition of BA could be observed in top part of the root, while higher WO could be observed in lower root part close to root hair. References: 1. Cole et al. (2008) Planta Med 74(4): 474 – 481. 2. Rhee J, Park H (1997) Analytical Science & Technology 10: 91 – 104.

PL70

Antioxidant activity of Jasminum malabaricum – A medicinal plant from Western Ghats
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Jasminum malabaricum Wight belonging to the family Oleaceae is endemic to Western Ghats of India. It is a climber with white flowers and fragrance, which is known for its ethnomedical importance as blood purifier and anti-tumor properties. The extensive exploitation of this species has led to reduction of its natural population. In the present study the leaves and stems were subjected for continuous shaking extraction (CSE) and microwave assisted extraction (MAE) using methanol. The antioxidant studies were performed with various concentrations of methanolic extract by DPPH and FRAP radical scavenging assay. The leaf extract showed significant activity (i.e. 92.86 ± 0.08% for CSE and 80.21 ± 0.01% for MAE) for DPPH method and for FRAP method the results showed significant activity (i.e. 4241.40 ± 212.07 for CSE and 80.21 ± 0.00% for MAE) at concentration 0.2% which was considered with the standard ascorbic acid. References: 1. Mann HH (2008) J. Linnean Soc (London) Botany 45:302: 155 – 8. 2. Thangavelu NR, Thomas S (2010) Int J Biol Med Res 1(4): 188 – 192.

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Antioxidant potential of Brazilian plants
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Oxidative stress, the imbalance between free-radical formation and elimination, is part of the pathophysiology of many diseases. Exogenous antioxidants, such as those from plants, can help to restore the normal redox state of tissues. The aim of this study was to evaluate the antioxidant potential of plant species found in the Brazilian cerrado. The ethanolic extracts of different anatomical parts of 22 plant species (13 botanic families) were evaluated in vitro using two distinct approaches: the DPPH free-radical scavenging method (determination of EC50) and the β-carotene bleaching test (125; 62.5 ± 31.25 µg/mL), both performed in microplates and with pyrogallol and quercetin as antioxidant standards. In this study, the sample was considered active when showed EC50 < 30 µg/mL in the DPPH method and inhibition of 50% of β-carotene oxidation at 31.25 µg/mL. All the 46 extracts evaluated showed concentration-dependent responses and 19 of them were considered active. Genetic showed the best antioxidant profile: Qualea grandiflora Mart. (Volchysiaeaceae), with EC50 of 4.62 ± 0.09 µg/mL (leaves) and 4.94 ± 0.34 µg/mL (barks) on the DPPH method and 71.6 ± 6.1% (leaves) and 74.7 ± 2.38% (barks) inhibition of β-carotene oxidation at 31.25 µg/mL; and Laphsenia pascual A.St.-Hill. (Lythraceae) with DPPH EC50 of 4.68 ± 0.07 µg/mL (leaves) and 4.62 ± 0.63 µg/mL (barks) and 62.4 ± 2.9% (leaves) and 64.5 ± 3.8% (barks) inhibition of β-carotene oxidation. These species will be further studied in vivo. In continuation of the search of extracts for use in the prevention and treatment of diseases in which oxidants or free radicals are implicated. Acknowledgement: Fapemig, for the financial support (FAPESPI/DEG-AC-43-10). Prof. Braga, F.C. and Prof. Castilho, R.O. for gently allowing the use of the laboratory facilities. References: 1. Valko A et al. (2007) Int J Biochem Cell B 39: 44 – 84. 2. Halliwell B. (2001) Free Radical Bio Med 46: 531 – 542.
Phenolic acids and free radical scavenging activity of Bulgarian endemic - Alchemilla jumrukczalica Pawl
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In the phytotherapy Alchemilla vulgaris L. complex is widely used as astringent, diuretic, anti-inflammatory agents, characterized by the presence of phenolic acids, flavonoids, tannins, triterpenes, etc. [1]. Alchemilla jumrukczalica Pawl. is rare plant, unstudied for chemical constituents and biological activity until now. The present study aims to establish the antiradical potential and phenol content of A. jumrukczalica (cultivated materials) and A. vulgaris complex. The antioxidant activity of the methanol extracts was evaluated by the scavenging effect on 1,1-diphenyl-2-picrylhydrazil radical (DPPH); radicals. The extracts showed significant antioxidant activity with IC50 Values of 12.05 and 19.62 µg/ml respectively for A. jumrukczalica and A. vulgaris complex. Commercial antioxidant butylated hydroxytoluene (BHT) and syringic acid were used as positive controls and their IC50 values are respectively 12.65 and 4.40 µg/ml. The methanol extracts of the studied samples were examined before and after acid hydrolysis for free and bounded phenolic acids. Ten free and seventeen bounded phenolic acids were identified and quantified by performed of gas chromatography mass-spectrometry (GC-MS). The extracts of the both samples contain phenolic acids in comparable amounts. Among the identified free phenolic acids gentisic, protocatechuic, caffeic and cinnamic acids are represented in the greatest amount. Salicylic, protocatechuic, caffeic, trans-cinnamic, gentisic and vanillic acids were the major bounded phenolic acids in the studied extracts. The present study revealed the extract of A. jumrukczalica as potential source of antioxidant activity. Acknowledgement: The authors are grateful to the National Science Fund, Ministry of Education, Youth and Science (Project DTK-02/623 – 629). 5 Nikoletti M et al. (1984) J Nat Prod 47: 953 – 957.

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In the present study, Achillea biebersteinii Anan. and Achillea teretifolia Willd. collected from Turkey were investigated for their in vivo angiogenic or antiangiogenic properties to correlate the folk medicine uses [1]. The essential oils were obtained from aerial parts by hydrodistillation, which were analyzed both by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS), simultaneously. The main constituents of the oils were identified as 1,8-cineole (36%, 34%) and camphor (22,11%), respectively. Using the in vivo chick Chorio Allantoic Membrane (CAM) assay the oils and their main constituents were tested at various concentrations (5 – 50 µg/pellet). As a result, A. teretifolia essential oil showed strong antiangiogenic effect with no irritation whereas, A. biebersteinii essential oil showed no antiangiogenic effect but slight irritation (10%) at 50 µg/pellet when compared with cortisone, suрамin, thalidomide and sodium dodecyl sulphate. 1,8-Cineole and camphor showed weak to strong antiangiogenic effect with no irritation at the same concentration in a scoring system [2]. Furthermore, none of the extracts showed any cytotoxicity confirming its safe use. Acknowledgement: The authors would like to thank TUBITAK – SBAG-107S262 (3756) for financial support of the project. References: 1. Baytop T (1999) Therapy with Medicinal Plants in Turkey 2nd Edition. Nobel Tıp, Istanbul, Turkey. 2. Wren RI (2010) Inhibition of angiogenesis and inflammation by an extract of red clover (Trifolium pratense L.). Phytotherapy Research 24(5): 567 – 572.
Assessment of antiradical activity of high-mountain medicinal plants in Bulgaria – Alchemilla achatarouwii, A. mollis, Gentiana lutea ssp. symphandra, Arnica montana

The present study protected, endemic and rare high-mountain medicinal plant in Bulgaria – Alchemilla achatarouwii Pawl., A. mollis (Buser) Rothm., Gentiana lutea L. ssp. symphandra (Murb.) Hayer, Arnica montana L. The species are widely used in modern phytotherapy, they are demand raw materials on national and international markets.

This requires their cultivation by conventional and biotechnological methods. The purpose of present study is to evaluate antiradical capacity and total phenols of which were grown in situ (A. achatarouwii, G. lutea), ex situ (A. mollis) and ex vitro (A. montana). The methanol extracts of examined species were estimated using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Folin-Ciocalteu assays. The extracts of aerial parts of A. mollis and A. achatarouwii showed significant antiradical activity with its IC50 values below 50 μg/ml. The lowest activity was found of extract of G. lutea > 200 μg/ml. The extracts of folia and flowers of ex vitro plants of A. montana revealed high radical scavenging activity too, their IC50 values are 64.01 and 85.73 μg/ml respectively. Commercial antioxidant butylated hydroxytoluene (BHT) was used as positive control and its IC50 value was 12.65 μg/ml. The antiradical properties of the studied extracts positively corresponded with total phenol content. The results obtained showed high antiradical qualities of the examined species. It is especially important that the ex situ and ex vitro grown plants kept its valuable properties. These results will be basis for future comparative analysis of antioxidant capacity and the content of active components of these species. Acknowledgement: The authors are grateful for the financial support provided by the Bulgarian National Science Fund, Ministry of Education, Youth and Science (Project DTK/02/38).

Determination of lycorine of Sternbergia candida by HPLC

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Sternbergia candida is one of the most widely used medicinal plant family [1]. Sternbergia candida Mathew & T. Baytop (Sc) is an endemic member of the family from Mugla, Turkey [2]. Lycorine is the major alkaloid of Sternbergia species. HPLC analysis of the bulbs of the species has been done previously [3]. In this study, it is aimed to determine the lycorine in chloroform and methanolic extracts of both bulbs (SCBC: SCBM) and leaves (SCLM) of Sc by HPLC. The analysis was performed with a shorter column (3 μm, C18 150 mm x 3 mm) in a gradient solvent system without acetonitrile (A: 97.5% 10 mM ammonium bicarbonate with 2.5% methanol; B: 2.5% 10 mM ammonium bicarbonate with 97.5% methanol pH: 7.8) with a flow rate of 0.3 ml/min. The results are given in Table 1. Lycorine was found in the leaves of the plant however it was higher in the methanolic extract of the bulbs.

PL80
Investigation of stability of Hypericum perforatum L. total extract due to temperature and humidity

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lanthus atropurpureus Bojer, revealed that its extracts is quite similar to silymarin. Both of them improve the parameters of CCl4-induced liver injury including serum AST and ALT. Among the extracts tested, root extract showed maximum activity as compared with aerial part extract relative to silymarin.

Response of germination and seedling growth of, hyssop (Hyssopus officinalis) and Marigold (Chrysanthemum x superbum) as medicinal plants to water stress
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In order to study the effects of five levels of water stress (0, -2, -4, -6 and -8 bar) on germination characteristics and seedling growth of two medicinal plants (Hyssopus officinalis L. and Chrysanthemum x superbum Bergmans ex L.) two experiments were conducted at physiology laboratory of Faculty of Agriculture Ferdowsi University of Mashhad as a Completely Randomized Design with four replications. The results showed that the effects of different levels of water stress were significant in all of the studied characteristics of two plants. Germination percentage was decreased and mean germination time were increased by increasing in water stress levels and, germination percentage was zero in levels of -6 bar in two types of plants. It is suggested that decrease in seed germination and depression in seedling growth under drought conditions related to limited hydrolysis of food reserves from storage tissues as well as de impaired translocation of food reserves from storage tissue to developing embryo axis [3]. Root length of Hyssop and Marguerite respectively, were increased and decreased by increasing in water stress levels. Plumule length had a decreasing trend in two studied plants, but amounts of this trend was less in hyssop and the root length/plumule length were increased in each of plants. Many researches were shown that an increased root/shoot ratio resulting in more efficient water and nutrient uptake [1,2]. Also, dry weight root had increased trend and dry weight plumule had decreasing trend but root dry weight/plumule dry weight was increased in two types of plants. References: 1- Fallahi J et al. (2008) Iranian J Environ Strt Agric Sei 1(1): 57 – 67. 2- Gorham J et al. (1999) Plant Soil 89: 15 – 40. 3- Misra N, Dwivedi UN (2004) Plant Sci 166: 1135 – 1142.

Omididun (corn liquor): an economic solution to xerostomia
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Xerostomia is dry mouth resulting from reduced or absent saliva flow associated with dehydration, use of drugs, various syndromes (Plummer-Vinson syndrome) and side effects of radiotherapy and chemotherapy in cancer treatment. It can affect nutrition and dental as well as psychological health. Omididun is the liquor obtained from fermented ground and sieved maize or sorghum. While the ground wet flour obtained is boiled into a semisolid cereal (Plum) for breakfast in Nigeria, Lactoperoxidase (LPO) presence was confirmed in five varieties of fermented corn [Zea mays Linn. (Poaceae) white and yellow varieties and Sorghum bicolor Linn. (Poaceae) white and red varieties] using the principle of LPO decomposition of hydrogen peroxide and the oxidation of colorless 1, 4-phenylenediamine into the purple indophenol. LPO was estimated with a reaction mixture of hydrogen peroxide and potassium iodide solutions, incubated at room temperature to achieve equilibrium and absorbance read at 350 nm in a UV spectrophotometer against a blank without omididun and procedure repeated for commercially available dry mouth wash and toothpaste. The colour intensity was proportional to the LPO’s concentration in the order of yellow corn> white corn> red sorghum> white sorghum. LPO content increases from 3.528±0.451 in white sorghum to 34.713±0.068 in yellow corn. It is proposed that omididun could be used as a mouth rinse or incorporated in tooth paste because of the natural LPO content to treat xerostomia and as well reduce oral bacteria and consequently the acid produced by those bacteria.

Fungal transformation of pimaradienoic acid and its schistosomicidal activity against Schistosoma mansoni
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In the present work, the microbial transformation of pimaradienoic acid (PA, 1) (Figure 1) was performed using submerged shaken liquid culture of Aspergillus ochraceus (1.8 x 106 spores/mL). The microorganism was grown by a two-stage fermentation procedure [1]. PA was added as a dimethylsulfoxide solution (0,1 g/L) and incubated for 3 days. The culture was filtered and the aqueous layer was extracted with ethyl acetate to furnish the extract codified as AoPA. Chemical and NMR studies of AoPA allowed us to isolate and to identify two PA derivatives (Figure 1: Compounds 2 and 3). The in vitro schistosomicidal activity of these metabolites was performed against male and female S. mansoni adult worms [2], and the results denote that PA is very effective with respect to the separation of coupled pairs, mortality, decrease in the motor activity and tegumental alterations. In addition, PA is able to reduce the percentage of eggs number and egg development. In this context, the schistosomicidal effects of PA indicate that ent-pimarane diterpenes could be considered a promising source for discovery of new agents to treat human schistosomiasis.

Figure 1: Chemical structures of PA (1) and its derivatives obtained through fermentation for 3 days with A. ochraceus.


Biotransformation of ent-8[14],15-pimaradiene and antimonial activity of the obtained derivatives against multi-resistant Gram-positive bacteria
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In the present work, the microbial transformation of ent-8[14],15-pimaradiene (Figure 1: 1, PI) was performed using submerged shaken liquid culture of Aspergillus ochraceus (1.8 x 106 spores/mL). The microorganism was grown by a two-stage fermentation procedure [1]. PI was added as a dimethylsulfoxide solution (0,1 g/L) and incubated for 7 days. The culture was filtered and the aqueous layer was extracted with ethyl acetate to furnish the extract codified as AoPi. Chemical and NMR studies of AoPi allowed us to isolate and to identify four PA derivatives (Figure 1: Compounds 2, 3, 4 and 5). The antimonial activity of these metabolites was evaluated against a panel of 14 multi-resistant Gram-positive bacteria. For this purpose, the broth microdilution method was applied and the minimal inhibitory concentration (MIC) values were determined [2]. Diterpene 2 displayed significant inhibitory effect on the growth of these pathogens, showing MIC values very promising [3].
Antioxidant capacity of Matricaria chamomilla L. extract and its effect on neural tube structure in diabetic rat offspring

Matricaria chamomilla L. extract may have a protective effect against diabetes-related embryopathy and it may be due to its antioxidant activity. Increased oxidant stress has been suggested to play a role in the pathogenesis of disturbed embryogenesis in diabetic pregnancies also cause several types of histopathologic changes in the placenta [1]. The present study was conducted to determine whether Matricaria chamomilla L. extract, a well-known medicinal herb with appropriate antioxidant activity, would reduce the incidence of diabetic embryopathy in the streptozotocin-induced diabetic rat model. Antioxidant capacity of extract was measured using DPPH method. Diabetic and control rats were administered 100, 300, 500, mg/kg chamomile extract. Mating condition was prepared by putting male rats and diabetic female rats together. All female rats were observed for the vaginal plug which was considered as a positive sign of pregnancy and treatment was given 100, 300, 500 mg/kg extract of Matricaria chamomilla. At 17th day, rats were scarified. The fetuses were isolated from different sacs and surrounding deciduas and were neural tube structure was examined by Light microscopy and electron microscopy. Results of the present analysis indicate that members of subg. Psychotria are promising sources of natural antioxidants due to the observed radical scavenging properties of their extracts. The antioxidant activity may be causal for the use of these species in folk medicine. The chemotaxonomic point of view, differentiation in the degree of antioxidant activity within Psychotria correlates well with current taxonomic views [3], confirming that species of subg. Heteropsychotria, devoid of strong activity, should be placed in a separate genus. References: 1. Berger A et al. (2010) In: Program and Abstracts, 19th International Symposium “Biodiversity and Evolutionary Biology”, German Botanical Society (DBG), Univ. Vienna, Austria, p. 76. 2. Sanz-Biset J, Campos-de-la-Cruz J, Epiquín-Rivera MA, Cañigueral S (2009)J Ethnopharmacol 122: 333 – 362. 3. Nepkroeff M, Bremer B, Systma KJ (1999) Syst Bot 24: 5 – 27.

Figure 1: Chemical structures of PI (1) and its derivatives obtained through fermentation for 7 days with A. ochraceus.

References:

Medicinal and aromatic plants in generating new values for the 21st century
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The population is growing exponentially. The needs for food and medicine is increasing. Regional and global poverty is increasing. A way to reduce the galloping growth of poverty is sustainable use of medicinal and aromatic plants (MAP). Especially in countries in transition [1,2]. The biggest global market of MAPs is China, Germany, France, Italy, Japan, Spain, United Kingdom and the United States. The International Council for MAPs has announced that global growth during 2001 and 2002 was 8 – 10% per year. The world market was estimated at 60 billion U.S. $ in 2003. Europe is a major world trader of MAPs. Today at the market are at least 2000 species of MAPs, of which 1200 to 1300 species are associated only to the European continent [3,4]. In current situation needs for herbal products at the international market is increasing. It is high opportunity for generating of sustainable benefit using of natural resources. There are more chances for regional and global economy to improve. Particularly it is great opportunity for global poor (especially in the Third and Fourth World). For the sustainable use of MAPs, it is necessary to develop programs of organic certification [5]. In addition, it is necessary to apply international law, particularly the Convention on Biological Diversity and the CITES Convention. Sustainable use of MAP is a prerequisite in generating ecologically sustainable benefit. MAPs are a great opportunity for new medicines and bio-materials [6] in both, developed and developing countries. Examplary references: 1. Redzic S (2006) Proc.1st IFOAM Intern. Conf. Organic Wild Production, 117 – 141. 2. Redzic SS (2007) Coll Antropol 31: 869 – 890. 3. Redzic S (2010) Med Plant Res 4(11): 1003 – 1027. 4. Redzic S (2008) Planta Med 74: 1143 – 1144. 5. Redzic S et al. (2009) Planta Med 75: 902 – 902. 6. Barudanovic S et al.(2009) Planta Med 75: 936 – 938.

Comparison of chemical composition of Artemisia annua volatile oil from Romania
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The aim of our work was to obtain and compare, quantitatively and qualitatively, the composition of volatile oil from the Artemisia annua L. (sweet wormwood). The fresh plant material, harvested from a Romanian natural population and Anamed (A3) cultivar, was distilled with the classical Clevenger (HD) and the microwave assisted (MWHD) laboratory hydrodistillation [1]. The MWHD apparatus, with original design, was equipped with a microwave oven (750 W), a rotating head and a Clevenger extension. The volatile oil samples were analyzed by thin layer chromatography (TLC) and gas chromatography (GC). The amount of volatile oil obtained was 0.57 respectively 0.58% v/w (HD) and 0.69% v/w (MWHD). The operation time for MWHD was 20 minutes and 180 minutes for HD. With TLC 11 spots were visualized; in case of MWHD 13 spots were identified by GC. For Anamed (A3) cv. the main components, for both HD and MWHD, were artemisia ketone (38.0/41.2%), L8-cineole (11.9/13.0%), bornol (8.7/8.8%) and camphor (8.4/9.1%). MWHD method was faster then HD and the quantity of volatile oil was higher by 19%. The main components ratio was also slightly higher in this case. Hence, the MWHD methods seem to be more efficient for essential oil hydrodistillation at laboratory scale. References: [1] Toth ET et al. (2010) Acta Medica Marisienis 56(2): 61. [2] Hethelyi EB et al. (1995) Essent Oil Res 7: 45 – 48.

Systemic Studies on Arctii Fructus
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Arctii fructus is the dry seeds of Arctium lappa L. and generally used as an herbal medicine in traditional Chinese medicine. The pharmacognosy and anticaner constituents of Arctii fructus as well as the ecological suitability of Arctium lappa and its suitable cultivation regions in China were studied. Our research established a method for distinguishing Arc- tii fructus from its adulterations. In Chinese patent medicine, the pressed Arctii fructus was mostly used. The optimal procedure for the processing of Arctii fructus were studied and the processing principle of Arctii Fructus was determined to be: 1) Protect arctin from degradation by hydrolytic enzyme in fructus arctii; 2) Extract the active constituents from arctii fructus easier by making the pericarp texture of arctii fructus crispy; 3) Abate the nature of arctii fructus by attenuating the purgative action by a decreasing content of lipids and arctin. Dao-Di-Yao-Cai means the best and highest quality of Chinese medicine materials. It is a unique quality index for evaluating Chinese medicine in traditional Chinese medicine and the result of long-time clinical experience of practitioners. The suitable cultivation regions for Arctii fructus in China were determined on the basis of its ecological suitability.

Determination of alkannin/shikonin derivatives in endemic Greek Alkanna species
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Alkannin and Shikonin (A/S) derivatives are optical antipodes of plant origin with a verified wide spectrum of antimicrobial, wound healing, anti-inflammatory and antioxidant activity. Although the aforementioned antipodes were originally introduced as wound healing agents, recent studies on cancer chemotherapy revealed that A/S also exhibit antitumor activity. A/S have been found in roots of several Boraginaceae species[1 – 3]. Determination of A/S, their esters and the total A/S content in Boraginaceae roots of different origin was reported in several epapers[4 – 7]. Alkanna species grown wild in Greece have been analysed for their A/S esters by LC-PDA-MS previously [5], whereas the total A/S content (A/S and their derivatives) has not been reported. In the present study endemic Alkanna species (tinctoria (L) Tausch, pindicola Hausskn., orientalis (L) Boiss., methanid Hausskn., callienis Boiss., gra- ca Boiss. & Spruner, primuliflora Griseb., strirbryi Velen., siebci DC., noneiformis Griseb.) grown in various Greek regions were collected and analyzed for their total A/S content for the first time. A comparison was additionally performed among species and different regions. Quantitative analysis revealed that specific root samples of A. tinctoria, A. pindi- cola and A. sieberi showed the highest amount of A/S and derivatives (1.41, 1.38, 1.00 mg/100 mg root respectively), but the A/S content varied from one region to another even within the same species. Yet, a significant difference in A/S content was observed among species. With this study it can be concluded that some of the examined Alkanna species of the Greek flora could serve as alternative sources for medicinally valuable A/S derivatives. Keywords: Alkannin, shikonin, Alkanna, Boragina- ceae, naphthoquinones, wound healing References: 1. Papageorgiou VP, Assimopoulou AN et al. (1999) Angewandte Chemie, Int. Edition 38(3): 270 – 301. 2. Papageorgiou VP, Assimopoulou AN et al. (2006) Current Organic Chemistry 10(16): 2123 – 2142. 3. Papageorgiou VP et al. (2008) Current Medicinal Chemistry 15(30): 3248 – 3267. 4. Papageorgiou VP, Assimopoulou AN et al. (2006) Current Organic Chemistry 10(5): 583 – 622. 5. Assimopoulou AN et al. (2006) Biomedical Chromatography 20: 1359 – 1374. 6. Pekin G, et al. (2007) Planta Medica 73: 267 – 272. 7. Akgun IA et al. (2009) Chromatographia 70: 963 – 967.
Mexican poppy (Argemone mexicana L.) is an annual thorny herb which belongs to Papaveraceae family. It is commonly spread in Mexico and in southern North America where it is growing in the wasteland. Argemone mexicana contains high variety of isoquinoline alkaloids which influence its activity (e.g. chologlucon, hipontine, antifebrile, antimarial, spasmolytic and depressive towards the CNS). In the course of current study main active constituents coming from methanolic extract of Mexican poppy’s roots were separated using Fast Centrifugal Partition Chromatography. Several solvent systems were elaborated according to their affinity to Mexican poppy’s alkaloids. Petroleum ether, ethyl acetate, methanol and water (15:30:21:20 v/v/v/v) was responsible for the most successful separation of its secondary metabolites. The obtained alkaloidal fractions were analyzed by means of ESI- octopole-orthogonal acceleration time-of-flight (oa TOF) mass spectrometry (MS) with high mass accuracy. Among well known and described alkaloids in this species (sanguinarine, chelerythrine, argemone, protopine, berberine or coptisine), magnoflorine, palmatine and galanthamine were confirmed in Argemone mexicana for the first time. Selective and sensitive TLC-bioautography screening test for natural acetylcholinesterase (AChE) inhibitors was performed for isolated alkaloids [1]. References: [1] Mroczek T (2009) J Chromatography A 1216(12): 2519 – 2528.

Antioxidant capacity and total phenolic content of Stachys aucheri endemc plant to Persia Namjooyan F1, Azemi M1, Hejazi H1, Soltani M2
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Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, oxidation-centered free radicals or other oxygen species (ROS), which are directly responsible in vivo, result in cell death and tissue damage. Anti-oxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress 1. Hence, compounds especially from natural sources capable of protecting against ROS mediated damage may have potential application in prevention and/or curing of diseases. The phenolic compounds in herbs act as antioxidants due to their redox properties. According to the studies about the presence of hypericin and qualitative and quantitative determination of hypericin and quantitative analysis of the phenolic compounds of the flowering aerial parts of five endemic Hypericum species of Turkey, namely, Hypericum kotschyanum Boiss., H. salignum Robson et Hub.-Mor., H. scabroides Robson et Poult., H. thymposis Boiss. and H. uniglandulosum Hausskn. ex Bornm. were performed by HPLC (1). The results were also compared to each other. It was observed that the H. salignum had the highest hypericin content among the others. In the phenolic compounds side of view, quercetin and isoquercitrin were determined in all species. The rutin was observed in the H. kotschyanum, different than the other species. References: 1. European Pharmacopoeia (2008). Herbal Monographs: St John’s Wort (Hyperic herba), EDPQ, Strasbourg, pp: 3839 – 3842.

New synthetic flavones of natural origin as antimarial agents Sén G1, Stiebling S2, Perrotsey S1, Collot V1, Schmitt M2, Njom G2, Weniger B3, Candoft E1, Venron Sénéchaux C1
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Recent reports of increased tolerance to artemisinin derivatives, the most recently adopted class of antimalarials, have prompted a need for new treatments. In this context, we tested the antimalarial activity of new treatments. In this context, we tested the antimalarial activity of...
Antioxidant compounds in food play an important role as a health protecting factor. In this study, the methanolic extracts of the aerial parts of nine Lamiaceae species: Mentha spicata L., Mentha aquatica L., Mentha piperita L., Stachys byzantina K.Koch, Marrubium vulgare L., Rosmarinus officinalis L., Salvia officinalis L., Thymus vulgaris L. and Melissa officinalis L. were investigated for their antioxidant activity and total phenolic and flavonoid contents using DPPH and Folin-Ciocalteu assays respectively. The IC50 of the methanolic extracts ranged between 38.27 – 59.14 mgGAEg⁻¹dw. R. officinalis and M. vulgare showed the most content of antioxidant activity. There was a direct correlation between total phenol and antioxidant activity which indicates that polyphenols are the main antioxidants.

Our aim was to obtain and compare, quantitatively and qualitatively, the polyphenols and flavonoids from six natural populations of Crataegus monogyna Jacq. harvested from the spontaneous flora of Eastern Carpathians. The Crataegus fructus and Crataea folium cum flore samples were collected from Neamt County -Cernegura Hill (no. 1), Batca Doamnei (no. 2), Cheile Bicazului (no. 6) and three other from Harghita County – Praid area (no. 3, 4, 5). The qualitative analysis of polyphenols and flavonoids was performed by TLC and HPLC. The quantitative analysis was performed by UV/VIS spectrophotometry [1] (rutoside for flavonoids in extracts ranged from 47.76 up to 78.82 mg of RU/g of extract. The content of polyphenols and flavonoids from Crataegus fructus has identified high amounts (2.11 – 2.70%)

Our aim was to obtain and compare, quantitatively and qualitatively, the polyphenols and flavonoids from six natural populations of Crataegus monogyna Jacq. harvested from the spontaneous flora of Eastern Carpathians. The Crataegus fructus and Crataea folium cum flore samples were collected from Neamt County -Cernegura Hill (no. 1), Batca Doamnei (no. 2), Cheile Bicazului (no. 6) and three other from Harghita County – Praid area (no. 3, 4, 5). The qualitative analysis of polyphenols and flavonoids was performed by TLC and HPLC. The quantitative analysis was performed by UV/VIS spectrophotometry [1] (rutoside for flavonoids in extracts ranged from 47.76 up to 78.82 mg of RU/g of extract. The content of polyphenols and flavonoids from Crataegus fructus has identified high amounts (2.11 – 2.70%). When the total quantity of polyphenols was compared (flowers, leaves and fruits) the most valuable populations were samples no. 3 and 4. Based on the obtained results, T. polium subsp. polium are rich sources of phenolic compounds and promising candidates for further development as natural antioxidant agents. Acknowledgement: Ministry of Science and Education, Republic of Serbia (III41010). References: 1. Sharifar F et al. (2008) Food Chem 112: 885 – 888. 2. Benzle IFF & Strain JF (1996) Analytical Biochem 239: 70 – 76. 3. Briggs TS & Rauscher WCJ (1973) Chem Educ 50: 496. 4. Stankovic SM et al. (2010) J Med Plant Res 5. Ellman GL et al. (1961) Biochem Pharmac 7: 88 – 95.

Antitubercular activity of pimaran and kaurene diterpenes against Mycobacterium tuberculosis Helena VC, Martins CG, Cabral MW, Silva AN, Matos PM, Souza MC, Veneziani RS, Ambrósio SR

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Tuberculosis (TB) is still a public health problem and causes millions of deaths every year [1]. According to the World Health Organization, 8.8 million new TB cases occurred in 2007 [2]. Since the increase of bacterial resistance and the emergence of new infections are common problems [3], the search for new antibacterial or antitubercular agents is a urgent matter. In the course of our investigation about diterpenes and their biological activities, we’ve performed some antimycobacterial assays against Mycobacterium tuberculosis H37Rv, ATCC 27294 with five diterpenes. All of them showed at least moderate activity a MIC of <= 31.25 µg/mL, that can be classified as promising, as according to Cantrell et al. [4] a MIC value below 64 µg/mL for isolated compounds can be considered to be of interest. In the present work, compound 1 was the less active with a MIC of <= 250 µg/mL. Compounds 2 and 4 are of moderate activity each with a MIC of 125 µg/mL. Most active compounds were 3 and 5 with a MIC of < 31.25 µg/mL. Further assays will determine detailed final MIC values for compounds 3 and 5 and other diterpenes of both classes will be investigated in the test system. Acknowledgement: FAPESP (Proc. 2009/09491 – 1), CAPES, CNPq. References: [1] Higuchi CT et al. (2008) Quim Nova 31: 1719 – 1721. [2] www.whqlibdoc.who.int/hq/2007/WHO_HTM_TB.2007.378_eng.pdf (accessed april/04/2011) [3] Porto TS et al. (2009) Fitoterapia 80: 432 – 436. [4] Cantrell et al. (2001) Planta Med 67: 1 – 8.

Bilological effects and phenol content of felfy germander (Teucrium polium L. subsp. polium) Stankovic MS1, Mila J2, Francko B2, Milos M2, Politeo O3

Carev P4

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Felty germander – Lamiaceae is popular species of Teucrium genus in the folk medicine and used for treatment of appetite loss and gastrointestinal ailments [1]. In the present study, antioxidative and anti-acetylcholinesterase activity, total phenolic content as well as flavonoid concentration of methanolic, acetone and ethyl acetate extracts were investigated. Ferric reducing/antioxidant power (FRAP) [2] was assayed and values were between 7.5% and 6.5%. Total phenol content have been evaluated using the Briggs-Rauscher oscillating reaction method [3], expressed as a time required for regeneration of oscillations in minutes and obtained values were: 43.5 for methanolic, 2.0 for acetone, while ethyl acetate extract did not show activity. The ability to scavenge DPPH radicals [4] was determined and expressed as IC50 values that ranged from 59.37 to 622.96 µg/mL. Acetylcholinesterase inhibition was measured using slightly modified Ellman’s method [5] and results indicate a weak inhibitory activity of extracts. Total phenolic content was determined using Folin-Ciocalteu reagent and the values ranged from 235 to 846 mgGAE/g of extract. The content of flavonoids in extracts ranged from 47.76 up to 78.82 mg of RU/g of extract. The content of flavonoids in extracts ranged from 47.76 up to 78.82 mg of RU/g of extract. Methanolic extract was most active in comparison with other extracts for all measurements. That indicates that the methanol, as a polar solvent, is the very effective for phenolic compounds extraction from T. polium L. subsp. polium. Based on the obtained results, T. polium subsp. polium extracts are rich sources of phenolic compounds and promising candidates for further development as natural antioxidant agents. Acknowledgement: Ministry of Science and Education, Republic of Serbia (III41010). References: 1. Sharifar F et al. (2008) Food Chem 112: 885 – 888. 2. Benzle IFF & Strain JF (1996) Analytical Biochem 239: 70 – 76. 3. Briggs TS & Rauscher WCJ (1973) Chem Educ 50: 496. 4. Stankovic SM et al. (2010) J Med Plant Res 5. Ellman GL et al. (1961) Biochem Pharm 7: 88 – 95.

Comparison of the antioxidant activity and total phenolic contents in some species of Lamiaceae family. Jomhidi M1, Fatihazad M2

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They were subjected to biological and phytochemical investigations. The ger and Aloe grandidentata extracts of A. ciliaris was performed according to the usual published methods. The ethanolic (colon) and HEPG2 (liver). Antimicrobial activity was investigated lines, U251 (brain), MCF7 (breast), H460 (lung), HELA (cervix), HCT116 formine. Antitumor activity was investigated using available human cell investigation for ethanolic extracts. Acute anti-inflammatory activity was tested microorganisms. Phytochemical screening for biologically active possessed significant antimicrobial and antifungal activity against the and 92%, respectively comparing to the standard. While A. ciliaris, has long been used in traditional medicine for the treatment of digestive system diseases, skin troubles, wounds and burns. Recently it was proved to have antitumor activity. About 19 species were cultivated in Egypt whereas Aloe arborescens Mill., Aloe ciliaris Haw., Aloe eru Ber- ger and Aloe grandidentata Salm-Dyck were found to be most abundant. They were subjected to biological and phytochemical investigations. The biological study includes toxicological (LD50) and pharmacochemical in- vestigation for ethanolic extracts. Acute anti-inflammatory activity was done using paw edema method in rats with standard indomethacin. Chronic anti-hyperglycemic activity was carried out using standard met- formine. Antitumor activity was investigated using available human cell lines, U251 (brain), MCF7 (breast), H460 (lung), HELA ( cervix), HCT116 (colon) and HEPG2 (liver). Antimicrobial activity was investigated against Gram -ve bacteria and fungi. Phytochemical screening was performed according to the usual published methods. The ethanolic extracts of A. ciliaris and A. grandidentata were found to produce sig- nificant dose-dependant anti-inflammatory effects equivalent to 85.7% and 92%, respectively comparing to the standard. While A. grandidentata showed potent anti-hyperglycemic effect equivalent to 95.7% and 87.5% after 4 weeks and 8 weeks respectively comparing to the standard. A. ciliaris showed significant antitumor effect on brain and liver cell lines. The ethanolic extracts of A. arborescens, A. ciliaris and A. grandidentata possessed significant antimicrobial and antifungal activity against the tested microorganisms. Phytochemical screening for biologically active extracts indicated that carbohydrates and/or glycosides, sterols and/or triterpenes, combined and free anthraquinones are the main constitu- ents present.

Biogenesis of Bioactive Secondary Metabolites in Herbs
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Plant secondary metabolites are the major source of bioactive compounds of Herb. Metabolic engineering has opened a new promising perspective for the improved production of these valuable secondary metabolites in plant cell factory. Apparently, the key to metabolic en- gineering is the detailed knowledge of pathways of interest. We have developed RACE (rapid amplification of cDNA ends) method for the iso- lation of genes involved in certain biosynthesis pathway or crucial reg- ulation process [1], which prompted the possibility of a key gene-based metabolic engineering for the synthesis of active compounds. In addi- tion, we have successfully developed several plant cell culture systems such as hairy root, suspension cell as well as Saccharomyces cerevisiae cultures [2], which not only facilitated gene manipulation such as trans- formation and knockout, but also feasible for the industrial production of desired compounds in the near future. In our study, several metabolic engineering strategies have been successfully used to channel metabo- lites into pathways leading to desired products, including overexpress- ion of rate-limiting enzyme genes, suppression or knockout of compet- itive enzyme genes, regulation of signal molecular pathway, and trans- formation of important transcription factors or transporters, etc [3]. Furthermore, through the unidentifed secondary metabolites, we are now using isotope tracing and 2-dimensional electrophoresis tech- nology to explore them [4]. The identification and isolation of the en- zymes involved will certainly help us to elucidate the whole biosyn- thesis pathway(s), and ultimately enable the possibility of metabolic en- gineering for the production of specific bioactive secondary metabolites in herbs. Acknowledgement: This research was financially supported by National Natural Science Foundation of China (20572130, 30800786) and Modernization of traditional Chinese medicine foundations (08021019502), Shanghai Science and Technology Committee. References: 1. Xiao Y et al. (2009) Mol Biol Rep 36: 2019 – 2029. 2. Huang BB et al. (2011) Metabo- lomics 7: 134 – 146. 3. Xiao Y et al. (2009) Physiol Plantarum 137: 1 – 9. 4. Xiao Y et al. (2010) Biosci Rep 30: 33 – 40.

Effect of Ailanthus altissima (Mill.) Swingle and Ailanthus excelsa Roxb. stem bark extracts on Streptozotocin Induced Diabetes
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The inhibitory effects of methanol (70%) extracts of Ailanthus altissima (Mill.) Swingle and Ailanthus excelsa Roxb. stem bark on streptozotocin (ST) – induced diabetes mellitus were studied using ST – treated dia- betic model. When the effects of the extracts on ST-induced ATP/ADP ratio of islets were assayed, the extracts were effective in restoring of ATP/ADP ratio and when the islets (200/condition) were treated with ST (5 mM for 30 min.) and then the extracts were added to the ST-treated cells, the extracts concentration (200 µg/ml) showed increased insulin production in pancreatic islet cells. Keywords: Ailanthus excelsa, Ail- anthus altissima, stem bark, Antidiabetic activity

Antibacterial potential of essential oil of medicinal plant Satureja bachtiarica Bunge against human pathogenic bacteria
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The essential oils of Ferula microleuca Boiss. collected from west of Iran during the flowering stage, were obtained by hydrodistillation and ana- lysed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC-MS). Under the optimum distillation and analysis condi- tions, 22 constituents (mainly monoterpens) were identified in Ferula microleuca which represented 93.6% of the oil. The main constituents were α-pinene (27.3%), β-pinene (16.4%), nonanal (8.7%), β-carophyl- lene (8.5%) and thymol (6.7%). The samples were also subjected to
screening for their possible antioxidant activity by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene-linoleic acid assays. In the first case, the free radical scavenging activity of polar sub-fraction of methanol extract was superior to all other extracts (IC₅₀ = 34.3 ± 0.3 μg/ml), non polar sub-fraction of methanol extract exhibited stronger activity than the essential oil. In the case of the linoleic acid system, oxidation of the linoleic acid was effectively inhibited by the polar sub-fraction of methanol extract, while the oil and non polar sub-fraction of methanol extract were less effective. Keywords: Ferula microcalcea, Antioxidant activity, Essential oil

Trilobolide and its analogues belong to guaianolide type of sesquiterpenes, widely distributed within families Asteraceae and Apiaceae [1]. Trilobolide [1], structurally related to thapsigargin (4), is quite specific in its structure and biological activities [2]. Certain guaianolides evoked attention for their promising anti-inflammatory, anticancer, anti-infectious and SERCA inhibitory activities. However, due to their cytotoxic properties, they are generally toxic. Search for compounds with significant immunobiological properties, but with minor cytotoxicity is a challenge for immunopharmacological research also in our case [3].

Figure 1

Acknowledgement: Supported by GACR grant No 305/07/0061

PM3

Targeted modification of trilobolide and search for related sesquiterpene with immunobiological properties

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Anthraquinones isolated from the roots of Rennellia elliptica Korth. demonstrated interesting antiplasmodial activity. The activity however, varies depending on substitution pattern of the anthraquinone skeleton which warrants further investigation. This paper reports preliminary structure-activity relationship of a series of 9,10-anthraquinones and their antiplasmodial activity. The natural anthraquinones were isolated from roots extract of R. elliptica. The analogues of bioactive anthraquinones were synthesized through Friedel-Craft reaction between phthalic anhydride and various benzene derivatives in the presence of eutectic mixture of aluminium chloride and sodium chloride. The antiplasmodial activity was determined by the 3D7 Plasmodium falciparum (3D7) growth in vitro. Combination of methyl and hydroxyl substituents at different positions on the anthraquinone skeleton caused strong antiplasmodial activity. The ortho-arranged substituents at 2,3 positions exhibited strongest activity with IC₅₀ value of 0.08 μg/ml followed by the compound with substituents at 1,2 positions. The para-arranged (1,4) and meta-arranged (1,3) substituted anthraquinones showed less potent activity. On the other hand, analogues of dihydroxyanthraquinones displayed a reverse order of activity with the strongest inhibition shown by 1,3-dihydroxyanthraquinone. The hydroxy-methyl anthraquinones and dihydroxyanthraquinones were substituted with additional methyl group at C-6-C-7 on ring A showed similar pattern of activity but much weaker than those substituted only on ring C. Protection of hydroxyl group via methylation reaction caused significant variation in antiplasmodial activity. Anthraquinones substituted at C-2 and C-3 and anthraquinones substituted at C-1, C-2 and C-6-C-7 promotes antiplasmodial activity. Structural differences due to different substitution pattern affects antiplasmodial activity of 9,10-anthraquinones. Keywords: Anthraquinones, Antiplasmodial, Structure-Activity Relationship, Rennellia elliptica

PM4

Assessment of Anti-angiogenic and Anti-tumoral Properties of Origanum onites L. Essential Oil

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Figure 1

Crude aqueous and methanolic extracts of 121 plant species belonging to 41 families collected from the west of Iran were screened for anti-fungal activity against mycelial growth of Phytophthora drechsleri. Bioassay was performed based on paper disc diffusion method with four replicates. Thirty eight of 121 (about 31%) plant species showed inhibitory activity against this phytopathogenic fungus, among which 23 species measurably inhibited the growth of Phytophthora drechsleri. Results indicated that methanolic extract of Xanthium strumarium L. showed the maximum activity (17.79 ± 1.35 mm) against P. drechsleri followed by Glycerizia glabra L, Hypericum perforatum L, Centaurea depressa M.Bieb, Lamium amplexicaule L, Haplophyllum perforatum (M.B.) Vved. The investigation on the effect of plant parts on mycelial inhibition of tested fungi using paper disc method indicated that inflorescence and fruits of cocklebur (Xanthium strumarium) has significantly more inhibitory effect against P. drechsleri. The study on the antifungal activity of two common species of cocklebur grown around city of Kermanshah, X. strumarium and X. spinosum L., showed that both of them have inhibitory activity on mycelial growth of tested fungus, but the X. strumarium showed significantly more inhibitory effect against P. drechsleri than X. spinosa. Keywords: Phytophthora activity, Xanthium strumarium, Iranian plants, Paper disc Results: Bahraminejad S et al. (2008) J Phytopathol 156: 1 – 7. Kim DK et al. (2002) Plant Pathol J 18(5): 288 – 292. Koko WS (2007) Nat Prod 15: 1 – 10. Qi LY et al. (2008) Agr Sci Tech-Hunan 8(4): 144 – 148.
analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). The antiproliferative activities (by MTT assay, 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2-H-tetrazolium bromide), the anti-angiogenic activities (by matrigel tube formation assay), cell migration inhibiting capability (migration assay) and apoptotic potential (DAPI staining) of the *Origanum* omotes essential oil (OOEO) were evaluated on rat adipose tissue endothelial cells (RATECs) and SRP7 (c-H-ras transformed rat embryonic fibroblasts) cells. Our experimental results revealed that OOEO could markedly inhibit cell viability and induced apoptosis of SRP7 cells and also could block in vitro tube formation and migration of RATEC. These results imply that OOEO having anti-angiogenic activity might be useful in preventing angiogenesis-related diseases and combating cancer. Keywords: Essential oil, *Origanum* omotes L, antiangiogenesis, cytotoxicity, apoptosis, cancer

**PM7**

Neuropharmacological Activity of *Pimenta Pseudocaryophyllus* (Gomes) L.R. Landrum

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*Pimenta pseudocaryophyllus* (Gomes) L.R. Landrum (Pp) is popularly used as a tranquilizer in the treatment of emotional tension in the city of Campos do Jordão, São Paulo, Brazil. The aim of this study was to evaluate behavioral changes induced by ethanol extract of the Pp leaves (PpEE), seeking to identify the most active fraction. PpEE was obtained by soaking the dried leaf powder in ethanol (95%;1:5). The hexane (HF), dichloromethane (DF), ethyl acetate (ACF) and aqueous (WF) fractions were prepared through PpEE Fractionation with solvents of different polarities. Swiss male mice (25 – 35 g) were treated orally with PpEE, HF10; DF160 mg/kg; ACF260 mg/kg; and WF 640 mg/Kg in proportion to their respective yield. After 1hr of treatment, anxiolytic activity was evaluated using the forced swim test (FST), and the open field (OF) and elevated plus maze (EPM) models. Group treated with Diazepam (1 or 5 mg/kg, i.p.) and vehicle 10 ml/Kg were used as a positive and negative control respectively. The PpEE increased in proportion to their respective yield. After 1hr of treatment, anxiolytic activity was evaluated in mice treated orally with PpEE, HF10; DF160 mg/kg; ACF260 mg/kg; and WF 640 mg/Kg. The results showed that PpEE had a significant anxiolytic activity in mice in different models.

**PM8**

Anti-Salmonella and anti-inflammatory activities of *Z-ligustilide from Ligusticum chuanxiong* Shin S, Lim H, Sim Y

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*Ligusticum chuanxiong* Hort. (Umbelliferae), a perennial herb cultivated mainly in Korea and China, is one of the main plant sources of *Cnidii Rhizoma* which were shown to exhibit cardiovascular, antiplatelet, anti-inflammatory, and also antimicrobial and insecticidal effects. To develop an effective agent against antibiotic-resistant *Salmonella* infections the essential oil was extracted from the rhizomes of *L chuanxiong* and analyzed by GC-MS to identify its composition. The antibacterial activity of the oil fraction and its main components against *Salmonella* species was estimated using the broth dilution method. Moreover, to determine the combined effect of essential oil components and antibiotics, checkerboard microtiter tests were performed. The fractional inhibiting concentrations (FICs) were calculated. In addition, the anti-inflammatory properties of *L chuanxiong* oil and its components were evaluated using RAW 264.7 cell viability was determined by an MTT assay after treatment with various dilutions of the compounds. Inhibition of nitric oxide production in cells treated with lipopolysaccharide (LPS) was evaluated by reaction with Griess reagent. The m-RNA expression levels of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) were investigated by PCR with corresponding primers. As the results, the minimum inhibiting concentrations (MICs) ranged from 1–4 mg/ml indicating differences between the tested *Salmonella* species and strains. The *L chuanxiong* oil and its components combined with antibiotics showed additive or synergistic activities with FICs between 0.28 and 0.63. The increases of m-RNA expression levels of iNOS and COX-2 were indentified by image analysis of the bands using Gel-doc system. Keywords: *Ligusticum chuanxiong*, essential oil, *Salmonella*, antibiotic-resistant, anti-inflammatory, and antioxidant activities. This research was supported by (applied by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2011 – 0011249) References: 1) Shin S (2010) Nat Prod Sci 16: 259 – 264 2) Shin S (2008) Arch Pharm Res 31: 497 – 502

**PM9**

Reduction toxicity by doxorubicin entrapped in liposomes nanocapsules Mezbah L, Mohamed A, Gillian B

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Doxorubicin induced an irreversible congestive heart failure, renal and hematological toxicity that are often fatal. The molecular mechanisms involved are only partially known and are complex and different from the anticancer mechanism involving oxidative stress. Encapsulation of doxorubicin in liposomes was elaborated in order to prevent the toxicity observed with the free form. The study was conducted in vivo by treatment of Wistar rats with doxorubicin encapsulated in liposomes or naked at different doses (10, 20 and 30 mg/kg) and in vitro on H9c2 cells. In case of control, different mitochondrial parameters (CR, Swelling, bioenergy...) were evaluated. The study is complemented by MTT and LDH tests. In vivo doxorubicin causes oxidative stress more pronounced than liposomal doxorubicin. A activity inhibition cytochrome c oxalate, depletion of tissue glutathione concomitant with increased production of ROS, swelling of mitochondria are observed. The mitochondrial dysfunction at origin of the cardiotoxicity is confirmed by the MTT assay and LDH test. We observed also renal dysfunction and aplasia in blood, spleen and bone marrow more serious with naked doxorubicin than with the encapsulated one. In conclusion, doxorubicin would be responsible for cytoxicity by inducing the mitochondria. Theses disorders may be prevented by its encapsulation in liposomes. Keywords: Cardiototoxicity, Nephrotoxicity, Haematoxicity, Doxorubicin, Liposomes, Oxidative stress References: 1 Plassat V et al. (2007) Int J Pharm 118 – 127. 2. Leite A et al. (2007) Life Sciences 80: 1327 – 34. 3. Lahouel M et al. (1987) Drugs Exp Clin Res 10: 593 – 599.

**PM10**

Total phenolic content, flavonoids and Superoxide radical scavenging activity of some Citrus peels Golfakhrabadi F, Shapoori A, Javdani A, Hassanzadeh A

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The Genus Citrus is a shrub from Rutaceae family. The main constituents of Citrus are phenolic compounds, acids, volatile oil, pectin, carote-noids, flavonoids and vitamin C. Polyphenols of citrus have antioxidative effect to prevent liperoxidation, increase serum antioxidant capacity and decrease oxidative stress in geriatrics. In this study three different extracts (methanol extraction, chloroform and flavonoids fraction) from peel of 8 species (orange, mandarin, sour orange, citron, grape fruit, lemon, sweet lime, lime) of citrus were prepared. The total phenolic content, flavonoids and superoxide radical scavenging activity of some citrus were evaluated using the Folin-Ciocalteu method, BNT, DPPH and FRAP assays. In NBT assay Citron flavonoids fraction IC50=0.035 mg/ml has the the highest capacity of inhibition and were comparable to vitamin C (IC50=0.058 μg/ml), in DPPH assay lime methanolic extraction IC50=9.40 mg/ml and in FRAP assay Lime methanolic extraction have the highest capacity of inhibition. In DPPH assay Vit C has IC50=0.072 μg/ml. The maximum amount of phenolic compound was observed in grapefruit flavonoids fraction (142.47 mg acid tannic/100g).

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1 g extraction). The maximum amount of flavonoids compounds was observed in Lime methanolic extraction (12.91 mg Rutin/1 g extraction).

Methanolic extract in DPPH and NBT method have the same results for all species, but in Folin-Ciucelute method all species except grapefruit and lemon have the results of DPPH and NBT methods. Methanolic extract of lime have maximum effects in DPPH, FRAP and NBT methods and almost the highest amount of polyphenol compounds. The results showed that lime peel have maximum antioxidant effects in citrus sp.

Keywords: Citrus, NBT, DPPH, Folin-Ciucelute, FRAP, Antioxidant, Flavonoids

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PM11
Antibacterial Activity of Different Extracts of Clidemia hirta (L.) D. Don leaves

Dianita R
d, Ramasamy K
d, Ab Rahman N
d
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Clidemia hirta (L.) D. Don (Melastomataceae), locally known as “senduduk bulu” by a local tribe in Malaysia, has been used traditionally to stop bleeding [1] and in the treatment of venom fever [2]. The use of this species as traditional medicine for several bacterial infections has also been recorded in several references [3, 4]. Thus, this study was conducted to investigate the antibacterial properties of this species and determine its MIC towards several bacteria, Escherichia coli, Enterococcus faecalis, Pseudomonas aeruginosa, and Staphylococcus aureus. Several extracts of the extract were chosen: cold-extracting (cold extraction) the leaves with different polarity of solvents such as hexane, ethyl acetate and methanol, respectively. The initial antibacterial property identification was done by using disk diffusion method. The active extract was further investigated for its mechanism and MIC’s value by dilution technique. Only the hexane extract was showing any antibacterial property against selected bacteria. Meanwhile, none of these extracts showed activity against E. coli. Interestingly, bactericidal activity was exerted against P. aeruginosa by ethyl acetate extract (MIC 0.625 μg/ml), followed by its bacteriostatic activity against E. faecalis (MIC 0.125 μg/ml). Conversely, the methanol extract showed bacteriostatic activity against P. aeruginosa (MIC 1.25 μg/ml) and bactericide activity against E. faecalis (MIC 1.25 μg/ml). Keywords: Clidemia hirta, Melastomataceae, antibacterial References: 1. Musa N (2007) The Forgotten Jungle Medicine of Taman Negara Pahang. Malaysian Pharmaceutical Association. Penang. 2. Kamarudin MS, Latiff A (2002) Tumbuhan Ubatan Malaysia. Pusat Pengurusan Penyelidikan UKM. Bangi. 3. Franca F Lago EL, Marsden PD (1996) Rev Soc Bras Med Trop 29: 229 – 232. 4. McClatchey W (1996)) Ethnopharmacology 50: 147 – 156.

PM12
Preventive Role of Cactus (Opuntia ficus-indica) Cladodes on methotrexate-induced Biochemical, Hematological and oxidative damage in rat liver

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Methotrexate is widely used in the therapy of various types of malignancy as well as in the treatment of various inflammatory diseases. Its efficacy is counterbalanced by severe side effects including blood damage. The ability of Extract of Cactus to restore MTX – liver and Hematological Damage was tested. Cactus cladode Extract (Opuntia ficus-indica Mill.) was injected intraperitoneally alone or simultaneously with methotrexate at a concentration of 0.625%. Conversely, the methanol extract showed bacteriostatic activity against P. aeruginosa (MIC 0.125 μg/ml) and bactericide activity against E. faecalis (MIC 1.25 μg/ml). Keywords: Clidemia hirta, Melastomataceae, antibacterial References: 1. Musa N (2007) The Forgotten Jungle Medicine of Taman Negara Pahang. Malaysian Pharmaceutical Association. Penang. 2. Kamarudin MS, Latiff A (2002) Tumbuhan Ubatan Malaysia. Pusat Pengurusan Penyelidikan UKM. Bangi. 3. Franca F Lago EL, Marsden PD (1996) Rev Soc Bras Med Trop 29: 229 – 232. 4. McClatchey W (1996)) Ethnopharmacology 50: 147 – 156.

Keywords: Cactus, Biochemical, Hematological Parameters, Prevention, Cladodes of Cactus

Acknowledgement: This research was funded by the Tunisian Ministry of Scientific Research and Technology through the Research Unit of Macromolecular Biochemistry and Genetics, Faculty of Sciences of Gafsa.

PM13
Antihyperglycemic effect of Derris reticulata Crab extract in alloxan-induced diabetic rats

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The global prevalence of diabetes mellitus (DM), a metabolic disorder characterized by chronic hyperglycemia, has been estimated to be increasing worldwide [1]. It is known that the present synthetic drugs available for treatment of DM can cause several undesirable side effects. As recommended by WHO, the quest for effective and safer anti diabetic plant drugs is an important topic [2]. In Thailand, Derris reticulata Crab (DC) which belongs to Leguminosae family has been traditionally used for diabetic treatment. However, limited scientific data are available. The aim of this work was to investigate the antihyperglycemic potential of the aqueous extract from DC stem in alloxan-induced diabetic rats. The result showed that administration of DC extract at the daily dose of 250 mg/kg for 15 consecutive days significantly decreased fasting blood glucose levels compared with diabetic control group. More gross pathological lesions were found on the pancreas of diabetic control rats than that of DC-treated rats. An association between antioxidant property and antihyperglycemcic activity of plant extract has been reported [3]. Total phenolic content and the IC50 of antioxidant potential against DPPH radicals of DC extract was 0.625% and 239.85 ± 0.13 mg/g/ml respectively. In conclusion, the DC extract exerts antidiabetogenic effect which may be associated with its antioxidant-mediated pancreatic protection. Keywords: diabetes mellitus, Derris reticulata, antioxidant References: 1. Wild S et al. (2004) Diabetes Care 27:1047 –1053. 2. Gupta S et al. (2009) J Ethnopharmacol 123: 499 – 503. 3. Alarcon-Aguilar FJ et al. (2010) J Ethnopharmacol 132:400 – 407.

PM14
Echinocystic acid inhibits acute-lung injury by inhibiting TLR4/LPS complex formation

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Orally administered lancemaside A isolated from Codonopsis lanceolata Traurt. (Campanulaceae) showed anti-inflammatory effects in vivo and produced 3 metabolites by the incubation with human intestinal microflora in vitro [1, 2]. Among lancemaside A and its 3 metabolites, echinocystic acid most potently suppressed the production of pro-inflamma- tory cytokines, TNF-α and IL-1β, as well as of the activation of their transcription factor NF-κB in LPS-stimulated alveola macrophages. Echinocystic acid also down-regulated the production of inflammatory markers, including inducible nitric oxide synthase and cyclooxygenase-2, as well as the inflammatory mediators, nitric oxide and prostaglandin E2 in LPS-stimulated macrophages. Echinocystic acid also inhibited the activation of IL-1 receptor-associated kinase, the phosphorylation of IKK-β and IκB-α, the nuclear translocation of NF-κB. Furthermore, echinocystic acid potently inhibited the interaction between LPS and TLR4. Echinocystic acid suppressed LPS-induced acute-lung injury in mice, as well as the expression of pro-inflammatory cytokines such as IL-1β and TNF-α, and the activation of their transcription factor, NF-κB. When lancemaside A was orally administered for mice, its metabolite echinocystic acid alone was detected in the blood. Based on these findings, echinocystic acid may express anti-inflammatory effects by inhibiting the binding of LPS to TLR4 on alveola macrophages in vitro and in vivo. Acknowledgement: This study was supported by a grant from World Class University Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (R33 – 2008 – 000 – 10018 – 0). References: 1. Jho EH et al. (2010) Int J Coloratal Dis 25: 545 – 551. 2. Jho EH et al. (2010) Chromatogr B Analyt Technol Biomed Life Sci 878: 1875 – 1880.
Antioxidant and antibacterial activities of the extract of Aquilaria crassna leaves

Acknowledgement:

myrton, Rhodomyrtus tomentosa, Streptococcus pyogenes, two-dimensional gel electrophoresis. It is concluded that the aqueous extract of young Aquilaria crassna are used to produce commercial herbal teas. In addition to its aroma, it is believed that Krissana leaves possess many interesting medicinal properties, such as antiinflammatory, antioxidative and antibacterial activities. However, scientific study on its pharmacological activity is very limited. Several in vitro studies were conducted to investigate the safety and antibacterial activity of the aqueous extract of Aquilaria crassna leaves. Acute toxicity test showed that even at high dose (15,000 mg/kg) the extract did not cause death or overt signs of toxicity when observed for 14 consecutive days in mice. It was found that the extract exhibited antibacterial activity against Staphylococcus aureus and Streptococcus epidermidis with MIC of 12.0 and 4.0 mg/ml, respectively. Since the correlation between antioxidant and antibacterial activities of plants has been reported [2], the total phenolic compound (TPC) and antioxidant property were also examined. The TPC of the extract was 162.4 ± 0.3 mg gallic acid equivalent/g. The extract showed strong antioxidant activity against DPPH radical with IC50 of 6.04 ± 0.18 mg/ml. It is concluded that the aqueous extract of Aquilaria crassna leaves may be beneficial for treatment of diarrhea caused by Staphylococcus aureus and skin infection associated with Streptococcus pyogenes. Key words: Aquilaria crassna, Acute toxicity, Antibacterial. Acknowledgement: The authors acknowledge the help of Dr. Vongkhit Pachara from the Photomyrtine and Its Antioxidant Activity Against DPPH Radical with IC50 of 6.04 ± 0.18 mg/ml (2). The extract showed strong antioxidant activity against the defined procyanidin fraction were shifted to the right in the presence of L-NAME (NG-nitro-L-arginine methyl ester), an inhibitor of eNOS. The observed relaxation was also abolished by wortmannin, an inhibitor of PI3K/Akt. Taking together, these findings indicate that rhodomyrtone might involve intracellular targets. In the present study, we found a strong antibacterial activity against the bacterial pathogens Streptococcus pyogenes. Our previous studies indicated that the bactericidal activity of rhodomyrtone might involve intracellular targets. In the present study, we followed a proteomics approach to investigate the mode of action of rhodomyrtone on S. pyogenes. For this purpose, S. pyogenes was cultivated in the presence of 0.39 μg/ml rhodomyrtone, which corresponds to 50% of the minimal inhibitory concentration. The results show that the amounts of various enzymes associated with important metabolic pathways were strongly affected, which is consistent with the growth-inhibiting effect of rhodomyrtone. Additionally, cells of S. pyogenes grown in the presence of rhodomyrtone produced reduced amounts of known virulence factors, such as the glyceraldehyde-3-phosphate dehydrogenase, the cAMP factor, and the streptococcal pyrogenic exotoxin C. Taken together, these findings indicate that rhodomyrtone has both antimicrobial and anti-inflammatory activities, which make it an interesting candidate drug. Keywords: glycolysis, proteomics, rhodomyrtone, Rhodomyrtus tomentosa, Streptococcus pyogenes, two-dimensional gel electrophoresis. Acknowledgement: We thank Jan Arens, and members of the Department of Medical Microbiology and Department of Molecular Genetics for strains and technical support. We thank Assoc. Prof. Dr. Wilawan Mahabasarakam and Mr. Asadahawat Hirunrat for rhodomyrtone isolation. The work was funded by the Thailand Research Fund through the Royal Golden Jubilee, Ph.D. Program (PHD/0029/2548). Funding was furthermore provided by the National Research University Project of Thailand’s Office of the Higher Education Commission, the Van Leer Foundation, The Netherlands (VEL/DA/3689), and the CEU projects LSHM-CT-2006-190164 and LSHC-CT-2006-037469. References: [1] Limsuwan S, Hesseling-Meinders A, Voravutikunchai SP, van Dijl JM, Kayser O (2011) Phytochemistry, in press [2] Limsuwan S et al. (2009) Phytochemistry 70: 1876 – 1883. In vivo evaluation of an herbal remedy for antimarial activity

Oluwamuyi OA1, Adeolu EA2, Adejimi AS1, Morohnfotun A1, Obugbeja IE1
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The antimarial activity of an herbal remedy (HR) formulated, based on ethnomedical claims followed by observational experiences, was investigated in Plasmodium berghei NK65-infected mice. The decoction of the HR was prepared, concentrated in vacuo and freeze-dried. Evaluation of the antimarial activity involved the use of early malarial (4-day test) and established infection models [1, 2]. The HR was tested at 15 – 240 mg/kg orally and amodiaquine (AQ) was tested at 1.25 – 10 mg/kg. For the established infection test, the HR was tested at 60 – 240 mg/kg with AQ (10 mg/kg) as positive control. Distilled water was used as negative control in both test. The HR and AQ gave ED50 of 40 and 3.8 mg/kg respectively, while for the established infection test, the highest dose of 240 mg/kg gave 54.45% clearance on day 5. The HR showed higher suppressive than curative activity. Keywords: Herbal remedy, Plasmodium berghei, Amodiaquine Acknowledgement: Prof. G. A. Ademowo, Department of Pharmacology and Therapeutics, University of Ibadan, Ibadan, Nigeria for access to the Plasmodium berghei NK 65 parasite References: 1. Peters W (1965) Exp Parasitol 18: 80 – 87 B. Ryley J, Peters W (1971) Am J Trop Med Parasitol 84: 209 – 211. Procyonidin C of Neloa meyeri SCHWANT. elicited endothelium-dependent relaxation in porcine coronary arteries by activation of the PI3/Akt signalling pathway

Kaufeld AM, Pertz H, Kolodziej H
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Neloa meyeri Schwant. (Mesembryanthemaceae) is a South African succulent known to contain procyanidins [1]. The aim of this study was to examine the molecular mechanism by which the extract from leaves of this plant elicits blood vessel relaxation. For this, a highly purified fraction comprised of bi- to tetramer flav-3-ols was applied to porcine coronary arterial rings suspended in organ chambers containing Krebs-Henseleit solution maintained at 37°C. In endothelium-intact rings pre-contracted with the thromboxane A2 mimetic U46619, the sample produced a concentration-dependent relaxation that was abolished by mechanical removal of the endothelium. Concentration-response curves to the defined procyanidin fraction were shifted to the right in the presence of L-NAME (NG-nitro-L-arginine methyl ester), an inhibitor of eNOS. The observed relaxation was also abolished by wortmannin, an inhibitor of PI3K (phosphoinositide 3-kinase). However, the relaxant response to the Neloa extracts remained unaffected in the presence of ICI 182,780, an estrogen receptor antagonist, and pertussis toxin, an inhibitor of Gi proteins. These observations confirm the essential role of EDRF in the relaxant response to Neloa procyanidins. In addition, relaxation to the Neloa fraction was abolished by MnTMPyP, a cell permeable mimetic of superoxide dismutase but not by tiron, a superoxide anion scavenger. The relaxation was insensitive to charybdotoxin plus apamin (Ca2+-activated K+ channel blockers) but was abolished by the combination of charybdotoxin plus apamin plus L-NAME. Taking together, these findings suggest that the endothelium-dependent relaxation induced by Neloa procyonidin is mediated by EDHF and EDRF following activation of PI3/Akt. Keywords: Neloa meyeri, procyanidins, endothelium relaxation, EDHF, EDRF References: 1. Kolodziej H (1984) Phytochemistry 23: 1745 – 1752.
**PM19**

**In vitro antileishmanial activity of resveratrol appears associated with cell cytotoxicity rather than antiparasitic properties**

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Recently, we reported the antileishmanial activity of resveratrol against Leishmania major GFP in infected BMMMs. In parallel we observed host cell cytotoxicity in a concentration-dependent manner, contrasting with claimed cell tolerability [1]. This apparent discrepancy prompted the present study using the reported resveratrol-tolerable J774-G8 cell line. When L. major GFP-infected J774-G8 cells were exposed to resveratrol (5 – 75 µg/mL), the resulting GFP signal was similarly reduced from 95% to 5% as a reflection of antileishmanial activity. However, host cell cytotoxicity invariably increased with sample concentrations as assessed by FACS analysis following staining with propidium iodide and apparent apoptosis-associated proteins [3]. Keywords: resveratrol, antileishmanial, cytotoxicity, BMMMs, J774-G8 cells References: 1. Kedzierski L et al. (2007) Parasitol Res 102: 91 – 97 2. Bhat et al. (2001) Antioxidants & Redox Signaling 3: 1041 – 1064 3. Li, G. et al. (2011) Phytomedicine

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**PM21**

The effect of Boswellia serrata on Giardia duodenalis

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Giardia duodenalis is a parasite that colonizes the small intestine of various mammalian hosts especially in humans. The common treatment for giardiasis includes metronidazole, furazolidone and benzimidazole-based drugs which cause many side effects besides an increasing resistance problem [1,2]. Having in mind that Boswellia serrata Roxb. is used for the treatment of chronic inflammatory disorders and that this parasite is known to facilitate these conditions [3], the gum resin of this plant source was tested for antigiardial activities. A crude extract standardized to 85% boswellic acids reduced the viability of the parasite by ca. 65% at a concentration of 20 µg/mL. At the highest concentration tested (80 µg/mL), the antigiardial effect was ca. 80% based on the metabolic conversion of resazurin [4]. Metronidazole (50 µg/mL) served as a positive control. In search for the active principle, the extract was subjected to HPLC separation showing two major peaks at Rt 12.3 and 14.7 min, respectively. The former, comprising a complex mixture of boswellic acids, exhibited pronounced antigiardial activity at 20 µg/mL, as evident from a ca. 80% reduction in parasite viability. HPLC analysis showed also the presence of oleanolic acid. Preliminary analyses proved this triterpenoid only moderately active (parasite viability ca. 65% at 45 µM corresponding to 20 µg/mL). This finding suggests boswellic acids as the active principle. Owing to the complexity of the fractions, the isolation of distinct boswellic acid members for antibacterial studies is still in progress. This is the first report on antigiardial effects of boswellic acids. Keywords: Boswellia serrata, antibacterial, boswellic acids, oleanolic acid References: 1. Gardner B and Hill D (2001) Clin Microbiol Rev 14: 114 – 128 2. Upcroft P and Mocray J (2001) Clin Microbiol Rev 14: 150 – 164 3. Layton MA et al. (1998) Brit J Rheumatol 37: 581 – 583 4. Bénére E et al. (2007) J Microbiol Methods 71: 101 – 106

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**PM20**

Targeting intestinal digestive enzymes by natural products: synergistic effect of flavonoids

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Diabetes is a common metabolic disorder that is caused by either inherited or acquired deficiency in insulin secretion or due to decreased responsiveness to insulin. One of the common approaches in the treatment of diabetes is decreasing postprandial hyperglycemia by inhibiting key enzymes for the hydrolysis of carbohydrates in the small intestine. In our laboratories, the effects of various natural products on key digestive enzymes, α-glucosidase and α-amylase, are routinely assessed [1]. It was found that some flavonoids including kaempferol-3-O-rutinoside (KR, Fig. 1) are potent inhibitors of these enzymes. A synergistic enzyme inhibitory effect: e.g. between KR and flavonoid aglycones, was observed for some flavonoids. The structure activity relationship established from the study and potential therapeutic implications are discussed.


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**PM22**

Anti-ulcerogenic Activity of the Standardized Water Extract of Phyllanthus emblica Linn

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Phyllanthus emblica Linn. (synonym: Emblica officinalis Gaertn.), Family Euphorbiaceae is native to the tropics of South and Southeast Asia. It is also called Emblic, Emblic myrobalan, Indian Gooseberry, Malacca tree and Myrobalan. In Thailand, it is known as Ma-kham-pom. P. emblica is an endemic plant commonly used in Asian traditional medicine systems for treatment of many disorders including anorexia, indigestion, and anemia (1, 2). The fresh or dry fruit is used in traditional medicine for the treatment of diarrhea, jaundice and inflammatory disorder (2, 3). The P. emblica water extract was prepared according to the Thai Herbal Pharmacopoeia and standardized. The phytochemical study, the P. emblica water extract contained tannins about 42.51%. The HPLC analysis of P. emblica water extract showed the presence of 20.48% gallic acid. Preliminary study, P. emblica water extract elicited the inhibitory effect on both COX-1 and COX-2 enzymes. Thus, the gastric ulcer may be one of the potential side effect of P. emblica water extract caused by its inhibitory effect on COX-1 enzyme. The oral administration of the P. emblica water extract at the dose of 600 mg/kg did not produce gastric lesions. On the contrary, the extract at the doses of 150, 300 and 600 mg/kg reduced ulcer formation in all tested acute gastric ulcer models i.e. ETOH/HCl-, indomethacin-, and stress-induced gastric lesions. These results indicate that P. emblica water extract possess anti-ulcerogenic effect. Keywords: Anti-ulcerogenic, Phyllanthus emblica Linn Acknowledgement: Royal Golden Jubilee Ph.D. Program and the National Research Council of Thailand. References: 1. Santisuk T et al. (2005) Flora of Thai-
Inhibition of angiogenic factors by laserolide, a sesquiterpene lactone from \textit{Laserium borhik.}

PM23

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Sesquiterpene lactones (SLs) are plant secondary metabolites, widely distributed within the families of Asteraceae and Apiaceae. SLs have received ever increasing attention for their beneficial effects in pathologies etiologically associated with angiogenesis, such as chronic inflammation and cancer. We have investigated the interference of SLs isolated from the non-pharmaeopoeia \textit{C.} European plant \textit{Laserium borhik. ex Gaertn.}, i.e. laserolide, isolaserolide, euclaserolide, archangelolide and 2-deangeloyl-archangelolide with production of angiogenic factors such as nitric oxide (NO), prostaglandin E2 (PGE2) and cytokines vascular endothelial growth factor (VEGF), interleukins IL-1 and IL-6.

We have employed the Griess reagent. Concentrations of PGE2 and cytokines were triggered by lipopolysaccharide (1 μg/mL RPMI-1640 medium for 24 h. The production of NO, PGE2 and cytokines was supported by the grant 305/07/0061 from GACR. PM25

\textbf{Screening on cytotoxicity, antioxidant and antimicrobial of stem bark from Malaysian \textit{Vatica odorata} and \textit{Vatica bella} (Dipterocarpaceae)}

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Dipterocarpaceae have proven to be rich sources of variety biological activities (Zain et al. 2010). In our continuing investigation on this family we wish to report the screening on three biological activities: cytotoxicity, antioxidant and antimicrobial of methanol and acetone extract from \textit{Vatica odorata} (Bgriff.) Symington and \textit{Vatica bella} Sooton. The antioxidant activity were evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH), Total Phenolic Content (TPC), Ferric reducing antioxidant power (FRAP) and Thiohibarbituric acid (TBA) method. The cytotoxicity activities were screened against Chang and HepG2 cells line (Mackeen et al. 1997). Meanwhile antimicrobial activity was conducted against six types of bacteria (Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Trichophyton rubrum, Pseudomonas aeroginosa and Klebsiella pneumonia) and four dermatophyte fungal species (Trichophyton mentagrophytes, Trichophyton tonsurans, Microsporum gypseum and Candida glabrata) by disc diffusion method (Barny et al.1979).

The results indicated that acetone extract of \textit{Vatica bella} displayed interesting biological activities. Aqueous and methanolic fractions were tested for cytotoxic, antioxidant and antimicrobial activities. The cytotoxicity assay were done using MTT colorimetric assay to determine the growth inhibition percentage of MT1, 6-farnesyl-3',4',5,7-tetrahydroxyflavanone. The biological activity of MT1 was evaluated for the first time. The cytotoxic activity of MT1 was evaluated by using MT assay, 6-farnesyl-3',4',5,7-tetrahydroxyflavanone exhibited strongly, moderately and very strongly the growth of HL 60, MCF-7 and HeLa cell lines with IC50 values of 14 ± 0.50 μg/mL and 20.5 ± 2.68 μg/mL respectively. Both the \textit{Vatica} extract displayed total phenolic content with range of 331.54 – 482.31 mg/g GAE and are weak DPPH scavenger as compared to standard with the range between 35.60 – 66.32. In TFC and TBA test, the Vatica extracts exhibited antioxidant potential with percent inhibition between the ranges 26.80 – 88.36%. The antimicrobial screening showed that both the crude extract inhibited moderately S. aureus except for acetone extract of \textit{Vatica bella}. Meanwhile methanol extract of \textit{Vatica bella} gave the active result where it inhibited moderately against \textit{Trichophyton mentagrophytes, Trichophyton tonsurans and Microsporum gypseum}. Keywords: \textit{Vatica bella, Vatica odorata, Dipterocarpaceae, oligostilbenoid, cytotoxicity, antimicrobial, antioxidant, antioxidant activity. We wish to thank to Ministry of Higher Education Malaysia for financial support via FRGS grant (011000070006) and UiTM for all the support References: 1-Zain WZWM, Ahmad N, Nawi L & Jusoh, K (2010) World Applied Science Journal 8(9):1050 – 1055. 2-Mackeen MM et al. (1997) Int J Pharmacognosy 35: 174 – 178 3-Berry AL et al. (1979). Journal of Clinical Microbiology 10: 885 – 889.

PM24

Participation of citral in the relaxation of isolated rat tracheal smooth muscle induced by ginger oil

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Ginger ( \textit{Zingiber officinale} ) Roscoe is a common food plant that has been used as alternative medicine for a number of ailments. The rhizome of this plant is well known for the treatment of gastrointestinal tract disorders, such as dyspepsia, nausea and vomiting, as well as respiratory illnesses [1]. Its hydroethanolic extract has been shown to inhibit hyperreactivity and remodelling, and lung inflammation [2 – 3]. The inhibitory effects of aequone and methanolic crude extracts of ginger on tracheal smooth muscle were described [4], however, the effects of ginger oil and its active compound on airway smooth muscle have never been reported. The aim of this study is to study the effect of ginger oil and its constituents on tracheal smooth muscle in vitro. Chemical compositions of ginger oil were determined by GC-MS. Citral and camphene appeared to be its major components. For the relaxation study, two-cartilage segments of rat trachea were prepared and mounted vertically in an organ bath. The contraction of tracheal smooth muscle was induced by acetylcholine (Ach) before ginger oil, citral or camphene were added. Ginger oil and citral, but not camphene, were found to reverse the Ach-induced airway contraction in concentration-dependent manner. This result indicates that citral is, at least partly, responsible for the myorelaxant effect of ginger oil on rat trachea. Keywords: Ginger oil, Citral, Tracheal smooth muscle, Relaxation Acknowledgement: This study is supported by the National Research Council of Thailand. References: 1. Gayyur MN et al. (2008) Can J Physiol Pharmacol 86: 264 – 271. 2. Kuo PL et al. (2011) Agric Food Chem (in press). 3. Amibire F et al. (2007) Prostaglandins Leukot Essent Fatty Acids 77:120 – 38 4. Gayyur MN, Gilani AH (2007) Eur Food Res Technol 224:477 – 481.

PM26

Isolation, cytotoxic and antiplasmodial activities of 6-farnesyl-3',4',5,7-tetrahydroxyflavanone from the flower of \textit{Macaranga triloba}

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The genus \textit{Macaranga} is one of the largest genera of the Euphorbiaceae, with approximately 300 species [1]. \textit{Macaranga} triloba Müll.Arg. locally known as “Mahang merah” is a tree endemic to Southeast Asia at forest and river margins and its water extract is used as pain relief for stomach trouble in Java [2]. The flower of \textit{Macaranga triloba} was collected from Pasir Raja, Hulu Terengganu (Malaysia), macerated successively with hexane, dichloromethane and methanol for 72 hours. The dichloromethane (DCM) extract (44.41 g) was dissolved in MeOH and subjected to vacuum liquid chromatography (VLC). The sub-fractions was further chromatographed on a reverse phase column chromatography (RPCC) that led to the isolation of MT1, 6-farnesyl-3',4',5,7-tetrahydroxyflavanone (58.1 mg) [3]. The biological activity of MT1 was evaluated for the first time. The cytotoxicity of MT1 was evaluated by using MTT assay. 6-farnesyl-3',4',5,7-tetrahydroxyflavanone exhibited strongly, moderately and very strongly the growth of HL60, MCF-7 and HeLa cell lines with IC50 value of 6.71, 11.38 and 2.64 μg/mL respectively. The DCM extract was also subjected to antiplasmodial screening which displayed an IC50 = 2.01 μg/mL indicating the potential as antiplasmodial agents. The antiplasmodial property of MT1 was evaluated using \textit{Plasmodium falciparum} with concentration of 10, 1, 0.1, 0.01 and 0.001 μg/mL. 6-Farnesyl-3',4',5,7-tetrahydroxyflavanone (MT1) was found to exhibit a strong antiplasmodial activity with an IC50 value of 0.06 μg/mL. This study
indicates the potential of MT1 anti-cancer and anti-plasmodium agents. Keywords: Macaranga triloba, 6-farnesyl-3’, 4’, 5, 7-tetrahydroxyflavanone, farnesyl, cytotoxic, antimalarial

6-Farnesyl-3’, 4’, 5, 7-tetrahydroxyflavanone (MT1)

Figure 1: MT1

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The role of rice bran extract on acetyl CoA carboxylase in liver of rats fed a high-fat diet Charkhonpunya C, Sireratawong S, Komindr S, Lerdvuthisopon N

The rice bran water extract (RBE) was shown to reduce fat mass in rats fed a high-fat diet [1]. The involvement of RBE in metabolic alteration were investigated in 7 groups of Sprague Dawley rats, 8 rats each. High-fat fed group 3 to 7 were either co-treated daily with RBE (2205, 2205, 4410 mg/kg) or metformin (15, 10, 38.20 mg/kg). Oral glucose tolerance was tested at the end of forth week. Rats were killed and specimens were collected. The results showed that the mean ± SEM of abdominal fat weight, epididymal fat cell size and triglyceride level in blood and liver were increased whereas cholesterol in high-density lipoprotein was decreased in rats fed a high-fat diet alone as compared to rats fed with chow (7.65 ± 0.29 vs. 4.73 ± 0.39 g; 4919.76 ± 453.59 vs. 2832.85 ± 249.15 µm; 44.67 ± 2.72 vs. 35.00 ± 2.02 mg/dl; 250.0 ± 0.26 vs. 1.48 ± 0.08 mg/dl; 67.14 ± 1.62 vs. 77.20 ± 2.82 mg/dl, respectively). At least 2205 mg RBE/kg or 19.10 mg metformin/kg were able to significantly reduce abdominal fat weight and triglyceride levels in liver. ACC activity was increased in high-fat feeding group but the activities returned to normal when they were also received RBE or metformin, though there was no statistic significance. In conclusion, fatty acid flux might induce fat synthesis in liver as an initial step in increasing fat accumulation [2]. Both RBE and metformin were able to reduce the alteration. Keywords: Rice bran, acetyl CoA carboxylase, high-fat diet.


PM27 Antifungal activity of the extract of Alpinia officinarum Hance rhizomes on Candida albicans Klahan K, Nantapong N, Chadaupongse N School of Biology, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima, 30000 Thailand

Alpinia officinarum Hance, known as lesser galangal, is a pungent and aromatic plant which is used as spice for flavoring food throughout Asian countries [1]. This plant has also been used as traditional medicine for several purposes such as relieving stomachache and pain, treating colds, invigorating the circulatory system, and reducing inflammation and swelling [2]. Candida albicans is a major causative microbe of fungal infection, especially in patients with endocrine disorders, immunosuppression, malignant disorders and AIDS [3]. Nowadays, choices of antifungal against candidiasis are quite limited due to drug toxicity and resistance. The crude extract of the rhizomes of Alpinia officinarum has been shown to possess antibacterial activity [4], however, antifungal activity of this plant has not been reported. In the present study, we found that lesser galangal exhibited antifungal activity against Candida albicans. The minimum inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) value were 1.2 mg/ml and 2.0 mg/ml, respectively. The assessment of cell damage produced by the crude extract of Alpinia officinarum rhizomes was conducted through scanning electron microscope (SEM) observation. SEM analysis showed that the extract induced deformation of Candida albicans. The treated cells had coarse surface and changed from oval to rounder shape. The result suggested that the extract damaged cell wall, causing Candida albicans to form spheroplast. This postulated mechanism may contribute to the antifungal activity of the crude extract of the Alpinia officinarum rhizomes against Candida albicans. Keywords: Alpinia officinarum Hance, Candida albicans, minimum inhibitory concentration, minimal fungicidal concentration, scanning electron microscope

PM28 Wound healing potentials of a herbal homeopathic remedy on NIH 3T3 fibroblasts in vitro Hostanska K, Rostock M, Saller R University Hospital Zürich, Institute for Complementary Medicine, Zürich, Switzerland

This study was aimed to investigate the effect of the commercial homeopathic remedy Similasan® Arnica plus Spray (Similasan AG, Zürich, Switzerland), which is an ethanolic (22% m/m) preparation of Arnica montana L. D4, Calendula officinalis L. D4, Hypericum perforatum L. D4 and Symphytum officinale L. D6 (0712–2), on wound healing in cultured NIH 3T3 fibroblasts. Wound healing requires the coordination of complex cellular and molecular interactions. Therefore we investigated the cell proliferation, migration and wound closure promoting effect of the preparation and its potentized hydroalcoholic solvent (0712–1) using BrdU uptake, transwell chamber assay and wound healing scratch assay, respectively. All assays were performed in a controlled, blinded manner at least in three independent experiments. The preparation (0712–2) exerted a stimulating effect on cell migration 31.7% vs. 15% solvent (0712–1) at 1:100 dilutions (p < 0.05, n = 3). Mean wound closure reached 59.5% by preparation in comparison to 22.1% by solvent (p < 0.05, n = 3) at the same concentration. Positive control (5% FCS) caused 63.0% closure. There was no effect of cell proliferation. In conclusion, the Similasan® Arnica plus homeopathic remedy showed wound healing activity in the NIH 3T3 fibroblasts scratch assay, which may be partly a result of fibroblasts migration and does not seem to be related to mitotic activity.

Figure 1: Wound closure of 3T3 fibroblasts by Similasan Arnica plus preparation Representative microphotographs of wound healing effect of preparation (1:100) on 3T3 fibroblasts after 24 h treatment. Indicated percentages of wound closure are normalized to the untreated control (medium). Keywords: wound healing, 3T3 fibroblasts, homeopathic preparation...
Antioxidant activity of crude extract from Algerian chemlal olive leaves and application in stored meat

**PM31**

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In this study an aqueous crude extract and oleuropein have been obtained from Algerian olive leaves (variety chemlal). The antioxidant effect of these compounds was determined in stored meat by TBA-RS methods. These compounds were added to chicken meat at 500 mg/kg. All meat samples have been stored in the aerobic conditions at 4–20°C for one week. The results showed that compounds of olive leaves have a remarkable antioxidant activity throughout the storage phase. However, crude extract showed higher activity on lipid oxidation. The sensory analysis showed that meat “off-flavour” added to these compounds reduced the possibility of using compounds of olive leaves as potent natural antioxidants in stored chicken meat.

Keywords: olive leaves, crude extract, oleuropein, Antioxidant activity, stored chicken meat
Acknowledgement: The authors are grateful to Ministerio de Asuntos Exteriores y Cooperación de España (AECID) and Ministerio de Salud, Consumo y Bienestar Social for financial assistance to this work within the Programa de Cooperación Interuniversitaria e Investigación Científica PCI/MED Algeria-Spain (grant ALI A/011170/07; A/019342/B; A/023365/C; A/033506/10) and CNEPRU (F00520090025), respectively.

Antioxidant potential, cytotoxic activity and phenolic content of Clematis flammula leaf extracts

**PM32**

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Five fractions of Clematis flammula L., a plant widely used in the Mediterranean traditional medicine, were isolated from the leaves using a selective extraction procedure and their total antioxidant capacity was measured by both the ABTS and ORAC tests. Furthermore, their capacities to inhibit microsomal lipid peroxidation and to scavenge the hydroxyl radical were assessed. The cytotoxic potential of the crude ethanolic extract and the aqueous fraction obtained from chloroform was also evaluated on three human hepatoma cell lines (HL-60, PLC and HuH7). The results showed a stronger antioxidant capacity for the two aqueous phases obtained from ethyl acetate and chloroform concerning ABTS (79 and 10.5 mmoles Trolox eq/g of plant extract, respectively), ORAC (487 and 387 mmoles Trolox eq/g of plant extract, respectively) and hydroxyl radical scavenging activity (IC50 = 36.5 and 48.4 µg/mL, respectively), compared to their organic counterparts which, however, inhibited microsomal lipid peroxidation more efficiently (IC50 = 390.7 and 523.5 µg/mL, respectively). The ethanol crude extract exhibited a fairly good cytotoxic potential on the two cell lines HL-60 and PLC (IC50 = 58.5 and 47.3 µg/mL, respectively), in contrast to the aqueous phase obtained from chloroform (IC50 = 457.7 and 304.9 µg/mL, respectively). A positive correlation was also found between the phenol content and the different activities. These results provide experimental support for the therapeutic virtues of Clematis flammula leaf extracts. Keywords: Clematis flammula, anti-cancer, antioxidant, phenolic compounds


Antioxidant capacity of Pistacia lentiscus and Fraxinus angustifolia extracts and their fractions

**PM30**

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Oxidative stress is thought to be the main cause of several pathologies; that is why research is focusing on the characterization of bioactive natural substances with antioxidant activity to replace synthetic molecules. The antioxidant activity of extracts of Fraxinus angustifolia Reut. ex Nyman and Pistacia lentiscus L., two medicinal plants used to treat inflammatory-related disorders was determined. The results indicated that aqueous chloroform extract of Pistacia lentiscus exhibited a great reducing power of 657.86 ± 35.25 mg Ascorbic Acid Equivalent/g of the extract, compared to that of the aqueous extract from Fraxinus angustifolia (260.73 ± 22.38 mg Ascorbic Acid Eq/g of extract). Extracts issued from both plants showed an outstanding capacity in scavenging the free radicals. In this study an aqueous crude extract and oleuropein have been obtained from Algerian chemlal olive leaves and application in stored meat.

Keywords: Fraxinus angustifolia, Pistacia lentiscus, Phenolic compounds, antioxidant, active fractions

**PM34**

**Hypoglycemic properties of banana pseudo-stems**

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Water extract of banana (Musa sapientum L.) pseudo-stems has been claimed by Lebanese herbalists to be efficient in the treatment of diabetes mellitus. This work aimed at verifying the alleged effect and at elucidating its possible mode of action. Administration of the extract in replacement of drinking water to streptozotocin-induced diabetic rats, did reduce significantly blood glucose levels. The mechanism of action of the extract was studied by investigating its involvement in intestinal glucose absorption and its effect on the Na+/K+ ATPase and glucose transporters SGLT1 and GLUT2 in the rat jejunum. Rat jejunum were perfused in situ with Krebs Ringer buffer containing [14C] 3-O-methyl-D-glucose, and the activity of the Na+/K+ ATPase in jejunal homogenates was assayed in vitro, by measuring the amount of inorganic phosphate released in presence and absence of inhibitors of the ATPase. The extract induced a significant reduction in glucose absorption and Na+/K+ ATPase activity, but did not affect the protein expression of SGLT1 and GLUT2 glucose transporters. It was concluded that the extract acts by reducing the Na+/K+ ATPase activity, but did not affect the protein expression of SGLT1 and GLUT2 glucose transporters. It was concluded that the extract acts by

**Acknowledgement:** This work was supported by a grant from the University Research Board. References: 1. K. Kellet GL et al (2000) Biochem J 350:155 – 162

**PM35**

**Radical scavenging activity and phenolic components in different plant parts of Saraca asoca**

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The therapeutic properties of herbal drugs depend on certain chemical constituents (secondary metabolites) which varies according to age and maturity of the plant. Phenolic compounds have multiple biological properties as antioxidants, they protect the human body against damage by reactive oxygen species. Medicinal plants have been focused for antioxidant compounds because of safety concerns associated with synthetic antioxidants. Saraca asoca (Roxb.) Wilde (Fabaceae) an important medicinal tree has been well known for its effectiveness in menorrhagia and dysmenorrhea. Its bark has stimulating effect on the endometrium and ovarian tissue and has been used traditionally for gynecological disorders. Different plant parts: bark, leaves and twigs of various aged group trees of Saraca asoca were evaluated for their total phenols (TP), total flavonoids (TF), tannins (T), phenolic acids contents and radical scavenging activity. TP varied from 5.27 – 8.65%, TF from 0.16 – 0.28%, T from 20.88 – 51.17%. This is the first study in which different phenolic acids were estimated in Saraca asoca; vanillic acid varied from 2.34 – 5.07%, caffeic acid from 1.37 – 7.15%, chlorogenic acid from 8.51 – 25.59%, gallic acid from 0.17 – 0.46% and catechin from 4.78 – 7.95 mg/100 g. Radical scavenging activity showed significant variation among different girth classes and IC50 values ranged between 2.29 – 4.82 mg/ml. Bark was found to contain maximum concentration of active ingredients. The results revealed that the optimum girth class to obtain quality produce is 61 – 90 cm as it contains maximum concentration of active ingredients and possess high radical scavenging activity. Thus it can be used for making various formulations containing natural antioxidants. Keywords: Antioxidants, phenolic acids, harvesting age, Saraca asoca Acknowledgement: The authors are thankful to the Director, Tropical Forest Research Institute for providing necessary facilities to carry out the research work. The work was supported by a grant from the National Medicinal Plant Board (NMPB), Govt. of India, New Delhi.

**PM36**

**Decrement of body fat and hypolipidemic effect of 3,4,5-trihydroxybenzaldehyde isolated from Geum japonicum in high fat diet-induced obese rats**

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3,4,5-trihydroxybenzaldehyde (THBA) is isolated from the aerial part of G. japonicum Thunb., a Korean herb belonging to the family of Rosaceae [1]. In a recent study we have shown that the ethyl acetate fraction of G. japonicum inhibited NO production by LPS-activated RAW 264.7 cells and the natural THBA showed a remarkable scavenging activity on the DPPH radical[2]. Being on these findings, we determined the preventive effect of 3,4,5-trihydroxybenzaldehyde (THBA) for adiposity and dyslipidemia using high fat diet-induced obese rats. As a result of the investigation for lipid and leptin metabolism in obese rats, body weight, adipocyte cell size and visceral fat mass was significantly reduced by feeding with THBA. The concentration of triglyceride and leptin in serum was also significantly reduced whereas the insulin level, the dried leaves of D. linearis were extracted with aqueous, chloroform and methanol. The extracts were tested against HL 60 (acute promyelocytic leukemia cell lines) using MTT assays. The methanol extract (Table 1) showed the promising cytotoxic activity against HL 60 (IC50 = 7.9ug/ml). The methanol extract was further partitioned in sequence with n-hexane, chloroform and methanol. The extracts and fractions

**Cytotoxicity activities of Dicranopteris linearis extracts and fractions**

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Cancer is currently a second leading cause of death in the world [1]. Therefore the newer anticancer drugs from natural product are needed to replace the synthetic drug from the chemical compound. Based on the traditional medicinal value, Dicranopteris linearis (Burm.f.) Underw. from the Gleicheniaceae family was shown to possess pharmacological potential such as cytotoxic activity. For initial investigations, the dried leaves of D. linearis were extracted with aqueous, chloroform and methanol. The extracts were tested against HL 60 (acute promyelocytic leukemia cell lines) and WRL 68 (normal liver cell line) using MTT assays. The methanol extract (Table 1) showed the promising cytotoxic activity against HL 60 (IC50 = 7.9ug/ml). The methanol extract was further partitioned in sequence with n-hexane, chloroform and methanol to determine which extract contain the most active constituents. The result showed the methanol fraction to be significantly active against HL 60 with the value IC50 = 12.88 µg/ml and non toxic to normal cell. The methanol fraction was then subjected to the bioassay-guided fractionation. The fractionation by vacuum liquid chromatography (VLC) gave eleven fractions labeled as F1, F2, F3, F4, F5, F6, F7, F8, F9, F10 and F11. The fraction F7 (Table 2) demonstrated cytotoxic activity with the best value (IC50 = 25.12 µg/ml). In addition, it was also found to be non-toxic against normal cell. Further work involving the isolation of active compounds in this potent ferns would be necessary to elucidate the actual source of the observed bioactivities. Keywords: Dicranopteris linearis, Gleicheniaceae, MTT assays, acute promyelocytic leukemia cell lines, normal liver cell line Acknowledgement: This study was supported by the research grant [02 – 01 – 01-SP002] from the Ministry of Science, Technology and Innovation, Malaysia. References: [1]. Pratt WB, Ruddon RW, Ensminger WD, Maybaum J [1994] The Anticancer Drugs. 2 Eds. Oxford University Press: New York, 3 – 16.
Antinociceptive activity of Muntingia calabura leaves
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The aims of the present study were to develop the in vivo antinociceptive profile of methanol crude extracts of Muntingia calabura L. leaves and its fractions. The antinociceptive activity of orally-administered test solutions was assayed using the formalin test in rats. Based on the data obtained, the methanol extract of M. calabura (MEMC) exhibited the most effective (P < 0.05) antinociception in the 1st and 2nd phases of the assay in a dose-dependent manner followed by the aqueous and chloroform extracts. The MEMC was then partitioned with petroleum ether (PEP) followed by ethyl acetate (EAP) and the remaining residue was dissolved in distilled water (AQW). Following subjectio of the formalin test, the PEP (100, 500 and 1000 mg/kg) exerted the most effective antinociception (P < 0.05) in both phases of the assay in a dose-dependent manner followed by the EAP and AQW. The PEP was subjected to the fractionation processes and yielded 7 types of fractions labelled as FA, FB, FC, FD, FF, FG and GA. All fractions, in the dose of 300 mg/kg, were subjected to the assay and only fractions FC, FD, and FF demonstrated significant (P < 0.05) antinociception at least in the 2nd phase of the test. In conclusion, the antinociceptive activity of M. calabura involved modulation of central and peripheral pain mechanisms and attributed to the presence of flavonoids. Keywords: Muntingia calabura; Elaeocarpacaeae; Antinociceptive activity; Formalin test; Flavonoids. Acknowledgement: The authors would like to thank Universiti Putra Malaysia for providing the Research University Grant Scheme (RUGS; Reference no: 04 – 02 – 10 – 0925RU) and Ministry of Science, Technology and Innovation (MOSTI) for granting the eScience Fund References: 1. Zakaria ZA et al. (2006) Fundamental and Clinical Pharmacology 20(4): 365 – 372. 2. Zakaria ZA et al. (2007) Journal of Natural Medicines 61(4): 443 – 448. 3. Zakaria ZA et al. (2011) American Journal of Chinese Medicine 39(1): 183 – 200.

Neural mechanisms and sedative effect of different fractions of Holarrhena floribunda (Apocynaceae) stem bark in mice
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Holarrhena floribunda G. Don (Apocynaceae) stem bark is used locally in the treatment of mental illness in Nigeria. This work examined the neural mechanism and the sedative properties of the crude extract and its fractions. Acute toxicity studies were carried out to establish the safety parameters of the crude extract and fractions by oral and intraperitoneal administration using Lorke (1983) method. The effect of the crude extract of Holarrhena floribunda and the fractions; hexane, chloroform, ethylacetate, butanol and aqueous was examined on novelty-induced rearing and grooming behaviors and on pentobarbital-induced sleeping time in mice. The results show that the crude extract and the fractions were not toxic with the exception of butanol fraction. The crude extract and fractions reduced novelty-induced rearing and grooming behavior in mice. The inhibitory effect of the crude extract and the fractions were not reversed by atropine, ciproheptadine, yohimbine and naloxone; however the crude extract and the fractions blocked the facilitating effect of flumazenil. This suggests that the crude extract and the fractions appear to facilitate GABA-ergic neurotransmission. The crude extract, aqueous, butanol and chloroform fractions prolonged pentobarbital-induced sleeping time in mice which were blocked by flumazenil a GABA(A) antagonist, indicating that the crude extracts, aqueous, butanol and chloroform fractions contain GABA agonist. The result suggests that the stem bark of Holarrhena floribunda possess sedative effects which may be mediated through GABA-ergic neurotransmission. Keywords: Holarrhena floribunda, Rearing, Grooming, Sedative, GABA References: Lorke D (1983) Arch Toxicol 54: 275 – 287.

Studies of analgesic activity of the different fractions of Holarrhena floribunda stem bark in mice
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The antinociceptive effect of crude extract of Holarrhena floribunda T.Durand & Schinz together with its fractions prepared with hexane, chloroform, ethylacetate, butanol and aqueous were investigated using hot plate, tail immersion and acetic acid-induced abdominal constriction tests in mice. Acute toxicity studies were carried out on the crude extract and fractions by oral and intraperitoneal administration using Lorke (1983) method. The crude extract was prepared by soaking the stem bark in 70% ethanol for 72 hours. It was filtered and evaporated under reduced pressure. The dry crude extract was dissolved in distilled water followed by butanol, ethylacetate, hexane and chloroform in a separating funnel to obtained the fractions. The result shows that the crude extract and the fractions were not toxic with the exception of butanol fraction. The crude extract and the fractions show dose dependent significant (P < 0.05) antinociception in the 2nd phase of the formalin test. They also reduced the abdominal constriction induced by acetic acid in mice. The analgesic effect of the extract and fractions were reversed by naloxone an opioid receptor antagonist in the hot plate and tail immersion test; while naloxone did not reverse the inhibitory effect of the extract and fractions in acetic acid-induced abdominal constriction test. The results show that the central analgesic effect of the crude extract and fraction is mediated through opioid receptor in the brain. The peripheral analgesic effect of the extract and fractions is not mediated through opioid receptors. Keywords: Analgesia, Holarrhena floribunda, hot plate, tail immersion, writhing, opioid References: Lorke D (1983) Arch Toxicol 54: 275 – 287.

Hepatoprotective effects of Artemisia monosperma and silymarin on carbon tetrachloride-induced hepatic damage in rat
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The hepatoprotective effect of aqueous ethanol extract of Artemisia monosperma Delile aerial parts was investigated against carbon tetrachloride-induced acute hepatotoxicity in rat. The hepatoprotective activity of A. monosperma was evaluated by determination of liver enzymes marker in the serum (aspartaat amino transferase AST; serum alanine transaminase ALT and alkaline phosphatase ALP). The histopathological studies were also carried out to observe the alteration in the liver sections. Oral administration of A. monosperma (100 and 200 mg/kg) markedly reduced the elevated values of marker enzymes caused by CCl4 – treatment. Glutathione (GSH) significantly decreases in the group treated with CCl4. The two doses of A. monosperma and silymarin (25 mg/kg) significantly increased GSH values when given in combination with CCl4. However, silymarin normalized liver enzymes and increased GSH levels than A. monosperma (two doses) when compared with the control group. A comparative histopathological study of liver of rat treated with A. monosperma exhibited almost normal architecture, compared to CCl4-treated group. Image analysis of liver revealed a marked reduction in the group treated with A. monosperma compared with CCl4-treated group. Phytochemical study of A. monosperma showed the presence of flavonoids, terpenoids, saponins and steroids. The results of the current studies suggest that the effect of A. monosperma is probably due to combined effect of flavonoids. Keywords: Artemisia monosperma, CCl4, enzymatic, silymarin.
**PM42**

**Kaerophyllin Suppresses Hepatic Stellate Cell Activation by Apoptotic Bodies and Ameliorates Hepatic Fibrosis in Rats**

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Hepatocyte apoptosis is a central feature of many liver diseases, leading to liver inflammation and fibrosis. In this study, we screened potential drugs inhibiting hepatic stellate cell (HSC) migration induced by hepatoxytic apoptotic bodies (ABSs) and evaluated the in vivo therapeutic effects in a rat model of hepatic fibrosis induced by thioacetamide (TAA). Rat HSCs were exposed to UV-irradiated hepatocyte ABS or TNF–α to investigate the anti-fibrotic effects of kaerophyllin. Liver fibrosis was induced by TAA injection into rats twice weekly for 6 weeks. Kaerophyllin (10 or 30 mg/kg) or curcumin (150 mg/kg, as a positive control) was given by gavage twice daily for 4 days starting 2 weeks after TAA injection. Kaerophyllin (α-(trans-3,4-dimethoxybenzylidene)-β-(3,4-dimethoxy-endoxy-benzyl)-γ-butyrolactone, a lignan isolated from a Chinese herb Bupleurum scorzonerifolium by bioactivity-guided fractionation) attenuated ABS- and TNF–α-induced HSC migration, protein levels of collagen I and α-SMA, and the mRNA levels of ICAM-1, MCP-1 and IL-1β genes, but elevated PAR-γ luciferase activity. Furthermore, kaerophyllin reduced TNF–α and ABS-induced NF-κB luciferase activity with decreased p65 phosphorylation and p65 nuclear translocation. In TAA rats, kaerophyllin and curcumin treatment significantly protected liver from injury by reducing serum AST and ALT levels, and improved the histological architecture and fibrosis score. In addition, kaerophyllin treatment suppressed α-SMA protein expression, and mRNA levels of collagen I, TIMP-1, TNF–α, IL-1β and MCP-1 genes in TAA rats. Our results demonstrated that kaerophyllin protected the rat liver from TAA-caused injury and fibrogenesis by suppressing hepatic inflammation and inhibiting HSC activation. Keywords: hepatic stellate cells, liver fibrosis, kaerophyllin, inflammation, Bupleurum scorzonerifolium. Acknowledgement: This work was supported by the National Science Council and the National Research Institute of Chinese Medicine in Taipei. References: [1] Friedman SL (2008) Gastroenterology 134: 1655 – 1669. [2] Marra F (2002) Front Biosci 7: d1899 – 1914. [3] Canbay A et al. (2003) Hepatology 38: 1188 – 1198.

**PM43**

**Bioassay-guided Isolation of Cytotoxic Fractions from Menthogastrica Leafe**

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M. calabura L. or locally known as “Kerukup Siam”, belongs to the family Elaeocarpaceae [1]. This plant is native to American continent and is widely cultivated in warm areas of Asian region, including Malaysia [2]. The leaf is used to provide relief from gastric ulcers and to reduce swelling of the prostate gland as reported in Peru folklore medicinal uses. The aim of the present study is to determine the in vitro cytotoxic activity of Menthogastrica leaf against cancer (HL60 and MCF-7) and normal (WRL68) cell lines using MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] assay as described by Mosmann [3] but with slight modifications. The crude medicinal extract of M. calabura (MCMC) was suspended in distilled water to afford an aqueous MeOH solution and then partitioned with petroleum ether and EtOAc to give petroleum ether, EtOAc and aqueous extracts. The EtOAc extract showed significant cytotoxic activity when tested against HL60 (IC50 = 27.48 ± 3.60 μg/ml) and was further fractionated using vacuum liquid chromatography with gradient mixture of hexane-EtOAc (from 9:1 to 1:9) as solvent systems. Seven fractions were obtained (F1-F7), and subjected to cytotoxic activity against HL60, MCF7 and WRL68 cell lines. The IC50 values of the M. calabura extracts and fractions are shown in Table 1. Fraction 5 tested against HL60 showed strong inhibition (IC50 = 3.98 ± 0.09 μg/ml) as compared to the other cell lines as well as other fractions. Fraction 5 will be isolated further and the bioactive compounds responsible for the activity will be determined in the future study.

**PM44**

**Pharmacokinetics of linalool and linalyl acetate, the two main constituents of silexan, an essential oil from Lavandula angustifolia flowers, in rats**

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Silexan is the active ingredient in Lasea<sup>®</sup>, which has recently been approved for the treatment of restlessness and mild anxiety in Germany. The naturally occurring enantiomers R-(-)-linalool and R-(-)-linalyl acetate (LA) are the main constituents of silexan representing 70–80% of the total oil. We investigated the bioavailability and organ distribution of L and LA in rats by headspace GC-MS after administration of silexan or the single constituents. The peak concentrations of L after 100 mg/kg silexan was 77 ng/ml in plasma, 228 ng/g in liver, 670 ng/g in kidney, 2085 ng/g in fat and 164 ng/g in brain tissue. LA was only measured in the brain (31 ng/g). Administration of 28.9 mg/kg L which corresponds to the amount contained in 100 mg silexan, resulted in peak concentrations of 33 ng/ml in plasma, 218 ng/g in liver, 541 ng/g in kidney, 1140 ng/g in fat and 45 ng/g in brain tissue. The gavage of 36.8 mg/kg of LA, the equimolar amount to 28.9 mg/kg L resulted in peak concentrations of 10 mg/ml in plasma, 274 ng/g in liver, 255 ng/g in kidney, 244 ng/g in fat and 0 ng/g in brain tissue. LA itself was only found in the brain and in fat tissue. The results indicate that the bioavailability of L is generally higher when applied as total as compared to the application of the single constituents. Interestingly, LA is very rapidly metabolized into L and can only be detected in the brain and in fat tissue. Keywords: Pharmacokinetics, Lavandula, Silexan, Bioavailability.

**PM45**

**Antiviral Activity of Aloe hijazensis against Some Haemagglutinating Viruses Infection and its Phytoconstituents**

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On our ongoing for investigating the bioactive compounds of A. hijazensis Lavrano & Collen. (Abd-Alla et al., 2009), one of 24 species of Aloe in Saudi Arabia (Collelnette, 1999); the flowers and flower-peduncules were selected for the present study. Thirteen compounds were isolated from both flowers and flower-peduncules. The isolated compounds were classified into: five hydroxyquinones; ziganin, Ziganin 5-methyl ether, aloesaponarin I, chrysophanol, aloe-emodin, one dihydroisocoumarin; feralodial, four flavonoids; homoplagmatin, isoorientin, luteolin 7-glu-
The use of a new phyto drug Sutiglian in gynaecology for treatment of inflammatory processes

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The aim of the research is an assessment according to the microbiological parameters of 3% suttiogin ointment on the basis of Suttiogin substance from a grass of Euphorbia soongarica Boiss., as vaginal swabs. In 185 female patients with inflammatory processes of the pelvic organs suttiogin ointment was applied. The levomekol ointment (55 patients) and 10% the metiluratsil ointment (43 patients) was applied in the control groups. Along with a local therapy there was a general treatment with antibiotics, desensibilization and detoxification drugs. During the treatment, a quantitative microbiological examination of smears from the cervical canal, urethra and vagina was carried out in women with inflammatory conditions of the pelvic organs. The intake of material was within the time of patients admission and continued in the dynamics for 2 – 3 days. Before the treatment, semination wounds of Staphylococcus aureus were 105 – 106 microbial bodies in genital habitats. On the 5th and the 6th days of the therapy with the use of this ointment was a completed result in a correcting completion of the genital habitat of Staphylococcus aureus. The content of Staphylococcus aureus in genital swabs in the control groups on the first day there were 105 - 106 microbial bodies, while on the third day 105 and on the fifth day – 103. In comparison with the ointments of levomekol and 10% metiluratsil 3% suttiogin ointment reduced the duration of a genital cleansing of biotopes from microflora to more than 1.3 – 1.5 times and improved therapy.

Mathematical model for Glucose-Insulin interactions after administration of the Arctium lappa extract in diabetic rats

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Diabetes is a widespread chronic disease which is increasing at an alarming rate in the world. It can lead to a variety of vascular, neurological or metabolic complications. Maintaining blood glucose levels within the normal range by exogenous insulin administration or oral administration of plant extracts which increase plasma insulin levels can decrease these effects. Mathematical models have provided one means of understanding diabetes dynamics. In this study we used one of these models "minimal model" (1) based on our experimental data for estimation of plasma insulin from plasma glucose. We used a modified Michaelis Menten equations (2) in our model. Diabetes was induced by intraperitoneal injection of streptozotocin (80 mg/kg). After 24 hours of food deprivation, blood samples were collected from the orbital sinus before, and at 1, 2, 3 hours after oral administration of Arctium lappa extract. Blood glucose and insulin level were determined by glucose oxidase and standard radioimmunoassay methods, respectively. In diabetic rats, plant extract increased blood insulin levels (p<0.05) and decreased blood glucose levels (p<0.01). Results showed the above model can predict plasma insulin level from plasma glucose value. Keywords: diabetics, Arctium lappa, insulin-glucose References: (1) Bergman RN, Cobelli C (1980) Federation Proc 39: 110 – 115. (2) Lin J (2007) Robust modeling of the glucose-insulin system for tight glycemic control of critical care patients, Ph.D. Thesis, Department of Mechanical Engineering, University of Canterbury, New Zealand.

PM48

Evaluation of acute toxicity of betulin

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Betulin is a pentacyclic triterpene alcohol belonging to the lupane series of compounds. It is extracted from the outer burch bark. The birch triterpenes have known antiallergic, antiviral, antimicrobial, antitumor and hepatoprotective effects [1-3]. Although to our knowledge no reports about acute toxicity of betulin were published. Acute toxicity of betulin was studied on rats and mice. Betulin (98%) was provided by VTT. Male and female outbred rats and mice were administered with betulin in single dose of 1000 – 16000mg/kg intragastric and rats in single dose of 250 – 4000 mg/kg intraperitoneally. No significant effect of betulin administration on body weight of animals and no lethal effect were observed during 14 days after administration in rats and mice in all doses tested. LD50 was not reached in all experiments. Skin irritation, edema or infiltration at the injection site after intraperitoneal injection was not observed. Irritation of the gastrointestinal tract of rats and mice, and peritonium in rats at the site of drugs injection has been not fixed. The form and location of all internal organs were without pathology both in rats and mice and there were no significant changes in mass coefficients of organs. In result of single intragastric administration or intraperitoneal injection of betulin in rats and mice no toxic effects were observed. Results of 14 days of observation of animals and data of necropsy evidenced about safety of this substance. Betulin is non-toxic and may be classified as substance of VI class toxicity [4]. Keywords: betulin, intragasric administration, intraperitoneal injection, mice, rats Acknowledgement: the study was done in frame of FORESTSPECs project, grant agreement 227239 References: 1. Alakurtti S et al. (2006) Eur J Pharm Sci 29: 1 - 13 2. Krasutsky PA (2006) Nat Prod Rep 23: 919 – 942 3. Shikov AN et al. (2011) Phyu Pharm Sin j.phymed.2011.01.021; 4. Gosselin RE et al. (1976) Clinical Toxicology of Commercial Products. Acute Poisoning, 4th edition. Baltimore: Williams and Wilkins.

PM49

Trigona laevisceps propolis: Chemical compositions and antiproiferative activity on cancer cell lines

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Cancer is a leading cause of death worldwide and continue rising. Many cancer patients resisting to recent chemotherapy agents, so it is very important to search for new compounds with antitumor activity and develop to be anticancer drugs. Propolis of stingless bee (Trigona laevicaps) is focused in this research. It was extracted by 95% ethanol and partitioned through their polarities with 40% MeOH, CH2Cl2 and hexane. All parts were tested for antitumor activities against five tumor cell lines (Chaga, Kato-III, SW620, BT-474 and Hep-G2) by MTT assay. In addition, the cytotoxicity against two normal cell lines (Fibroblast and CH-liver) was performed by the same assay. Due to IC50 value, the hexane part had the highest antiproliferative activity and the lowest cytotoxicity on normal cell lines. The hexane part was therefore purified further by column chromatography. Eight fractions (100HEX, 100DCM, 30DCM, 50DCM, 70DCM, 100DCM, 5MET and 10MET) were obtained. After MTT assay, the most effective fractions were 30DCM and 100DCM. Both were separately purified by size exclusion chromatography, yielding totally 8 fractions (30DCM-F1, 30DCM-F2, 30DCM-F3, 30DCM-F4, 100DCM-F1,
Ascorbic acid content, phenolic compounds and antioxidant capacity of Brazilian exotic fruits açai (Euterpe oleracea Mart.) and cupuçu (Theobroma grandiflorum Schum.)

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It is well-known that diets high in fruits and vegetables may decrease the risk of chronic diseases, due to their low fat content and high levels of fiber and antioxidant substances, such as ascorbic acid and polyphenols. Current work describes the characterization of two Brazilian exotic fruits namely açai (Euterpe oleracea Mart.) and cupuçu (Theobroma grandiflorum Schum.), for their: antioxidant capacity; ascorbic acid content; and total polyphenolic compounds. Antioxidant capacity was determined in pulps by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Ascorbic acid was quantified by ultra-fast liquid chromatography using a Shimadzu™, UFLC-20A chromatograph with a reversed-phase octadecylsilylane column XR-ODS/C27, and 0.025 M of a dihydrogen potassium phosphate solution as the mobile phase. Polyphenolic compounds were determined by the Folin-Ciocalteau method. Antioxidant capacity, expressed in terms of grams of pulp per 100 g of DPPH, was 166.76 for the açai fruit, and 4366.71 for the cupuçu fruit. Ascorbic acid was not detected in açai pulp, and its content was 7.04 mg per 100 g of pulp in cupuçu pulp. Total phenolic compounds content, was 108.5 expressed in terms of gallic acid equivalent per 100 grams of pulp for the açai and 91.85 for the cupuçu pulp. Results pointed out the nutritional and therapeutic potentials of these exotic fruits, for their antioxidants properties. Keywords: food phenolics, data base, liquid chromatography, nutritional properties

Acknowledgments: We thank the INCT/CNPq (National Council for the Development of Science & Technology, Brazil) for the financial support received while the fourth and fifth co-authors also thank CAPES for fellowships.

Phenolic metabolites from Acacia nilotica flowers and evaluation of its free radical scavenging activity

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Reactive oxygen species (ROS) have been recognized as playing an important role in the initiation and/or progression of various diseases such as atherosclerosis, inflammatory injury, cancer and cardiovascular disease. Many antioxidant compounds, naturally occurring from plant sources, have been identified as a free radical or active oxygen scavengers. Additionally, it has been determined that the antioxidant effect of plant products is mainly due to phenolic compounds, such as simple phenolic acids, flavonoids, isoflavonoids, hydrolyzable tannins and condensed tannins. The present study deals with the isolation and identification of the phenolic metabolites from Acacia nilotica (L.) Delile (Leguminosae) flowers and evaluation of its free radical scavenging activity. The aqueous alcoholic extract (MeOH: H2O, 8:2) of Acacia nilotica flowers was subjected to extensive repeated column chromatography on polyamide, cellulose and Sephadex LH-20 resulted in catechin, catechin-7-O-gallate, gallic acid, naringenin 7-O-β-glucopyranoside, quercetin 3-O-β-glucoside (2→1) glucopyranoside, quercetin 3-O-β-glucopyranoside, chalconaringenin 4′-O-β-glucopyranoside, naringenin and quercetin. The structure of the isolated compounds was elucidated on the basis of spectral analysis (UV, HRESI-1D/2D NMR). The radical scavenging activity of the extract was quantified spectrophotometrically, using DPPH radical. The total polyphenols showed excellent antioxidant potency when tested by radical scavenging methods. Keywords: Acacia nilotica, Phenolic compounds, antioxidant activity, DPPH

Chemical Constituents and pharmacological activities of Zilla spinosa

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Zilla spinosa D.Durand & Schinz is very widely distributed in the Egyptian deserts, it is used by the natives for the treatment of the kidney stones. The present study deals with the isolation and identification of chemical constituents of the aerial parts of Zilla spinosa and evaluation of pharmacological activities of its extract. Chemical investigation of the n-hexane and methanolic extract of the aerial parts of Zilla spinosa lead to the isolation of campesterol, spinasterol, β-Sitosterol, α-amyrin, β-amyrine, squalene as well as quercetin 3-O-α-rhamnopyranoside (6→1) β-glucopyranoside, kaempferol 3-O-α-rhamnopyranoside (6→1) β-glucopyranoside, quercetin 3-O-β-glucopyranoside, kaempferol 3-O-β-glucopyranoside, quercetin, kaempferol. Structures of the isolated compounds were established by chromatography, UV, HRESI-MS and 1D/2D 1H/13C NMR spectroscopy. The methanolic extract showed potent anti-inflammatory, anti-inflammatory and hepatoprotective activities. Keywords: Zilla spinosa, Flavonoids, hepatoprotective, analgesic, anti-inflammatory activity

Phenolic metabolites from Euterpe oleracea and evaluation of their free radical scavenging activity

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Salvia species belongs to the Lamiaceae are widely distributed in Turkey, 50% of the 89 Salvia species is endemic (1). Various parts of some Salvia species have been reported to have traditional uses (2.3). The common indications include GIT symptoms/disorders (colic, diarrhea, indigestion, and abdominal pain), respiratory tract symptoms/disorders (colds, sore throat, and cough), infections (tuberculosis, bacterial infections, influenza, and parasitic infections), pain (headache and arthralgia), and miscellaneous disorders (diabetes mellitus, liver diseases, barrenness, urticaria, and hemorrhage). In this study the analgesic activity of ethanol, butanol, chloroform and water extracts of S. wiedemannii Boiss. has been evaluated by using tail flick and acetic acid induced writhing tests. The chloroform extract (500 mg/kg, i.p.) obtained from S. wiedemannii showed significant analgesic activity on tail flick assay, its efficacy was very close to morphine. The water, ethanol and butanol extracts showed analgesic activities similar to that observed with aspirin. Chloroform extract (500 mg/kg, i.p.) also inhibited number of writhings induced by acetic acid. Chloroform extract provided analgesic effects similar to morphine. Its effect was quick and durable. This in vivo study demonstrates that S. wiedemannii has strong analgesic effect in accordance with the public belief. Keywords: Salvia wiedemannii, analgesic activity, folk medicine

Antihyperglycemic and antioxidative potential of Acacia nilotica pods in streptozotocin-induced diabetic nephropathy

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Although a wide array of medicinal plants plays a role in the prevention and treatment of diabetes, there are few reports of the application of herbal remedies in amelioration of renal damage. The present study examined the effect of methanolic extract (25 and 50 mg/kg body weight) of Acacia nilotica (L.) Delile (Leguminosae) pods in streptozotocin-induced diabetic rats for 30 days, and its biochemical, histopathological and histochemical study in the kidney tissues. Diabetic rats exhibited loss of body weight, hyperglycemia, elevated of serum urea and creatinine. Significant increase in lipid peroxidation (LPO), superoxide dismutase (SOD) and reduced glutathione (GSH) was observed in diabetic kidney. Histopathological examination revealed infiltration of the lymphocytes in the interstitial spaces, glomerular hypertrophy, basement membrane thickening and tubular necrosis with loss of their brush border in some of the proximal convoluted tubules in diabetic rats. Daily oral administration of Acacia nilotica extract reversed the adverse effect of diabetes in rats. Acacia nilotica extract lowered blood glucose levels, restored serum urea and creatinine and body weight loss. In addition, Acacia nilotica extract attenuated the adverse effect of diabetes on LPO, SOD and GSH activity. Treatment with Acacia nilotica was found to almost restore the normal histopathological architecture of kidney of streptozotocin-induced diabetic rats and ameliorate mitochondrial succinic dehydrogenase. However, glomerular size and damaged area showed ameliorative effect after treatment with the extract. In conclusion, the antioxidant and antihyperglycemic properties of Acacia nilotica extract may offer a potential therapeutic source for the treatment of diabetes. Keywords: Acacia nilotica, streptozotocin, biochemical, histopathological, antioxidant activity

PM56

Anti-proliferative Effect of Brucea javanica Fruit Extract Against Human Hepatocarcinoma Cell Lines and Its Mechanism

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The fruits of Brucea javanica (Linn.) Merr (Simaroubaceae) are used as herbal remedies for treatment of human anemia, as well as for cancer in Chinese folklore. Here, we studied the anti-proliferative activity of the extract from fruit of B. javanica (BJE) and the fraction (BJEF5) against Hep G2 human hepatocarcinoma cells, and explored their mechanisms. From these studies, BJE showed growth inhibitory activity effect by the MTT assay in a dose-dependent manner with an IC50 value of 1.56 ± 1.02 μg/mL, and by HP20 resin chromatography, the fraction (BJEF5) of elute washing by 50% EtOH showed more potent effect in a dose-dependent manner with an IC50 value of 0.44 ± 0.02 μg/mL. However, cell cytotoxic activity was not observed in the peripheral blood mononuclear cell (PBMC) treated with BJE or BJEF5 less than 30 μg/mL. Results from flow cytometry analysis also showed, BJE and BJEF5 induced cell cycle arrest in G1 phase as compared with the control groups, and the β-catenin transcription activity was inhibited in Hep G2 cells when treated with BJE and BJEF5, respectively. Furthermore, western blot analysis indicated that BJE and BJEF5 significantly reduced c-Myc, cyclin D1 protein levels leading to cell cycle arrest, and survivin protein level leading to apoptosis in a dose-dependent manner, respectively. Therefore, the BJE or BJEF5 deserves further exploration for its use as a potential agent in the therapy for hepatocellular carcinoma (HCC).

Keywords: antiproliferation, Brucea javanica, hepatocarcinoma Acknowledgement: The authors would like to thank the Ministry of Economic Affairs for the financial support of this research under contract No. MOEA 99-EC-17-A-02 – 04 – 0317.

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Antibacterial effects of two Iranian plant extracts and their synergistic effects on Staphylococcus aureus in laboratory medium

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The aim of this work was to evaluate the antimicrobial effects of wild garlic (Allium hirtifolium Bois.) and peppermint (Mentha piperita L.) extracts and their combination on Staphylococcus aureus. In the present work the antimicrobial effects of the mentioned plant extracts were evaluated using disk diffusion method as a preliminary step and microdilution method. The mentioned extracts were introduced into TSB Broth at ten concentrations from 50% to 0.09%(v/v) in order to determine minimum inhibitory concentration (MIC) for Staphylococcus aureus using Bioscreen C device, which is based on optical density measurements. Results indicated that wild garlic and peppermint extracts showed MIC of 3.17% (v/v) and 12.5% (v/v) respectively for Staphylococcus aureus. The antimicrobial activity was enhanced in response to extract mixture than individual extracts of each species, as no growth was observed at the concentrations from 50 to 0.09%(v/v). In conclusion, edible plants can be a potential source for inhibitory substances for some pathogens. Both extracts studied in this research were effective on Staphylococcus aureus and the combination of them showed synergistic effect on the inhibition of the growth, so the potential of plant extracts when combined with each other can be used as a more effective barrier for preservation. Keywords: antimicrobial, extract, Staphylococcus aureus, Mint, Wild Garlic

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Antihyperglycemic and antioxidative potential of Acacia nilotica pods in streptozotocin-induced diabetic nephropathy

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Although a wide array of medicinal plants plays a role in the prevention and treatment of diabetes, there are few reports of the application of herbal remedies in amelioration of renal damage. The present study examined the effect of methanolic extract (25 and 50 mg/kg body weight) of Acacia nilotica (L.) Delile (Leguminosae) pods in streptozotocin-induced diabetic rats for 30 days, and its biochemical, histopathological and histochemical study in the kidney tissues. Diabetic rats exhibited loss of body weight, hyperglycemia, elevated of serum urea and creatinine. Significant increase in lipid peroxidation (LPO), superoxide dismutase (SOD) and reduced glutathione (GSH) was observed in diabetic kidney. Histopathological examination revealed infiltration of the lymphocytes in the interstitial spaces, glomerular hypertrophy, basement membrane thickening and tubular necrosis with loss of their brush border in some of the proximal convoluted tubules in diabetic rats. Daily oral administration of Acacia nilotica extract reversed the adverse effect of diabetes in rats. Acacia nilotica extract lowered blood glucose levels, restored serum urea and creatinine and body weight loss. In addition, Acacia nilotica extract attenuated the adverse effect of diabetes on LPO, SOD and GSH activity. Treatment with Acacia nilotica was found to almost restore the normal histopathological architecture of kidney of streptozotocin-induced diabetic rats and ameliorate mitochondrial succinic dehydrogenase. However, glomerular size and damaged area showed ameliorative effect after treatment with the extract. In conclusion, the antioxidant and antihyperglycemic properties of Acacia nilotica extract may offer a potential therapeutic source for the treatment of diabetes. Keywords: Acacia nilotica, streptozotocin, biochemical, histopathological, antioxidant activity

PM55

Antibacterial activity of Salvia hydroæana extract against oral bacteria and comparison with vancomycin antibiotic in vitro

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The three oral bacteria (members of the normal flora in the mouth) including Streptococcus mutans, Lactobacillus and Streptococcus sanguis play a major role in the production of dental caries so that the first two bacteria accelerate dental caries while the third reduce this process. The purpose of this study was to determine the antibacterial activity of hydro and methanolic extract from Salvia hydroæana DC. ex Benth. against the three oral bacteria and comparison with vancomycin antibiotic in vitro. At first, a sample of hydro and methanolic extract of the Salvia hydroæana was prepared and then its antibacterial activity against S. mutans (PTCC 1601), Lactobacillus (PTCC 1608) and S. sanguis (PTCC 1449) was evaluated by well diffusion (with concentration of 100 mg/ml) and agar serial dilution methods for determining of MIC (minimum inhibitory concentration) with dilution of 0.195 to 100 mg/ml. Also, we studied the antibacterial activity of vancomycin antibiotic on them by disk diffusion method. The results from the antibacterial tests Salvia hydroæana hydro and methanolic extract had not been affected against any of the bacteria. While S. mutans was sensitive to vancomycin and Lactobacillus and strep. Sanguis were resistant to it. This study demonstrated that hydro and methanolic extract of Salvia hydroæana may not be an effective antibacterial medication in the prevention of dental caries. Keywords: Salvia hydroæana, hydro and methanolic extract, antibacterial activity, vancomycin, oral bacterial
**Inula** species are widespread in the world and used traditionally for ages by different cultures due to their various biological activities. The members of this genus contain terpenic compounds, especially sesquiterpene lactones, flavonoids, glycolipids and anthranilic acid derivatives (1.3). *I. viosa*, suffrutescous and rank-smelling herb up to 1–2 m, is widespread in Mediterranean area (4). In this study, antioxidant activity of freeze dried water, methanol and ethyl acetate extracts of flower, leaf and radix of *I. viosa* were evaluated via DPPH and ABTS methods. All the extracts showed antioxidant activity in different concentrations. Water extract of *I. viosa* flower expressed strong antioxidant activity with IC50 values which were lower compared with the other extracts. Ethyl acetate extracts of the investigated parts of the plant showed less antioxidant activity compared with the water and methanol extracts. It’s obvious that phenolics are responsible for the antioxidant potential of the plants. For this reason, phenolic compounds such as chlorogenic acid, caffeic acid, rutin, myricetin, quercetin, luteolin and kaempferol were analyzed qualitatively and quantitatively in the flower, leaf and radix methanol extracts of *I. viosa* by RP-HPLC. Chlorogenic and caffeic acids were found in all the investigated parts of the plant. Only myricetin was absent in the flower extract and chlorogenic acid was found in significant amount in radix extract. While myricetin was not determined in the plant, kaempferol was found only in the flower extract. Therefore, most of the investigated phenolics could be responsible for the potent antioxidant activity of *I. viosa*. Keywords: Inula, Antioxidant activity, DPPH, ABTS, RP-HPLCReferences: 1. Zhao Y-M et al. (2006) Chem Biodivers 3: 371–384. 2. Danino O et al. (2009) Food 1414.
PM62

Diuretic activity of Olive (Olea europaea L.)

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Diuresis is important in the treatment of many diseases ranging from acute cases as renal failure to chronic cases as hypertension. Olive, Olea europaea L., is a species of a small evergreen tree in the family Oleaceae, native to the coastal areas of the Mediterranean region. Olive leaves are used as anti-rheumatic, anti-inflammatory, anti-oedematous, diuretic and hypoglycemic agents in traditional medicine. Recently, it has been shown that olive leaf extract (OLE) has calcium channel blocker property. The mechanism of the diuretic activity was studied through determination of saluretic, natriuretic and carbonic anhydrase inhibition indices, in addition to assessment of vasodilatory, hypotensive, diuretic and hypoglycemic agents in traditional studies.

PM63

Antiradical Activity and Total Phenolic Contents of Wild and Cultivated Myrtle (Myrtus communis L.) Fruits

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Myrtle is a typical Mediterranean ecosystem plant. White fruit types are more common compared to black fruited ones in Turkey. Myrtle fruits and leaves are considered commercial commodity and are sold in different regions of the country for using as antiseptic, disinfector and hypoglycemic agent. Myrtle leaves are mainly used for myrtle oil production in Turkey (1). Fruits of wild and cultivated plants with both black and white in color were used as plant materials. Total phenolic contents which were expressed as gallic acid equivalents in milligrams per 100 gram of fresh fruit weight (mg GAE/100 g FW) were measured with Folin Ciocalteu method (4) while antioxidant activity (EC50) was assayed by DPPH method (5). Antiradical activity and total phenolic content were either UVB radiation or lipopolysaccharide. The results indicate the use of the presented extract as an anti-aging cosmetic ingredient.

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Anti-inflammatory evidence of a standardized Passiflora alata dry extract

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It is well established that aging of human skin is enhanced by continuous inflammatory process triggered by intrinsic and extrinsic factors, such as ultraviolet radiation, pollutants and reactive oxygen species (ROS) (1). Interleukins play a pivotal role in skin inflammation (2). IL-6 contributes to production of matrix metalloproteases 2, that degrades collagen type IV (3). IL-8 induces the expression of urokinase-type plasminogen activator (uPA) that enhances extracellular matrix degradation (4). After these statements, the search for cosmetic ingredients showing anti-inflammatory activity is of great value to prevent or reduce the process of skin aging. The objective of this study was to evaluate in vitro anti-inflammatory activity of a Passiflora alata Curtis extract, a Passiflora species endemic to Brazil. The extract was standardized in vitamin-2-O-rhamnose, the major component responsible for the biological activity. The content of vitexin-2-O-rhamnose in the dried extract is of 12%. The anti-inflammatory activity was evaluated by ELISA analysis for IL-6 and IL-8 in an in vitro model of human fibroblasts. The inflammatory stimuli were either UVB radiation or lipopolysaccharide. The results indicate the use of the presented extract as an anti-aging cosmetic ingredient.

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Activity of (-) mamea A/BB from the Leaves of Calophyllum brasiliense on Mycobacterium tuberculosis

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Despite the development of a number of effective treatments over the past half century, tuberculosis remains one of the most destructive bacterial infections in humans. The emergence of multidrug-resistant Mycobacterium tuberculosis leads to research of new classes of antimycobacterial agents (1). Calophyllum brasiliense Cambess (Clusiaceae) is a tree popularly known as “guanandi” being a rich source of bioactive substances, including coumarins (2). Previous studies of (-) mamea A/BB reported leishmanicidal (3) and anti-HIV (4) activities. This compound is a coumarin-type mammea was purified from a dichloromethane crude extract of Calophyllum brasiliense leaves by chromatographic methods. The current study, we evaluated the cytotoxicity and in vitro antimycobacterial activity of the (-) mamea A/BB. The compound was identified by spectroscopic methods and comparison with literature data. Antimycobacterial activity was calculated out using resazurin microtiter assay plate (REMA) to determine the minimum inhibitory concentration (MIC) of (-) Mamea A/BB against M. tuberculosis H37Rv (ATCC 27294). The cytotoxicity assay was carried out by Sulforhodamine B colorimetric method (7). The cytotoxicity for J774G8 macrophages was compared using the selectivity index (SI). The coumarin (-) Mamea A/BB showed significant activity against M. tuberculosis with MIC value of 31.2 μg/mL. The cytotoxicity against J774G8 macrophages showed SI of 0.823. These results provide new perspectives on the development of novel drugs obtained from natural products with anti M. tuberculosis activities. Keywords: Calophyllum brasiliense, Mycobacterium tuberculosis, antimycobacterial Acnowledgement: The authors are grateful to CNPq for providing a research grant and fellowships References: 1. Luciani F et al. (2009) PNAS 106: 14711 – 14715. 2. Ito C (2002) J Nat Prod 65: 267 – 272. 3. Brenzan MA et al. (2008) Pharmaceutical Biology 46: 380 – 386. 4. Reyes-Chilpa R (2004) Life Sci 75: 1635 – 1647. 5. Brenzan MA et al. (2008) Biomedicine & Pharmacotherapy 62: 651 – 658. 6. Palomino J C et al. (2002) Antimicrob Agents Chemother 46: 2720 – 2722. 7. Papazis KT (1997) J Immunol Methods 208: 151 – 158.
**PM66**

Anti-biofilm Activity of Pimarane Diterpenoids Against Anaerobes

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One of the greatest challenges in endodontic treatment is the presence of bacteria as a biofilm, which confers stronger bacterial resistance to antimicrobial compounds [1]. In the present work, the in vitro anti-biofilm activity of two natural pimarane-type diterpenes and one semi-synthetic derivative were investigated against nine bacteria responsible for dental root canal infections. The following anaerobic bacteria were evaluated in the present study: Porphyromonas gingivalis (ATCC and clinical isolate), Prevotella nigrescens (ATCC), Prevotella intermedia (clinical isolate), Prevotella buccae (clinical isolate), Bacteroides fragilis (ATCC), Actinomyces naeslundii (ATCC), Peptostreptococcus micros (clinical isolate), and Aggregatibacter actinomycetemcomitans (ATCC). The diterpenes ent-pimar-8(14),15-dien-19-oic acid (1), its sodium salt (2), and ent-8(14),15-pimaradien-3β-ol (3) (Figure 1) were used for determination of the minimum biofilm inhibition concentration (MBIC\(_{50}\)) [2]. MBIC\(_{50}\) results varied between 6.25 and 25.0 µg/mL for the studied compounds. All the examined compounds displayed 50% or higher inhibition activity concerning biofilm formation. A maximum value of approximately 20-fold the MIC was attained for P. gingivalis (ATCC) [3] in the case of compound 1, and a minimum value of approximately one fold the MIC was achieved for most of the tested bacteria. The present results suggest that pimarane-type diterpenes are able to inhibit biofilm formation in vitro, and that their structure influence this anti-microbial activity [3]. So this class of diterpenes should be considered in the search for new irrigating substances in the area of endodontic infections treatment. Studies of antibacterial activity linked to biofilm formation versus cytotoxicity of these compounds are being undertaken by our research group.

![Chemical structure of diterpene type-pimarane](image)

**Figure 1:** Chemical structure of diterpene type-pimarane


**PM67**

Chemical analysis and biological activities of methanol extracts from Astragalus gombiformis Pomel (Fabaceae)

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In the present study, wild Astragalus gombiformis Pomel extracts were tested for their biological activities and phenolic amounts. Antibacterial activity of this species against various bacteria was tested by the paper disk agar diffusion method and determination of the minimal inhibitor concentration. DPPH and ABTS assays were used to evaluate the antioxidation activity of methanol extracts. These extracts were also chemically investigated by spectrophotometric and HPLC analysis. For DPPH test, inhibitor concentrations 50% were 473.3 ± 64.29 and 626.66 ± 64.29 µg/mL, respectively, for aerial part and roots methanol extracts. Ascorbic acid, used as positive control, showed an inhibitor concentration 50% of 7.36 ± 0.70 µg/mL. ABTS test showed that roots and aerial part extracts contain respectively, 471.3 ± 0.05 and 79.81 ± 1.31 µmol of Trolox equivalents per g of dry plant material weight. Chemical investigation showed that total polyphenols and flavonoids were three folds higher in aerial part methanolic extracts. The antioxidant potential seems to be correlated to the phenolic contents. Five among the tested extracts exhibited a diameter of inhibition zone equal or above 12 mm and with a minimal inhibitor concentration ranging between 233 and 1250 µg/mL. However, no insecticidal effect of aerial part extracts was shown against Culex pipiens. It appears that both roots and aerial part of A. gombiformis extracts possess antioxidant and antibacterial effects and should be more studied for identification of active compounds. Keywords: Astragalus gombiformis, Antioxidant, insecticidal, Antibiotic, phenolic amounts

**PM68**

Phenolic amounts, antioxidant and antimicrobial potential of Crithmum maritimum L. cultivated in Tunisian arid zones

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Polyphenols are bioactive molecules exhibiting a lot of scientific attention due to their multiple biological activities. The present study aimed at assessing the phenolic content, antimicrobial and antioxidant activities of different organs (leaves, flowers, seeds and stems) of Crithmum maritimum L. cultivated in the south east of Tunisia (Medenine). The analyzed organs exhibited different total polyphenol amounts (8.60 ± 2.64 to 24.89 ± 2.44 mg of gallic acid equivalents per g of dry weight), flavonoids (2.04 ± 0.64 to 16.58 ± 0.64 mg of quercetin equivalents per g of dry weight) and tannins (0.532 ± 0.488 to 1.06 ± 0.77 mg of catechin equivalents per gram of dry weight). Evaluation of antioxidant potential showed that seeds extracts displayed the highest DPPH scavenging ability with the lowest IC\(_{50}\) value (406 ± 11 µg/mL). The antioxidant activity of C. maritimum was evaluated by method disc diffusion against different pathogens and expressed as diameter of zone inhibition. Results showed that the bacteria tested responded differently according to each organ. Among tested bacteria, E. coli and P. aeruginosa are the most sensitive strains to C. maritimum extracts. Tested extracts showed also antifungal effect against Candida albicans. We can conclude that C. maritimum has an antioxidant activity, which is correlated to their phenolic amounts. This species exhibited also antimicrobial effects against several human pathogens. Thus, the identification of the bioactive compounds is under progress in our laboratory to reinforce the notion of a potent valorization of such plant extracts. Keywords: Crithmum maritimum, Phenolic content, Antioxidant, antimicrobial

**PM69**

Seaweeds: new source of MAO-A inhibiting compounds

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The intense phytochemical study of terrestrial ecosystems, in the past decades, resulted in the identification and characterization of several new bioactive compounds. However, along the years, the scientific community became motivated to change its route of investigation into aquatic systems, which are known to possess a rich and unexplored biodiversity. Therefore, seaweeds are now being exploited by the pharmaceutical industry. Since they have to overcome highly competitive habitats, seaweeds developed some morphological and chemical survival strategies, and these last include the biosynthesis of a wide variety of primary and secondary metabolites that shall be studied in order to assess their bioactivities. In the present study the antidepressant capacity of 11 seaweed belonging to Rhodophyta, Phaeophyta and Chlorophyta phyla was evaluated in vitro, by the inhibition of MAO-A enzyme. Their aqueous
extracts were tested, revealing five very active brown seaweeds: Cystoseira usneoides (EC50=0.084 mg/mL), C. tamariscifolia (EC50=0.073 mg/mL), C. nodicola (EC50=0.041 mg/mL), Stypocaulon scoparium (EC50=0.265 mg/mL), and Fucus spiralis (EC50=0.110 mg/mL). After liquid-liquid partitioning, activity was found in the ethyl acetate fractions. These fractions were further analysed by HPLC-DAD, which showed compounds absorbing at 254, 280 and 350 nm. An attempt to identify these compounds by HPLC-NMR is currently in progress. Keywords: seaweeds, MAO-A inhibition, HPLC-NMR Acknowledgement: Clara Costa thanks the Fundação Para a Ciência e a Tecnologia for the Post-Doc fellowship (SFRH/BPD/63922/2009).

PM70

Diterpenes from Copaifera langsdorffii oleoresin against anaerobic oral pathogens

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Anaerobic bacterial infections are the major cause of pulp and periodontal diseases [1] and Phorophyromonas gingivalis can be considered as one of the beginning of these pathological processes. Our research group has demonstrated that some diterpenes isolated from Copaifera langsdorffii Desf. oleoresin are able to inhibit the growth of various aerobic and anaerobic pathogens with very promising MIC values [2]. In view of these significant results against oral bacteria, we decided to investigate the effect of these compounds on a panel of representative microorganisms responsible for root canal infections using the microdilution broth method [3]. The results indicate that (-)-copalic acid was the most active compound, displaying very promising MIC values against the main pathogens associated with these diseases (P. gingivalis). The other compounds also displayed some activity against the tested microorganisms. Keywords: Phorophyromonas gingivalis, diterpenes, Copaifera langsdorffii Acknowledgement: FAPESP (Proc. 2009/99438 – 3 and Proc. 2009/12796 – 9). References: 1. Gomes BFPA et al. (2004) Oral Microbiol Immunol 19: 261 – 267. 2. Souza AB et al. (2010) Phytother Res 25: 215 – 220. 3. NCCLS (2007) Methods for antimicrobial susceptibility testing of anaerobic bacteria, approved standard.

PM71

Observations with rapid micro-colony assay to screen antifungal activity of Origanum vulgare L., Zostera marina L. and Centaurea enisaefolium P.H. Davis extracts

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Currently, synthetic antifungal drugs are the main option to treat fungal pathogens e.g. Candida albicans C.P. Robin in humans. In turn, fungal pathogens generate resistance upon clinical treatments. Moreover, human pathogen Aspergillus fumigatus Fresenius isolates were suggested to generate resistance toazole antifungal drugs due to the necessity to control plant fungal diseases in the field conditions. Thus, antifungal agents derived from natural products have vital importance for sustainable control of fungal infections. Plants accumulate plethora of antimicrobial compounds e.g. alkaloids, iridoids, flavonoids and lignans which could target the different sites e.g. cell wall formation and protein biosynthesis in fungi. Alternative antifungal agent screening methods should be assayed for faster and sound detection. Previously, the micro-colony method i.e. the measurement of the early fungal development using microscopy was developed to screen dose response in the filamentous fungal species Fusarium efusum Winter. The micro-colony assay was tested to detect dose response in Candida albicans (ATCC 10231), A. fumigatus, Fusarium oxysorum Schlecht. emend. Snyders-Hansen on Origanum vulgare L. (Lamiaceae), Zostera marina L. (Zosteraceae) and Centaurea enisaefolium P.H. Davis (Asteraceae) extracts and reference fungicide flucanazole. The D. vulgaris essential oil was toxic in all testing concentrations, but Z. marina methanol extracts were ineffective. C. enisaefolium methanol extract showed slight growth inhibition on C. albicans and A. fumigatus. This method provides a dose response in 24 hours. Additionally, method could be used to evaluate topical treat-

PM72

Can it be possible to use Caulerpa species for the treatment of some diseases

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Many algae species have important secretions which are used for defensive purposes. These secretions generally have significant potential in terms of pharmaceutical industry. Among these secretions caulerpenyne (CYN) which is the main secondary of Caulerpa sp. holds an important position in medical investigations as a result of its determined properties such as cytotoxic, antiviral, antiproliferative and apoptotic effects (Lemée et al., 1993; Fischel et al., 1995; Galgani et al., 1996; Nicoletti et al., 1999; Barier et al., 2001; Cavas et al., 2006). In the present study, the inhibitory effects of CYN isolated from Caulerpa prolifera (Forskàll) J.V. Lamouroux (Caulerpaceae) on mononacetylglucol lipase and lipoxgenase were investigated. The results of the study presented that purified caulerpenyne inhibited soybean lipoxgenase with an IC50 of 5.1µM. The results of the works aimed to investigate mononacetylglucol lipase revealed that the inhibition of this enzyme were well in line with CYN concentration. The IC50 value of mononacetylglucol lipase inhibition with CYN was also determined as 98.4µM. In conclusion, Caulerpa species can be a promising material for the treatment of mononacetylglucol lipase and lipoxgenase related diseases. Keywords: Caulerpa species, caulerpenyne, lipoxgenase, mononacetylglucol lipase, inhibition Acknowledgement: We thank Prof. Dr. Georg Pohnert, Institute for Inorganic and Analytical Chemistry, Friedrich Schiller University of Jena for sharing his valuable knowledge about CYN purification with us. The authors are grateful to the Research Foundation of Dokuz Eylül University (Project No: 2008. KB. FEN. 019) for financial support. Sevilay Cengiz thanks to The Scientific and Technological Research Council of Turkey (TÜBİTAK) for the scholarship. TÜBİTAK Project (109T152) is also acknowledged for financial support. References: 1. Barber P et al. (2001) Life Sci 70: 415 – 429. 2. Cavas L et al. (2006) J Exp Mar Biol Ecol 339: 111 – 119. 3. Fischel JL et al. (1995) Anticancer Res 15: 2155 – 2160. 4. Galgani L et al. (1996) J Biochem Toxicol 11: 243 – 250. 5. Lemée R et al. (1993) J Appl Phycol 5: 485 – 493. 6. Nicoletti E et al. (1999) Phytotherapy Res 13: 245 – 247.

PM73

Screening of Indian medicinal plants for their antimicrobial property

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Medicinal plants play a key role in human health care. Over the side effects of allopathic drugs, the medical world moves towards the plant kingdom for the treatment of various ailments1. Many people are infected by venereal diseases, where most of them are causing by infectious microbial agents. Indigenous people tend to use several medicinal plants to treat these infectious diseases rather than western medicines. Therefore, the present study was carried out to screened the different herbs to evaluates their antimicrobial properties. The dried plant materials i.e. flowers, stem, fruit, seeds and leaves of ten medicinal plants (Aloe vera (L) Burm.f., Annona squamosa L., Cremoscreta L., Mangifera indica L., Morinda dioica Wall., Picrocarpus mar- supium Roxb., Rosa centifolia L., Thevetia peruviana K.Schum. and Zamia furfuracea L.) were extracted with 100% methanol (MeOH). The extracts were evaluated for their antimicrobial properties against Gram-positive (Bacillus coagulans), Gram-negative (Escherichia coli) bacteria, using Well diffusion method2. The presented results offer supporting evidence for effective use of selected plant extracts. It also supports the wealth of nature and shows that most of the plant materials possess antimicrobial property. They have different inhibition zones but gives a basic idea of the uses of these plants as antimicrobial agents. However, more in vitro confirmatory tests using other assays and/or in vivo tests are required.

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Juglans sigillata Dode., a fast growing deciduous tree species in the family Juglandaceae, is indigenous to mountain slopes and valleys of Tibet, Yunnan, Sichuan, and Guizhou provinces of southwest China [1, 2]. The green husk, cortex, kernel, nutshell, root and leaf of J. sigillata have a long history of being used in folk medicines to treat oxidative, inflammatory, rheumatic and nociceptive diseases, as well as to relief eczema, cancer, kidneys and stomach disorders. In the present study, antibacterial properties of J. sigillata green husk extracts were studied by hole-plate diffusion assay method described by Rios et al. [3] against Gram-negative bacteria, including Salmonella enterica and Escherichia coli and Gram-positive bacteria, such as Bacillus subtilis and Staphylococcus aureus. Aqueous EtOH (95%, v/v) extracts from green husk were successively partitioned with a series of polar solvents to get fractions soluble in n-hexane, CH2Cl2, EtOAc, n-BuOH and H2O. Results, expressed by diameter of inhibition zone, revealed that the 95% aqueous EtOH extracts and all resulting soluble fractions from J. sigillata green husk revealed moderate or significant antibacterial effects against the four Gram bacteria, which indicated that J. sigillata green husk extracts have potential to destroy bacteria or suppress their growth or their ability to reproduce and could be used as excellent antibacterial agents. Keywords: antibacterial capacity, green husks, Juglans sigillata, Juglandaceae, hole-plate diffusion assay Acknowledgement: This work was financially supported by Project for New Century Excellent Talents in University (NCET 2010), Foundation for the Development of Science and Technology in Tianjin Universities (No. 20080616), National Natural Science Foundation of China (No. 31070279) and National Science Foundation of Tianjin City (No. 09JCJOC15800). References: 1. Wu ZY, Raven PH (1999) Flora of China, Vol. 4. Science Press, Beijing. 2. Si CL et al. (2009) Planta Med 75: 922 – 922. 3. Rios JL et al. (1998) Ethnopharmacol 23: 27 – 149.

Gastroprotective and anti Helicobacter pylori activities of propolis


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Role of polyphenolic compounds on biological activity of collagenous materials

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Collagen, a unique connective tissue protein, is extensively used as bio-compatible biomaterial in wound healing [1], cosmetics [2] and tissue engineering [3]. Due to its high sensitivity to enzymatic degradation, cross-linking of collagenous materials must be a compulsory step in their fixation. Chemical cross-linking agents are able to form new covalent bonds in collagen structure, but are cytotoxic. The ability of natural plant polyphenolic compounds to stabilize collagen structure while preserving its cytocompatibility is now established [4]. This study aimed to investigate the interaction of small molecules from plants with collagen and their effect on its biological properties. Three mixtures of collagen with a polyphenolic extract of Urtica dioica L., a plant-derived flavonoid (quercetin) and a flavin from milk (riboflavin), respectively, were conditioned as porous materials by freeze-drying technique. A collagen-gluutaraldehyde mixture was used as control. Free amino groups within the mixtures were spectrophotometrically assessed. An in vitro experimental model using bacterial collagenase was used to mimic the enzymatic attack on the collagenous materials implanted in vivo. The swelling capacity and in vitro cytocompatibility tested according to ISO 10993 – 5 on fibrobasts from NCTC cell line were evaluated. Results showed a good correlation between the free amino groups and the biodegradability of each mixture. The values of swelling capacity were at least 70-fold higher than the initial weight. Fibroblast viability and morphology showed a high cytocompatibility of the polyphenol-collagen mixtures. In conclusion, all these tests indicated an improved applicability of these mixtures for further wound healing applications.

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Abnormal Balgham Munziq (ABMq) is a traditional Uighur mediche herbal preparation commonly used to preventing and treating the vitiligo. To study the mechanism of this preparation its effect on the activity of mushroom tyrosinase, the melanogenesis and proliferation of B16 murine melanoma

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Abnormal Balgham Munziq (ABMq) is a traditional Uighur medicinal herbal preparation commonly used to preventing and treating the vitiligo. To study the mechanism of this preparation its effect on the activity of mushroom tyrosinase, the melanogenesis and proliferation of B16 murine melanoma cell were estimated using non-cell vitro system as well as in culture. The results showed that at concentration of 0.5 – 200 μg aqueous biotest extracts of ABMq significantly activate the mushroom tyrosinase, melanogenesis in the non cells system in vitro. The ethanol extracts strongly activate the tyrosinase activity and increase melanogenesis than aqueous extracts, also show dosage depend manner. The aqueous extracts and ethanol Isolation from ABMq also has
a strong potential to promote the proliferation and melanogenesis of B16 cell, also can promote cell tyrosinase activity. The results of the present study suggest that the putative mechanisms of ASmg in treat-
ment vitiligo may at least involve enhance the proliferation of melano-
cyte, melanogenesis, the tyrosinase activity of melanocyte. This activity may play an important role in its treatment of vitiligo. Keywords: Ab-

Cockscome (Celosia cristata L.) is a traditional medicine herb used for treatment of fatigue, atherosclerosis, diabetes (1). The chemical constituents of this plant include mainly flavonoids (2). The purpose of this study was to evaluate the antioxidant compounds and antioxidant activities of the methanolic extracts and solvent fractions from cockscome flowers. To determine the antioxidant compounds in the methanolic extract and solvent fractions, the content of total polyphenol, flavonoid and tannin were measured by spectrophotometric methods. These were evaluated for antioxidative activities by DPPH and ABTS radical scavenging activities. The yield of methanolic extracts, hex-
ane, chloroform, ethyl acetate, butanol, and water fractions of cocks-
come flowers were 23.33, 10.27, 20.00, 13.63, 17.55 and 38.54%, respec-
tively. The total polyphenol, flavonoids and tannin contents of metha-
nolic extracts on the cockscome flowers were 6.80, 2.34 and 6.23 mg/g extract residue, respectively. The DPPH and ABTS radical scavenging activities of the methanolic extracts on the cockscome flowers were 52.43 and 107.01 mg Trolox equivalent antioxidant capacity per g extract residue, respectively. The results of this study show that notable antioxidant activities in L. cuneata G. Don are considered to have signif-
ificant health benefits. Keywords: Lespedeza cuneata G. Don, polyphe-

Lespedeza cuneata G. Don is a member of the Pea or Fabaceae family. This invasive plant has spread throughout the eastern Asia. L. cuneata is used as a medicinal herb to treat ailments such as skin ulcerations, dysentery, enteritis, and hernias (1). The purpose of this study was to evaluate the antioxidant compounds and antioxidant activities of the methanolic extracts and solvent fractions from L. cuneata. To determine the antioxidant compounds in the methanolic extract and solvent fractions, the content of total polyphenol, flavonoid and tannin were measured by spectrophotometric methods. These were evaluated for antioxidative activities by DPPH and ABTS radical scavenging activities. The yield of methanolic extracts, hexane, chloroform, ethyl acetate, butanol, and water fractions of L. cuneata were 19.52, 3.85, 21.00, 6.73, 7.31 and 61.11%, respectively. The total polyphenol, flavonoids and tannin contents of methanolic extracts on the L. cuneata were 12.44, 2.94 and 8.75 mg/g extract residue, respectively. The DPPH and ABTS radical scavenging activities of the methanolic extracts on the L. cuneata were 206.15 and 338.64 mg Trolox equivalent antioxidant capacity per g extract residue, respectively. The results of this study show that notable antioxidant activities in L. cuneata G. Don are considered to have signif-
ificant health benefits. Keywords: Lespedeza cuneata G. Don, polyphe-
lipopolysaccharide (LPS)-triggered nitric oxide (NO) production and inducible nitric oxide synthase (iNOS) expression [1]. To further understand the underlying molecular mechanisms of PFs activity, the effect of Cy polyphenolic fractions was evaluated on the LPS-induced nuclear factor (NF)-κB pathway in the mouse microphage cell line Raw 264.7. In Western blot assays, we observed that PFs inhibited the degradation and phosphorylation of inhibitory protein-κB (IκB). Next, the NF-κB transcriptional activity was assessed using cells transiently transfected with a NF-κB-dependent luciferase reporter plasmid. In addition to the interference with LPS-induced NF-κB activation observed in Western blot, PFs inhibited the LPS-induced NF-κB transcriptional activity. In summary, these results demonstrate that PFs from Cymbopogon citratus inhibited the NF-κB pathway and therefore could be used as a natural anti-inflammatory agent. Keywords: Anti-inflammatory, Cymbopogon citratus, polyphenols, NF-κB Acknowledgement: Research supported by FCT PhD fellowship SFRH/BD/46281/2008, FCT project PTDC/SAU-FCF/105429/2008 and FEDER/COMPETE (FCOMP-01 – 0124-FEDER-010996). References: 1. Francisco V et al. (2011) Ethnopharmacol 133: 818 – 827

PM82

Evaluation of antinitrosative activities of selected plant polyphenols

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The involvement of free radicals as reactive oxygen (ROS) and reactive nitrogen species (RNS), specially their increased production, appears to be a common feature to most human diseases, including cardiovascular disease, neurodegeneration and cancer [1, 2]. The treatment with antioxidant substances and other strategies leading to the reduction of oxidative and nitrosative stress may represent a therapeutic intervention that could reduce the progression of the pathological process. As such, plant polyphenolics have been suggested to play particularly important role to fight against these diseases, by affording protection towards free radical damage in cellular DNA, lipids and proteins[3 – 7]. Our goal was herein to investigate the scavenging capacity of some plant polyphenolic derivatives using different antinitrosative assays at different concentrations (from 0 to 300μM). In addition, the anti-proliferative activity against different human tumor cell lines was estimated using the MTT assay. The seventy-kilo Dalton heat shock protein (Hsp70) plays an important role in the deterrence of protein damage during aging and their expression is required for longevity [1]. Recently, we demonstrated that ADAPT-232, a fixed combination of the extracts of three adaptogenic plants – Rhodila rosea L., Schisandra chinensis K.Koch and Eleutherococcus cunicularius Maxim., significantly increases the levels of circulating Hsp70 in the blood of rats [2]. Further, the long term treatment of aged rats with ADAPT-232 diminished or prevented a range of age-related disorders including malfunction of the central nervous system, loss of memory and loss of learning ability [3]. Similarly, ADAPT-232 improves cognitive function and mental performance in humans [4]. In this study, for the first time we demonstrate that ADAPT-232 stimulates the release of the heat shock factor-1 (HSF-1). Taken together, our data suggests that the stimulation of HSP expression by adaptogens is associated with their anti-aging activity. Keywords: Adaptogens, Heat Shock Proteins, Neuroglia Cells, ADAPT-232, Hsp70 Acknowledgement: This work was supported in part by the Swedish Herbal Institute; Scott & White Memorial Hospital and Clinic; the Texas A&M Health Science Center College of Medicine, the Central Texas Veterans Health Administration and an Endowment from the Cain Foundation. References: 1. Calderwood et al. (2009) Gerontology 55: 550 – 558. 2. Panosanian et al. (2009) Phyto- medicine 16: 617 – 622. 3. Makarov et al. (2007) Abstract of International Congress Stress, Budapest, p. 242. 4. Aslanian et al. (2010) Phyto- medicine 17:499 – 494.
oxidative-related disorders, such as inflammatory diseases and cancer. In this work, a phenol-rich fraction from Agrimonia eupatoria dry aerial parts (AePRF) was studied. Results revealed p-coumaric and elagic acid derivatives, flavon and flavone glycosides, and monomers and oligomers of flavan-3-ols (proanthocyanidins). Some key features in molecular structure of flavonoids seem to be crucial to anti-inflammatory mechanisms: 4-oxo functional group and C2-C3 double bond at C-ring, 5- and 7-OH on A-ring and also OH functions on B-ring.1 Polycondensation degree of proanthocyanidins plays a significant role in bioactivity, since dimers and higher oligomers are more effective than monomers, in inhibiting NO production. On the other hand, catechol moiety increases antioxidant activity, leading to presume that catechin-type proanthocyanidins are very active.2 Anti-inflammatory effect was evaluated in LPS-stimulated Raw 264.7 macrophage cell line by measuring the nitric oxide (NO) production through the Griess assay and AePRF cytotoxicity was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Moreover, antioxidant capacity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. Total phenols and total flavonoids were also evaluated, and phenolic compounds were identified by high performance liquid chromatography, coupled to photodiode-array and electrospray ionization mass spectrometry detectors. Anti-inflammatory and antioxidant activities verified, as well as the phenolic profile established corroborate the traditional use of AePRF in inflammatory-related pathologies, since generous amount of the cited compounds were found in the phenol-rich fraction studied. Keywords: anti-inflammatory, antioxidant, phenolic, agrimony, eupatoria


**PM86**

Chilean medicinal plants as a source of norA efflux pump inhibitors against resistant Staphylococcus aureus colonies

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Staphylococcus aureus is a highly encountered pathogen in skin infections and multidrug resistance in this strain caused by native norA efflux pump. A growing clinical problem. A path to solve this problem is the synergistic combination of an antibiotic with an efflux pump inhibitor (EPI) providing an effective drug. Ethnopharmacological knowledge on treatment of infected wounds may prove valuable in the search for anti-staphylococcal compounds. 24 plants traditionally used by the Huilliche people in southern Chile for wound healing therapy were used. Plant extracts were tested for norA efflux pump inhibitory activity in an assay based on fluorometric measurement of ethidium bromide transport by norA. Synergy studies were performed using the microtiter plate method and MIC to rule out intrinsic activity. A total of 24 plant species were collected. Seven crude extracts was active (> 50% inhibition) at 100 µg/ml, compared to reference drug reserpine at 20 µg/ml. None of the seven plants revealed antimicrobial activity in the concentration range tested. The two most potent efflux inhibitors were tested for dose-response activity and showed similar profile as reserpine, but had higher IC50-values of 11 and 14 µg/ml compared to 6 µg/ml of reserpine. Synergy studies of the two extracts G and M showed a 4-fold reduction in Moxifloxacin MIC at an extract concentration of 100 µg/ml. Extracts of Huilliche medicinal plants is likely to inhibit S. aureus norA facilitated EtBr efflux. Combination of extracts G and M with Moxifloxacin enhanced antibiotic action 4-fold.Acknowledgement: Glenn W. Kaatz of John D. Dingell VA Medical Center, Detroit for encouraging help on setting up the assay. Luca Guardabassi at LIFE-faculty University of Copenhagen for hosting our experiments.

**PM87**

STW 5 (Iberogast®) is a fixed combination of nine plant extract with Iberis amara L. (STW 6) as its main component. It is successfully used for treatment of functional dyspepsia or irritable bowel syndrome (IBS). Because an clinical data suggest an inflammatory etiology of IBS the influence of STW 5 and STW 6 on tone and acetylcholine (ACh)-induced contractions of intact and inflamed intestinal preparations was examined. We used 1 – 1.5 cm long ileum and colon preparations of male Wistar rats to analyze region specific differences. Inflammation was induced by intraluminal instillation of 2,4,6-trinitrotoluene sulfonic acid (TNBS, 10 mM, 30 min). STW 5 (500.5 µg/ml) inhibited the increase in gene expression and reduced significantly the release of TNF-α by 87% in LPS (100 ng/ml)-stimulated human monocytes, while having no effect in untreated cells. In equivalent concentrations to STW 5, caraway, milk thistle, lemon balm and greater celandine had no effect on the LPS-induced increase in TNF-α release. Bitter candystuff, peppermint, chamomile, liqueur and angelica reduced the TNF-α release, though less pronounced as compared to STW 5. STW 5 (500.5 µg/ml) reduced TNBS (100 µM)-induced cell death significantly by 51.2%. Apart from caraway all other compounds revealed a significantly decreased cell death in differentiated THP-1 cells after co-incubation with TNBS. Lemon balm had the strongest effect and caused a reduction to 2.64%. The results indicate that the herbal components contribute differently to the effect of STW 5. Keywords: Iberogast®, Rat ileum, Inflammation, TNF alpha, THP-1 cells, LDH-test

**PM88**

Effects of STW 5 and STW 6 on rat ileal and colonic preparations: A comparative study

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STW 5 (Iberogast®) is successfully used in therapies of functional dyspepsia and irritable bowel syndrome. Given that clinical data suggest an inflammatory etiology of IBS, STW 5 and its components were examined on the production of the pro-inflammatory cytokine TNF-α and inflammation-induced cell death. The inflammation was induced by 2,4,6-trinitrotoluene sulfonic acid (TNBS, 10 mM, 30 min). The gene expression of TNF-α was determined in rat ileum preparation by real-time-RT-PCR. The release of TNF-α was measured in LPS-stimulated human monocytes using a commercially available ELISA. The cell death of THP-1 cells was determined using a commercially available LDH (lactate dehydrogenase)-assay. The TNBS-induced inflammation in ileal preparations was accompanied by increased TNF-α gene expression. STW 5 (500.5 µg/ml) inhibited the increase in gene expression and reduced significantly the release of TNF-α by 87% in LPS (100 ng/ml)-stimulated human monocytes, while having no effect in untreated cells. In equivalent concentrations to STW 5, caraway, milk thistle, lemon balm and greater celandine had no effect on the LPS-induced increase in TNF-α release. Bitter candystuff, peppermint, chamomile, liqueur and angelica reduced the TNF-α release, though less pronounced as compared to STW 5. STW 5 (500.5 µg/ml) reduced TNBS (100 µM)-induced cell death significantly by 51.2%. Apart from caraway all other compounds revealed a significantly decreased cell death in differentiated THP-1 cells after co-incubation with TNBS. Lemon balm had the strongest effect and caused a reduction to 2.64%. The results indicate that the herbal components contribute differently to the effect of STW 5. Keywords: Iberogast®, Rat ileum, Inflammation, TNF alpha, THP-1 cells, LDH-test
PM98

Agar-overlay assay: a useful and cost benefit method for detection of antibacterial peptides in plant seeds

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Multi-drug resistant bacteria are considered as a worldwide problem. Plant material have been an attractive candidate for overcoming human pathogens and amongst them plant defensins has been noticeably identified as a new class of promising antimicrobial substances. In this study, antimicrobial activity of some plant seeds components were assessed in two series of experiments. 8 different plant seeds were chosen according to data obtained from screening experiments which had been planned for accession of antimicrobial potential of different plant seeds methanolic extract. Then an agar-overlay method, using fully separated proteins on SDS-PAGE gels was used for initial determination of active putative proteins in total water soluble proteins of seeds. 4 different gram positive and gram negative bacteria were subjected to the assay. For 2 of 8 selected plant seeds, there were clear and remarkable zones of inhibition in a region correspond to low molecular weight proteins in agar-overlay assays for all of tested gram positive bacteria but a smaller inhibition zone with several colonies for the gram negative bacterium. Clear and noticeable inhibitory zone in the case of our gram positive strains would be promising results and characterization of the effective peptides is now progressing in our laboratory. Even more important this approach may be possible to estimate the antimicrobial activities of the peptides in a semi-quantitative manner which was not possible in routine screening test by using organic solvent extracted materials which are very complex matrix with unknown antagonistic or synergistic effects on their components. Keywords: Plant defenses, Multi-drug resistant bacteria, Bioassay Acknowledgement: We acknowledge the financial support of Medicinal Plants and Drug Research Hungarian Research Fund (OTKA K72771), the New Hungary Development Institute of Shahid Beheshti University.

PM99

Effect of methanolic extract of Harmla (Peganum harmala L.) on greenhouse whitefly Trialeurodes vaporariorum (Westwood) (Homoptera: Aleyrodidae)

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The greenhouse whitefly, Trialeurodes vaporariorum is one of most serious pests of vegetables in the world [1]. The current insecticides mainly may be used as adjuvants for therapy of multidrug resistant cancer.

PM90

New pentacyclic diterpene polyesters isolated from Euphorbia falcata L. as resistance modulators in cancer cells

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Resistance is a major cause of failure of chemotherapy and efflux is one of its major mechanisms that render the cancer cell resistant to more than one anti-cancer agent. The use of antagonists that restore the activity of already existing chemotherapeutics is a promising way to overcome resistance. Four new premyrsinane- (1 – 4) and three cyclomyrsinane- (5 – 7) diterpene polyesters isolated from E. falcata L. were tested for their activity as efflux modulators. The compounds were identified as diterpene polyesters, tetra- (1), tetra- (2), penta- (3), hexa- (4) and heptaesters (5 – 7) derivates of a polyfunctional diterpenic acid, acylated with acetic, propa-noic, isobutanoic, 2-methyl-butanolic acid, n-hexanoic and benzoic acids. Compounds 2, 3, 4, 6 and 7 increased the reduction of rhodamine 123 in L5178 mouse T-cell lymphoma cells transfected with pHA MDR1/A resistance gene yielding fluorescence activity ratios between 12.63 and 46.15 at 0.002 mM. The compounds when used in combination with doxorubicin synergistically decreased resistance of the cells yielding combination index values at ED50 between 0.13 and 0.34 and at ED90 between 0.03 and 0.3. Among premyrsinanes the presence of 7-OBz and 15-Ac groups seem to be important for the activity of the compounds. The acetyl group at position C-17 slightly decreases activity. In case of cyclomyrsinanes, compounds with benzoyl group have lower activity while lack of substitution at C-17 increases activity. The results of this work contribute to the understanding of important structural elements of diterpene polyesters for the design of new effective compounds that may be used as adjuvants for therapy of multidrug resistant cancer. Keywords: Diterpene polyesters, Resistance modulator, Cancer therapy, ABCB1, efflux pump Acknowledgement: This work was supported by the Hungarian Research Fund (OTKA K72771), the New Hungary Development Plan (TÁMOP-4.2.2-08/1-2008-0013 ad TÁMOP-4.2.1/B-09/1-KOV- 2010-005) and the Szeged Foundation of Cancer Research. A. Vasas acknowledges to the János Bolyai Scholarship from the Hungarian Academy of Sciences. References: 1. Syloky E et al. (2010) Planta Med 76: 1257 2. Kars MD et al. (2006) Anticancer Research 26: 4559 – 4566.

PM92

Potent anti-inflammatory compounds identified in Zingiber officinalis Roscoe var. rubrum Thelland: Mechanisms of action in psoriasis

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Psoriasis is an autoimmune inflammatory skin disease associated with aberrant activation of T and B-lymphocytes. Increasing evidence indicates that T-helper 1 (Th1) and Th17 lymphocyte subsets play key roles in the immunopathogenesis of the disease. In such a setting, activated Th1/Th17 cells interact with keratinocytes leading to their proliferation and hyperplasia. Our studies are focused on developing new approaches for targeted therapy for psoriasis. Recent studies from our laboratories have identified therapeutic effects for compounds extracted from the ginger species Zingiber officinalis Roscoe var. rubrum Thelland, on pathogenic mechanisms in psoriasis. Initially, the therapeutic effects of chloroform extract (HB02) and selected fractions were assessed for their ability to suppress the production of pro-inflammatory mediators produced by macrophage. Four fractions, F5, F6, F7 and F10 with dual sup-
pressive effects on NO and PGE2 production were identified. The fractions had higher potency than L-NAMe, a specific inhibitor of iNOS, and exhibited comparable effects to indomethacin in inhibiting of PGE2. F6 had particularly potent inhibitory effects on inhibiting NO (IC50=6.7 ± 2.7 µg/ml) and suppressing iNOS gene transcription by 82.3 ± 3.73% at 20 µg/mL. Two compounds, 6-shogaol and a ferulate derivative were isolated from F6. Interestingly, the 2 compounds had additive effects in down-regulating iNOS and iG2 gene transcription. These compounds may be key components responsible for the anti-inflammatory effects of HB02. Current experiments are focused on mechanisms of action and therapeutic efficacy of these compounds on suppressing psoriasis involving chemokine and cytokine production and keratinocytes proliferation using an in vitro human psoriatic skin model. Keywords: Psoriasis, Zingeribe officinale Roscoe var. rubrum Thellae. 6-shogaol, ferulate derivative. Acknowledgement: 1. Standards and Industrial Research Institute of Malaysia (SIRIM Berhad), Malaysia 2. Ministry of Science, Technology & Innovation (MOSTI), Malaysia

**PM93**

Effect of some medicinal plants on nymphal development and mortality of *Brevicoryne brassicae* (L.) (Homoptera: Aphididae)

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The misuse and excessive use of synthetic insecticides may cause some undesirable effects not only to the agricultural ecosystem but also to human health due to insecticide residue in food. Therefore, several efforts have been created to control the use of synthetic pesticides particularly the use of synthetic insecticides. One of the efforts is the development of botanical insecticides as a novel and safer alternative strategy. Botanical insecticides, which contain plant extracts as active components, are safer as well as environmentally friendlier than synthetic insecticides [1]. Botanical insecticides are easily biodegradable and their use in crop protection is a practically sustainable alternative. Ethanolic solution of three plant species, clove flower buds (*Syzygium aromaticum* L.), haramal seeds (*Peganum harmala* L.), and Persian lilac fruits (*Melia azedarach* L.) were tested for their activities on duration of development and mortality of cabbage aphid (*Brevicoryne brassicae* [L.] in the laboratory. The first instars (one day old) were sprayed with ethanolic solution (30 mg/ml) of different plant extracts, and nymphal duration as well as mortality were estimated. In the control treatment, the insects were sprayed with ethanol (95%). In haramal treatment, the duration of nymphal development was compared with a mean of 8.0 days. Total mortality (%) during the development of the aphid from N1 to adult emergence were 50.0%, 36.6%, 30.0% and 21.4% in clove, haramal, Persian lilac and control treatment, respectively. Keywords: *Brevicoryne brassicae*, plant extract, nymphal development, mortality. References: 1. Sadeghi GD et al. (2008) JISSAAS 15(1); 42 – 51. 2. Devlin JF, Zettel T (1999) Ecoculture: Initiatives in Eastern and Southern Africa. Weaver Press, Harare.

**PM94**

An approach to studying the mechanism of action of STW 5 in functional dyspepsia using the restraint stress model in rats

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While the clinical efficacy of medicinal plants as therapeutic options in treating functional gastrointestinal diseases is widely accepted, the understanding of their mechanisms of action still remains uncertain. Two models for stress-induced functional dyspepsia were performed in order to choose the more adequate one for testing sensitivity changes of the fundus to various mediators. In one model, maternal separation (1) was performed on weanling rats starting from postnatal day 2 for 3 h each day for 3 weeks. Rats were then allowed to mature to an adult age. The other model was that of restraint stress (2.3). Adult animals were restrained for 90 min/day for 1 week. The animals were eventually sacrificed, the stomach fundus was isolated and its sensitivity in vitro to carbachol, potassium chloride, serotonin and adrenaline was tested. The sensitivity of the fundus strips from restrained rats towards these agents was more depressed than those from maternally separated ones. That model was therefore chosen to test the efficacy of STW 5 in restoring sensitivity to the agents mentioned. A group of animals received STW 5 orally once daily for 2 weeks before subjecting them to restraint stress. Treatment with STW 5 was effective in normalizing the depressed responses exhibited by animals subjected to restraint stress. Samples of blood were taken to assess levels of CRF and ghrelin. The findings throw further light on the mechanisms underlying the therapeutic usefulness of STW 5 in functional dyspepsia, especially when triggered by psychological stress. Keywords: Functional dyspepsia, STW 5, Stomach fundus, restraint-stress References: 1. Cheung CK et al. (2010) Gastroenterology 138: S-766–2. Zhang H et al. (2008) Phytomedicine 15: 602 – 611. 3. Zheng J et al. (2009) Am J Physiol Regul Integr Comp Physiol 296: R1358–R1365.

**PM95**

Reproduction and longevity of the cabbage aphid (*Brevicoryne brassicae* [L.]) after exposure to ethanolic extract of clove (*Syzygium aromaticum L.*)

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Botanical pesticides are an important group of naturally occurring, often slow-acting crop protection that are usually safer to humans and the environment than conventional pesticides, and with minimal residual effects. Therefore the use of plant pesticides has been recommended ever more as a suitable alternative of plant protection with minimum negative risks [1, 2]. Especially botanical insecticides have long been a subject of research in an effort to develop alternatives to conventional insecticides. Therefore, this research was conducted to assess the effectiveness of ethanolic solution (30 mg/ml) of clove flower buds extract (*Syzygium aromaticum* L.) on reproduction and longevity of the cabbage aphid (*Brevicoryne brassicae* [L.]) in the laboratory. In this experiment, 50 newly 1st nymphal instars of the cabbage aphid were placed together into the round plastic Petri dishes on rape leaves and sprayed with the ethanolic solution. In control treatments only ethanol (95%) were applied. After 24 hours, the nymphs were transferred into the new Petri dishes with fresh leaves and reared until adulthood. Afterwards, the adults were confined singly in other Petri dishes and reared until death. During the longevity experiments, reproduction of the adults was estimated during one week. The ethanolic plant extract caused a significant reduction in longevity of the adults (8.7 days) when compared with the control treatments. Moreover, the plant extract had a significant deleterious effect on the mean total number of laid nymphs during the seven days with the mean of 5.8 nymphs. Keywords: *Brevicoryne brassicae*, plant extract, *Syzygium aromaticum*, Reproduction, longevity References: 1. Isman MB (2006) Ann Rev Entomol 51: 45 – 66. 2. Pavela R (2007) Pest Technol 1: 47 – 52.

**PM96**

Toxic effect of three medicinal plant extracts on *Myzus persicae* (Sulzer) (Hom.: Aphididae)

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The peach potato aphid *Myzus persicae* (Sulzer) (Hom.: Aphididae) is one of the most noxious species [1]. It can infest plants of over 40 different families including many economically important ones world wide, and it is able to transmit over 100 plant viruses [2]. Therefore, in the present study the efficacy of acetic leaf extracts from three medicinal plants

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were evaluated against 3–4-day-old individuals of the *M. persicae*. The plants were included *Eucalyptus globulus* Labill. (Myrtaceae), *Teucrium polium* L. (Lamiaceae) and *Onoestea persica* Boiss. (Labiatae). In order to obtain the crude extracts, the dried leaves were powdered and extracted with acetone. Experiments were carried out at 25 ± 1 °C temperature, relative humidity of 60 ± 10% and 16 hours of artificial light at an intensity of about 4000 lux. In control treatments only distilled water and DMSO (dimethyl sulfoxide) were applied. Topical treated aphids with three acetone extract emulsion (in distilled water with DMSO) were placed on the broad bean leaf discs (4.5 cm diameter) in the round plastic Petri dishes (5.5 cm diameter), filled with a 0.5-mm-thick agar gel layer. The highest percentage of mortality (55.6%) was observed in the acetone extract of *O. persica* in the concentration of 80 μg/ml after 48 hours. While, it was less than 10% in the acetone extract of *E. globulus*. The acetone leaf extract of *T. polium* caused 14.5% mortality of *M. persicae*. It is concluded that *O. persica* is the most promising for future development and use as botanical pesticide. Keywords: Myzus persicae, medicinal plant, Toxic effect, Topical test References: 1. Blackman RL, Eastop VF (1984) Aphids on the World’s Crops. John Wiley and Sons. New York. 2. Clements KM et al. (2000) RevToxicol 3: 1 – 23.

**PM97**

**Evaluation of antioxidant and antibacterial properties of Ziziphus vulgaris (Rhamnaceae)***

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Ziziphus vulgaris Lam. is a deciduous shrub, native of the Mediterranean region and used to treat sore throats, alleviate stress and helps in the common colds. In present work 10 g of the dried plant material was soaked in 100 ml methanol and shaken for 24 h and clear filtrate was obtained. The fresh methanolic crude extracts were qualitatively screened for secondary metabolites. Results showed that: flavonoids, hydrolysable tannins, alkaldoids, terpenes and saponins had reasonably high contents but anthraquinones and coumarines were low. In vitro antimicrobial assay and MIC determination growth inhibition activities of methanolic leaves extract of *Z. vulgaris* against gram-positive and gram-negative bacterial species using the conventional paper disc assay showed good inhibitory effects only against gram-positive with no antagonistic effects against gram-negative bacterial species tested. The MIC values of the crude methanolic *Z. vulgaris* extract on gram-positive was in range to 12.5 – 25.0 μg/mL, whereas extract exhibited very weak antimicrobial activity against gram-negative with very high values (1000 μg/mL) of MICs. Total phenolics in the Folin-Ciocalteu method in plant extract also showed reasonably high contents of polyphenolics (300 mg/gm extract). Results collectively suggest that *Z. vulgaris* is not only a reliable natural source of antimicrobials but also potential sources of phenolic compounds and hence could be nominated for intensive studies. Keywords: Ziziphus vulgaris, antimicrobials, phenolic antioxidants.

**PM98**

**Antioxidant activity and phenolic content of different extracts of Gentiana cruciata L**

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*Gentiana cruciata* L. is a perennial plant belonging to the genus *Gentiana* (fam. Gentianaceae) [1]. *Gentiana* species are widely used throughout the world as potential stomachic and hepato-protective agents [2]. *G. cruciata* is used in the traditional medicine for loss of appetite, as a stomachic as well as component in preparations showing beneficial effects in gall and liver diseases [3]. The aim of this study was to evaluate the antioxidant and radical-scavenging activities of methanol extract, chloroform, ethyl acetate and n-butanol fractions obtained from the methanol extract of aerial part of *G. cruciata*. Amounts of total phenolics and flavonoids were also determined. The total phenolics contents in the fractions and extract were determined as gallic acid equivalent (GA) using Folin-Ciocalteu’s reagent, while the spectrophotometric method with aluminum chloride was used for the determination of total flavonoids. The total amount of flavonoids was calculated as the rutin equivalent (RU). The extracts were investigated for antioxidant capacity using two different assays: DPPH assay and inhibitory activity toward lipid peroxidation. The highest content of total phenolics (109.8 mg GAE/g) and flavonoids (110.9 mg RU/g) was determined in the n-butanol fraction. The most effective DPPH radical scavenger was n-butanol fraction (IC_{50}= 114.7 μg/mL), while the methanol extract showed the highest inhibitory activity toward lipid peroxidation (IC_{50}= 69.9 μg/mL). The results show a significant antioxidant activity of the investigated extracts compared to relevant antioxidant compounds, such as butylated hydroxytoluene (BHT), ascorbic acid (AA), gallic acid (GA) and α-tocopherol. Keywords: Gentiana cruciata, antioxidant activity, phenolic content Acknowledgement: This was supported by the Ministry of Science and Technological Development of the Republic of Serbia (project No. III 43004). References: 1. Struwe L, Albert V (2002) Gentianaceae-systematics and natural history, Cambridge University Press, Cambridge. 2. Jiang R et al. (2005) Phytochemistry 66: 2674–2680. 3. Menkovski E et al. (2011) J Ethnopharmacol 133: 97–107.

**PM99**

**Melissa officinalis: an important dietary source of phenolic compounds with high antioxidant capacity**

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Balm, *Melissa officinalis* L. a perennial herb native to southern climates of Europe and North America and is cultivated in Mediterranean and central Asian areas [1]. Oil of balm has been shown to have antiviral, antibacterial and antispasmodic activity [2,3]. In this research, total phenolic content and related total antioxidant capacity of plant infusions was analyzed. Infusions were prepared in common way in which teas are prepared for human consumption. The antioxidant activity were measured by Folin-Ciocalteau assay. The total antioxidant capacity was estimated by Ferric Reducing/Antioxidant Power (FRAP) assay. Also, the phenol antioxidant coefficient (PAC) was calculated for plant infusion. The obtained results for Melissa infusions showed: high phenolic concentration, very high FRAP (> 20 mM/L) and PAC > 3. The fect of infusion time and temperature on the phenolic content, FRAP, and free radical scavenging ability was tested. Preparation of Balm infusions with hot (98°C) and cold (20°C) revealed that although antioxidants were liberated from leaves into the water at both of the temperatures studied, infusions prepared at higher temperature had more than 2-fold higher antioxidant capacity determined as FRAP. DPPH radical scavenging ability of Balm phenolics was similar to (+)-catechin but not as good as for quercetin. Compared to Trolox and vitamin C, Melissa phenolics were more efficient free ABTS radical scavengers. The results indicate that Melissa officinalis infusions could be an important dietary source of phenolic compounds with high antioxidant capacity comparable with red wine or beverages like tea. Keywords: Phenolic compound, antioxidant capacity; Infusions; Melissa officinalis; FRAP; DPPH; ABTS References: 1- Kennedy D, Little OW, Haskell CF, Scholey AB (2006) Psychopharmacol Res 2006:90 – 102 2-Weiman Z, Golfer E, Biehler H, et al. (2006) Pediatr 122:650 – 652 3-Wake G, Court J, Pictering A, Lewis R, Wilkins R, Perrey E (1999) J Ethnopharmacol 69: 105 – 114.

**PM100**

**In vitro and In vivo Antitumor Effects of Deoxylepholephantopin on Human Breast Cancer Cells**


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Breast cancer is one of the most common cancers in women, and a leading cause of death worldwide. It is often highly resistant to chemotherapy, and there is often no effective cure for patients in the advanced stages of the disease. In this study, we evaluated the effect of deoxylepholephantopin (DET), a phyto-compound extracted from *Elephantopus scaber* L. (Asteraceae) for possible anti-tumor activities in the human breast cancer cell line MDA-MB-231. Cell-apoptosis assay showed that DET treatment was able to effectively suppress the growth of test tumor cells in vitro. In addition, DET treatment significantly decreased expression level of transforming growth factor-beta (TGF-β), effectively inhibited cell growth by inducing G2-M phase cell cycle arrest and apoptosis, and reduced the clonogenicity in a concentration-dependent manner in MDA-MB-231 cells. DET also significantly inhibited the invasion and migration of test breast tumor cells. The effect of DET on suppression of NF-κB, via activation by TNF-α, was examined using electrophoretic mobility shift analysis (EMSA). Decreased levels of expression of phosho-NF-κB and the downstream molecules of the NF-κB signaling path-
Artemisia afra Jacq. is one of the oldest, most well known and widely used traditional medicinal plants in South Africa. It is used to treat many different medical conditions, particularly respiratory and inflammatory ailments (Liu et al., 2009). There is no reported evidence of its use for the treatment of cancer but due to its reported cytotoxicity (Fouche et al., 2008; Mativandlela et al., 2008), we investigated the effect of A. afra extracts on 2 cancer cell lines. IC50 values of 18.21 µg/mL and 31.88 µg/mL of ethanol extracts were determined against U937 and HeLa cancer cells, respectively. An IC50 value of the aqueous extract was greater than 250 µg/mL. Dose response assays were also performed using confluent HeLa cells, yielding an IC50 value greater than 250 µg/mL. The effect of the cytotoxic ethanolic A. afra extract (20 µg/mL) on U937 and HeLa cells, progression through the cell cycle, apoptosis and mitochondrial membrane potential was investigated. Melphan was used as a positive control. After 24 hours of treatment with melphan using U937 cancer cells, an increase in sub G1 phase was evident. Treatment of cells with A. afra showed a delay in G2/M phase of the cell cycle. Apoptosis was confirmed using the TUNEL assay for DNA fragmentation, which was evident with the positive control and A. afra treatment at 24 and 48 hours. JC-1 staining showed a decrease in mitochondrial membrane potential at 24 hours. The results obtained suggest that A. afra potentially has medicinal and anticancer properties. Keywords: Artemisia afra, apoptosis, cytotoxicity References: 1. Liu et al. (2009) S Afr J Bot 75: 185 – 195. 2. Fouche et al. (2008) J Ethnopharmacol 119: 455 – 461. 3. Mativandlela et al. (2008) Phytother Res 22: 841 – 845.

Bioactivity of in vitro glycoalkaloids from Solanum nigrum
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Glycoalkaloids were produced from callus and regenerated plants of Solanum nigrum L. (Solanaceae) using different concentrations of auxins and cytokinins. The glycoalkaloids were separated by acid base precipitation, and determined by high performance liquid chromatography. The produced glycoalkaloids found to have antioxidant activity against the free radical DPPH. Also, they exhibited cytotoxic activity against the selected carcinoma cell lines including liver, breast and lymphohlastic leukemia cell lines. Examination of the antiviral activity showed that glycoalkaloids had virucidal effect against the tested virus strains. In addition, callus glycoalkaloids were found to have antischistosomiasis and antifasciolas activities. Keywords: In vitro glycoalkaloids, Solanum nigrum, cytotoxicity, antiviral, antiparasitic

Determination of in vitro antioxidant potential of Coriopsis L. and its polyphenol content
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Coriopsis L. (Cornaceae) is one of the two species of the genus Coriopsis represented in the Turkish flora [1]. Its leaves have been widely used in Anatolian folk medicine against diarrhea and diabetes [2]. This study was designed to investigate the antioxidant potential of the 80% methanolic extract prepared from the leaves. The antioxidant properties were examined by using different in vitro systems. Ascorbic acid and BHT were used as reference substances. In the DPPH test IC50 value was
found to be 418 ± 2.26 μg/mL. The extract showed a strong superoxide scavenging activity (IC50 = 114.75 ± 3.12 μg/mL). Moreover, the result of total antioxidant capacity as equivalent to ascorbic acid was 228 ± 5.08 mg AA/g dry extract. Ferric-reducing antioxidant power and metal-chelating activities of 1 mg/mL extract were 1487 ± 7.46 μmol FeSO4/g dry extract and 32.7%, respectively. Total phenolic content 248 ± 0.41 μg gallic acid/mg extract, the flavonoid content was 75 ± 0.24 μg quercetin/mg extract. These results suggest that Corinns mas L. leaf extract exert significant in vitro antioxidant potential and further in vivo studies are in progress. References: [1] Chamberlain DF (2001) Flora of Turkey and The East Aegean Islands, Davis P.H (ed.), Edinburgh, Vol 4, pp. 539 – 540 [2] Yeşilada et al. (1999) Journal of Ethnopharmacology 64: 195 – 210

PM06

Mechanism of action of action of Fragaria vesca leaf extract on LPS treated macrophages

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Fragaria vesca L., commonly known as Strawberry, has been used over the years by traditional medicine for the treatment of several diseases. However, scientific reports of its molecular action mechanism are lacking. Thus, this work aims to investigate the anti-inflammatory effects, of a Fragaria vesca leaf extract obtained by successive extractions with ethanol and 50% aqueous ethanol, on the macrophage cell line, RAW 264.7, stimulated with lipopolysaccharide (LPS). For this purpose nitric oxide (NO) production, scavenging activity and cytotoxicity of the extract were assessed. Furthermore, was evaluated the expression of proteins that are potential targets to prevent or treat chronic inflammation, namely iNOS, COX-2, phospho-IkBα and Jnk-α. The results demonstrated that Fragaria vesca leaf extract was not cytotoxic and inhibit the production of NO triggered by LPS. Using (S)-Nitroso-N-acetylpenicillamine as NO donor, the extract promoted a significant decrease of NO in the culture medium. Western Blot analysis showed that LPS triggered a significant increase in iNOS and COX-2, though no significant differences were observed between cells treated with LPS or co-treated with the extract. Furthermore, in cells stimulated with LPS we observed a strong decrease on the content of IkBα, while phosphorylated IkBα strongly increased. However, an increase on the phosphorylation of Jnk-α occurred in cells co-treated with the plant extract and LPS, suggesting a potential reduction of proteasome degradation, since phospho-Jnk-α is a target for the ubiquitin-proteasome pathway. In conclusion, our data show that Fragaria vesca decreased the level of nitrites, mainly through direct NO scavenging activity of the extract. Keywords: Fragaria vesca, strawberry, anti-inflammatory properties, scavenger activity, proteasome inhibition

Acknowledgement: Research supported by FCT PhD fellowship SFRH/BD/72918/2010 and FCT project PIDD/SAU-FCF/105429/2008 and FEDER/COMPETE (FCOMP-01 – 0124-FEDER-010996).
Hyperalgesia is considered as one of the marked signs of subchronic diabetes mellitus that could affect the life style of the patients. This study was designed to investigate the antinociceptive effect of chronic feeding of Allium schoenoprasum L. (AS) leaf in streptozotocin-diabetic rats using formalin and hot tail immersion tests. Rats were divided into control, AS leaf-treated control, diabetic, sodium salicylate (SS)-treated diabetic, and AS leaf-treated diabetic groups. The treatment groups received oral administration of AS leaf-mixed pelleted food (3%) for 8 weeks. Finally hyperalgesia were assessed using standard formalin and hot tail immersion tests. AS leaf treatment of diabetic rats reduced pain score in chronic phase of formalin test from 2.41 ± 0.14 to 2.01 ± 0.12 (p < 0.05). Regarding hot tail immersion test, diabetic rats showed a significant reduction (5.9 s) in tail flick latency as compared to control ones (p < 0.05) and AS leaf treatment of diabetic rats did not significantly increase this latency relative to untreated diabetics. Taken together, 8-week administration of AS leaf could attenuate nociceptive activity on COX-2 expression.

Historical analysis of rat cutaneous wounds treated with a semi-solid formulation of linseed (Linum usitatissimum L.) oil

Melanin is a pigment that is distributed widely in bacteria, fungi, plants and animals (1). Melanogenesis is initiated with oxidation of L-tyrosine by tyrosinase that is rate-limiting step in this process. Next reactions can proceed spontaneously (2). Tyrosinase that is a key enzyme in formation of melanin pigments, widely exists in animals and plants. Tyrosinase has a monophenolase activity as well as diphenolase activity that oxidizes o-diphenols to o-quinones (3). This study evaluated inhibitory effect of four plants including Physalis alkekengi L., Alcea rosea, Bunium persicum B. Fedtsch. and Marrubium vulgare L. on mushroom tyrosinase. In this study L-Dopa (Dihydroxyphenylalanin) is used as substrate, so diphenolase activity of mushroom tyrosinase was evaluated. Kojic acid was used as positive control. Extracts of Physalis alkekengi L. Alcea rosea L., Bunium persicum B. Fedtsch. and Marrubium vulgare L. on mushroom tyrosinase. In this study L-Dopa (Dihydroxyphenylalanin) is used as substrate, so diphenolase activity of mushroom tyrosinase was evaluated. Kojic acid was used as positive control. Extracts of Physalis alkekengi, Alcea rosea, Bunium persicum (Total), Bunium persicum (defatted) and Marrubium vulgare showed IC50 values of 0.09, 0.38, 0.37, 0.38, 2.76 mg/ml respectively. IC50 values were defined as concentration of inhibitor that inhibited 50% of tyrosinase activity (4). Extract of Physalis alkekengi showed greatest inhibitory effect on mushroom tyrosinase activity with IC50 value of 0.09 mg/ml. Kinetic and Inhibition parameters (Km, Vm, Ki) were calculated. In this study kinetic parameters (Km, Vm) are evaluated and Ki evaluated for substrate, so diphenolase activity of mushroom tyrosinase was evaluated. Kojic acid was used as positive control. Extracts of Physalis alkekengi L. Alcea rosea L., Bunium persicum B.Fedtsch. and Marrubium vulgare L. on mushroom tyrosinase. In this study L-Dopa (Dihydroxyphenylalanin) is used as substrate, so diphenolase activity of mushroom tyrosinase was evaluated. Kojic acid was used as positive control. Extracts of Physalis alkekengi, Alcea rosea, Bunium persicum (Total), Bunium persicum (defatted) and Marrubium vulgare showed IC50 values of 0.09, 0.38, 0.37, 0.38, 2.76 mg/ml respectively. IC50 values were defined as concentration of inhibitor that inhibited 50% of tyrosinase activity (4). Extract of Physalis alkekengi showed greatest inhibitory effect on mushroom tyrosinase activity with IC50 value of 0.09 mg/ml. Kinetic and Inhibition parameters (Km, Vm, Ki) were calculated. In this study kinetic parameters (Km, Vm) are evaluated and Ki evaluated for P. alkekengi, A. rosea and B. persicum. Data has revealed that these three plants display a mixed- type inhibition. However Marrubium vulgare shows an uncompetitive inhibition (Table1). P. alkekengi that had the greatest tyrosinase inhibitor showed Ki value of 0.52 mg/ml comparing another study on total extract of Lavandula stoechas L. showed Ki value of 0.183 mg/ml (4). Finally calcul-
ation methods, types of inhibition (herbs & pure compounds) will be discussed.

Table 1: effect of Extracts and kojic acid on the kinetic parameters of mushroom tyrosinase.

<table>
<thead>
<tr>
<th></th>
<th>Initial velocity (Vmax)</th>
<th>Kt (μM)</th>
<th>n</th>
<th>IC50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>0.28</td>
<td>1.5</td>
<td>0.3</td>
<td>0.11</td>
</tr>
<tr>
<td>Proteose</td>
<td>0.3</td>
<td>1.6</td>
<td>0.13</td>
<td>0.14</td>
</tr>
<tr>
<td>Kojic acid</td>
<td>0.4</td>
<td>2.1</td>
<td>0.12</td>
<td>4.1</td>
</tr>
<tr>
<td>Kojic acid</td>
<td>0.2</td>
<td>1.5</td>
<td>0.3</td>
<td>0.11</td>
</tr>
</tbody>
</table>


PM113

Antioxidant capacity of Bifora radians Bleb OECD O. Hürkul MM, Çavuşev Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Tandâoûk, Ankara, 06100, Turkey

The genus Bifora Hoffm. (Apiaceae = Umbelliferae) is represented by two species in Turkey, namely Bifora testculata (L.) Sprengel and B. radians Bleb. [1] B. radians is an annual herb with typical odor in the fieldsdes especially chalky of Central Anatolia and known locally as “yabani kisste ve Buglun”. 2nd ed. Nobel Tıp Kitabevleri, İstanbul. pp. 151 – 152. [2]. B. radians is rich in alkanals and alkenals [3]. The phenolic contents of the samples were determined using Folin-Ciocalteu's phenol reagent. The antioxidant activity of diethy ether and methanol extracts were studied by two different techniques: qualitative DPPH (11-diphenyl-2-picrylhydrazyl radical) and the TBA assays. Both extracts showed a slightly antioxidant activity with the qualitative DPPH test. Methanol extract of the plant was found to possess moderate inhibitory activity (IC50 = 89.43 ± 3.09) on the lipid peroxidation whereas the nonpolar fraction (IC50 = 261.32 ± 2.72) showed a slightly antioxidant activity. Propyl gallate (IC50 = 0.09 ± 0.18) was used as a positive control. Keywords: Bifora radians, antioxidant activity, Apiaceae References: 1. Hedge IC, Lamond JM (1972) Bifora Hoffm. in Flora of Turkey and the East Aegean Islands, Ed. Davis, P.H., University Press, Edinburgh, Vol. 4, pp. 332 – 333. 2. Baytop T (1999) “Türkiye’de Bitkiler ile Tedavi. Geçiçme ve Buglun”, 2nd ed. Nobel Tıp Kitabevleri, İstanbul. pp. 332 – 333. 3. Baser HC, Demircakmak B, Ermin N, Demirci F, Boydag I (1998) Essent Oil Res 10(4): 451 – 452.

PM114

Evaluation of the hypoglycemic activity of extracts from Boldoa purpurascens Cav González DM1, Hernández Y1, Boradý B1, Vicet L1, Saeuco Y2, Pieters L2, Appers S3; 1University of Marta Abreu Las Villas, Santa Clara, Cuba; 2University of Antwerp, Antwerp, Belgium

Boldoa purpurascens Cav. (1), a plant belonging to Nyctaginaceae family, is traditionally used for its diuretic effect comparable to furosemide (2). In the species described the presence of several flavonoid compounds was confirmed for its diversity of actions and therapeutic. The phytochemical analysis by 1 H and 13C NMR spectroscopy of such elements as iron (24,25*10 – 4%), silicium (8,99*10 – 4%), magnesium (0,15%), sodium (0,14%), aluminium (9,94*10 – 4%) – the given elements contained in the most concentration in the plant. A separation of biologically active substances in the leaves, flowers and stems were determined: flavonoids (0,70%, 2,25%, and 0%) [5], carbohydrates (3,60%, 1,01%, and 0%) [3] and tannins (2,03%, 2,06%, and 0,08%); by method of titrimetry- organic acids (2,59%, 3,16%, and 0,58%).[2]. Determination of the mineral composition of ash from boldoa purpurascens reveals that the plant was determined by mass spectrometry with inductively coupled plasma. There was found 31 elements in samples under analysis. As a result it was found that the plant is prone to the accumulation of such elements as iron (24,25*10 – 4%), silicium (8,99*10 – 4%), calcium (0,07%), potassium (0,14%), strontium (7*10 – 4%), magnesium (0,015%), sodium (0,14%), aluminium (9,94*10 – 4%) – the given elements contained in the most concentration in the plant. A separation scheme was developed for the study of flowers. Water-alcohol extract was concentrated to a complete removal of ethanol. The resulting aqueous extract was separated from the sediment and then the liquor was exhaustively extracted with ethyl acetate. The separated sediment was processed with petroleum ether, benzene, ethanol, aqueous alcohol, water coherently [1]. Quercetin was identified in the ethyl acetate extract while rutin was found in the alcohol extract. References: 1. Fedoseeva L et al. (2005) Chem Plant Substances 3: 45 – 50. 2. State Pharmacopeia USSR (1990) 11: 296 – 297. 3. Zaporozvet M et al. (2003) BiochemCarcinohydrides 1: 324 – 326. 4. Grinkevich N et al. (1983) Chem Anal Med Plants 1: 87 – 118. 5. Khaled A et al. (2004) Quantitative Content of Flavonoids 1: 356 – 358.

PM115

Influence of a semi-solid formulation of Persea americana oil fruit on the healing of cutaneous wounds in rats

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Persea americana Mill. (Avocado) oil fruit presents polyunsaturated (oleic (ω-9) and linoleic (ω-6)) and monounsaturated fatty acids (linolenic (ω-3)) [1,2]. Several studies have shown a role for ω-3, ω-6 and ω-3 in the process of tissue repair [3,4,5]. The purpose of this study was to investigate the effects of a semi-solid formulation of avocado oil (SSFAO) on cutaneous wound healing of rats. Wistar rats (200 – 250 g) were anesthetized with intraperitoneal injection of ketamine (75 mg/kg) plus xylazine (15 mg/kg) followed by shaving of the skin at wounding site and an circular area (78.5mm2) of skin was surgically removed from dorsal region of the animals. After surgery, the animals were divided in groups (n = 6) and treated with topical application ofSSFAO (1%, 5%, 10% or 50%), avocado oil (AO), Curare/Ace (positive control) and petroleum jelly negative control) once daily for 14 days. Concerning to wound area (mm2) evolution, in the second day of treatment a statistically significant difference was observed between the AO group (118.88 ± 14.37) compared to positive control (86.56 ± 16.23), and in the fifth day the difference was observed between SSFAO 1% (59.52 ± 9.74) compared to negative control (92.09 ± 14.91). No difference was observed on the qualitative aspects (crust color and presence of fibrin, exudates, granulation and re-epithelialization) between SSFAO groups (1%, 5%, 10%, 50%) or AO when compared to controls. In conclusion, the topic use of SSFAO (1%, 5%, 10% or 50%) or the AO appears to have no influence over the wound healing of rats. Keywords: Persea americana, wound healing References: [1] Salgado JM et al. (2008) CTA 28: 20 – 26. [2] Tongo JS et al. (2004) RBF 26: 17 – 23. [3] Manhezi AC et al. (2008) RBE 61: 620 – 629. [4] Hatanaka E Curi R (2007) RBF 88: 53 – 58. [5] Cardoso RB et al. (2004) WRR 12: 235 – 243.
The genus Michauxia L’Hérit (Campanulaceae) is represented by five species in Turkey, namely Michauxia campanuloides L’Hérit ex Aiton, M. laevigata Vent., M. tchichatchewii Fisch. et Mey. (E); M. thyrsoides Boiss. & Heldr. (E), and M. nuda A.D.C. The fresh stem and roots of M. campanuloides and M. tchichatchewii have been used as a vegetable. In the present study, the phenolic contents and antioxidant activities of the water, methanol (MeOH), dichloromethane (DCM), Ethyl acetate (EtOAc) and butanol (BuOH) extracts obtained from the root and herb of 5 species of Michauxia collected in different parts of Turkey were compared. The phenolic contents of the samples were determined using Folin-Ciocalteu’s phenol reagent. Antioxidant activities of the extracts were studied by qualitative and quantitative DPPH· (1,1-diphenyl-2-picrylhydrazyl radical) assay to detect the free radical scavenging activity and by thiobarbituric acid (TBA) assay to detect their liposome lipid peroxidation. The total phenolic contents of extracts in the five different polarity were found the EtOAc extracts ranged from 108.1 to 439.1 mg/g in dry weight expressed as gallic acid equivalents (GAE). All extracts showed a moderately antioxidant activity with the qualitative DPPH· test. In the quantitative DPPH· method the highest activity were determined in EtOAc extracts of five plants. High activity was observed in the herb EtOAc extracts of M. tchichatchewii (IC_{50}=4.94 ± 6.46) and the MeOH extracts of M. campanuloides herb (IC_{50}=97.58 ± 6.03) when compared to the other extracts in the TBA test. Propyl gallate (IC_{50}=0.09 ± 0.18) is used as a positive control. Keywords: Michauxia campanuloides; M. laevigata; M. tchichatchewii; M. thyrsoides; M. nuda; Antioxidant activity

Digitonin reverses doxorubicin resistance in cancer cells

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A decrease of the intracellular concentration of doxorubicin by activation of ABC-transporters, mainly P-glycoprotein, leads to a reduction of its chemotherapeutic efficacy. To overcome multidrug resistance, digitonin, a steroidal saponin, was selected to enhance cell permeability, increase intracellular accumulation, and anticancer effect of doxorubicin. We investigated the cytotoxicity and P-glycoprotein modulatory effect of digitonin in combination with doxorubicin in resistant leukemia and colon cells. MTT assay was applied to evaluate the cell viability and reversal effect of this combination. Rhodamine123 and calcine efflux assays were used for investigate P-glycoprotein function by flow cytometry. At the molecular level, RT-PCR confirmed the data obtained. Digitonin exhibits a significant effect on viability of Caco-2 and CEM/ADR5000 cells with IC_{50} values 15.17 ± 1.2 μM and 16.02 μM, respectively. The co-incubation of doxorubicin with non-toxic concentration of digitonin (5 μM) resulted in an enhancement doxorubicin cytotoxicity in Caco-2 and CEM/ADR5000 cells by 1.9- and 1.2-fold, respectively. Digitonin increase Rhod123 and calcine accumulation in Caco-2 and CEM/ADR5000 cells in dose dependent manner. Moreover, 5 μM digitonin increases the accumulation of Rhod123 and calcine 1.3- and 1.1-fold of verapamil activity in Caco-2 cells. RT-PCR data indicate that 5 μM digitonin down regulated P-gp/MDR1 mRNA to 80% of the control level. In conclusion, digitonin enhances the antitumor effect of doxorubicin and exhibits P-glycoprotein modulatory effect, so it considered as an efficent additive to the chemotherapeutic principle. Keywords: digitonin, anticancer, doxorubicin resistance

Modulation of P-glycoprotein, cytochrome P450, and glutathione-S-transferase by resveratrol in human cancer cells

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Resistance of cancer cells to chemotherapy is controlled by a decrease of intracellular drug accumulation, increase of detoxification, and diminished propensity of cancer cells to undergo apoptosis. ABC-membrane transporters together with intracellular metabolic enzymes contribute to the complex and unresolved phenomenon of multidrug resistance (MDR). Resveratrol, a polyphenol of Fallopia japonica (Houtt.) Ronse Decr., has antiinflammatory and antioxidant properties [1]. However, it is also interesting in the field of cancer therapy [2]. The mechanisms by which resveratrol might produce anticancer effects are not well understood. In this study, resveratrol was shown to increase intracellular doxorubicin and calcine accumulation in a concentration dependent manner (1–500 μM) in Caco-2 cells by 3–167% and 5–361% of verapamil. Moreover, the treatment of CEM/ADR5000 with 10–100 μM resveratrol significantly inhibited the Rhod123 and calcine efflux by 107–407% and 164–460% as compared with verapamil (100% respectively). The cytotoxicity of doxorubicin was enhanced by using 20 μM resveratrol; IC_{50} values were decreased from 4.15 to 1.23 μM, and from 33.67 to 1.81 μM, respectively. Furthermore, resveratrol significantly inhibited GST and cytochrome P450 enzyme activity in a dose dependent manner with IC_{50} values 33.30 μM and 11.49 μM, respectively. RT-PCR reveals a significantly down-regulation of ABC-transporters and of smetabolic enzymes mRNA levels in Caco-2 cell lines in response to resveratrol treatment. In conclusion, the inhibition of both ABC-transporters and of metabolite enzymes could explain the advantages of resveratrol in cancer therapy. References: 1. Harmsen S et al. (2007) Cancer Treat Rev 33: 369 – 380. 2. Szakacs G et al. (2006) Nat Rev Drug Discov 5: 219 – 234.
PM121

Variability of phenolic contents, antioxidant and antimicrobial activities of *Inula crithmoides* from Tunisia

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*Inula crithmoides* L. is a spontaneous halophyte thriving on waterlogged zones. This species is harnessing edible, medicinal, aromatic and economic potentials. In fact, this plant is known for its richness on bioactive compounds, mainly on essential oils. In this study, we tried to carry out the richness of this species on phenolic compounds and to evaluate their biological activities. Different parts of the plant were collected from Kairouan (center of Tunisia), air dried, grounded to a fine powder then subjected to a selective extraction with petroleum ether, acetone 60% then ethyl acetate in order to have a phenolics enriched fraction. Dried extracts were dissolved in methanol to be used in the colorimetric quantification of phenolics and to estimate their antioxidant activities (DPPH, trolox equivalent antioxidant activity, reducing power and inhibition of the β-carotene bleaching tests) and antibacterial activity against four human pathogenic bacteria. Results revealed that *I. crithmoides* extracts contain interesting amounts of these phytochemicals, significantly variable within the different plant parts, with highest amounts recorded in flower extracts. Besides, the entire investigated antioxidant test showed that *I. crithmoides* extracts exhibited high antioxidant activities, especially flower extracts. The effect of *I. crithmoides* extracts on the degree of inactivation of selected food borne pathogenic bacteria was variable and depended on the strains in question and on the part of the plant. These finding suggest that *I. crithmoides* is an interesting source of phenolics having antioxidant and antibacterial potentialities allowing them to be used as preservative ingredients in the food, pharmaceutical, and cosmetic industry. Keywords: *Inula crithmoides*, phenolic compounds, biological activities

PM122

Inhibitory activities of selected medicinal plants on mushroom tyrosinase

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Tyrosinase is a key enzyme in melanin synthesis from tyrosine. Using tyrosinase inhibitors has become increasingly important in medicinal and cosmetic products to prevent or treat pigmentation disorders (1). To evaluate inhibitory effects the extracts of *Urginea maritima* (L.) Baker [1], *Zizania mays* Rech.f. & Wendelbo [2] and *Physalis divaricata* D.Don [3] on mushroom tyrosinase this study was designed. L-Dopa as used as substrate. Ethanolic extracts of *U. maritima* [bulb], *Z. mays* [leaves] and *P. divaricata* [air organs] were used for their inhibitory effect in vitro on diphenolase activity of tyrosinase, using a spectrophotometric method. The extracts showed anti tyrosinase activity weaker than positive control (Kojic acid). The inhibitory activity of tested plants *Urginea maritima*, *Zizania mays* and *Physalis divaricata* against mushroom tyrosinase is expressed using IC50 (concentration of inhibitor that inhibited 50% of tyrosinase activity) values of 2.79, 2.37, 3.34 mg/ml respectively. The kinetic study indicated that all extracts were uncompetitive inhibitors for tyrosinase. Keywords: *Urginea maritima*, *Zizania mays*, *Physalis divaricata*, mushroom tyrosinase, inhibitor Acknowledgement: This research is a part of a project granted by Ahvaz Jundishapur University of Medical Sciences. References: Zhang X Hu et al. (2009) Biological and Pharmaceutical Bulletin 32(1): 86 – 90.

PM123

Anti-inflammatory activity of ointments with dry extracts of rhizome and herb of *Aremonia agrimonoides*, (L.) DC (Rosaceae)

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*Aremonia agrimonoides*, (L.) DC (Rosaceae), also known as Bastard Agrimony, is a native plant species in the central Europe. The aqueous extracts of rhizome and herb were prepared by method of percolation with water. The liquid extracts were then evaporated in stream of nitrogen. The content of phenolics was determined by the method with Prussian Blue (1). The ointments with 1% dry extract of rhizome and 1% dry extract of herb were prepared in paraffin ointment. The anti-inflammatory effect of ointments was tested through the model of mouse ear model. Inflammation of both ears of albino mice (both sexes) was induced by applying 10 μl of 3% solution of acetone-based croton oil (2). As control we used 1% hydrocortisone ointment. Ointments were applied once a day in the period of three days on the left ear, two hours after inducing inflammation. The right ear was not further treated. The appearance of the ears observed during the experiments was measured in scores on 0 – 14 scale. The mean values recorded on the third day after the causing of ear inflammation were: for ears treated with ointment with extract of rhizome 6 ± 1, for ears treated with the extract of aerial part of plant 7 ± 1, for untreated ears 12 ± 2, and for ointment with 1% hydrocortisone 6 ± 1. Content of phenolic compounds in the extracts was 12,69% for the rhizome and 12,76% for the herb. The pharmacological response to both ointments was similar with the ointment with 1% hydrocortisone. Keywords: antiinflammatory, aremonia, mouse ear References: 1. Price ML, Butler LG (1977) Agric Food Chem 25,1268 – 1272. 2. Williamso EM, Okpako DT, Evans FJ (1996) Selection, Preparation and Pharmacological Evaluation of Plant Material in: Pharmacological Methods in Phytotherapy Research; John Wiley Sons p.131 – 153.

PM124

Change of total anthocyanins content and kernel lightness according ripening days after silking date in black waxy corn

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This study was carried out to evaluate changes of total anthocyanins content and kernel lightness according ripening days after silking date in black waxy corn. Black waxy corns have pericarps colored black and Black pericarps contain anthocyanins. Anthocyanins relate to antioxidant activities. Thirty black waxy corn inbred lines were planted at upland crop fields of National Institute of Crop Science in Korea, 2009. They were evaluated total anthocyanins content, respectively. Base on these results, they were classified by 3 groups. Three groups were mutually crossed. Their F1 seeds were planted in upland crop fields of National Institute of Crop Science in Korea, 2010. They were classified 8 crossing groups by crossing combinations. These crossing groups were harvested at 19 days, 21 days, 23 days and 25 days after silking date respectively. And their products were evaluated by total anthocyanins content and lightness. As increasing of harvest days, total anthocyanins content were increased, but lightness was not. The total anthocyanins content and lightness were analyzed correlation by SAS Enterprise Guide 4.2. They have negative correlation and coefficient of determinant (R2) was 0.7151. Keywords: black, waxy, corn, anthocyanin, days after silking date References: 1. Lopez Martinez et al. (2009) Food Science and Technology 42: 1187 – 1192

PM125

Quercetagetin, a component of premature *Citrus unshiu* (Swingle) Marcow., suppress the chemokines related with atopic dermatitis by regulating STAT1 signal

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Atopic dermatitis (AD) is an itchy and relapsing inflammatory skin disease. It was known that a predominant systemic Th2 dysbalance with...
increased IgE levels and eosinophilia is widely accepted in the pathogenesis of AD [1]. Thymus and activation-regulated chemokine (TARC/CCL17) and macrophage-derived chemokine (MDC/CCL22) are related with AD and are elevated in serum and lesional skin of AD patients [2, 3]. Citrus unshiu (CIT) contains various flavonoids that have various bioactive effects [4]. In present study, we investigated the effect of a component of premature CU, quercetin, on the production of TARC and MDC in HaCaT human keratinocytes. As results, quercetin inhibited the inflammatory activity on the protein production and mRNA expression of TARC and MDC in IFN-γ and TNF-α-stimulated HaCaT human keratinocytes. Also, quercetin inhibited the phosphorylation of STAT1 protein in IFN-γ signaling pathway, in a time- and dose-dependent manner. These results suggest that quercetin, a component of premature CU, may have an anti-atopic activity by inhibiting the inflammatory chemokines (TARC and MDC) via the STAT1 pathway. Keywords: quercetin, Citrus unshiu, Atopic dermatitis, TARC/CCL17, MDC/CCL22, Jak-STAT pathway References: 1. Bieber T (2008) N Engl J Med 358: 1483 – 94. 2. Tajiri D et al. (2004) J Allergy.}

**PM126**

Solvent extracts of *Carpinus tschonoskii* suppress the expression of atopic inflammatory cytokines and chemokines in RAW264.7 macrophages and HaCaT keratinocytes

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Atopic dermatitis (AD) is a chronic, chronic relapsing, inflammatory skin disease characterized by pruritus and inflammation and accompanied by cutaneous physiological dysfunction through chemokine-mediated infiltration of numerous mononuclear cells in lesional skin [1]. TARC (thymus and activation-regulated chemokine/CCL17) and MDC (macrophage-derived chemokine/CCL22) that bind to the chemokine receptor CCR4 which is highly expressed on T-helper 2 cells lead to preferential influx of Th2-type lymphocytes to the lesional skin in AD [2]. Furthermore, cytokines are another triggers of AD and the expressions of inflammatory cytokines (TNF-α, IL-2) and IL-6 increase in lesional skin macrophages of AD patients [3]. In present study, we investigated the anti-inflammatory effects of *Carpinus tschonoskii* Maximin in RAW264.7 murine macrophage and HaCaT human keratinocytes. As results, the CHC13 subfractions (C-4, -5, and -6 fr.) dose-dependently inhibited the production of TNF-α, IL-2, and IL-6 in the LPS-stimulated RAW264.7 murine macrophage. Also, they inhibited the mRNA expression and protein level of TARC and MDC via suppressing the phosphorylation of STAT1 protein in IFN-γ-stimulated HaCaT human keratinocytes. These results suggest that *C. tschonoskii* may be an effective source for improving anti-atopic dermatitis by inhibiting the inflammatory cytokines and chemokines. Keywords: *Carpinus tschonoskii*, Atopic dermatitis, TARC/CCL17, MDC/CCL22, RAW264.7 macrophages, HaCaT keratinocytes References: 1. Bieber T (2008) N Engl J Med 358: 1483 – 94. 2. Sekiya T et al. (2000) J Immunol 165: 2205 – 2213. 3. Grossman RM et al. (1989) Proc Natl Acad Sci USA 86: 6367 – 71.

**PM127**

Effect of *Moringa oleifera* extract on experimental reflex esophagitis in rats

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*Moringa oleifera* Lam. (Family: Moringaceae) is commonly known as drumstick tree or horseradish tree. The leaves are highly nutritious, being a significant source of beta carotene, Vitamin C, protein and antioxidants [1]. In the present study, we investigated the effect of *M. oleifera* on experimental reflex esophagitis in rats as well as the antioxidant activity. Rats received *M. oleifera* extract (200, 400 mg/kg, p.o.), omeprazole (30 mg/kg) given at 1 h prior to surgery [2]. *M. oleifera* extract at doses 200, 400 mg/kg significantly inhibited the esophagitis index (P < 0.001) as compared to control. Further, acid and pepsin out put of gastric contents were significantly decreased in treated groups. *M. oleifera* extract (400 mg/kg) significantly inhibited the lipid peroxidation (from 58.3 ± 0.3 to 38.3 ± 0.02 nmol of malondialdehyde (MDA)/mg protein) (P < 0.001) and increased in levels of catalase to 25.4 ± 2.8 units of catalase activity/mg protein and superoxide dismutase (SOD) to 712 ± 5.8 units/mg protein (P < 0.001). *M. oleifera* extract (200 mg/kg) and omeprazole also showed significant inhibition in lipid peroxidation (P < 0.05) and enhanced the activities of catalase (P < 0.01) and SOD activity. Further, it altered the elevated levels of sialic acid and hexose contents in esophageal tissue. Indeed, *M. oleifera* significantly decreased the elevated plasma histamine content (P < 0.05). The results suggested that antioxidants potential of *M. oleifera* could attenuate the severity of reflux esophagitis and prevent the esophageal mucosal damage. Keywords: *Moringa oleifera*, Reflux Esophagitis, Antioxidant References: 1. Verma A R (2009) Food Chem Toxicol 47: 2196 – 2201. 2. Rao ChV, Vijayakumar M (2008) Eur J Pharmacol 589: 233 – 238.

**PM128**

In vitro antiangiogenic effects of polyphenolics from *Potentilla recta*

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Most pharmacological studies have confirmed the traditional use of Potentilla species in many diseases. These pharmacological effects of Potentilla species can be explained by the high amount of polyphenolic compounds present in all plant parts [1,2]. *Potentilla recta* L. (sulfur cinquefoil) is a long-lived invasive perennial plant from Eurasia that has become one of the most serious invaders of natural area grasslands in North America and Canada. It has been reported to contain 2-pyrene-4,6-dicarboxylic acid, which seems to be a chemotaxonomic marker for the Potentilla species. Ranges of seasonal changes in the content of chlorophyll, carotenoids, free organic, ascorbic and triterpenic acids, neutral triterpenoids and fat oils in some organs and overground phytomass of *P. recta* have also been determined. More recently, from the aerial parts of *P. recta*, ten compounds including a neolignan glycoside and flavonol derivatives have been isolated [3,4]. The purpose of the current study was to investigate in vitro the antibacterial activity (by determination of the MICs) of selected polyphenolic compounds: methyl brevifolin-3奢侈-(e)-p-coumaroyl)-glucopyranoside (II), ellagic acid 3, 3’-di-O-methyl ether 4-0-β-D-xylopyranoside (III), apigenin 7-O-glucopyranoside (IV) against cariogenic microorganisms. This study is financially supported by the Polish Ministry of Science and Higher Education (Grant No. N N405 626138) References: 1. Tomczyk M, Latté KP (2009) Ethnopharmacol 122: 184 – 204. 2. Tomczyk M, Pleszczyńska M, Wiater A (2010) Molecules 15: 4659 – 4651. 3. Tomczyk M, Wiater A, Pleszczyńska M (2011) Phytother Res 25: 343 – 350. 4. Soleretoń D, Krmiezklez H (2011) Biochem Syst Ecol 39: 132 – 134.

**PM129**

Phytoestrogenic activity of *Sophora flavescens* extract and its constituents

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*Sophora flavescens* Ait. is monographed in the Chinese Pharmacopoeia as Radix *Sophorae flavescents* (Kushen) [1]. The crude drug is traditionally used for the treatment of diarrhoea, gastro-intestinal haemorrhage and eczema. *S. flavescens* roots contain quinolinidin alkaloids, non-glycosidic flavones, and triterpene glycosides. Although some of the flavones found in this medicinal plant have potent estrogenic properties, surprisingly, still up to now, very few reports on the estrogenic activity of extracts from this drug have been published [2]. Due to the presence of the alkaloids, *S. flavescens* roots have some toxic potential. Thus, we have engaged in the development of a special extract from this drug...
which is characterized by the absence of alkaloids [3]. This extract was tested for estrogenic activity in a panel of suitable test models. Besides a significant competitive binding to estrogen receptors alpha (ER alpha) and ER beta, induction of alkaline phosphatase in Ishikawa endometrial adenocarcinoma cell was observed. Unfortunately, the extract did not display any estrogen receptor selectivity and promoted uterine growth in ovariectomized rats. Hence, it was considered inappropriate for the treatment of climacteric complaints and precluded from further product development. Keywords: Sophora flavescens, antimicrobial activity References: 1. Kuang L, Zhang K (2005) Pharmacopoea of the Peoples Republic of China, VoI, People’s Medical Publishing House, Beijing, 2. Hillrens PI, Wink M (2005) Planta Med 71: 1065. 3. Dr. Willmar Schwabe GmbH & Co., European Patent EP 1294388 B1 (granted 2004)

PM130

Antibacterial activity of plant extracts highly depends on extraction solvent Sprei C, Mader E, Henkli S, Teichmann K, Schatzmayer G. Biomim Research Center, Tulln, Austria

As an alternative to antibiotic growth promoters in animal nutrition, that have been banned in the EU in 2006, the demand for plant derived substances (phytogenics) is emerging to counteract bacterial infections in swine and poultry. In contrast to antibiotics, phytogenics are expected to refrain from causing transmissible bacterial resistances and leaving critical residues in animal tissue. Looking for potential phytogenics, five different plant raw materials (Berberis aristata DC. root, Sophora flaves- cens Aiton root, Holarrhena antidysenterica (L.) Wall. bark, Bridelia ferruginea Benth. bark, and leaves) were selected. Dry extracts were produced from each material using different extraction solvents (ethanol abs., water and 50/50 (v/v) ethanol/water). The antibacterial activity of the extracts on two pathogenic bacteria, Salmonella typhimurium and Clostridium perfringens Type C, was examined with a turbidimetric microdilution method. The bacterial cultures with defined microbial concentration were incubated together with different concentrations of the extracts. The change in optical density of the bacterial culture led to a quantitative result, indicated as the MIC50 value. The lowest MIC50 values were reached by the ethanol extracts of B. aristata (78 mg/l) and S. flaves- cens (156 mg/l) against C. perfringens Type C. The ethanol and ethanolic extract of H. anti- dysenterica showed higher activity against S. typhimurium. In fact, the ethanol extracts of all plant materials were most effective, except for the extracts of B. ferruginea bark, whereof the water extract was most effective against C. perfringens (MIC50 value 156 – 625 mg/l). Based on these findings about extraction solvent-dependent activity, further investigations towards active substance identification will be accomplished.

PM131

Phytochemistry and biological activities of the ethanolic extract of Onosma australianum Maškovic P1, Niciforović N2, Soljačić S2, Manojlović N2, Cvičović M1, Mladenovic F1, Acimović Djoković G2, Radijoković M1

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This study was aimed at evaluating the antioxidant activity and efficacy of the ethanolic extract of the endemic plant species Onosma australianum DC. in inhibiting the development of selected fungi and bacteria. The highest susceptibility to the ethanolic extract of O. australianum among the bacteria tested was exhibited by B. subtilis and S. aureus (MIC = 15.62 µg/ml). Among the fungi, A. niger (MIC = 15.62 µg/ml) showed the highest susceptibility. Total phenolic, flavonoid, condensed tannin and galloantannin contents were 90.26 ± 0.00 mg GAE/g, 35.24 ± 0.55 mg RE/g, 74.65 ± 0.75 mg CA/g and 31.74 ± 1.05 mg GA/g, respectively. Total antioxidant capacity was 78.45 ± 0.98 µg AA/g, 36.46 ± 1.68 µg/g for inhibited activity against lipid peroxidation, 99.11 ± 0.23 µg/g for hydroxyl radical scavenging activity and 45.91 ± 0.88 µg/g for inhibiting the ros- manic acid was found to be the dominant phenolic compound of the extract. Keywords: antimicrobial activity, antioxidant activity, Onosma australianum, HPLC analysis, phenolic compounds

PM132

Topical anti-inflammatory activity of Plantago lanceolata L. leaves: the relevance of triterpenic acids Sosa S1, Faudale M1, Zacchigna M2, Cateni P2, Del Favero G2, Tubaro A1, Bella Loggia R2

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The leaves of Plantago lanceolata L. (Plantaginaceae) are used in traditional medicine for the topical treatment of skin inflammatory affections [1]. Although P. lanceolata leaf extracts and some of their constituents have been shown to inhibit in vitro enzymes involved in inflammation [1, 2], the in vivo topical anti-inflammatory properties of the leaves have not been investigated. Therefore, P. lanceolata leaves have been studied for their topical anti-inflammatory activity by the Croton oil-induced ear dermatitis assay in mice [3]. P. lanceolata leaves were sequentially extracted with n-hexane, chloroform and methanol and the relevant ex- tracts were evaluated for their ability to inhibit the mouse ear edema induced by Croton oil. Each extract (300 µg/cm2) provoked a significant edema reduction, the croton oil being the most active. Its potency was only two fold lower than that of the reference non steroid anti-inflammatory drug indomethacin: their ID50 (dose inducing 50% edema inhibition) values were 186 and 97 µg/ml, respectively. By column chromatography, the chloroform extract was separated in five fractions (A-E), concentrating its activity into fraction C, which was constituted mainly by ursolic acid (44%) and oleanolic acid (27%). These compounds induced a dose-dependent edema inhibition, and ursolic acid (ID50 ≈ 56 µg/cm2) was more active than oleanolic acid (ID50≈ 132 µg/ cm2) and indomethacin. The two triterpenes, which give a significant contribution to the anti-inflammatory activity of the parent extract, can be proposed as parameters in the quality control of P. lanceolata leaf preparations for the topical use against skin inflammations. References: 1. Beara IN et al. (2010) Pharm Biomed Anal 52: 701 – 706. 2. Vigo E et al. (2005) Pharm Pharmacol 57: 383 – 391. 3. Tubaro A et al. (1985) Agents Actions 17: 347 – 349.

PM133

Cyathula prostrata inhibits in vitro cancer cell growth via multiple targets Van De Venter M1, Schnablegger GE1, Baatjes L1, Koekemoer TC1, Sowemimo A2

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The in vitro anticancer activity of an 80% ethanol extract of Cyathula prostrata (L.) Blume, an annual branching shrub used by traditional healers in Nigeria to treat cancer was investigated. IC50 values were 100.8 µg/ml and 64.4 µg/ml for HeLa (cervical cancer) and U937 (mye- lo-monocytic) cell lines, respectively. Further experiments were performed using 125 µl C. prostrata leaf extract and 0.5 µM cisplatin as positive control. More than 80% of the cells were arrested in the G1 phase after 48 hours of C. prostrata treatment. The annexin V-FITC/PI assay revealed an increase in percentage apoptotic cells from 4.9% to 53.1% at 24h. Cell cycle arrest was not accompanied by increased levels of the cyclin-CDK inhibitor p21. Increase in caspase-8 activation was observed in response to treatment with the extract with no cyt-c release from the mitochondria. The lack of cyt-c release was due to no change in mitochondrial membrane potential, which was investigated with the aid of fluorescent mitochondrial dyes and flow cytometric techniques. The results therefore show that C. prostrata extract induces apoptosis via the extrinsic pathway and this activation is independent of the mitochon- dria. Levels of p53, the catalytic subunit of telomerase, were also shown to decrease upon C. prostrata treatment. The findings from this study suggest that the extract acts through multiple targets, by induc- ing: cell cycle arrest in the G1 phase through an unknown mechanism; apoptosis through an extrinsic death receptor pathway and replicative senescence through inhibition of telomerase. Keywords: Cyathula prostrata, apoptosis, caspase 8, telomerase, cell cycle arrest Acknowledgement:

PM134

Total Phenols, Antioxidant potential and Antimicrobial Activity of the Methanolic Extracts of Ephedra sarcocarpa

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The antioxidant activities of the methanolic extracts of Ephedra sarcocarpa Aitch. & Hems. growing in Iran was evaluated using ferric reducing antioxidant power (FRAP) [1] and 2,2-diphenyl-1-pircyhydrazyl (DPPH) [2] free radical scavenging assays. FRAP values 2.1 mmol eq quercetin/g extracts, and 2.6 mmol eq trolox/g in the DPPH assay 4.6 mg/mL. This plant showed the highest antioxidant activities. This plant showed the highest antioxidant activities. FRAP and DPPH assay results showed good correlations with the total phenolic contents [3] of the plants, measured by the Folin-Ciocalteau assay (r2= 0.920 and 0.893, respectively, p < 0.0001). The antimicrobial activity [4] was screened against Gram-positive and Gram-negative bacteria and fungi. The extract inhibited the growth of Gram-negative bacteria, being pseudomonas aeruginosa the most susceptible one with MIC of 16 mg/mL for the extract. The results obtained indicate that Ephedra may become important in the obtainment of a noticeable source of compounds with health protective potential and antimicrobial activity. Keywords: Antioxidant(s), FRAP, DPPH, Total phenol, Antimicrobial, Ephedra sarcocarpa References: 1. Benzie I F, Strain J (1996) Journal of Analytical Biochemistry 239: 70 – 76. 2. Hwang BY et al. (2001) Journal of Natural Products 64: 82 – 4. 3. Singleton VL, Rossi JA (1965) American Journal of Enology and Viticulture 16: 144 – 158. 4. Bauer AW, Kirby WMM, Sherries JC, Turck M (1966) American Journal of Clinical Pathology 45: 493 – 496.

PM135

Is the inhibition of STAT3 phosphorylation in vascular smooth muscle cells by indirubin-3'-monoxime redox-dependent?

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Indirubin is a natural product found in the traditional Chinese anti-inflammatory recipe, Danshen Longhu. Wang. Its reported anti-proliferative activity makes it a promising candidate in the treatment of cardiovascular diseases (CVDs). We showed recently that the derivative indirubin-3'-monoxime (13MO) inhibits the proliferation of vascular smooth muscle cells (VSMC) by inhibition of STAT3 phosphorylation. The importance of reactive oxygen species (ROS) in STAT3 activation has been demonstrated. However, the role of ROS as a putative target of I3MO acting upstream of STAT3 has not been investigated. Here, we examine the role of ROS as a putative target of I3MO acting upstream of STAT3. Employing the fluorescence probes 2',7'-dichlorodihydrofluorazyl (Hoechst 33258) and 5-carboxy-2',7'-dichlorodihydrofluorazyl, we examined the role of ROS as a putative target of I3MO acting upstream of STAT3 phosphorylation. The results obtained indicate that I3MO may become important in the obtainment of a noticeable source of compounds with health protective potential and antimicrobial activity. Keywords: Antioxidant(s), FRAP, DPPH, Total phenol, Antimicrobial, Ephedra sarcocarpa References: 1. Benzie I F, Strain J (1996) Journal of Analytical Biochemistry 239: 70 – 76. 2. Hwang BY et al. (2001) Journal of Natural Products 64: 82 – 4. 3. Singleton VL, Rossi JA (1965) American Journal of Enology and Viticulture 16: 144 – 158. 4. Bauer AW, Kirby WMM, Sherries JC, Turck M (1966) American Journal of Clinical Pathology 45: 493 – 496.

PM136

Antioxidant, antimicrobial, anti-inflammatory and anticancer activities of Cardhusmus tinctorius flowers

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Cardhusmus tinctorius L. (Asteraceae) is an aromatic and folkloric medicinal plant thanks to its multiple virtues. However, few scientific studies investigated its biological activities. For that, this study aimed to investigate antioxidant, antibacterial, anti-inflammatory and anticancer activities of methanolic flower extracts of Cardhusmus tinctorius in order to validate some of its ethnopharmacological claims. Antioxidant activity was assessed via the ABTS radical scavenging and β-carotene inhibition tests, the antibacterial capacity were tested against human pathogen strains. Whereas, anti-inflammatory activity were estimated using inhibition NO release in LPS-stimulated Raw 264.7 macrophages, in comparison with N(G)-nitro-L-arginine methyl ester (L-NAME) which used as a positive control. In addition, anticancer activity was evaluated against Human lung carcinoma (A-549) and Human colorectal adenocarcinoma (DLD-1) cell lines. Main results showed that the flowers exhibit interesting biological activities. Indeed, flower extract displayed an inhibition percentage against ABTS equal to 30% and over 40% for the β-carotene inhibition assay. Antimicrobial activities were important especially against S. aureus strains (100% inclusion). Concerning anti-inflammatory activity, methanolic extract was able to inhibit NO release by 80% at 160 μg/mL. Furthermore, C. tinctorius extract showed an anticancer activity against tumor cell lines DLD-1 with an IC50 value of 7.9 μg/mL. These findings demonstrate the interesting potentiality of Cardhusmus tinctorius flowers as valuable source of antioxidant compounds which exhibit novel biological activities as antibacterial, anti-inflammatory and anticancer activities. Keywords: Cardhusmus tinctorius, antioxidant capacity, antibacterial activity, anti-inflammatory activity, anticancer activity.

PM137

Evaluation of Hydroalcoholic extract of Astragus fasciculifolius Boiss. on Immunological factors IFN-γ, IL-4 in early sensitized mice induced by Ovalbumin

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The genus Astragus is a very large group of more than 2,000 species and about 800 species in Iran. Currently, much of the pharmacological research on Astragus is focused on its immune-stimulating polysaccharides and other active ingredients useful in treating immune deficiency conditions. Astragus has demonstrated a wide range of potential therapeutic applications in immunodeficiency syndromes, as an adjunct cancer therapy, and for its adaptogenic effect on the heart and kidneys. Astragus can modulate the balance of Th1/Th2 cytokines; it decreases IL-4 and increases IFN-γ. Since, allergy conversely disturbs the balance of Th1/Th2, increases IL-4 and decreases IFN-γ; we decided to use Astragus fasciculifolius Boiss to balance the hydroalcoholic extract of Astragus fasciculifolius assessed by phytochemical tests to recognize the main active constituents. Mice were sensitized with subcutaneous injection of 100 μg of ovalbumin, 1 μg aluminum hydroxide, days 1 and 7. Efficiency of sensitization was assessed by blood IgE levels. then 14
PM138

Betulinic acid enhances glucose uptake in 3T3L1 adipocytes after long term treatment
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The metabolic syndrome including hyperglycaemia and insulin resistance is on the rise worldwide and consequently also cardiovascular diseases and Diabetes Mellitus Type 2. Currently used drugs for these indications are effective, but possess side effects when used chronically. Nature could provide a variety of compounds with undeserved potential to treat and prevent these disorders. In this study, we tested betulinic acid (BA), a naturally occurring pentacyclic triterpenoid, in two diabetes-related assays, namely inhibition of the Protein Tyrosine Phosphatase 1B (PTP1B) in vitro and 2-deoxy-D-glucose (3-D-Glu) uptake in 3T3L1 adipocytes. We found no inhibition of PTP1B activity, despite its close structural similarity to uricosuric agent, a known natural PTP1B inhibitor. However, in differentiated 3T3L1 adipocytes, BA (10 µM) elicited a 1.8-fold increase of the basal glucose (3-D-Glu) uptake rate after 48 hours of treatment. The observed increase in glucose uptake was further enhanced by insulin stimulation and not accompanied by a decrease in cell viability as evident by unaltered cell morphology under the microscope and lack of procaspase 3 cleavage shown by immunoblots. Interestingly, incubation of RAW264.7 macrophages and immortalized human umbilical vein endothelial cells (HUVECs) with 100 µM BA also increased their basal glucose uptake rate approximately 1.4-fold and 1.7-fold, respectively. Given the vast number of so far reported anti-fungal, anti-viral, anti-bacterial and anti-cancer properties of BA [1], our data indicate that BA may be successfully repurposed also for metabolic disorders (hyperglycaemia), and warrant further investigations concerning the underlying mode of action. Keywords: adipocytes, hyperglycaemia, triterpenoids, metabolic syndrome References: [1] Mul-lauer FB et al. (2010) Anticancer Drugs 21(3):215 – 227

PM139

Evaluation of antidiabetic and anti-inflammatory properties of Malaysian Rubiaceae and correlation to their antioxidant potential
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We have previously reported the antioxidant activities of methanolic extracts of 22 species of Rubiaceae plants (family Rubiaceae) [1]. In this paper, we now report the antihyperglycemic and anti-inflammatory properties of the 22 species. The assays employed were α-glucosidase inhibition assay for antidiabetic potential and Griess assay for the measurement of nitric oxide (NO) inhibition in lipopolysaccharide (LPS) and interferon-γ (IFN-γ)-treated RAW 264.7 cells. In the α-glucosidase inhibitory assay, extracts of Hydrophytum formicarum Jack, Psychotria griffithii Hook.f. and Urophyllum griffithianum Hook.f. were shown to be effective inhibitors against α-glucosidase. The results indicated that H. formicarum and P. griffithii showed high percent inhibition in the α-glucosidase inhibitory assay with percent inhibition of 89.8% and 87.7%, respectively. U. griffithianum showed moderate activity with percent inhibition of 68.4% while other species showed no activity. In the anti-inflammatory assay, Hedyotis philippinensis (Wild. ex Spreng.) Merr. ex C.B. Rob. [leaves and stems], Spermacoce exilis (L.O. Williams) C.D.A.dams and Spermacoce latifolia Aubl. showed potent inhibitory activity on NO production in LPS and interferon-γ (IFN-γ)-treated RAW 264.7 cells. We have also found a good correlation (R² = 0.684) between DPPH radical-scavenging activity and α-glucosidase inhibition. Our results support the findings that antioxidants (specifically, radical-scavengers) play an important role in the control and management of diabetes [2]. Keywords: Rubiaceae, Rubioidae, antiadipogenic, anti-inflammatory, α-glucosidase, NO inhibition Acknowledgements: Research and Management Institute (RMI), Universiti Teknologi MARA and Ministry of Higher Education (MOHE) for research grant 600-RMJST/FRGS 5(3)/FSTE(35/2009) and Dr Shamsul Khamis from Universiti Putra Malaysia for identification of plants. References: 1. Ahmad R et al. (2010) African Journal of Biotechnology 9: 7948 – 7954. 2. Rahimi R, Nikfar S, Larijani B & Abdollahi M (2005) Biomedicine and Pharmacotherapy 59: 365 – 373.

PM140

Anti-sickling studies of Nigerian plants
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Sickle cell disorder is a public health problem in many countries particularly in Africa. It is one of the most prevalent haematologic genetic disorders which results from a single point mutation of βGlu6 in Hb to βVal6 in Hbs (1). No drug could effectively cure the disorder but a potentially useful drug if available should effectively provide relief by alleviation of its symptoms. Nonetheless, there are few anti-sickling agents to date available for clinical use (2). In view of its genetic origin, advocacy remains the only option for the prevention of the disorder. However, with over 1 million individuals worldwide with sickle cell disorder, the search for ideal anti-sickling drugs is a major priority. This study reports the anti-sickling properties of eight Nigerian plant species with inhibitory and reversal properties. Extracts of the Colea species tested, in particular, showed the same order of activity as p-hydroxybenzoic acid, the positive control. Keywords: Sickle cell disorder, plants, Colea species, anti-sickling properties References: 1. Quattara B et al. (2009) Phytomedicine 16: 125 – 129 2. Martin KS et al. (2004) J Med Chem 47: 4665 – 4676

PM141

In vitro COX-1, COX-2 and 5-LOX inhibitory assay of their wild family Ranunculaceae
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The arachidonic acid metabolism is the main target for non-steroidal anti-inflammatory drugs (NSAIDs). Two cyclooxygenases (constitutive COX-1 and inducible COX-2) and lipoxygenase (5-LOX) enzymes are responsible for transformation of arachidonic acid into the potent biologically active lipid mediators that are intimately involved in inflammation [1]. The newly developed COX-2 selective inhibitors seem to possess lower risk of unwanted side-effects than traditional NSAIDs. In our previous study, we identified potential COX-2 inhibiting plant material in Ranunculaceae family [2]. It is now being perceived, that dual blocking of both COXs and 5-LOX is promising approach to treatment of inflammatory diseases [3]. Thus we decided to evaluate the in vitro inhibitory activity against both COXs and 5-LOX of ethanolic extracts prepared mainly from roots of more than 30 plant species belonging to plant family Ranunculaceae using method previously described by Re-ininger and Bauer [4] and Adams et al. [5], respectively. The amounts of prostaglandin E2 (for COX) and leukotriene B4 (for 5-LOX) were determined by commercial EIA kits (Assay Designs). The highest prevention against production of COXs and 5-LOX derived eicosanoids possessed extract from roots of Helleborus purpurascens Waldst. & Kim., where COX-1/COX-2/5-LOX scavenging rate 1,5/1/1,2 was recorded. The consequent bioactivity guided fractionation showed, that isomers of linoleic acid seem to be responsible for blocking of even COXs or 5-LOX. Cimicifuga racemosa (L.) Nutt. and Trollius alifissimus Crantz were also determined as other promising plant materials, suggesting these species potential for further research for new anti-inflammatory substances. Keywords: cyclooxygenases, lipoxygenase, anti-inflammatory. Ranunculaceae, in vitro Acknowledgement: This research was supported by Czech Science Foundation (Project No. 525/08/1179). References: 1. Claria J.
Sutherlandia frutescens (L.) R.Br. ex W.T.Aiton is an indigenous South African medicinal plant traditionally used to treat a number of ailments including diabetes. While previous in vivo studies have confirmed its anti-diabetic properties, the precise molecular mechanism of action has not been elucidated. In the present study we have established that S. frutescens treatment specifically attenuates a number of adipose tissue related parameters, including circulatory and adipose tissue free fatty acid and triglyceride levels. The lack of any significant changes in adipose tissue nitrotyrosine and plasma MCP-1, both classical markers for adipose inflammation, indicates that these effects are not attributable to anti-inflammatory properties. In 3T3-L1 preadipocytes, treatment led to a significant increase in the rate of glucose consumption despite the complete absence of triglyceride accumulation. This increased glucose consumption is reflected by a corresponding dose dependent increase in lactate production, suggesting an increased glycolytic flux in treated cells. Taken together our in vivo and in vitro findings are consistent with a hypothesis in which S. frutescens induces mitochondrial uncoupling in adipose tissue, resulting in a reduced efficacy of oxidative phosphorylation and a consequent up-regulation of glycolysis. In this manner the carbon flux is redirected away from lipid synthesis resulting in both adipose tissue nitrotyrosine and plasma MCP-1, both classical markers for adipose tissue inflammation and a consequent up-regulation of glycolysis. In this manner the adipose tissue, resulting in a reduced efficacy of oxidative phosphorylation, mitochondria serve for the ointment formulation prepared with 1% M. nuda extracts.

Michauxia L’Herit (Campanulaceae) species are used as vegetable and medicinal plants in Turkey, and five species of this genus grow naturally in Turkey. The leaf of M. capitifoliae L’Herit ex Aiton is used in traditional medicine the treatment of wounds in Kahramanmaras (Turkey). The present study was designed to investigate the wound healing and anti-inflammatory activities of the water and methanol extracts obtained from the root and herb of five Michauxia species. The incision was performed on the right and left flanks of C57BL/6 mice. The surgical wounds were categorized into four groups: (A) control, (B) fodder, (C) ethanolic extracts, and (D) methanolic extracts. The grafting was performed using pedicle type. Effects of the extracts on the viability of RAW 264.7 macrophages were determined by using WST-1 cell viability assay. Effects on NF-κB activation on LPS-induced RAW 264.7 macrophages were determined by using Image J program. The hexane, chloroform and remaining water subextracts of C. laurifolius exhibited the highest activity (54.9%, 54.8% and 27.7%, respectively). Only hexane extract of C. nigra exhibited an inhibition of inhibition zones was done by spraying the surface of culture media with MIT solution. Clear zone in dark purple background was the positive and the negative anaerobic bacteria. In the study, the hexane, chloroform and remaining water subextracts of C. laurifolius which is used in Turkish folk medicine for treatment of rheumatism and related inflammatory problems, were evaluated for NF-κB inhibitory activity.

P. aeruginosa and S. aureus exhibited the highest activity (9.3%) and remaining water subextracts of C. nigra exhibited the lowest activity (9.3%). Only hexane extract of C. nigra exhibited an inhibition of inhibition zones was done by spraying the surface of culture media with MIT solution. Clear zone in dark purple background was the positive and the negative anaerobic bacteria. In the study, the hexane, chloroform and remaining water subextracts of C. laurifolius which is used in Turkish folk medicine for treatment of rheumatism and related inflammatory problems, were evaluated for NF-κB inhibitory activity.

PM144

Antimicrobial evaluation of hydroalcoholic extract and Volatiles components of aerial part of Kelussia odoratissima Moazzaf.

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Kelussia odoratissima Moazzaf, is a sweet-smelling, wild plant which is traditionally consumed in Iran as a garnish. Only Little, is known about its potential antioxidant activity. In this study, the antibacterial activities, against Staphylococcus aureus, Escherichia coli, Bacillus cereus and Pseudomonas aeruginosa were investigated by paper Disc and immersion bioautography Methods. Separately Ethanol extract was taken by maceration method and Volat. components was taken by cold solvent extraction method using petroleum ether. Inhibitory zones of blank discs, impregnated with defined amounts of extract were observed and MIC were measured using E-Test method. In bioautographic Methods, extract and volatile components were separated on silica gel TLC plates to the best resolution then were placed on agar plate inoculated with bacterial suspension. After incubation for 16–18 hr at 37°C, visualization of inhibition zones was done by spraying the surface of culture media with MIT solution. Clear zone in dark purple background was the positive and the negative anaerobic bacteria. In the study, the hexane, chloroform and remaining water subextracts of C. laurifolius which is used in Turkish folk medicine for treatment of rheumatism and related inflammatory problems, were evaluated for NF-κB inhibitory activity.

NF-κB is a transcription factor mediating the expression of several genes involved in inflammation and its inhibition might be a valuable strategy to develop effective anti-inflammatory agents [1]. Sambucus ebulus L, S. nigra L (Caprifoliaceae) and C. laurifolius (Cistaceae) which are used in Turkish folk medicine for treatment of rheumatism and related inflammatory problems, were evaluated for NF-κB inhibitory activity.

Effects of the extracts of the ointment formulation prepared with 1% M. nuda extracts.

PM145

Effects of the extracts from Turkish medicinal plants on NF-κB activation on LPS-induced RAW 264.7 macrophages

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PM143

Wound healing and anti-inflammatory activities of the Michauxia L’Herit (Campanulaceae) species native to Turkey

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Some of the potentially beneficial ingredients which are traditionally used in wound healing are obtained from some plants but their effectiveness has not been scientifically evaluated yet (1, 2). In this study, new cream formulations (Levant storax and Complex creams) with the same cream base were developed. The composition of the cream base was as follows: (1) Butyrospermum parkii (shea butter) (2) squalane, (3) cetyl oleate and sorbitan olate, (4) tetrasodium EDTA, (5) methylparaben, ethylparaben, propylparaben, butylparaben and isocapric triglyceride, (6) petrolatum, (7) glycerine, (8) petrolatum, (9) benzyl alcohol, (10) isopropyl alcohol and, in addition to the ingredients used in the cream base, Levant storax cream also contained balsam of oriental sweet gum, while Complex cream contained (1) calendula oil, (2) petrolatum, (3) glycerine, (4) cetyl stearyl alcohol, (5) caprylic/capric triglyceride, (6) petrolatum, (7) glycerine, (8) tetrasodium EDTA, (9) methylparaben, ethylparaben, propylparaben, butylparaben and isocapric triglyceride, (10) deionized water. In addition to the ingredients used in the cream base, Levant storax cream also contained balsam of oriental sweet gum, while Complex cream also contained balsam of oriental sweet gum, while Complex cream contained...
The standardized rose hip powder LitoMove® (Rosa canina L.) is a widely used herbal remedy. Consumption of LitoMove® has been shown to reduce pain in patients with osteoarthritis [1]. The dichloromethane extract of LitoMove® possesses in vitro immunomodulating effects which have been correlated to the presence of triterpene acids [2]. To establish if the clinical effect of LitoMove® is caused by anti-inflammatory or analgesic effects, the dichloromethane extract was tested in the paw edema model of inflammation and the 1:1 methanol: dichloromethane extract in the hot plate test of acute pain. In both models, extracts were administrated orally once daily in the indicated period. Treating rats with 100 mg dry extract/kg for three weeks did not result in a significant reduction of the paw edema compared to the control group. In the hot plate test, mice were treated with 500 mg dry extract/kg for five days. No significant difference in pain threshold between the treatment and the control group could be observed. It thus appears that the tested extracts neither possess anti-inflammatory nor analgesic effects in the chosen animal models. However, the paw edema model and the hot plate test are general models of inflammation and pain and thus, may not represent the inflammation and pain in arthritis. Therefore, further studies in specific animal models of arthritis inflammation and pain are needed before the clinical effects of LitoMove® can be understood. Keywords: Rosa canina, arthritis, inflammation Acknowledgement: HybenVital and the Danish Rheumatism Association are thanked for financial assistance. References: 1. Davis PH (1982) Flora of Turkey and Ege University, 35100 Bornova-Izmir, Turkey; 2. Department of Biology, Faculty of Science, Ege University, Bornova, 35100 Izmir, Turkey; 3. Department of Pharmacology, Faculty of Pharmaceutical Sciences, Universiteitsparken 2, 2100 Copenhagen, Denmark

Ulmus L. (Ulmaceae) is represented by three species in Turkey. Ulmus minor Mill. subsp. minor is a tree to 30 m but often much smaller, suckering. Twigs glabrous or sparsely pubescent [1]. Although there are no reports of the medicinal uses of Ulmus minor Miller subsp. minor in Turkey, the bark of the root and stem of Ulmus davidiana var. japonica has been used as a traditional Korean medicine to treat inflammatory disorders. This plant reportedly exhibits antioxidant, anticancer, and anti-inflammatory effects [2]. Ulmus species contain biologically active compounds, such as sesquiterpenoids, triterpenes and flavonoids [3,4]. In this study, n-hexane, dichloromethane, ethyl acetate, methanol, and methanol:water (20:80) extracts of leaves and stems of Ulmus minor subsp. minor were tested separately against selected Gram-positive, Gram-negative bacteria and Candida albicans, an unicellular yeast, using a broth microdilution broth susceptibility assay. All of the extracts exhibited antihelmintic activity against Enterococcus faecalis and Salmonella typhimurium resulting MIC values 0.81 and 2.5 mg/ml. Ethyl acetate extract of the stems of the plant was found to be active against all tested microorganisms with a range of MIC values extended from the concentration of 6, 25 – 0, 2 mg/ml. Keywords: Ulmus minor, Ulmaceae, anti-microbial activity References: 1. Davis PH (1982) Flora of Turkey and East Aegean Islands, University Press, Edinburgh. 2. Choi SY et al. (2010) J Med Food 13: 1019 – 1023. 3. Zheng MS et al. (2010) Biomol Ther 18: 321 – 328. 4. Lee CY et al. (2008) Planta Med 74: 1800 – 1802.

Semecarpus kathalekanesis Dassapa & Swaminath, an evergreen tree with very large simple leaves, which attains a height of about 30 m belonging to the family Anacardiaceae which is critically endangered and belongs to the family Anacardiaceae. It is a swamp tree and consists major chemical compounds like phenols, bioflavonoids and traditionally having high medicinal importance also used as an antimicrobial, antioxidant, and as an anticancer. The endophytic fungi were isolated from plant species and subjected for antimicrobial studies which showed significant results against gram positive and gram negative bacteria. Keywords: Endemic, Endophytic and Semecarpus kathalekanesis

The influence of extracts from Potentilla species on normal human colon cells

The biological activity of extracts obtained from aerial parts of Potentilla species: P. ereta (L.), P. argentea, P. argentea L., P. rupestris L. and P. grandiflora L. was analyzed. Extracts were tested using MTT, NR and DPPH tests on two human normal cell lines: CDD 841 CoT and CDD-18Co. Fluorescence staining of the cellular cytoskeleton after rhodamine-phalloidin addition and IL-6, IL-10 (ELISA) in culture supernatants after 24 h of incubation with Potentilla extracts and nitric oxide (NO) analysis with a Griess method were performed. Extracts were tested at the range of 25 – 225 µg/ml concentrations while to the ELISA two non-toxic doses (15 and 30 µg/ml) were chosen. We found that all extracts stimulated metabolism of epithelial cells while myofibroblasts’ mitochondrid dehydrogenase activity was stimulated at concentrations higher than 125 µg/ml. Activity of P. grandiflora which activated succinyl dehydrogenase just at low extract dose (25 µg/ml). Extracts from P. ereta and P. argentea had no toxic effect on colon epithelial cells while other extracts significantly decreased viability of cells even when added at 25 µg/ml concentration. Only P. grandiflora and P. rupestris significantly decreased viability of myofibroblasts. All extracts showed free radical scavenging effect in a concentration dependent manner. Potentilla extracts inhibited IL-6 and IL-10 production by myofibroblasts while in epithelial cells slightly induced or had no effect on the cytokine level. Potentilla extracts influenced F-actin filament composition and changed the cellular cytoskeleton and morphology of cells. Modulation of NO production after plant extracts addition has also been observed. Keywords: Potentilla, cytotoxicity, normal human colon cells

Antimicrobial and cytotoxic properties of roots of Centaurea cadmea Boiss.

Centaurea cadmea Boiss. is an endemic taxon for Anatolia, growing wild in N, W & SW of Turkey [1]. Phytochemical studies revealed the presence of a sesquiterpene lactone, ivalin, which is known cytotoxic com-
pound on several tumor cell lines (2), together with equatorin, 5-hydroxy-3',4',6,7- tetramethoxyflavone and β-sitosterols from the aerial parts of C. cadmea (3). In vitro anti-inflammatory, antioxidant, antiprotozoal and antimicrobial activities of the aerial parts of C. cadmea extracts have been reported before (4, 5), but no bioactivity study has been performed on roots of the plant, yet. The present study aims at investigating the antimicrobial and cytotoxic activities of roots of C. cadmea. The antimicrobial activities of extracts of the plant were investigated by MIC method. The antimicrobial activities of the extracts were tested against four gram negative (Escherichia coli ATCC 23999, Pseudomonas aeruginosa ATCC 27853, Salmonella typhimurium CCM 5445 and Klebsiella pneumoniae CCM 2318) and four gram positive (Staphylococcus aureus ATCC 6538P, S. epidermidis ATCC 12228, Enterococcus facialis ATCC 29212 and Bacillus cereus ATCC 7064) bacteria strains. While the chloroform extract of the plant has no activity against the tested microorganisms, the methanol extract has weak anti-bacterial activity (MIC = 64 – 256 µg/ml). The cytotoxic activity were analyzed by cell proliferation assay using WST-1 reagent against three human cancer cell lines; MCF7 (human breast cancer), A549 (human lung cancer), U2OS (human osteosarcoma) and one non-cancer cell line; 293 (human embryonic kidney). C. cadmea extract was found active against U2OS (IC50 = 138 µg/ml).

Keywords: Centaurea cadmea, Asteraceae, cytotoxicity, antimicrobial

Acknowledgement: Authors are appreciated to U. Karabay-Yavasoglu, PhD. and P. Ballar, PhD. for their scientific contribution.

Antibacterial activity of the essential oil and main components of two Dracocephalum species from Iran

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Antibacterial activity of Dracocephalum polychaetum Bornm. and D. surmacum species essential oils and two main components were investigated. Essential oils of the plants were analyzed by GC and GC-MS[1]. The oil was rich in oxygenated compounds (73.1%) and Oxygenated aldehydes (54.3%) and limonene (30.1%) were the main constituents. The 25 identified compounds (97.8%) in the oil of D. polychaetum were investigated. Essential oils of the plants were analyzed by GC and GC-MS.[1] Adams RP (2007) Identiﬁcation of essential oils components by Gas Chromatography/Quadrupole Mass Spectrometry. Allured Publishing, Carol Stream, IL.

References:

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Antibacterial activity of the essential oil and main components of two Dracocephalum species from Iran

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Antibacterial activity of Dracocephalum polychaetum Bornm. and D. surmacum species essential oils and two main components were investigated. Essential oils of the plants were analyzed by GC and GC-MS[1]. The oil was rich in oxygenated compounds (73.1%) and hydrocarbons (25.0%). Monoterpenes including perilla aldehyde (63.4%) and limonene (22.1%) were among the 25 identified compounds (97.8%) in the oil of D. surmacum perilla aldehye (54.3%) and limonene (30.1%) were the main constituents. The bioassays exhibited that all of the Gram-positive and Gram-negative bacteria tested were highly inhibited in the presence of the oils and main compounds investigated. The most sensitive microorganism to the oils was found to be Staphylococcus epidermidis with the lowest MIC value of 0.3 mg/ml. The resistant Gram-negative Pseudomonas aeruginosa was highly inhibited by the oil of D. polychaetum with MIC value of 2.4 mg/ml. The resistant Gram-negative Pseudomonas aeruginosa was highly inhibited by the oil of D. polychaetum with MIC value of 2.4 mg/ml. Keywords: Dracocephalum, antibacterial activity, essential oil, perilla aldehyde, limonene References: [1] Adams RP (2007) Identification of essential oils components by Gas Chromatography/Quadrupole Mass Spectrometry. Allured Publishing, Carol Stream, IL.

Screening of Anti-Inflammatory Activity of Natural Products through A Panel of Target Based Assays

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Inflammation is considered as a risk factor for several types of cancers, obesity and metabolic disorders. Chronic inflammation has been linked to various steps involved in tumorigenesis, including cellular transfor-
Long-term effects of the rhapontic rubarb extract ERY 731® on estrogen-regulated targets in the uterus and on the bone in ovariectomized rats

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The efficacy of the commercially available extract ERY 731® from Rheum rhaponticum L. regarding attenuation of menopausal complaints like hot flushes, depression, anxiety and vaginal dryness has been proven in a two-year clinical study. Further, no undesired side effects like uterotrophy or proliferation of the endometrium became apparent while testing ERY 731® in a 3-day uterotropin assay. The present study aimed at further substantiating the safety of application of ERY 731® regarding endometrial hyperplasia and at the same time test for potential bone sparing effects in the preclinical ovariectomized (ovx) rat model. For this purpose we performed a 90 d dietary feeding study in ovs rats. The impact of estrogen receptor modulation was investigated by assessing the mRNA levels of proliferation marker genes (Mki67, PCNA) in the proximal tibia metaphysis using peripheral computed tomography and quantified bone homeostasis marker in the serum. With this study we report for the first time about phenolic profile, antioxidant and anti-inflammatory agents.

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Anti-inflammatory property of Juniperus communis L. var. communis needles and cones extracts and essential oils
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All over the world plants from the Juniperus genus have always been regarded as a well-known traditional remedy and spice. These plants are extensively used in the folk medicine for healing various disorders: common cold, urinary and kidney infections, dermatological disorders, bronchitis, pneumonia, dysentery, hemorrhage, rheumatic arthritis, stomachache, diarrhea and for regulation of the menstruation and in relieving menstrual pain [1]. However, there are only few literature data about their pharmaceutical activity and chemical composition. In this study anti-inflammatory properties of methanol extracts and essential oils of leaves and cones of the Juniperus communis L. var. communis, were determined using assays which measure the inhibitory potency toward COX-1 and 12-LOX enzymes in human platelets, by novel optimized method which was based on method previously described [2].

References:

Regulatory effect of 4-O-methylhonokiol on TGF-β1-induced cell cycle arrest in human keratinocyte cell line (HaCaT)
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Transforming growth factor-β (TGF-β) signal pathway has a pivotal role in the progression of catagen phase in hair growth cycle. 4-O-Methylhonokiol, a neolignan compound from Magnolia Officinalis, has various biological activities such as anti-inflammatory, neurite outgrowth activity and anti-acetylcholinesterase activity. Recently we have reported the hair-growth promoting effect of 4-O-methylhonokiol. However, the hair-growing mechanisms of 4-O-methylhonokiol on the TGF-β signal pathway have not yet been elucidated. We thus examined whether 4-O-methylhonokiol has an inhibitory effect on TGF-β signal pathway in HaCaT cells. When HaCaT cells were pretreated with 4-O-methylhonokiol, the expression of TGF-β1-induced p21 was decreased. Moreover, 4-O-methylhonokiol attenuated the nuclear translocation of Smad2/3, Smad4 and Sp1 activation. 4-O-Methylhonokiol reduced TGF-β1-induced activation of ERK. On the other hand, TGF-β has been reported to increase reactive oxygen species (ROS), and TGF-β1-induced growth arrest have been known to be mediated by oxidative stress. 4-O-methylhonokiol inhibited TGF-β1-induced ROS production and suppressed mRNA expression of NOX4. These results suggest that hair-growing activity of 4-O-methylhonokiol might be at least related to its modulatory way have not yet been elucidated. We thus examined whether 4-O-methylhonokiol has an inhibitory effect on TGF-β signal pathway in HaCaT cells. When HaCaT cells were pretreated with 4-O-methylhonokiol, the expression of TGF-β1-induced p21 was decreased. Moreover, 4-O-methylhonokiol attenuated the nuclear translocation of Smad2/3, Smad4 and Sp1 activation. 4-O-Methylhonokiol reduced TGF-β1-induced activation of ERK. On the other hand, TGF-β has been reported to increase reactive oxygen species (ROS), and TGF-β1-induced growth arrest have been known to be mediated by oxidative stress. 4-O-methylhonokiol inhibited TGF-β1-induced ROS production and suppressed mRNA expression of NOX4. These results suggest that hair-growing activity of 4-O-methylhonokiol might be at least related to its modulatory way have not yet been elucidated. We thus examined whether 4-O-methylhonokiol has an inhibitory effect on TGF-β signal pathway in HaCaT cells. When HaCaT cells were pretreated with 4-O-methylhonokiol, the expression of TGF-β1-induced p21 was decreased. Moreover, 4-O-methylhonokiol attenuated the nuclear translocation of Smad2/3, Smad4 and Sp1 activation. 4-O-Methylhonokiol reduced TGF-β1-induced activation of ERK. On the other hand, TGF-β has been reported to increase reactive oxygen species (ROS), and TGF-β1-induced growth arrest have been known to be mediated by oxidative stress. 4-O-methylhonokiol inhibited TGF-β1-induced ROS production and suppressed mRNA expression of NOX4. These results suggest that hair-growing activity of 4-O-methylhonokiol might be at least related to its modulatory way have not yet been elucidated. We thus examined whether 4-O-methylhonokiol has an inhibitory effect on TGF-β signal pathway in HaCaT cells. When HaCaT cells were pretreated with 4-O-methylhonokiol, the expression of TGF-β1-induced p21 was decreased. Moreover, 4-O-methylhonokiol attenuated the nuclear translocation of Smad2/3, Smad4 and Sp1 activation. 4-O-Methylhonokiol reduced TGF-β1-induced activation of ERK. On the other hand, TGF-β has been reported to increase reactive oxygen species (ROS), and TGF-β1-induced growth arrest have been known to be mediated by oxidative stress. 4-O-methylhonokiol inhibited TGF-β1-induced ROS production and suppressed mRNA expression of NOX4. These results suggest that hair-growing activity of 4-O-methylhonokiol might be at least related to its modulatory
action on TGF-β-induced cell cycle arrest and ROS production. Keywords: 4-O-methylirected; Magnolia officinalis; TGF-β; HaCaT cells; cell cycle arrest, NOX4

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Chemical composition, antioxidant and antimicrobial activities of the lichen Toninia candida (Weber) Th. Fr. [Cetrarialesae].
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In the present investigation, methanol, chloroform and petroleum ether extracts of the lichen Toninia candida (Weber) Th. Fr. were assayed for their antioxidant and antimicrobial activities. The phenolic composition of the extracts was determined by HPLC-UV analysis. The predominant phenolic compound in all the extracts was the depsidone norstictic acid. Apart from the norstictic acid, the tested extracts of T. candida contain different amounts and ratios of artranor and stictic, protoberanteic and usnic acids. The lichen extracts showed comparable and strong antioxidant activity, exhibited higher DPPH and hydroxyl radical scavenging capacity, chelating activity and inhibitory activity towards lipid peroxidation. The lichen extracts demonstrated major antimicrobial activity against 8 strains with MIC values ranging from 16.62 to 62.50 μg/ml. This is the first report of the chemical composition, antioxidant and antimicrobial activities of the lichen Toninia candida. Keywords: Toninia candida, HPLC-UV, chemical composition, antioxidant activity, antimicrobial activity. Acknowledgement: This work was supported by the Serbian Ministry of Science and the Environment, Project No. 172015

PM163

An in vitro approach to neuroprotective activity of Rosa damascena Mill., a medieval age traditional medicine used for memory enhancement
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Rosa damascena Mill. was recorded to be used traditionally for memory enhancement in the medieval age. Therefore, neuroprotective effects of the essential oil and aromatic waters of R. damascena was investigated by in vitro and in silico methods. The essential oil and its components (citronellol, geraniol, nerol, and phenylethyl alcohol), and two samples of the aromatic water (Eau de rose) of R. damascena were tested for their inhibitory activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) at 100, 200, 500, and 1000 μg/ml. Since oxidative damage is associated with neurodegeneration, antioxidant activity of the samples was determined by DPPH radical scavenging, metal-chelation, and ferric-reducing antioxidant power (FRAP) assays. Chemical composition of the samples was elucidated by GC-MS. The rose essential oil showed a noteworthy inhibition against AChE (60.86 ± 1.99%) and BuChE (51.08 ± 1.7%) at 1000 μg/ml, whereas the aromatic waters did not have any inhibition. The essential oil exhibited moderate activity in antioxidant assays. Phenylethyl alcohol exerted higher cholinesterase inhibition than other components. None of the double and triple combinations of citronellol, geraniol, nerol, and phenylethyl alcohol could reach at inhibition level of phenylethyl alcohol. Phenylethyl alcohol was theoretically studied utilizing molecular docking simulations into the active site gorge of AChE and BuChE and the data revealed that this compound is more selective towards BuChE than AChE. Our findings confirmed traditional use of R. damascena for memory enhancement, which is suggested to come into view through mainly cholinesterase inhibition, and antagonistic interaction presumably exists between phenylethyl alcohol and other components. Keywords: Rosa damascena, rose water, memory enhancement, enzyme inhibitory activity

PM164

Studies on anticholinesterase and DPPH radical scavenging effects of 41 species of Fritillaria L. genus of Turkish origin.
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The genus Fritillaria L. (Liliaceae) is a member of geophytes with attractive flowers, which are cultivated as ornamental plants. Many of the European Fritillaria species are found in the Alps, the Pyrenees, the Balkans, and northern Turkey. There are 41 Fritillaria species growing in Turkey, 26 of which are endemic. The research carried out on Fritillaria species are focused on the alkaloid content of the plant. In the present study, the dichloromethane and methanol extracts prepared from the bulbs of 59 samples belonging to 41 Fritillaria cultivated in Turkey have been investigated for their cholinesterase inhibitory activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), the enzymes linked to Alzheimer’s disease, at 50, 100, and 200 mg mL⁻¹ using ELISA microplate reader. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging effect of the extracts was also tested 12.5, 25, and 50 mg mL⁻¹ final concentrations. According to our results, the highest inhibition against AChE was caused by the dichloromethane and methanol extracts of F. persica (21.03 ± 0.34% and 27.39 ± 2.26%, respectively). The most active extracts against BuChE were found as the dichloromethane extract of F. pinardii Boiss. (49.72 ± 2.56%) and two samples of F. persica (48.27 ± 1.98% and 47.29 ± 1.72%). Among the methanol extract, the best BuChE inhibitions were found to be caused in F. minima Rix (54.69 ± 3.40%), F. persica (51.85 ± 4.68%) and F. carusca J.F. Adam (46.14 ± 2.96%). On the other hand, all of the extracts displayed low profile of DPPH scavenging effect below 25%. The present results indicate that especially F. persica could be a potential source of natural compounds with anticholinesterase activity. Keywords: Fritillaria, anticholinesterase activity, free radical scavenging effect

PM165

Methanolic Alnus glutinosa bark extract affect ROS and TNF-α production.
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Alnus glutinosa (L) Gaertn., commonly known as ‘European alder’, presents several uses as secondary plant metabolites, including antiinflammatory, antifungal, and antimicrobial properties. The traditional uses suggest that AGSB may contain active metabolites related to the inflammation process. Inflammation is associated with the progression of numerous diseases and is accompanied by the chronic release of cytokines and reactive oxygen species (ROS), which may be involved in tissue injury increment. Moreover, ROS, as well, contribute to the expression of a variety of different inflammatory cytokines such as TNF-α, which is considered to be a primary mediator of the inflammatory response. In this sense, the present study was designed to evaluate the capacity of the AGSB extract to reduce ROS generation in H2O2-induced oxidative stress in HeLa cells. In addition, the extract effect on TNF-α production using HL60 cell line, was also tested. Results show that the AGSB extract is able to protect cells from induced oxidative stress and may be able to decrease TNF-α production. These biological properties are linked to a successful reduction in inflammatory processes and may support, in part, its ethnopharmacological use. Keywords: Alnus glutinosa, TNF-α, ROS.
The genus Paeonia L. (Paeoniaceae), known as "sakaykik, ayí gúlú, bocur, etc." in Turkey, was recorded to be used in Chinese traditional medicine against amnesia. Paeonia species were recorded in Turkey, which is the most important gene center worldwide for this genus. Consequently, the ethanol extracts of the defatted seeds of 7 Paeonia taxa; (P. anitaei Anders., P. dautria Andrews, P. mascula Miller subsp. bodnarii N. Ozhatay, P. cf. mascula L. (Mill.) subsp. mascula, P. peregrina Miller, P. tenuifolia L., and P. khayae N. Ozhatay) were screened against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), linked to Alzheimer’s disease and tyrosinase (TYRO), connected to Parkinson’s disease using ELISA microplate reader. As amnesia is a neurodegenerative situation associated with oxidative damage, antioxidant activity of the extracts was also measured by radical scavenging activity tests against 2,2-diphenyl-1-pircylhydrazyl (DPPH), N,N-dimethyl-p-phenylendiamine (DMPD), and nitric oxide (NO) as well as metal-chelation capacity and ferric-reducing antioxidant power (FRAP) tests. Total phenol and flavonoid contents were determined spectrophotometrically. All of the extracts strongly inhibited AChE (85.69 € 0.58% – 96.68 € 0.44%), BChE (73.34 € 1.92% – 84.21 € 0.21%), and DMPD radicals, whereas they were not able to quench NO. They exhibited moderate FRAP values and very low metal-chelation capacity. As conclusion, our findings reveal that Paeonia species possess potent anti-amnesic activity in vitro via enzyme inhibition associated with neurodegeneration. The present study confirms the claimed utilization of the amnesic activity in vitro via enzyme inhibition associated with neurodegeneration. The current study confirms the claimed utilization of the amnesic activity in vitro via enzyme inhibition associated with neurodegeneration. The present study confirms the claimed utilization of the amnesic activity in vitro via enzyme inhibition associated with neurodegeneration. The present study confirms the claimed utilization of the amnesic activity in vitro via enzyme inhibition associated with neurodegeneration.
Salvia species are rich in terpenoids [2,3], steroids, flavonoids and other phenolics. In our continuing studies on Salvia species, we have isolated many oleanane and ursane type triterpenoids, namely oleanolic and ursolic acids in addition to diterpenoids, flavonoids and phenolics. Besides oleanane and ursane triterpenoids, we have also isolated lupane triterpenoids from Salvia species, but with poor biodiversity. In this presentation, a number of lupane triterpenoids, obtained from the extracts of several Salvia species (S. montbretii Benth., S. cedronella Boiss., S. macrochlamys Boiss. & Kotschy ex Boiss., S. strichocladta Benth., S. hypargera Fisch. & Mey.)[4,5] will be presented with their promising activity results. Structures of the pure triterpenoids were identified by spectroscopic analysis using extensive NMR (1D and 2D), UV, IR, and mass spectroscopic techniques. Antioxidant activity of the triterpenoids was established by β-caroten bleeding and radical scavenging methods. The most promising activity was found for globolides, and those were tested against AChE and BChE enzymes by Ellman Method [6]. Monogynol A and its three natural derivatives, isolated from S. macrochlamys were also found to be highly active in a metal chelating test system on ferrous ion [5]. Keywords: Salvia species, S. montbretii, S. cedronella, S. macrochlamys, S. strichocladta, S. hypargera, antioxidant activity, Ellman Method.

In-house pharmacophore Figure 1: In-house pharmacophore

Cancer is one of the diseases with increased prevalence in the 21st century. Since the 1970s a class of transmembranar proteins called ABC transporters are known, being P-Glycoprotein (Pgp) the most representative one. Several pharmacophores were already published that failed to detect our in-house macrocyclic diterpenes [4]. Inspired on the published literature and based on the lathyrane-type scaffold, we developed a new pharmacophore capable of detecting not only the published literature-derived ones, but also our in-house macrocyclic diterpenes [4]. Improved pharmacophore description of P-glycoprotein modulators

Figure 1: In-house pharmacophore

Osteoporosis is a major disease in postmenopausal women. There is compelling evidence that the special extract from Cimicifuga racemosa (L.) Nutt. (CR) BNO 1055 influences positively bone metabolism and prevents induced osteoporosis by ovariectomy (ovx). Aim of the study was the investigation of CR BNO 1055 extracts and their protective effects on bone metabolism. Via liquid-liquid extraction, extracts were separated into two groups, i.e. a lipophilic fraction in saponins and a hydrophilic rich in sugars and phenylpropanoids. Preparative fractions were characterized by TLC and by HPLC-UV-ELSD and HPLC-MS. Analytical data clearly prove the presence of triterpene glycosides in the lipophilic fraction and the presence of carbohydrates and phenylpropanoid acid derivatives in the hydrophilic fraction. To investigate the activity on bone metabolism of CR, ovx rats received either nutrition containing CR, 17β-oestradiol or food without any active component. All types of food were soy-free food. QCTs were performed at the level of the metaphysis of the tibia and the trabecular density measured prior to the ovariectomy and the end of the four weeks of the extracts. Within these four weeks the bones of the ovx rodents lost nearly half of their cancellous density. This effect did not appear by rats fed with nutrition including 17β-oestradiol and CR. The lipophilic fraction shows a significant high activity on bone metabolism while the hydrophilic fraction was inactive. The QCT results and the prevention effect of CR BNO 1055 on bone is up to the lipophilic compounds of the extract. Keywords: Black Cohosh, Cimicifuga racemosa, bone metabolism

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PM171
Black Cohosh and the protective effects on bone metabolism as measured by computer tomography (qCT) in ovariectomized (ovx) rats

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PM172
Biologically active lupane triterpenoids from Anatolian Salvia species

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Salvia species are used in perfumery, food and drug industry, belonging to Lamiaceae family which contain annual or perennial plants with nice fragrance. Totally 900 Salvia species grow widely all over the world, and Turkey has over 90 Salvia species, half of them being endemic [1]. Since ancient times, Salvia species have been traditionally used as antiseptic, digestive, carminative, and sedative as well as in the treatment of bronchitis, tuberculosis, menstrual, and neurological disorders. Salvia species

Figure 1: In-house pharmacophore

Enhancement of MHC-II peptide loading by low-molecular weight chemicals is of twofold interest in immunological research. Compounds that elicit an increased loading of MHC-II molecules with immunogenic peptides (MLe) may be involved in the pathophysiology of autoimmune diseases. On the other hand, such compounds might be of potential use to enhance the activity of vaccines and of antitumour immunotherapies [1]. We have now discovered that some natural essential oils and their constituents are able to increase the loading of MHC-II allele HLA DR1 to a very significant extent. In a screening based on Dissociation-Enhanced Lanthanide Fluorescent Immunoassay (DELFIA) [2], we found that a variety of essential oils as well as isolated constituents could increase the spontaneous loading of soluble HLA DR1 [3] with an influenza A haemagglutinin peptide (HA 306 – 318) [4]. Quite interestingly, structurally simple and widespread monoterpenes (citronellol, geraniol) showed the strongest activity among >40 pure compounds tested. Of 28 essential oils tested so far, chamomile oil (German Chamomile, Matricaria recutita L.) showed the strongest effect, comparable with the reference compound, adamantylethanol, a potent MLE [1]. Activity-directed isolation led to the identification of E-ene-yne-dicycloether as the strongest MLE compound, about 3 times stronger than the Z-isomer. Bisabololoxides A and B were also significantly active but much less potent than the E-spiroether. These findings indicate that MHC-II loading enhancement might be involved in the immunological activities of essential oils and may also open new perspectives with respect to potential applications. Keywords: Major histocompatibility complex-II; MHC-loading enhancers MLE; essential oil, German Chamomile, Matricaria recutita L.; HLA-DR1; influenza A haemagglutinin

An ethnopharmacological study on Verbascum species: From conventional wound healing use to scientific verification

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The leaves, flowers, and whole aerial parts of Verbascum L. (Scrophulariaceae) species are used to treat eczema and other types of inflammatory skin conditions for desiccating wounds in traditional Turkish medicine. The methanolic extracts prepared with thirteen Verbascum species growing in Turkey, including V. chionophyllum Boiss. & Heldr. (Dia.), V. dudleyanum (Hub.-Mor.), V. laurinum Boiss., V. latisepalum Hub.-Mor., V. mucronatum Lam., V. olympicum Boiss., V. pterocalyx var. mutense Hub.-Mor., V. pycnostachyum Boiss. & Heldr., V. salvifolium Boiss., V. splendidum Boiss., V. stachydifolium Boiss. & Heldr and V. uschackense (Murb.) Hub.-Mor. were assessed for their in vivo wound healing activity. In vivo wound healing activity of the plants were evaluated with linear incision and circular excision experimental models subsequently histopathological analysis. The healing potential was comparatively assessed with a reference ointment Madecassol PM174

MHC-II loading enhancement (MLE) – a new immunological activity of natural essential oils and their constituents

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Saponins from Astragalus pycnocephalus var. pycnocephalus FISCHER and their α/β-glucosidase inhibitory effects

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The genus Astragalus belonging to the Leguminosae family is widely distributed throughout the temperate regions of the world. In the flora of Turkey, this genus is represented by 445 species [1,2]. The roots of Astragalus are used in traditional medicine as an antispasmodic, diuretic and tonic drug and has also been used in the treatment of diabetes mellitus, nephritis, leukemia and uterine cancer [3]. Astragalus species are known to be rich in two major classes of biologically active compounds, polysaccharides and saponins. Also the indolizidine alkaloids, the nitro compounds and flavonoids were isolated from the genus [4 – 6]. Inhibition of glycoside hydrolases has widespread application in the treatment of diabetes, viral infections, lysosomal storage diseases and cancers. As part of our ongoing research of new bioactive compounds from Turkish Astragalus species, we carried out a study on A. pycnocephalus Fischer var. pycnocephalus. In the present work, four known secondary metabolites namely trojanoside H, astragaloside IV, astragaloside VIII, and astreaiservianin X were purified by various chromatographic techniques and their inhibitory effects on α- and β-glucosidases were investigated. The compounds showed strong inhibition against α-glucosidase whereas they had moderate activity against β-glucosidase. This is the first phytochemical and biological activity investigation reported on A. pycnocephalus var. pycnocephalus.
Protective Effects of Astragalisde IV and Cycloastragenol in 6-hydroxydopamine (6-OHDA)-Induced Neurotoxicity in PC12 Cells

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Astragaloside IV (AST-IV), one of the bioactive constituents of Radix Astragali, was extracted from the roots of Astragalus trojanus Bunge (Leguminosae). Cycloastragenol (CG), which is a minor metabolite mostly found in its glycosidic form, was obtained from AST-IV via hydrolysis reaction. CG has been shown to extend T cell proliferation by increasing telomerase activity showing that it may also help delay the onset of cellular aging (1). Indeed, recently, CG has been introduced to the market as a new generation antiaging molecule. Moreover our study was to investigate the protective effects of AST-IV and CG on neurotoxicity induced by 6-hydroxydopamine (6-OHDA) in PC12 cells, an accepted in vitro model for Parkinson’s disease. The cells were seeded on tissue culture plates for 24 h. After 24 hours, they were incubated with AST-IV (0.1 μM-1 μM) and CG (0.1 μM-1 μM) for 30 min before the insults with 200 μM 6-OHDA. The cells were incubated for 24 h. Cell viability and cells death were assessed by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) MTT assay and lactate dehydrogenase (LDH) assay kit, respectively. AST-IV and CG inhibited the apoptosis of PC12 induced by 6-OHDA at 0.001 and 0.0001 μM concentrations. On the basis of these results, we propose AST-IV and CG as potential neuroprotective agents in the treatment of Parkinson’s disease.

Figure 1

agents with higher specificity and efficacy still remains [1]. In previous studies, we have reported the isolation of several macromycyclic lathyrane and jatrophane-type diterpenes, which were found to have a potent inhibitory activity against P-glycoprotein of human MDR1 gene-transfected mouse lymphoma cells [2–4]. The purpose of this work was to study the ability of lathyrane and jatrophane derivatives to modulate the transport activity of P-glycoprotein on human colon adenocarcinoma cell lines (COLO 205 and COLO 320). The reversal of MDR was investigated by flow cytometry, measuring rhodamine-123 accumulation. Several of the compounds tested have shown to be strong Pgp inhibitors. Furthermore, some of these modulators, which presented a significant MDR reversal activity, were assayed, in vitro, for their antiproliferative effects in combination with doxorubicin. Some of the compounds synergistically enhanced the effect of the antitumor drug. According to these results, macromycyclic diterpenes may be valuable as lead compounds for the development of Pgp modulators in different multidrug-resistant cancer cells and to further study their effect in animal experiments. Keywords: Cancer, multidrug resistance, macromycyclic diterpenes, adeno carcinoma cells Acknowledgement: This study was supported by FCT, Portugal (project PTDC/QUI-QUI/99585/2008; grant SFRH/BD/72915/ 2010) and Szeged Foundation for Cancer Research, Hungary:

References:


Richness of extremophile plants in phenolics with interesting biological activities

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Extremophile plants are remarkable plants that tolerate severe environmental conditions which may trigger oxidative stress. Able to overcome oxidative stress, these species have developed potent antioxidant systems. Among them, polyphenols constitute the main powerful compounds, owing to their strong biological activity. Therefore, the need exits for safe, natural antioxidants to replace synthetic ones. In this context, Tunisian extremophile species known for their ethno-pharmacological uses was making them good candidates for industrial application. This study aimed to investigate antioxidant activities, to estimate the antimicrobial capacities, to examine anti-inflammatory and antiancer activities, to identify phenolics, and to valorize extremophile plants in industry. Results showed that some halophytes (Limoniastrum monopetalum L. and Suaeda fruticosa Forssk. ex J. F. Gmelin) showed an interesting in vivo antioxidant activity in fibroblast cell, anti-inflammatory ability in (LPS)-stimulated RAW 264.7 macrophage and an anticancer power against carcinoma cell lines. Indeed, for the contribution of these halophytes in the field of cosmetics, cell suspensions culture in bioreactors for the production of cells enriched in phenolic as active principle in cosmetic formulation was made. Acknowledgement: This work was supported by the Tunisian Ministry of Higher Education and Scientific Research (LR10CBBC02).

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Iberis amara L. desensitizes low-threshold mechano-sensitive afferents of the colon

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Medicinal plants used in irritable bowel syndrome include Iberis amara L. (1). Its extract (STW 6) is component of the herbal combination preparation STW 5 (Iberogast®) (2), for which an antagonizing effect on gastrointestinal hypersensitivity has been shown (3). The colon of C57Bl6 mice was isolated and recordings of the mesenteric nerve were taken in an organ bath, while perfusing lumen and bath with Krebs solution (32 °C, 10 ml min⁻¹) containing 1 μM nifedipine to eliminate contractility. STW 5 (lyophilized, 57.1 x 10²μg/ml) or STW 6 (lyophilized, 21.2 x 10²μg/ml) were applied 10 minutes prior to stimulation with bradykinin (0.5 μM), 5-HT (10 μM) or luminal ramp distension from 0 to 80 cm H2O. Intestinal afferent nerve discharge to 5-HT was reduced to 0.2 ± 0.2 imp s⁻¹ after STW 6, compared to 5 ± 2 imp s⁻¹ following vehicle and 3 ± 1 imp s⁻¹ following STW 5 (p < 0.05 STW 6 vs. vehicle). The response to bradykinin was 36 ± 5 imp s⁻¹ after STW 6 and 38 ± 6 imp s⁻¹ after vehicle (n.s.). Following STW 5 it was reduced to 9 ± 2 imp s⁻¹ compared to vehicle (p < 0.05). Values at 80 cm H2O were 14 ± 3 imp s⁻¹ after STW 6, 22 ± 3 imp s⁻¹ after vehicle and 4 ± 3 imp s⁻¹ after STW 5 (p < 0.05 for both versus vehicle). The Iberis amara extract STW 6 has a particular desensitizing effect in low-threshold mechano-sensitive afferents, while STW 5 acts on both low- and high-threshold afferents. Therefore the broader profile of STW 5, possibly mediating its therapeutical effects in irritable bowel syndrome, is in part, but not entirely, based on the effects of STW 6. Keywords: Iberis amara, Colon, Colon irritable, Irritable bowel syndrome, Intestinal hypersensitivity, Bradykinin References: 1. Reichling J, Saller R (2002) Klass Naturheilkd, 9 (Suppl. 1): 21 – 32; 2. Kroll U, Cordes C (2006) Phytomedicine, 13 (Supp. V): 12 – 19; 3. Müller MH et al. (2006) Phytomedicine 13: 100 – 106.

PM183

Larvicidal activity of Eugenia uniflora in Aedes aegyptii

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In Brazil, the leaves of Eugenia uniflora L. are crushed and spread on the floor for its flavour and fly-repellent property [1]. Insecticidal activity of the oil [2] and larvicidal activity of the extract has been reported[3]. The larvicidal activity of the leaf methanol extract was therefore investigated to determine the most active subfracion from which the active compounds could be isolated. The leaf methanol extract was partitioned into n-hexane, chloroform, ethylacetate, butanol and aqueous phase and fractions and subfractions of the leaf methanol extract were therefore subjected to 5-HT it was reduced to 9 ± 2 imp s⁻¹ compared to vehicle (p < 0.05). Values at 80 cm H2O were 14 ± 3 imp s⁻¹ after STW 6, 22 ± 3 imp s⁻¹ after vehicle and 4 ± 3 imp s⁻¹ after STW 5 (p < 0.05 for both versus vehicle). The Iberis amara extract STW 6 has a particular desensitizing effect in low-threshold mechano-sensitive afferents, while STW 5 acts on both low- and high-threshold afferents. Therefore the broader profile of STW 5, possibly mediating its therapeutical effects in irritable bowel syndrome, is in part, but not entirely, based on the effects of STW 6. Keywords: Iberis amara, Colon, Colon irritable, Irritable bowel syndrome, Intestinal hypersensitivity, Bradykinin References: 1. Reichling J, Saller R (2002) Klass Naturheilkd, 9 (Suppl. 1): 21 – 32; 2. Kroll U, Cordes C (2006) Phytomedicine, 13 (Supp. V): 12 – 19; 3. Müller MH et al. (2006) Phytomedicine 13: 100 – 106.

PM183

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In Brazil, the leaves of Eugenia uniflora L. are crushed and spread on the floor for its flavour and fly-repellent property [1]. Insecticidal activity of the oil [2] and larvicidal activity of the extract has been reported[3]. The larvicidal activity of the leaf methanol extract was therefore investigated to determine the most active subfracion from which the active compounds could be isolated. The leaf methanol extract was partitioned into n-hexane, chloroform, ethylacetate, butanol and aqueous phase and tested for larvicidal activities, using the larvae of Aedes aegypti, the most active n-hexane fraction (B₁) was sequentially subjected to Va-cuum Liquid Chromatography (VLC) to yield 10 fractions C₁ to C₁₀ that were equally tested. The calculated percentage mortalities of the extract, fractions and subfractions of the leaf as well as of endosulphan, positive control, were used to determine their LC₅₀ and LC₉₀ values. The result showed that methanol extract had larvicidal activity that was statistically comparable (P > 0.05) to that of endosulphan. Fraction B₁ had LC₅₀ and LC₉₀ values that were insignificantly different from those of metha-nol extract and endosulphan. Fractions C₁ and C₂ showed significantly greater activity than B₁ and comparable to that of endosulphan, making them to be the most active fractions. Work is in progress in order to isolate the active compounds from these most active fractions. Keywords: Eugenia uniflora, larvicidal, extract, leaf References: 1. Morton J (1987) Surinam Cherry. In: Fruits of Warm Climates, JF Morton, Miami Florida. 2. Gbo-lade AA, Adebayo T (1993) Insec Sci Appl 4(5/6): 631 – 636. 3.Luna JS et al. (2005)j Ethnopharmacol 97(2):199 – 206.
Diabetes mellitus (DM) results in severe metabolic imbalances and pathological changes in many tissues (1). Diabetes was induced by Streptozotocin in this research. Mating condition was prepared by putting male rats and diabetic female rats together and vaginal plug was taken as a positive sign of pregnancy and treatment started with three doses: 100, 300, 500 mg/kg chamomile extract or vehicle from 1st to 17th day of gestation by gastric gavages. Blood glucose was measured during 17 days. At 17th day, rats were sacrificed. The fetuses were released from the yolk sacs and surrounding decidua and were examined for absorption rate. Results show that level of blood glucose was reduced about 11.52-fold (p < 0.05) in comparison to vehicle-treated diabetic rat group. In diabetic group that received no treatment fetal's spontaneous abortion was 15%. Percentage of absorbed fetuses in chamomile groups received 100, 300, 500 doses and control group were 0.2%, 0.8% and 0% respectively. The percentage of absorptions was significantly reduced in vehicle-treated diabetic rats, in comparison with vehicle treated healthy rats and treated diabetic rats. Treatment with Matricaria chamomilla L. significantly reduced re-absorption rates in diabetic rats. Also in placetas cause reduction of defects such as Artesia and immature trophoblast in treated diabetic rats. In the Diabetic group, all signs, such as separated necrosis of hepatocytes, anacrosis of liver plates, and lymphocytic inflammation were improved. Matricaria chamomilla was found to have protective effects on spontaneous abortion and histopathological changes of placenta and liver associated with STZ diabetes in chambomile treated pregnant female rats. Keywords: Matricaria chamomilla, fetal absorption, placenta, liver, diabetes, pregnancy, Ultrastructure, STZ, fetal absorption, placenta, liver, diabetes, pregnancy.

Acknowledgement:


Keywords: PPARs, anti-inflammatory and immunomodulatory, Ficus, steroidal compounds, stem-leaf, diabetes, pregnancy.

Figure 1: Chemical structures of the polyacetylenes from N. incisum.

PM185

Radical scavenging effects of fruit extracts from two Ficus species
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Ficus gnaphalocarpa L. and Ficus dekdekana (Miq.) A Rich. belong to the well-known plants of Moraceae family growing in tropical regions. Their fruits are traditionally used as dietary wild fruits in West Africa [1]. The aim of our study was to evaluate the in vitro radical scavenging activities (RSA) of the fruit hydroalcoholic extracts, using two different free radicals: hydroxyl radical (HO·) and superoxide anion (O2·−). At doses of 100 and 25 mg.mL−1 the percentage of inhibition of O2·− values obtained for extracts were 61.41 and 24.02 for F. dekdekana, 23.3 and 31.77 for F. gnaphalocarpa respectively. At 10 and 2 μg.mL−1 the percentage of inhibition of HO· values obtained for extracts were 58.57 and 52.51 for F. dekdekana, 87.09 and 50.28 for F. gnaphalocarpa. This result shows the presence of protocatechuic acid, chlorogenic acid, ferulic acid, homorutin, rutin, hyperoside, catechin, epicatechin and other catechin derivatives well known as radical scavenging compounds [2]. These results suggest that F. dekdekana and F. gnaphalocarpa fruits possess radical scavenging activity, which could be attributed to the presence of phenolic compounds. Keywords: Ficus, hydroxyl radical, superoxide anion, phenolic compounds References: 1. Lockett et al. (2000) J Food Sci Nutr 51(1): 105 – 208. 2. Nango F et al. (1999) Biosci Biotechnol Biochem 63(9):1621 – 1623.

PM186

Polyacetylenes from Notopterygium incisum as a novel class of specific PPARgamma activators
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In the course of our search for PPARs active anti-inflammatory natural products we have investigated the dichloromethane extract of the dried rhizome and root of Notopterygium incisum Ting ex H. T. Chang (Umbelliferae). PPARs are one of the three Peroxisome Proliferator-Activated Receptor (PPAR) subtypes and is involved in the regulation of glucose and lipid metabolism and therefore an important target for metabolic diseases. Additionally, PPARs plays a role in other chronic diseases such as inflammation, cancer and atherosclerosis [3]. We have isolated six polyacetylenes, which were structurally characterized by means of multidimensional NMR and mass spectroscopy, and which were shown to inhibit NO production in RAW 264.7 macrophages. Now, the potency of these polyacetylenes as PPARs agonists has been evaluated. The EC50 of 8-acetoxy-10β-hydroxyl, 10β-falcariol, 9-epoxy-falcariol, crithmumol, 9-epityciklopeen-4,6-diyne-1-ol and 2,9-diptycyan-4,6-diyne-1-ol were determined as 2.36-fold activation (EC50 of 3.59 μM), 2.29-fold activation (EC50 of 4.25 μM), 1.88-fold activation (EC50 of 2.03 μM) and 2.29-fold activation (EC50 of 4.56 μM), 1.92 fold activation (EC50 of 11.31 μM) and 1.73-fold activation (EC50 of 4.18 μM), respectively. Moreover, the positive control pioglitazone exhibited 7.96-fold activation (EC50 of 0.31 μM). Therefore, these polyacetylenic derivatives contribute to the anti-inflammatory activity of Notopterygium incisum by selectively activating PPARγ without affecting the other two PPAR subtypes [4].


PM187

Characterization of glycosidic triterpenoids of Scilla litardierei and investigation of its potential cytotoxic effects
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Scilla litardierei Breistr. (syn. Chouardia litardierei Speta) (Hyacinthaceae) is a bulbous geophyte, which is part of the original flora of southeast Europe and the Balkans. In this study the separation and purification of saponin-rich fractions obtained by partition of bulbous extracts from S. litardierei was carried out. The glycosidic natural products were elucidated by chromatographic and spectrometric methods. Pure substances were gained by purification using preparative HPLC. Sugar moieties were identified after hydrolysis and derivatisation by gas chromatography. Several triterpenoids, which differ in the aglycons, were detected. African green monkey kidney cells (COS-7) and human embry-
Effect of fumarprotocetraric acid isolated the Lichen Cladonia verticillaris on tracheobronchial phenol red excretion in mice

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The lichen Cladonia verticillaris is very common in the northeast of Brazil. It has as essential biologically active secondary metabolites the fumarprotocetraric (FUM) and protocetraric acid. Similar chemical composition is found in Cetraria islandica used in Turkish folk medicine for treatment of bronchitis and tuberculosis. This study shows the effect of FUM isolated from C. verticillaris on tracheobronchial phenol red excretion in mice. FUM administered by oral gavage (50 and 100 mg/kg) or intraperitoneally (25 and 50 mg/kg) and Ambroxol (1 mg/kg) were administrated in male Swiss mice (n=6 animals/group) thirty minutes before the administration of phenol red (200 mg/kg; i.p.). Administration of vehicle (solution saline 0.9%) was used as control. Sixty minutes after the dye injection, the mice were euthanized and a bronchial lavage (BL) was realized with saline. The lavage fluid was mixed with NaOH 0.01N, and the quantification of phenol red in BL was analysed photometrically at 535 nm. The expectorant effect was determined by comparing the phenol red concentration (mg/mL) in BL of treated and control group. It was shown that oral gavage (50 mg/kg and 100 mg/kg) or intraperitoneal (25 and 50 mg/kg) administration of FUM increased phenol red excretion in BL in a dose-dependent manner in comparison to control group (p<0.05). However, there was no statistical difference between phenol red excretion in the BL after intraperitoneal (3.2±0.38 µg/ml) or oral gavage (4.0±0.68 µg/ml) administration of the same dose (50 mg/kg). The results suggest that expectorants action of FUM is not mediated by a vagal reflex initiated by stimulation of the gastric mucosa following oral administration. Keywords: Cladonia verticillaris, fumarprotocetraric acid, excretion, Acknowledgement: CAPES References: [1] Dulger B et al. (1998) TJB 22:11 – 118. [2] Santos N. P, et al. (1997) RUASC91(2): 23 – 43.

Non-host interactions to detect anti-Fusarium substances

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Fusarium are ubiquitous filamentous fungi and a major threat for numerous plant or animal species [1]. They are often considered as highly resistant to conventional antifungal treatments. The Fusarium genus also contains isolates considered as endophytes or saprotrophic strains. These lifestyles are dispersed over a large phylogenetic spectrum [2], which suggests either multiple evolution of the same trait or enormous capacity of each strain to adapt to the available environment or [2], which suggests either multiple evolution of the same trait or enormous capacity of each strain to adapt to the available environment or [2], which suggests either multiple evolution of the same trait or enormous capacity of each strain to adapt to the available environment or [2], which suggests either multiple evolution of the same trait or enormous capacity of each strain to adapt to the available environment or [2], which suggests either multiple evolution of the same trait or enormous capacity of each strain to adapt to the available environment or [2] [2] [2] [2]. They have antioxidant effects, and they protect cells against oxygen radical-related damage at the basis of various diseases. The concept evolves that the human and animal health condition could be partly controlled through the dietary intake of plant polyphenols. Plant polyphenols are recognized as naturally occurring antioxidants but also catalyze oxidative DNA degradation of cellular DNA either alone or in the presence of transition metal ions such as copper. In this paper we show that similar to various other classes of polyphenols, delphinidin is also capable of causing oxidative degradation of cellular DNA [1]. The antifungal activity and major anthocyanin pigments were determined of Vitis vinifera L. and the extracts of Vitis vinifera were studied with association of its antiradical activity [2]. Anthocyanin content varied from 85.7 to 1914.0 mg/kg fresh berry weight. Assessment of the antiradical activity of extracts suggesting that other constituents are likely to exert strong antioxidant effects in grapes. Our work is focused to study antimicrobial and antioxidant activity of anthocyanins from the selected grape species in Slovakia. Keywords: anthocyanines, antioxidant activity, health, animal Acknowledgement: This research is supported by the Agency of Ministry of Education SR for the Structural Funds of the EU, the project: ITMS 2622012003, ITMS 2622012004, ITMS 26220220013. References: [1] Hanif S et al. (2008) DNA Toxicology 249: 19 – 25. [2] Kallithraka S et al. (2005) J Food Comp Anal 18: 375 – 386.

Nitrotoxins or nitroglycosides are aliphatic nitro compounds, which chemically or structurally glucose esters of nitropionic acid and nitropropanol, which were detected in some legumes (Papilionoideae). They are important due to management of pollinators or seed disperses repulsion or inhibition of herbivores and microorganisms and has a role in plant defense [1]. In this study six weeks aged grown Corinonila varia L. plants in equal growth condition were treated with different concentrations of Zn and Ni for 24 and 72 h. The Qualitative test and quantitative determination for aliphatic nitro toxins of control and treated plants examined was done [2, 3]. Increasing nitrotoxins concentration ranging from 4 – 25 mg NO2 mg g-1 in all treated plants were observed in compared to control (1 – 4 mg NO2 mg g-1). This study showed nitro compounds may have a protective defensive role against some environmental stressors such as heavy metals pollution [4, 5]. Keywords: Nitrotoxins, Corinonila varia, heavy metals, Zn, Ni, legumes Acknowledgement: Authors wish to thank Biology Department of University of Arak. References: [1] Majak M (2001) Range Management 54: 494 – 498. [2] Cooke AR (1955) Arch Biochem Biophys 55: 114 – 120. [3] Williams MC and Parker R (1974) Weed Sci 22: 259 – 262. [4] Noori M et al. (2007) Toxicol and Environ Chem 89 (3): 479 – 485. [5] Noori M et al. (2010) Toxicol and Environ Chem 92 (1): 97 – 105.
Two matrix metalloproteinase inhibitors from *Scrophularia striata* Boiss.

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Because the activation of metalloproteinases (MMPs) is a key factor in the metastatic process, compounds with the ability to inhibit MMP activity have a potential in treatment of tumor. The genus Scrophularia, consisting of about 300 species, is one of the genera belonging to the Scrophulariaceae. In this study, two active substances of *S. striata* Boiss. were identified by using bioassay-guided fractionation and their chemical structures were deduced by nuclear magnetic resonance and mass spectrometry. Neptin at lower doses showed progressive MMP inhibitory effect with negligible cytotoxicity, whereas acteoside at higher doses (up to 80 μg/ml) revealed most MMP inhibitory effect preserving low cytotoxicity. Keywords: Scrophularia striata, neptin, acteoside 1, wehi-164, zymoanalysis.

Effect of an alpha – glucosidase inhibitor from the seeds of *Adenanthera pavonina* on gonadal weight and function in mice

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The compound responsible for the alpha – glucosidase inhibitory activity of seeds of *Adenanthera pavonina* L. was isolated from ethanolic extract (70% v/v), purified by ion exchange chromatography and partially characterized by UV, 1 H NMR, and 13 C NMR to be an unresolved nitrogenous five- membered lactone structure, that is possibly pavonin. In vivo treatment of mice with isolate at sub acute levels (10 – 100 mg/kg body weight) for 28 days decreased significantly the relative weight (expressed as percentage of body weight) of the testes and ovaries of male and female rats respectively at p < 0.05. The glutathione-S-transferase activities of both organs also decreased significantly (p < 0.05) with time when compared with the control, suggesting reduced gonadal function. Histopathological studies on testes and ovaries showed a moderate reduction of spermatogenesis and oogenesis respectively. However, resorption of foetus was observed in pregnant mice in the course of the experiment. Conclusively, the isolate may interfere with gonadal function by affecting spermatogenesis, oogenesis and possibly the production of sex hormones in both sexes. Keywords: *Adenanthera pavonina*, five membered lactone, gonadal weight, function Acknowledgement: Dr A.I. Gray, Department of Chemistry, University of Strathclyde, Glasgow, UK for spectral elucidation of isolate References: 1. Ali MS (2005) Nat Prod Res 19: 37 – 40 2. Awasthi S et al. (1993) Arch Biochem Biophys 301: 143 – 150. 3. Macedo MLR et al. (2010) Arch Insect Biochem Physiol 73: 213 – 231

Brazilian Amazon plant extract active against head-and-neck tumor cell line tested for toxicity

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Plant extract obtained from *Pentaclethra macroloba* (Willd.) Kuntze (Pepm), Simaroubaceae, showed antiproliferative activity against head- and-neck human tumor cell line. Toxicity assays were carried out in order to obtain its toxic tendency, as well as its L50. Acute toxicity assay was carried out using groups of three young-adult Balb-c male mice. Animals received i.p. administration of Pepm suspended in almond oil, also used in the control group. Doses were tested starting up from 5 g/kg and subsequent ½-fold-decreasing doses until the observation of non- lethality. General activity including evaluation of more than 20 parameters was also accessed. Statistics was done using ANOVA. Parameters observed after i.p. administration of Pepm show that general activities was not significantly affected by treatment, as well as the surface-righting- reflex, body tone and grip reflex. Hindquarter-fall showed was significantly different from control after Pepm-administration of 5 and 2.5 g/kg. LD50 for Pepm was 37 mg/kg (medium-toxicity). Toxicity of Pepm was being investigated and some degree of toxicity was found, although the effective cause of death in mice is unknown so far. Substances causing both the antiproliferative and the toxicity is being isolated and elucidated, as well as the hystopathological analysis of some organs as liver and kidneys. Keywords: toxicity, Pentaclethra macroloba, Amazon plant extract, general activity Acknowledgement: FAPESP grant#2008/58706 – 8, UNIP References: Brito AS (1994) Manual de ensaios toxicológicos in vivo. Editora da UNICAMP. São Paulo. Botham PA (2004) Toxicol in vitro 18: 227 – 30.

Triterpenoids as inhibitors of *Plasmodium* liver-stage development

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Malaria is one of the foremost public health problems in Africa. It is endemic in 90 countries, affecting nearly 40% of the global population. The increasing prevalence of drug-resistant *Plasmodium falciparum* strains is one of the greatest challenges in malaria control. In order to overcome drug-resistance, new antimalarial drugs are urgently needed. Most of the available antimalarial agents kill blood stage parasites, while liver-stage parasites are not targeted by these drugs. In fact, only a limited number of drugs act on liver stages. In fact, the study of *Plasmodium* liver stage development has been hampered by limitations in the experimental approaches required to quantify hepatocyte infection by the parasite. Therefore, the development of new drugs targeting the *Plasmodium* liver stage represents an important and under-exploited site of intervention [1, 2]. Previously, bioassay-guided fractionation of the methanol extract of the aerial parts of *Momordica balsamina* L led to the isolation of several cucurbitane-type triterpenoids. Several of those compounds and acylated derivatives displayed in vitro antimalarial activity against blood schizonts of chloroquine-sensitive and -resistant strains of *Plasmodium falciparum* [3 – 5]. In this study some of the isolated compounds from *Momordica balsamina* and several alkanyl and aroyl ester derivatives were evaluated for their in vitro tissue-schizontocidal activity on *Plasmodium berghei* infected hepatoma cells. Inhibition of liver stage infection was determined by measuring the luminescence intensity in Huh-7 cells infected with a firefly luciferase-expressing *P. berghei* line, PbsGFP-Lucim, as previously described [1]. Most of the compound tested displayed a dose-dependent antimalarial activity with IC50s 5 μM. Keywords: *Malaria*, *Plasmodium*, liver-stage, *Momordica balsamina* Acknowledgement: This study was supported by FCT, Portugal (SRH/BD/22321/2005). References: 1. Ploem et al (2009) PLoS One 4: e7881. 2. Prudêncio et al. (2008) Cell Microbiol 10: 218 – 24. 3. Ramalhete et al (2009) Bioorg. Med Chem 17: 6942 – 51. 4. Ramalhete et
Anthelmintic activity of \textit{Cymbopogon schoenanthus} and \textit{Cymbopogon martini} essential oils evaluated by four different in vitro tests

\textbf{Table 1:} \textit{C} (\mu L) and confidence limits of \textit{Cymbopogon schoenanthus} and \textit{Cymbopogon martini} essential oils in egg hatch assay (EHA), larval development assay (LDA), larval exsheathment assay (LEA) and larval feeding inhibition assay (LFI A) against \textit{Haemonchus contortus} (EHA), \textit{Listeria monocytogenes} (LDA), \textit{Leishmania donovani} (LEA) and \textit{Escherichia coli} (LFI A).

\begin{tabular}{|c|c|c|}
\hline
 & C. schoenanthus & C. martini \\
\hline
EHA & 0.05 (0.04 – 0.06) & 0.15 (0.13 – 0.17) \\
LDA & 0.07 (0.06 – 0.08) & 0.18 (0.17 – 0.19) \\
LEA & 27.10 (21.37 – 32.38) & 32.02 (29.87 – 34.47) \\
LFI A & 0.01 (0.01 – 0.02) & 0.04 (0.03 – 0.05) \\
\hline
\end{tabular}


**PM199**

Simultaneous determination of four auxins in cyanobacterial extracts using HPLC-ESI-MS

**Keywords:** Comparison of the cytotoxicity and antimicrobial activity of several isohexenylnaphthazarins

**PM197**

Anti-Zygomycetes activity of 7-hydroxyxalamenene isolated from \textit{Croton cajucara}

The leaves and bark from \textit{Croton cajucara} Benth. (family Euphorbiaceae), a shrub from the Amazon, have been used locally used in folk medicine to treat diabetes, malaria, gastrointestinal and liver disorders [1]. The essential oil from the leaves is rich in linalool [2] and presented antilistemic and antimicrobial activities [3,4]. A chemotype of this species was found, with an essential oil rich in 7-hydroxyxalamenene [5]. This substance is reported to have antifungal activity against \textit{Botrytis cinerea}, \textit{Cladosporium cucumerinum}, \textit{Phytophthora infestans}, \textit{Pyricularia oryzae} and \textit{Septoria tritici} [6]. During our studies with \textit{C. cajucara} oil, we isolated 7-hydroxyxalamenene by silica gel column chromatography followed by preparative TLC. The pure compound (>98% by GC) was tested against some zygomycetes. A minimum inhibitory concentration (MIC) of 9.76 \mu L/mL was found for \textit{Aspergillus oryzae}, \textit{Aspergillus niger}, \textit{Candida albicans} and \textit{Mucor circinelloides}. A reference drug used, amphotericin B, presented a MIC of 43.9 \mu g/mL for \textit{C. elegans} and \textit{M. circinelloides}, and 0.3 \mu g/mL for the other species tested. From these data, it was observed 7-hydroxyxalamenene is a compound with good activity against zygomycetes. Keywords: \textit{Zygomyces, Croton cajucara, essential oil}, antifungal activity


**PM198**

Simultaneous determination of four auxins in cyanobacterial extracts using HPLC-ESI-MS

**Keywords:** Prokaryotic cyanobacteria are important source of structurally bioactive secondary metabolites[1]. Besides having considerable pharmacological impacts such as antibacterial, antifungal and also cytoxicity, the potential biofertilizer activity of cyanobacteria make them an attractive alternative to chemical fertilizers. Most paddy soils have a natural population of cyanobacteria. Treatment of rice seedlings with collected cyanobacteria from paddy fields of Iran showed positive growth effect in vitro [2] which could be partly attributed to the possible presence of phytohormons in their composition [3]. Herein, we report a new approach for the simultaneous determination of four important auxins including, indole-3-acetic acid (IAA), indole-3-propionic acid (IPA), indole-3-butyric acid (IBA) and 1-naphthalene acetic acid (NAA) with HPLC equipped with PDA detector at 220 nm after extraction of the microalgae using ultrasonic and microwave assisted extraction. The identities of the auxins were further confirmed using liquid Chromatography-ESI-Mass spectrometry. Under the optimized conditions, a complete separation of four auxins was achieved within a short time with a good reproducibility. The comparison of auxin chromatographic profile of this cyanobacteria with others and also their concentration levels will be reported and further discussed. Keywords: Cyanobacteria, Auxin, HPLC-ESI-MS References: 1. Tan LT (2007) Phytochem 68: 475 – 479 2. Saadatnia H, Riahi H (2009) Plant Soil Environ 55:207 – 212 3. Rosa MSCS et al. (2000) J Essent Oil Res 12: 705 – 708. 3. Rosa MSCS et al. (2000) J Ethnopharmacol 70: 41 – 45. 4. Alviano WS et al. (2005) Oral Microbiol Immunol 20: 101 – 105. 5. Pereira AQ et al. (2010) J Essent Oil Res 23: 20 – 23. 6. Scher JM et al. (2004) Phytochemistry 65: 2583 – 2588.

**PM199**

Comparison of the cytotoxicity and antimicrobial activity of several isohexenylnaphthazarins

**Keywords:** Shikonins, alkannins and derivatives thereof are natural, lipophilic red pigments and found in many species of the Boraginaceae family. Since many centuries, they are traditionally used for the treatment of wounds and have been shown to possess wound-healing, anti-inflammatory, anti-microbial, anti-thrombotic and anti-cancer activities [1]. The cytotoxicity of several shikonins (shikonin, acetylschikonin, \(\beta\)-hydroxyisovalvalallylshikonin, isobutyrylalkannin, a-hydroxyisobutyrylalkannin, b-hydroxyisovalvalallylshikonin, methyl-\(n\)-butyrylshikonin, deoxyshikonin, dimethylacylschikonin, \(n\)-eicosanol and isovaleralshikonin) and alkannins (alkannin, acetyllalkannin, \(\beta\)-hydroxyisovalvalallylkarlynin, isobutyrylkarlynin, \(\alpha\)-methylbutyryl-alkannin, dimethylctylykarlynin propionylalkannin and teracylylalkannin) was determined using the XTT viability assay and human CCRF-CEM leukemia, MDA-MB-231 breast cancer, U251 glioblastoma and HCT 116 colon cancer cells. Most IC50 values of shikonins were in a range of 0.1 to 10\mu M, where by, the highest activity was found for shikonin. IC50 values of alkannins varied from 0.4 to 70\mu M indicating that shikonin derivatives possess a higher cytotoxic potential than alkannins. Dimethylctylykarlynin exhibited no

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activity up to 100 pg/ml in contrast to dimethylacylshikonin. Moreover, the anti-microbial activity of the allamin derivatives and acetylshikoonin against nine microorganisms (Staphylococcus aureus, S. epidermidis, Escherichia coli, Enterobacter cloacae, Klebsiella pneumonia, Pseudomonas aeruginosa, Candida albicans, C. tropicalis and C. glabrata) was examined by the disc diffusion method. The most active derivatives were allamin, acetylshikoonin, beta-hydroxyisovaleralkannin and isobutyralkannin. Also in this case, acetylshikoonin exhibited higher activity than the respective allamin derivative. Keywords: Isoheteropyrhapthazarins, shikonins, alkannins, cytotoxicity, antimicrobial activity Acknowledgement: This work was supported by the “TWF – Fonds zur Foerderung der Wissenschaftlichen Forschung” P21114. References: 1. Papageorgiou VP et al. (1999) Angew Chem Int Ed. 38: 270

Lately in Brazil there is an appreciate increase in the consumption of non-traditional tropical fruits. However, very little information is available on the presence of bioactive compounds and antioxidant properties in these fruits. Some locally grown tropical fruits such as acerola (Malpighia emarginata DC), mangaba (Harcorena speciosa Gomez), siriguela (Spondias purpurea L) and umbu (Spondias tuberosa Arruda) were analyzed for their antioxidant capacity and L-ascorbic acid content. Antioxidant capacity was measured by using 2,2-diphenyl-1-pircyldihydrazil (DPPH) standard and L-ascorbic acid content was determined by ultra-fast liquid chromatography using a Shimadzu UFLC-20A chromatograph with a reverse-phase octadeclysilane column XR-ODS, and 0.025 M of a dihydrogen potassium phosphate solution as the mobile phase. Antioxidant capacity, expressed as concentration of antioxidant required to reduce the original amount of free radicals by 50% and values expressed as g of pulp per g of DPPH, was 7,256.8 for acerola; 15,163.9 for the mangaba; 9,415.3 for the siriguela and 14,100.4 for the umbu fruit pulp. Ascorbic acid was not detected in siriguela pulp, and its content (in mg per 100 g of pulp) was 1,719.63 in acerola; 22.62 in umbu fruit pulp. Ascorbic acid was not detected in siriguela pulp, and its content (in mg per 100 g of pulp) was 1,719.63 in acerola; 22.62 in umbu fruit pulp. Ascorbic acid was not detected in siriguela pulp, and its content (in mg per 100 g of pulp) was 1,719.63 in acerola; 22.62 in umbu fruit pulp. These results indicate the nutritional and therapeutic potential of these tropical fruits for their antioxidants properties. Among the fruits evaluated, the decreasing order of antioxidant activity was ranked as mangaba followed by umbu, siriguela and acerola fruits. Keywords: Bioactivity, bioactive compounds, food phenolics Acknowledgments: This work was supported by the FWF – Fonds zur Foerderung der Wissenschaftlichen Forschung “P21114. References: 1. Papageorgiou VP et al. (1999) Angew Chem Int Ed. 38: 270

Recently by expose the health requirement as pridal needs of mankind and people interested to use of organic production, investigation to find out effective natural compounds to replace of synthetic materials is increasing [1, 2, and 3]. In this context, the use of plant extracts as natural materials is a new strategy for management of weeds. In present study the allelopathic effects of aqueous and hydro-alcoholic extracts of Ruta graveolens L., Artemisia sieberi Besser, Allium sativum L., Tribulus terrestris L. and Peganum harmala L. were evaluated on Portulaca oleracea L. and Lepidium sativum L. seed germination at planting emergence. Percentage, germination rate (GR) and mean days to germination (MDG) obtained in 1–2 day intervals for 12 days. The results showed that hydro-alcoholic were effective in prevention of seed germination in compare with aqueous extract. Aqueous extract of R. graveolens with (76%), A. sieberi (89.33%) and A. sativum (44%) showed preventive effect on L. sativum seeds germination in compared with control (100%). All aqueous extract had shown allelopathic effect (64–73%) on P. oleracea in Compared with control (100%). Hydro alcoholic extract of plants had shown less than 40% germinated seed corresponding to both of evaluated plants. Generally both kinds of extracts cause to decrease (GR) and increase (MDG). In conclusion Terrestres extracts is introduce as natural compound with high allelopathic effect that can be used as herbicide. However other studies are necessary to fully understand the reasons by which some medicinal plants may affect as herbicide in order to commercial application. Keywords: natural herbicides, medicinal plants, germination, organic production, allelopathic References: Bogatek R and Yanekcko A (2006) Journal of Chemical Ecology 32: 2569 – 2583 Chauhan B S et al. (2006) Weed Science 54: 854 – 860. Fattahi B et al. (2010) Planta Med 76: 1271.

Frankenia thymifolia Desf. is an endemic xero-halophyte species in the salted and arid region of Tunisia [1]. In this study, two shoot fractions (methanolic and chloroformic) of Frankenia were assessed on their polyphenol contents and biological activities [2]. Then, the main phenolic and fatty acid compositions were identified. Results showed that polar fraction contains a highest amount of polyphenol, flavonoid and condensed tannin contents (14.2 mg GAE g-1 DW, 4.8 and 4.6 mg CE g-1 DW respectively). The higher phenolic content in this fraction reflect the best total antioxidant capacity (8.8 mg GAE g-1 DW), antioxidative activity against DPPH, ß-carotene bleaching and Fe-reducing tests with the lowest IC50 and EC50 values as compared to apolar fraction. However, chloroformic fraction was more efficient against human pathogens strains. In fact, this fraction was active against all strains. The HPLC analysis showed that salicylic and trans-cinnamic acids were the major pheno- lics. The major fatty acids identified by GC/MS were palmitic, elaidic and linoleic acids. Such variability in biological capacities between the 2 fractions can be explained by different bioactive compounds contain in each fraction and might be of great importance in terms of valorizing this halophyte as a source of bioactive molecules for cosmetic and pharmaceutical industries. Keywords: biological activities, fatty acids, Fran- kenia thymifolia, Haba H, Marcourt L, Long C, Benkhaled M (2007) Biochem Syst Ecol 35: 176 – 179. 2. Meot-Duros L, Le Floch G, Magn C (2008) J Ethnopharma- col 116: 258 – 262.
Reducing power (EC50 = 410 μg mL-1) were more important in leaf non-polar fraction as compared to polar fraction. Besides, chloroform fraction was more efficient against all human pathogen strains mainly Escherichia coli and Staphylococcus aureus. The HPLC analysis showed two major phenolic compounds: trans-4-hydroxy-3-methoxyecinnamic acid (ferulic acid) and p-coumaric acid. The major fatty acids identified by GC/MS were palmitic acid (28.04%), and polyunsaturated acids (48.78%) are characterized by linolenic acid (29.69%) and α-linolenic acid (omega 3) (19.0%). These results indicate that selective extraction of bioactive molecules from natural sources as halophyte species, by appropriate solvents, is important for obtaining fractions with high biological activities which can be used as preservative ingredients in food, cosmetic and/or pharmaceutical industries. Keywords: Nitraria retusa, phenolic content, biological activities, phenolic composition, fatty acid, GC/MS, RP-HPLC. References: 1. Ghade M, Boukhir M (1998) Association pour la protection de la Nature et de l’environnement, 178 – 179. 2. Meda A et al. (2005) Food Chem 91: 571 – 577.

PM205
Protective effect of (-)-α-bisabolol on markers of oxidative stress in erythrocytes subjected to oxidative stress
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(-)-α-bisabolol is a sesquiterpene alcohol found as a major component of essential oil of chamomile (Matricaria recutita L., Chamaomilla recutita L., Matricaria chamomilla L; Family Asteraceae). Chamomile, one of the most ancient and widely recognized herbs to mankind, has been used traditionally for centuries as an anti-inflammatory, antispasmodic, carminative, mild astringent and healing medicine [1,2]. It is also known to be very helpful as an external agent for encouraging the rapid healing of ulcers and burns without infection, as well as persistent skin problems such as eczema and psoriasis [3]. Since clinical trials and human studies are limited, we have investigated the effect of (-)-α-bisabolol on markers of oxidative stress in human erythrocytes by incubating with hydrogen peroxide (2mM) and tert-butyl hydroperoxide (10μM). Subjecting erythrocyte to oxidative stress caused a significant alteration in reduced glutathione GSH), malondialdehyde (MDA) concentration as well as superoxide dismutase and catalase activity compared to control. Presence of (-)-α-bisabolol as low as 0.1μM in incubation medium protected the erythrocytes from oxidative stress and helps to maintain the basal level of GSH and MDA. The activity of superoxide dismutase and catalase were also restored in a concentration-dependent manner (0.01 – 100μM). The effect was also compared with L-Ascorbic acid, quercetin and BHT. Our findings provide evidence for the protection of oxidative stress in erythrocytes by (-)-α-bisabolol that could be considered for further studies. Keywords: (-)-α-bisabolol, GSH, MDA, Superoxide dismutase, Catalase, Hydrogen peroxide, tert-butyl hydroperoxide Acknowledgement: Council of Scientific and Industrial Research, New Delhi, Council of Science and Technology, Government of Uttar Pradesh, India. References: 1. Srivastava JK, Shankar E, Gupta S (2010) Mol Med Report 3(6): 895 – 901. 2. McKay DL, Blumberg JB (2006) Phytother Res 20(7): 710 – 730. 3. Martens D (1995) Chiropractic Acud Homeopathy 6:15 – 18.

PM206
Cytotoxic activity of selected plants extracts on normal and cancerous oral mucosal cells
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Oral cancer is associated with cells of the oral mucosa that have become malignant and destroys healthy tissues of the lips, tongue, gingiva and other intra-oral locations (1). Many plants from the tropical forest of Malaysia have been used in local medicaments in the prevention and treatments of various types of cancer. The active polyphenolic compounds are often obtained through vigorous heating and then consumed in the form of decoction. The objective of this study was to determine the toxic effect of extracts from three plants on oral mucosal cell lines. Fibroblast and two cancerous oral mucosal cell lines which include the KB (ATCC) and ORL-48 (CARIF, Malaysia) cells were tested for their responses to the extracts of Brueca sp., Typhonium sp. and Azadirachta sp. using the neutral red cytotoxicity assay (2). The effective concentrations (EC50) of the extracts were observed using Giemsa staining procedure. Results revealed the extracts from all three plants to exhibit cytotoxic activity towards KB cells with EC50 at less than 100μg/mL. Potent cytotoxic activity on ORL-48 cells at an EC50 of 6.67 ± 11.5μg/mL was only displayed by Brueca sp. extract. Extracts of all plants did not produce toxic effect on normal fibroblast cells. Based on its strong cytotoxic activity, Brueca sp. extract is worthy of further investigation to isolate and identify the active compounds. Keywords: Brueca sp.; Typhonium sp.; Azadirachta sp. Acknowledgment: The research was financially supported by the University of Malaya Research Grant (RG020/09HTM) and Post Graduate Grant (PS164/2010B). References: 1. Kumar V, Cotran RS, Robbins S.L (2010) Pathology of Cancer, 5th ed. Elsevier, W.B. Saunders Asia P.L. 2. Fathiah AR, Sujata R, Norhanom WA, Ilham MI (2010) J Med Plants Res 4(11):987 – 990.

PM207
Fatty acid composition and antioxidant activity of Pistacia lentiscus L. fixed oil
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Pistacia lentiscus L. is known by its essential oil and its mastic. The fixed oil extracted from fruits is used in traditional medicine for stomach diseases and wound healing. This study aims to determine the fatty acid composition and the antioxidant activity of Pistacia lentiscus fixed oil extracted from different parts of mature fruits. The work was performed on three different parts of the fruit: the envelope, seed and whole fruit harvested from Nefza located in North West of Tunisia. The extraction was done by the Soxhlet Apparatus. The determination of the fatty acid composition, done by GC/MS, showed the presence of five principal fatty acids: oleic, palmitic, linoleic, palmitoleic and stearic acids. The major fatty acid is the oleic that present more than 53%, palmitic and linoleic acids present respectively 25 and 15%. The difference between fatty acid composition of oil extracted from envelope, seed and whole fruit was not significant. The antioxidant activity was determined by the DPPH test and the Trolox Equivalent Antioxidant Capacity (TEAC) for oils extracted from the different parts of fruit. A significant difference was
determined and the highest antioxidant activity value was reached by oil extracted from whole fruit with a percentage of inhibition of DPPH about 9.67% and about 7% for both envelope and seed oil. Similarly, the highest value of TEAC was reached by whole fruit oil with about 2.32 ng of Trolox of oil. Acknowledgement: This study was financially supported by IRDC – Canada (105568 – 006) and WWF–Tunisia

The genus *Trifolium* is represented by 96 species in Turkish Flora (1). Among these species *Trifolium pratense* L. is used in Turkish folk, for wound healing (6). Also, it is used for chronic skin diseases in worldwid

In Vivo Healing Potential of *Trifolium* L. species on Excisional And Incisional Dermal Wounds

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The medicinal plant “Velame” (Macrosyphonia velame (A. St.-Hil.) Müll. Arg. – Apocynaceae) has ornamental and medicinal potential. In the southwestern state of Mato Grosso (MT-sw), Brazil, this plant is native. Between the years 2005–2009, it was found in specific locations (savannah sparse tracks without the presence of cattle). Scientific studies have found that the “Velame” root extract intensely inhibits tyrosinase, an enzyme that is derived from the population ancestors. In the nursery, it was found that the seeds of Quina have low germination. The seedlings and plants show an extremely slow growth. Incentives to Quina growing are needed to prevent its regional extinction. Laboratory study showed that the extract EMEQ interferes with larval development of *S. frugiperda*, preventing the insect from completing its cycle, causing its death. In addition to the medicinal value of Quina, its biocidal effect is presented as an alternative for pest control. Keywords: Mato Grosso, Brazil, Bioactivity, Strychnos pseudoquina, Spodoptera frugiperda (Lepidoptera: Noctuidae) in the larval stage

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PM212

Plant known as Sarsaparilla [Herreria sarsaparilla Mart. [Herreriaceae]] and its medicinal use in southwestern Mato Grosso, Brazil

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The medicinal herb Sarsaparilla (Herreria sarsaparilla Mart [Herreriaceae]), native in Caceres (MT, Brazil), was studied in our laboratory and the field, between 2003 – 2009. The species also has ornamental potential. It has roots thickened tuberiform and elongated stems cylindrical. The roots and leaves of the plant, in tea form, are used in folk medicine for many different diseases, such as sweat, blood purifying, diuretic, and also for the treatment of skin diseases, gout, syphilis, gonorrhea, arthritis, fevers, coughs, and hypotension. They mention that the root tea also help fight obesity; tea leaves and branches are used to aid digestion and relieve stomach pains. To treat influenza, colds and rheumatism use the infusion. The healers in the city of Caceres sell the plant root, especially, for purifying and anti-rheumatic. There are few scientific studies of this species relationship with the indicated uses in folk medicine. In the laboratory, to evaluate the digestive tract in various concentrations, the effect of the raw methanol extract of leaves of the plant on mining of Spodoptera frugiperda not was no larvicidal effect and also non-interference in the remaining duration of the larval stage. Alert treatments, that even though natural medicine, without guidance from qualified health professionals may harm due to inadequacies in its implementation. Keywords: Medicinal Plant, Bioactivity, Mato Grosso, Brazil Acknowledgement: For Fapemat – financial support, and for UNEMAT – institutional support; To the collaborators colleagues from the research group FLOBIO – (Plants carrying Bioactive substances)

PM213

Xanthine oxidase-inhibitory and hypouricemic action of Black poplar bud extract

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Our study aimed to investigate the effect of the extract from Black poplar (Populus nigra L.) buds on xanthine oxidase (XO) activity in vitro and its hypouricemic action in rats. Poplars have been traditionally used in goats- and arthritis-treatment practices in medieval Europe. Besides, phenolic-rich rains from poplar buds are usually the main constituents of honey bee propolis that has a wide traditional use. Other preliminary in vitro evaluation, extract was administered for 3 consecutive days to potassium oxonate-induced hyperuricemic rats in concentrations of 100 and 500 mg/kg. Allopurinol (10 mg/kg), was used as a reference drug. Uranic acid/serum urate, urea, creatinine and electrolytes Na¹, K, Cl were determined in daily collected urine and in serum at the end of the experiment. ALT, AST activities in serum and XO activities in the liver homogenate were determined. The extract inhibited XO activity in vitro, showing a mixed-mode inhibitory action and IC₅₀ value of 8.2 µg/mL. In rats, poplar extract at 500 mg/kg significantly (P < 0.05) reduced serum urate levels by 27% compared to hyperuricemic control group which the effect similar to that of allopurinol at a dose 10 mg/kg. The mode of action still needs to be further elucidated as it did not exhibit effect on liver X0 but the observed effect on Na¹, K, and Cl excretion suggest the uricosuric action. Further research is needed to fully elucidate the potential of poplar extract in management of hyperuricemia. Keywords: Xanthine oxidase, hypouricemic effect, hyperuricemia, gut, enzyme inhibition, Populus nigra, black poplar extract Acknowledgement: This research was supported by MSM 6046070901 and GACR 525/08/P053

PM214

n-Hexane extracted compounds of Bromus inermis with excellent anti-MRSA activity

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Bromus inermis L. is a grass which belongs to family Poaceae. It is a potent weed. Weeds could be a potent source for finding antibacterial compound regarding their characteristics such as living in a divers conditions and situations. In a large screening program for antibacterial activity screening of some weed plants extracts, we finally find a great anti-MRSA activity of B. inermis (MIC of smaller than 100 µg/mL for crude extract in triplicate assays). We used HPTLC and a simple biographical assay in parallel for initial characterization and identification of effective substance(s) in the extract, and finally it was concluded that the effective anti-MRSA substance is a relatively polar substance which has MIC of smaller than 100 µg/mL. Susceptibility tests with standard antibiotics had been shown the MIC of 35 for chloramphenicol against this strain. Further investigations with HPLC Fractionation and subsequent NMR analysis could reveal the identity of the effective substances in our continuing work.

PM215

The antioxidant activity and free radical scavenging potential of two medicinals plants Salvia officinalis L. and Phlomis samia L.

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In recent years considerable attention has been devoted to medicinal plants with antioxidant properties. The properties are commonly postulated to play an important role in preventing diseases caused by oxidative stress, such as cancer, coronary arteriosclerosis, and the ageing process. And there is much literature concerning the antioxidant properties of many species of the genus Salvia, for their 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activities. Among them, Salvia officinalis L. leaf extracts have been shown to be the most active with effective dose (EC₅₀) of 17 µg/mL followed by the genus Phlomis by (EC₅₀: 32 µg/mL). This is for the first time studied in our search. For its high antioxidant activity, S. officinalis is begin against our MRSA strain. Susceptibility tests with standard antibiotics had been shown the MIC of 35 for chloramphenicol against this strain. Further investigations with HPLC Fractionation and subsequent NMR analysis could reveal the identity of the effective substances in our continuing work.

PM216

Bioassay guided fractionation of extracts from flowers of Bells perennis L. for their anticancer activity

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Common daisy (Bells perennis L.) is a member of the cosmopolitan family Compositae (Asteraceae). It is native to western, central, and northern Europe, but is commonly found as an invasive plant in North America [1]. B. perennis has been used in the treatment of gastritis, enteritis, diarrhoea, bleeding, rheumatism, inflammation and infections of the upper respiratory tract (2 – 3). In this study, anticancer activity of crude hexane, dichloromethane, methanol, water extracts, also n-butyl-N-methylacetamide fractions (after separation of methanol extract) of flowers from Bells perennis were investigated. Cytotoxic activities were carried out on human lung cancer (A549), human colorectal cancer.
Antimicrobial activity of some *Teucrium* species from Serbia

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In the Serbian flora, genus *Teucrium* is represented by six species. In this work, for the first time, antimicrobial properties of methanol, ethyl acetate and acetone extracts of *T. scordium* subsp. *scordium*, *T. scordium* subsp. *scordioides* (Schreb.) Arcang, and *T. botrys* L. were examined [1]. Antimicrobial activity was tested by microdilution method determining minimum inhibitory (MIC) and microbicidal concentration (MMC) against standard and clinical strains of bacteria and fungi. 

Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans ATCC 10231, Candida albicans and Aspergillus niger [2]. The activity of extracts varied depending on the microorganism species, plant species and type of extract. The extracts of *T. scordium* subsp. *scordium* and *T. scordium* subsp. *scordioides* showed inhibitory effects towards tested bacteria while *T. botrys* inhibit only *S. aureus* ATCC 25923. Antifungal activity was recorded for ethyl acetate extracts of *T. scordium* subsp. *scordium* and *T. botrys*. MIC and MMC values were in range from 0.3 mg/ml to > 20 mg/ml. Among tested extracts, the methanolic showed the greatest inhibitory effect. ATCC 25923 was the most sensitive strain. It was inhibited by extracts of tested plants, except ethyl acetate extract of *T. botrys*. The inhibitory and bactericidal concentrations were lower than 1 mg/ml. Susceptibility of *S. aureus* was inhibited by extracts of tested plants, except ethyl acetate extract of *S. aureus* in inhibitory effects.

The extracts did not show any significant activity against A-549 and DLD-1.

Oxidative stress in the heart tissue was estimated by measuring the glutathione (GSH) levels homogenous and malondialdehyde (MDA) in the cardiac cytosolic fraction. The pretreatment of rats with the GABE orally at a dose of 100 mg/kg for one month resulted in a decrease in cardiac cytosolic MDA and a maintenance of cardiac cytosolic GSH level as compared to ADR treated animals at a dose of 20 mg/kg intrapertioneally. In this study we have investigated the cardioprotective effect of GABE against ADR induced acute cardiotoxicity. We report here that the pretreatment of GABE is able to reduce the ADR induced acute cardiotoxic manifestations in GSH, MDA. Analytical chemical study revealed that GABE contains phenolics and flavonoids, which are responsible for its potent antioxidant activity. Our studies have shown that ADR induced considerable increase in lipid peroxidation. In conclusion, the present study shows that chronic administration of butanolic fraction of *Globularia alypum* has cardioprotective potential against ADR induced cardiotoxicity.

Keywords: Adriamycin, *Globularia alypum*, Cardiotoxicity, Antioxidants, Lipid peroxidation, Histopathology

**PM217**

**Brenodaida salicina (Rubieaceae) extracts are as effective as a commercial fungicide in post harvest protection of oranges against *Penicillium* infections**

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After examining the antifungal activity of several medicinal and aromatic plants *Brenodaida salicina* (Vahl) Hepper & J.R.I.Wood was selected for in depth compound (ursolic acid) isolation and characterised. Acetone extracts had good in vitro antifungal activity against *P. janthinellum* (MIC 0.08 mg/ml) but *P. digitatum* and *P. expansum* were more resistant (MIC 1.25 mg/ml). *Penicillium* species cause serious post harvest problems in the citrus industry. To test the in vivo efficacy, oranges were infected with the fungi and treated with the extracts, ursolic acid and a commercial fungicide amphotericin B. The crude leaf extract had the same level of protection as ursolic acid indicating synergistic activities within the crude extract. The acetone extract had an MIC of 0.16 mg/ml compared to the MIC of 0.08 mg/ml of amphotericin B. The acetone extract therefore had sufficient antifungal activity against these organisms to consider its use in the citrus industry especially since it could be produced at a very low cost. The extract was however more toxic to the kidney cells that to the fungi. The results show the potential use of plant extracts to combat plant fungal infections if extracts with lower cellular toxicity can be found or if the toxicity of the extract can be decreased without changing the antifungal activity. Keywords: antifungal extract, *Brenodaida salicina*, Penicillium, post harvest, orange, antifungal compound Acknowledgement: The National Research Foundation provided funding References: 1. Mahlo SM, McGaw LJ and Eleff JN (2010) Crop Protection 29: 1529 – 1533

**PM220**

**The Study on the Effect of Different Manure and Plants Density on the Growth and some Quantitative Characteristics of Milk Thistle (Silybum marianum L.)**

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Milk thistle (*Silybum marianum* L.) is an annual medicinal plant. *Silybum marianum* has been recognized as an antihepatotoxic plant. The active constituents of *S. marianum* include a group of flavonolignans known collectively as silymarin. To investigate the effects of different manure and plant density and the interaction between manure and density on the grows and some quantitative characteristics of this plant an experiment were examined. The completely Randomized design was installed in the experimental field, College of Agricultural of Ferdowsi University located in Mashhad. The treatments were included of two factors. The first factor included 3 different manure: cow, sheep and multiple manure (1.65 t/ha) and the second factor was three level of plant density (5, 7 and 10 pl.m-2) with 3 replications. At the end of fullbloom stage, morphological characteristics included plant height, number of shoots, number and diameter of capitule, percentage of leaf dry matter, percentage of healthy and free seeds, mass of 1000 grains of main and secondary capitule and chlorophyll content of leaf. Mean comparison was carried out using LSD test (at 5% level). Results showed a significant effect

**PM218**

**Cardioprotective effect of Algerian medicinal plant *Globularia alypum* extract against cardiotoxicity of Adriamycin in rats**

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Adriamycin (ADR) is an efficient chemotherapeutic agent used against several types of tumors; however, its use is limited due to severe cardiotoxicity. Since it is accepted that ADR induced myocardopathy is the consequence of Oxidative stress through the mediation of free radicals. The aim of this study was to investigate the effect of *Globularia alypum* L. butanolic extract on the acute cardiac toxicity induced by ADR in rats. GABE represent a significant source of phenolic compounds. It has been recognized that polyphenols and flavonoids show antioxidant activity. Oxidative stress in the heart tissue was estimated by measuring the
on number of capitula and shoots and plant height. By increase in plant density: plant height, number of shoots, number of capitula and mass of 1000 grains were decreased. The results showed that the best kind of manure was ship manure and the most suitable plant density was 40 × 40 cm (5 plant per m²). Keywords: Milk thistle, Silybum marianum, Animal Manure, Plant Density

PM221

Chemical profile and biological activities of Allium melantherum Panč. extracts

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Members of the genus Allium have been used and cultured for thousands of years for their medicinal properties and characteristic flavor. Only two species of genus, A. cepa L. and A. sativum L., are well researched, while data on chemical composition and biological activities of other species, including Allium melantherum Panč. (subgen. Allium, sect. Codonoprasum) are very scarce. In the present study we investigated chemical composition, antioxidative and anti-inflammatory properties of methanolic extracts of Allium melantherum wild growing in Serbia. Phychochemical profile was determined by measuring total phenolic, total flavonoid and total anthocyanin contents and by LC-MS/MS analysis of the extracts and headspace GC/MS analysis of fresh buds volatiles. The antioxidant activity was evaluated by measuring radical scavenging capacity towards 2,2-diphenyl-1-picrylhydrazide (DPPH) and NO radicals and effect on lipid peroxidation (LP) [1]. In addition, the anti-inflammatory activity considering inhibitory potency toward production of 12-HETE, 12-HHT, PGE2 and TXB2 was observed [2]. High contents of total phenolics (5.13 – 5.31 mg gallic acid equivalents/g of dry extract), total flavonoids (1.15 – 2.82 mg quercetin equivalents/g of dry extract) and total monomeric anthocyanins (33 – 169 µg cyanidine-3-glucoside equivalents/g of dry extract) were found. The dominant phenolic compounds in the extracts are ferulic and p-coumaric acids and flavonol quercetin-3-O-Glc and kaempferol-3-O-Glc. D-methyl-disulphide was detected as only volatile compound. The extract inhibited production of 12-HETE, 12-HHT, PGE2 and TXB2 in a dose-dependent manner. Antioxidant activity was weak compared with synthetic antioxidants. Keywords: Allium melantherum, antioxidant, anti-inflammatory activity, GC/MS, LC-MS. Acknowledgement: Science and Technological Development, Republic of Serbia, grant No. 172658. References: [1] Lesjak M et al. (2011) Food Chem 124:580 – 586. [2] Beara IN et al. (2010) Pharm Biomed Anal 52: 701 – 706.

PM222

Tectona grandis Linn. (Verbenaceae) leaf ethanol extract in renal artery occluded hypertensive rats

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Hypertension is one of the principal health problems and leading cause of cardio-vascular deaths in various communities worldwide. An elevated arterial pressure is an important public health issue. Although it is common, asymptomatic and readily detectable, but it can often lead to lethal complications, if left untreated. Many new drugs have been introduced which may demonstrate better efficacy but posses side effects. Recently attention has been focused towards herbal preparations which are traditionally used as potential therapeutic agents in the prevention and management of cardiovascular diseases. Ethanol extract of Tectona grandis Linn. leaf (Family: Verbenaceae) was evaluated for its antihypertensive activity in renal artery occluded hypertensive rats. Wistar rats (160 – 250 g) were pretreated with ethanol extract of T. grandis for 6 weeks. Hypertension was induced in animals by clamping the renal artery with renal bulldog clamp for 4 h. Ischemia of the kidney caused elevation of blood pressure by activation of the renin-angiotensin system. Elevated blood pressure of the animals was significantly (p < 0.05) decreased by the ethanol extract of T. grandis at the dose levels of 20, 40 and 80 mg/kg, i. v. Captopril, angiotensin converting enzyme inhibitor (ACE-I) at the dose of 1 mg/kg, i. v. showed significantly (p < 0.05) reduction in the elevated blood pressure. The antihypertensive activity of ethanol extract of T. grandis may be due to the action on rennin-angiotensin system. This result would tend to justify the traditional use of the herb for the management hypertension. Keywords: Tectona grandis, antihypertensive activity, rennin-angiotensin system

PM223

The use of metabolomics for the discovery of antimicrobial biomarkers from Plectranthus species indigenous to southern Africa

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For many years a reductionist approach has been followed in the evaluation of the bioactivity of medicinal plants and the subsequent isolation and identification of active compounds. Synergism and prodrug effects cannot be detected with this approach [1]. It is also a tedious and time consuming process involving isolation, dereplication of known compounds and structure elucidation [2]. A holistic approach is more appropriate in the study of herbal and traditional medicines [1]. This means that the phytomedicine is evaluated as one active ingredient or as a set of poly-phytochemicals acting synergistically [3]. The research method best suited for the holistic research concept of phytomedicine is metabolomics. Plectranthus is the largest genus of the Lamiaceae family in South Africa. The main recorded ethnobotanical use of these species is as traditional medicine for the treatment of various ailments such as digestive ailments, skin conditions and a range of infections [4]. A total of 93 leaf samples from different Plectranthus species, indigenous to southern Africa, were collected and extracted with dichloromethane followed by methanol. The antimicrobial activity of the samples was determined against various micro-organisms including Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis, Candida albicans and Cryptococcus neoformans. One-dimensional NMR and LC-MS metabolic fingerprints were acquired for all samples. Chemometric tools were employed to identify active and less active extracts by combining and correlating NMR and LC-MS metabolomic profiles of the different samples with the MIC assay results. Putative biomarkers responsible for the antimicrobial activity of active samples were also identified. References: 1. Verpoorte R, Choi YH, Kim HK (2005) J Ethnopharmacol100: 53 – 56. 2. Heinrich M (2008) Phytochemistry Letters 1: 1 – 5. 3. Li et al. (2008) Trends in Analytical Chemistry 1: 66 – 76. 4. Lukhoba GW, Polglase RJ, van der Hoff G, Nkabinde K. Ethnopharmacol 103:1 – 24. 5. Gibbons S (2004) Nat Prod Rep 21: 263 – 277.

PM224

Experimental evaluation of the potential of Bridelia ferruginea stem bark in wound management

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The methanol extract (ME) of Bridelia ferruginea Benth. stem bark obtained by 48 hr maceration, was subjected to solvent guided fractionation in a silica gel column using petroleum ether, dichloromethane and methanol successively to yield the petroleum ether (PE), dichloromethane (DCMF) and methanol (MF) fractions. The extract (ME) and fractions (DCMF and MF) were assessed for hemostatic activity using bleeding/clotting and coagulation time in rats. The effects of the extract and fractions on wound contraction and rate of epithelialization of excision wounds in rats were evaluated. Also the antimicrobial effects of the extracts and fractions were studied. Data collected were analyzed using one way ANOVA and further subjected to LSD Post Hoc tests. The extract and fractions significantly (P < 0.05) reduced bleeding/clotting time in rats and also reduced the coagulation time of whole rat blood. They also significantly (P < 0.05) increased rate of wound contraction and epithelialization of excision wounds compared to control rats; on day 17, extract treated rats showed 99.5 – 100% wound contraction. The extracts also exhibited antimicrobial activity against Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi and Klebsiella pneumonia. The results suggest that the stem bark of Bridelia ferruginea exerted beneficial effects in wound management through hemostatic, wound contraction and antimicrobial activities. Keywords: Bridelia ferruginea, hemostatic, wound contraction, epithelialization, antimicrobial
In Turkish traditional medicine, the species under Juniperus section are frequently employed to treat several diseases [12]. This work was designed to define and compare the antiproliferative and antimicrobial activities of berries methanol extracts of five Juniperus species from Turkey: J. communis L. var. communis (jcc), J. communis L. var. sauvatilis (Pallas) A.E.Murray (jcs), J. drupacea L. sp. oxycedrus (joo), J. oxycedrus L. sp. macrocarpa (Sibth. et Sm.) Ball (jom). The effect of Juniperus extracts on cell proliferation was tested “in vitro” on human hepatocellular carcinoma (HepG2) cells. A decrease in HepG2 cells viability after 24-hour exposure to jcc, jcs, and jom extracts was observed. Based on IC50 values, the activity of the extracts decreased in the order jcs > jom > jcc (IC50= 6.62 ± 0.61 μg/mL, 7.61 ± 2.25 μg/mL, and 8.42 ± 1.32 μg/mL, respectively). Jom and jom extracts inhibited the growth of HepG2 cells approximately of 40% at the lowest tested dose (1.25 μg/mL), while the activity diminished with increasing concentrations, resulting close to zero at the dose of 10 μg/mL. The antimicrobial activity was evaluated by standard methods on gram-positive, gram-negative bacteria and fungi. The efficacy was appreciable on gram-positive only. Among the extracts jom showed the higher bacteriostatic activity (MIC: 78.12 – 312.50 μg/mL) than jcs and jcc (156.25 – 1250.00 μg/mL), followed by jom and jom (25.00 – 1250.00 μg/mL). The obtained results give support to the ethnopharmacological use of these Turkish Juniperus species and suggest their potential use in the prevention and/or treatment of infections and cancer. Acknowledgement: The authors are grateful to Federica Messina for financial support.

**References:**


**Topic N: Veterinary Applications**

**PN1**

Traditional Ethnoveterinary Phytotherapies from Pakistan

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Present investigation was conducted to document plant based Traditional Ethnoveterinary Phytotherapies to cure various ailments in remote areas of Pakistan including Indo-Pak, Pak-Afghanistan, Pak-China, and Pak-Iran borders. Questionnaires based on semi-structured interviews and observations were used to collect data from traditional veterinary healers residing in these remote sites. Eighty seven medicinal plant species belonging to 46 families were recorded for their applications against veterinary diseases. All plant species were indigenous to the study areas. Diarrhea, dysentery, dysentery, gas trouble, constipation, colic, worms, ulcers, warts, scabies, sores, infections of mouth, throat, lungs, foot, hooves; fever, cough, lactation, unequal memory, glands, weakness, mastitis, arthritis and urethra prolapse were frequently reported veterinary ailments. Forty one plant species were reported in more than two conditions. Commonly used routes of drug administration were oral and dermal. Validation of these ethnoveterinary practices for their quality, efficacity and standardization of doses and screening for active substances that may lead to the discovery of some new, safer and cost effective medicines.

**PN2**

The effects of the different levels of Aloe vera gel on oocysts shedding in broilers with coccidiosis

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Coccidiosis is the most important parasitic disease in poultry. The disease may result in losses, indigestion, and increased feed conversion ratio in chickens. Resistance against anti-coccidiosis drugs is among the major problems resulting from chemical therapy. Therefore, it seems necessary to replace chemical substances with herbs. Thus, the present study aims to identify the effects of different levels of Aloe vera L. gel on performance and oocysts shedding in broilers with coccidiosis.

The study was carried out on 200 one-day-old male broilers from Ross 308 strain on a completely randomize design with four treatments each with five replicates each composing of ten chickens. The groups included control group (basal diet), three group with basal diet mixed different level of Aloe vera gel (1.5, 2, and 2.5%). On the day 28, all chickens with oocysts were challenged by *Eimeria maxima*. Feed conversion ratio was calculated for the whole farming period (42 days). The findings suggested that groups treated by Aloe vera gel had improved feed conversion ratio compared to the control group. The group treated by 2.5% Aloe vera gel showed significant difference from the control group. In addition, significant reduction was observed in oocysts per gram of feces in the Aloe vera gel groups in comparison with the control group. 2.5% Aloe vera gel group showed the lowest level of oocysts per gram of feces. The results of this study indicate that Aloe vera gel can improve feed conversion ratio in broilers with coccidiosis and reduces oocysts shedding.

**Keywords:** Aloe vera, coccidiosis, broiler.

**PN3**

Acaricidal activity of the essential oil from *Tetradenia riparia* (Lamiaceae) on the cattle tick *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae)

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This experiment was carried out to study the bioacaricidal activity of *Tetradenia riparia* (Hochst.) Cord essential oil against engorged females of *Rhipicephalus (Boophilus) microplus* (Acari; Ixodidae). For this purpose, nine serial concentrations (12.50, 6.25, 3.75, 1.80, 0.90, 0.45, 0.22, 0.11, and 0.05% w/v) of *T. riparia* were used for the adult immersion test (AIT). For the larval packet test (LPT), we used 14 serial concentrations (100.00, 50.00, 25.00, 12.50, 6.25, 3.75, 1.82, 0.91, 0.45, 0.22, 0.11, 0.057, 0.028, and 0.014% w/v). The results for AIT showed that the LC50 and LC99.9 were 1.222 g/mL (0.655 – 1.788) and 11.382 g/mL (1.27 – 2.248); and for hatchability inhibition were 0.114 g/mL (0.0 – 0.31) and 2.462 g/mL (1.501 – 3.422), respectively. The LPT showed that the LC50 and LC99.9 were 1.222 g/mL (0.655 – 1.788) and 11.382 g/mL (7.86 – 14.91), respectively. A positive correlation between *T. riparia* EO concentration and tick control, was observed by the strong acaricidal effects against R. (B.) microplus, and the mortality rate of ticks was dose-dependent. Our results showed that *T. riparia* is a promising candidate as an acaricide against resistant strains of R. (B.) microplus. Keywords: Tetradenia riparia, Acari; Ixodidae; Tick; Rhipicephalus (Boophilus) microplus Acknowledgement: The authors are grateful to CNPq for providing a research grant and fellowships References: 1. Gazim ZC et al. (2010) Molecules 15: 5509 – 5524.

**PN4**

In vitro activity of different plants essential oils against the yeast-like alga *Prototheca*

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Species of the genus *Prototheca* (family Chlorellaceae) are unicellular aclorophyllous microalgae, spherical, oval or kidney shaped with diameters ranging from 3 to 30 μm. They are ubiquitous in nature and have a worldwide distribution [1]. Of the five known species of the genus, *P. wickerhamii* causes human infection and *P. zopfii* is considered pathogenic for animals, particularly cows and dogs [2,3]. These algae do not respond to classic therapy so introduction of new therapeutic agents for
treatment or prophylaxis is an important goal. Therefore this study aimed to investigate the antimicrobial activity of Mentha piperita L. (peppermint), Melaleuca alternifolia Cheel (tea tree), Origanum compactum Benth. (oregano) and grape seed essential oils, compared with Amphoterin B (A2942, Sigma-Aldrich). Ten P. zopfii isolates from cow mastitic milk samples and two P. zoophil isolates from bovine feaces were submitted to antifungal susceptibility testing by broth microdilution assay following the CLSI guidelines for yeasts. The inhibitory effects, depends on the plant species from which the product was obtained, on their chemical composition as well as on the tested concentration. Peppermint (MIC of 0.125 – 0.5 μg/mL) and tea tree (MIC of 1 – 2 μg/mL) essential oils demonstrated the strongest antifungal efficacy against all tested strains. In contrast Amphoterin B showed efficacy at MIC of 25 μg/mL. All tested isolates were resistant to oregano and grape seed essential oils. Difficulties in treating protothecosis and the treatment in vitro activity of peppermint and tea tree essential oils demonstrated here raise the interest for further investigations on the therapeutic use of these natural products. Keywords: Prototheca, essential oil, Mentha piperita, Melaleuca alternifolia, Origanum compactum, grape seed Acclerias. This work was supported by the NCNSU-UEFISCU grant number PN II RU 175/2010. References: [1]. Pore RS (1985) Mycopathologia 90: 129 – 139. [2]. Roesler U et al. (2006) Int J Syst Evol Microbiol 56: 1419 – 1425. [3]. Lass-Fiori C et al. (2007) Clin Microbio. Rev 20: 230 – 242.

Antibacterial activity of the essential oil of Mountain Savory (Satureja montana) against Arcanobacteriuym pyogenes

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The pharmaceutical properties of aromatic plants are partially attributed to essential oils (EOs) which are widely used to prevent and treat human infections. EOs are lipophilic and thus interact strongly with many types of cell membranes. The EO of the cultivated SM (Serbia), was extracted by hydrodistillation and analyzed by gas chromatography. According to compositional analysis of the EO (GC, FID), major compounds were linalool (24.57%) and p-cymene (19.85%) were found as predominant compounds in oil. Antibacterial sensitivity of AP (ATCC 19411) and other lactobacilli was tested by agar dilution method. Inhibitory concentration (MIC) for each sample in accordance to CLSI guidelines was determined obtaining mimimum inhibitory concentration (MIC) of 15 μg/mL. All tested isolates were resistant to oregano and grape seed essential oils. Difficulties in treating protothecosis and the treatment in vitro activity of peppermint and tea tree essential oils demonstrated here raise the interest for further investigations on the therapeutic use of these natural products. Keywords: Prototheca, essential oil, Mentha piperita, Melaleuca alternifolia, Origanum compactum, grape seed Acclerias. This work was supported by the NCNSU-UEFISCU grant number PN II RU 175/2010. References: [1]. Pore RS (1985) Mycopathologia 90: 129 – 139. [2]. Roesler U et al. (2006) Int J Syst Evol Microbiol 56: 1419 – 1425. [3]. Lass-Fiori C et al. (2007) Clin Microbio. Rev 20: 230 – 242.

Inhibition of chemically induced mammary and non-mammary carcinogenesis by astaxanthin in Wistar rats

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Astatxanthin is a fat-soluble, oxygenated pigment called a xanthophyll and a member of the carotenoid family. It has a unique molecular structure that gives it powerful antioxidant function. Astatxanthin is extracted from microalgae, salmon and Pelofia (a yeast) (1, 2). The aim of the study is to follow up the effect of astatxanthin (ASTA) in chemoprevention of the chemically induced mammary carcinogenesis in immature Wistar female rats. There were established five groups of 37 days old Wistar rats: group I inoculated with the carcinogen MNU (N-methyl-N-nitroso-urea) (n = 9), group II with MNU and ASTA in diet (n = 8), group III which received oil in diet (oil was used as solvent for ASTA) (n = 4); and group IV with ASTA in diet (n = 4). The ASTA was administrated orally in a dose of 50 μg astatxanthin/rat/day, during 7 months. The experiment was finished at 14 months from MNU intake. Mammary tumor induction determined by MNU was reduced, representing 33.3% respectively 37.5% from all cases in groups I and II. There were diagnosed several other tumor types in several organs (nephroblastoma, liposarcoma, heamangio- sarcoma, squamous carcinoma, pulmonary carcinoma, cholangiocarcino- noma). Involvement of oxidative stress in (mammary and non-mammary) carcinogenesis was revealed by partial protection conferred by astatxanthin in cancer chemoprevention. Present study is one of the few long term experiments (420 days) that resemble the effect of astat- xanthin in chemically induced carcinogenesis in rats. Concluding, a diet enriched in astatxanthin yield beneficial effects in cancer chemoprevention, minimizing the bad effects of oxidative stress induced by MNU. Keywords: astatxanthin, carcinogenesis, mammary, rat Acknowledge- ments: This work was supported by the NCNSU-UEFISCU grant number PN II RU 185/2010. References: [1] Guerin M et al. (2003) Trends Biotechnol. 21(5):210 – 6. [2] Hyouko N et al. (1999) Pure Appl Chem. 71(12): 2273 – 2278.

Researches Regarding in vitro Antimicrobial Effect of Some Types of Honey from Transylvania on Staphylococci Isolated from Animals and Humans

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Despite the pharmaceutical industry development in recent years, resistance of microorganisms to antibiotics is increasing [1,2,3]. Under these conditions, the alternative of natural products with similar effect, must be considered. This study aimed to test in vitro antimicrobial activity of four types of honey obtained in Central Transylvania, on staphylococci isolated from lesions in both animals and humans, as well as to test comparatively the effect of the most frequently antibiotics used in treating the lesions caused by staphylococci. The products with potentially antimicrobial effect were represented by forest honey, multi-flower honey, lime honey and acacia honey. Tests were done on 38 strains of staphylococci form species S. aureus (8 strains), S. intermedius (10 strains), S. xilosus (7 strains), S. hominis (5 strains), S. chromogenes (4 strains) and S. sciuri (4 strains). Comparatively seven antibiotics commonly used to treat staphylococci were also tested. The sensibility was determined using the microdilutions method obtaining minimum inhibitory concentration (MIC) for each sample in accordance to CLSI standards. Forest honey had a good antimicrobial effect (MIC 15 μg/mL) on S. intermedius and S. chromogenes strains and multi-flower honey had good effect (MIC 15 μg/mL) against S. sciuri. Lime honey had a decreased...

In vitro antimicrobial efficacy of honeydew honey and Calendula officinalis L. against Pseudomonas aeruginosa

Niculae M1, Spini M1, Rindt K1, Sandru CD1
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The complex therapeutic potential of honey and medicinal plants is well documented by the literature, but discrepancies may be observed when comparing research results [1, 2]. Twelve honeydew honey samples from different Transylvanian geographical locations were investigated for their antibacterial properties based on the results of two diffusion assays that included Pseudomonas aeruginosa (n = 10, strains isolated from bovine mastitis, and the reference strain Ps. aeruginosa ATCC 27853) as a relevant antibiotic resistant pathogen. Both screening tests indicated a strong inhibitory effect for eight samples when compared to the artificial honey and also that the development of growth inhibition zones was dose dependent. The most active samples were subjected alone and in combination with Calendula officinallis L. essential oil to minimum inhibitory concentrations assay using the broth microdilution method that pointed out values below those recorded for well or disc diffusion assays (MICs for honeydew honey ranged from 5% to 10% (v/v) and were inhibitory concentrations assay using the broth microdilution method for honeydew honey 0.5%). The layers were bred in a three-storey terraced cage battery; feed and water were at disposal ad libitum. The dry extract contained Eleutheroside B (0.71%), Eleutheroside E (1.14%), and 30% ethanol was used as an extraction medium. The weight of the eggs and the layers was sampled weekly. The quality of the eggs was assessed on the basis of the weight and shape of the eggs, the strength and thickness of the eggshells. In the 2nd experimental group, statistically significant changes (P < 0.05) in the strength (EGII = 30.93 ± 5.00) and thickness (EGII = 0.41 ± 0.03) were detected in comparison with the control group (CG = 26.71 ± 4.42, g; EG = 0.39 ± 0.02 thickness of the eggshells). Keywords: honeydew, Eleutheroside, Eleutherococcus senticosus, egg Quality, immunostimulant effects and in the production of biofood, which is an important component of the food chain [1, 2]. In the model experiment a dry extract of Eleutherococcus senticosus Maxim. was applied to the layers of Hisex braun breed. The layers were divided into three groups, a control group (CG, n = 10), 1st experimental group (EGI, n = 10) with the addition of the extract in the concentration of 0.1%, the 2. experimental group (EGII, n = 10) with the addition of the extract in the concentration of 0.5%. The layers were bred in a three-storey terraced cage battery; feed and water were at disposal ad libitum. The dry extract contained Eleutheroside B (0.71%), Eleutheroside E (1.14%), and 30% ethanol was used as an extraction medium. The weight of the eggs and the layers was sampled weekly. The quality of the eggs was assessed on the basis of the weight and shape of the eggs, the strength and thickness of the eggshells. In the 2nd experimental group, statistically significant changes (P < 0.05) in the strength (EGII = 30.93 ± 5.00) and thickness (EGII = 0.41 ± 0.03) were detected in comparison with the control group (CG = 26.71 ± 4.42, g; EG = 0.39 ± 0.02 thickness of the eggshells). Keywords: honeydew, Eleutheroside, Eleutherococcus senticosus, egg Quality, immunostimulant effects and in the production of biofood, which is an important component of the food chain [1, 2]. In the model experiment a dry extract of Eleutherococcus senticosus Maxim. was applied to the
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