April 6, 2009

Dear Friends,

On behalf of the National Center for Natural Products Research, School of Pharmacy, and the University of Mississippi, we would like to welcome you to our conference entitled “8th International Conference on the Science of Botanicals” This conference is supported through a cooperative agreement between the NCNPR and the Center for Food Safety and Applied Nutrition (CFSAN) of the Food and Drug Administration. Co-sponsors are: CFSAN/FDA, Shanghai Institute of Material Medica/CAS, China; The Council of Scientific and Industrial Research (CSIR-India); the Society for Medicinal Plant Research (GA); Institute of Indigenous Medicine (IIM), Sri Lanka, and the American Society of Pharmacognosy (ASP).

We are excited to present a program featuring a roster of internationally recognized experts and researchers in the field of botanicals. We wish to extend our thanks to our speakers for their willingness to participate in and contribute to the success of the meeting.

We invite you to visit the website of the National Center for Natural Products Research at http://www.pharmacy.olemiss.edu/cnpr to learn more about our research program. Oxford and the Ole Miss campus are a beautiful setting, and we hope you will get to explore them, especially if this is your first time to visit here. If there is anything we can do to make your visit more enjoyable, please contact us.

Sincerely,

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Invited Speakers

A. P. G. Amarasinghe, Ph.D.
Institute of Indigenous Medicine, Sri Lanka

Rudolf Bauer, Ph.D.
University of Graz

Mike Balick, Ph.D.
New York Botanical Garden

Y. S. Bedi, Ph.D.
Institute of Integrative Medicine (CSIR), Jammu Tawi, India

Amy Boileau, Ph.D.
Regulatory and Scientific Affairs, Cargill

Josef Brinckmann
Traditional Medicinals

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Victoria Kuyeye
Health Canada Natural Health Products Directorate
was less than 15%. In the final analysis, Red Clover was shown to
However, the study was powered sufficiently if the dropout rate
this, only 88 subjects were recruited of the 128 initially planned.
was a possibility that there would be taking Prempro. Because of
Prempro and many women were reluctant to enter the trial if there
study had four arms, i.e. Black Cohosh, Red Clover, Placebo and
added difficulty in the ability to recruit suitable women since the
subjected to accelerated stability studies. During the recruitment of
in vitro. The study preparations had to be formulated and were sub-
of the study would be interaction with certain serotonin receptors,
study was delayed for more than a year in order to prepare a bota-
Menopause, for purposes of FDA is not considered a disease. The
measured was reduction in hot flashes in menopausal women.
an IND application was not required since the end point being
the trial, a Phase 1 study was required and approval from FDA that
1 Department of Pharmacognosy, Section Metabolomics, Universitätsplatz 4, 8010 Graz, Austria
2 Institute of Pharmaceutical Sciences, Department of Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang, China

A Phase 2 clinical trial with Black Cohosh and Red Clover was conceived in 2000 within our UIC/NIH Center for Botanical Dietary Supplement Research on Women’s Health. Prior to implementing the trial, a Phase 1 study was required and approval from FDA that
that an IND application was not required since the end point being measured was reduction in hot flashes in menopausal women. Menopause, for purposes of FDA is not considered a disease. The study was delayed for more than a year in order to prepare a bota-
nally authenticated and chemically and biologically standardized extract. It was ascertainment that the biological endpoint for purposes of the study would be interaction with certain serotonin receptors, in vitro. The study preparations had to be formulated and were sub-
ected to accelerated stability studies. During the recruitment of suitable subjects the results of the WHI (Women’s Health Initiative) caused difficulty in the ability to recruit suitable women since the study had four arms, i.e. Black Cohosh, Red Clover, Placebo and
and many women were reluctant to enter the trial if there was a possibility that there would be taking Prempro. Because of this, only 88 subjects were recruited of the 128 initially planned. However, the study was powered sufficiently if the dropout rate was less than 15%. In the final analysis, Red Clover was shown to positively affect cognition but neither test preparation reduced hot flashes. A discussion of these results will be presented.

Evaluation of the Anti-inflammatory Potential of Chinese Botanicals

Traditional Chinese medicine (TCM) has been used about 1300 plants for anti-inflammatory purposes. Activity guided isolation in a medium throughput approach has resulted in the discovery of a number of new drug leads. From the fruits of Evodia rutaecarpa (Juss.) Benth. (Rutaceae), several quinoline alkaloids, like 1-methyl-2-nonyl-4(1H)-quinoline, 1-methyl-2-(6Z)-6-undecenyl-4(1H)-quinoline, 1-methyl-2-(4Z,7Z)-4,7-tridecadienyl-4(1H)-quinoline, evo-carpine and 1-methyl-2-(6Z,9Z)-6,9-pentadeca-
There are estimated to be 420,000 species of higher plants on earth, about half of which are found in the tropics. Over millennia, people have learned to use plants to sustain their lives. Ethnobotany is a science that studies the relationship between plants, people and traditional culture. This presentation discusses the study of plants used in traditional healing, with examples from Belize, Central America, the Pacific Island region of Micronesia, and New York City by a Dominican immigrant community. Traditional knowledge in many parts of the world is inextricable from living a lifestyle, and when it is not taught to younger generations, there is a risk of its loss.

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Plants are still an important resource for the discovery of new drugs, such as those used in traditional medicine. In search for novel antimalarial compounds, we focused on neocryptolepine (5-methyl-5-dielenyl-4(1H)-quinolinone), one of the minor alkaloids of Cryptolepis sanguinolenta, a plant used in traditional medicine in Central and West Africa. A series of chloro- and aminoalkylamino-substituted neocryptolepine derivatives were synthesized and evaluated as antimalarial agents.

Neocryptolepine Derivatives and Standardised Extracts from Traditional Medicine

Antimalarial Agents from Plants: Neocryptolepine Derivatives and Standardised Extracts from Traditional Medicine

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Triterpenoids as Anti-inflammatory Compounds of Natural Origin

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Despite the progress in understanding the molecular mechanisms underlying chronic inflammation, the current treatment options are not satisfactory. The transcription factor NF-κB, a key player in the development and progression of chronic inflammation, is considered a promising target for therapeutic intervention. In Ayurvedic medicine, extracts from the oleogum resin from Boswellia serrata are being used as anti-inflammatory remedies. After purification to chemical homogeneity, we have identified a number of pentacyclic triterpenoids including acetyl-boswellic acids (ABAs). Using LPS as an activator of human monocytes, we found that ABAs inhibit NF-κB signaling. We identified specific inhibitory effects on NF-κB kinase (IKK), which is pivotal for the degradation of the NF-κB inhibitor IκB, as well as the phosphorylation of p65, two steps essential for NF-κB activation and the subsequent cytokine expression. Using active human recombinant IKKα and IKKβ, we positively confirmed the direct effect of the ABAs on the IKK complex. We further studied the effects of systemically applied AKβBA on the development of atherosclerotic lesions in apolipoprotein E-deficient (apoE−/−) mice. Atherosclerotic lesion formation was accelerated in those animals by weekly intraperitoneal lipopolysaccharide (LPS) injections. LPS alone increased the atherosclerotic lesion size by two-fold and treatment with AKβBA significantly reduced it by about 50%. Daily treatment of the mice with AKβBA potently inhibited the NF-κB activation in atherosclerotic plaques and led to significant down-regulation of several NF-κB-dependent genes such as MCP-1, MCP-3, IL-1α, MIP-2, VEGF and TF. By contrast, AKβBA did not affect the plasma concentrations of triglycerides, total cholesterol, and various subsets of lymphocyte-derived cytokines. Thus, the inhibition of NF-κB signalling by constituents of the oleogum resins from Boswellia species might represent an alternative for conventional treatments of chronic inflammatory diseases such as atherosclerosis.

Acknowledgements: This work was supported by the Deutsche Krebshilfe.

Scientific Studies of a Popular Sri Lankan Indigenous Therapeutic Agent Rathakalka Used in Pediatric Practice

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The test drug Rathakalka, selected for these studies, is a popular Sri Lankan indigenous medical recipe specially used for children. A clinical study of the Rathakalka recipe revealed significant changes in serum immunoglobulins (IgG, IgM, and IgA) and serum complements (C3, C4) levels in infants and young children. Animal experiments with albino rats showed its highest anti-inflammatory activity 3 hours after induction of edema. In-vivo experiment demonstrated that Rathakalka reduced yeast induced elevation of the body temperature in rats. In-vitro experiment revealed that the recipe has anti-bacterial effect on Staphylococcus aureus, Pseudomonas aeruginosa, and Listeria monocytogenes. In-vivo experiment showed that the prolonged administration does not produce any toxicity changes in rabbits. Microbiological study indicated that the microbial colony counts observed in this study were with in the limits acceptable by the World Health Assembly (W.H.A.). These results scientifically evaluate that the drug samples are tested and deemed microbiologically safe and up to the microbial quality standards. These studies confirmed the presence of immune enhancing effect, anti-pyretic and anti-inflammatory effect, non toxicity, and microbiological safety in Rathakalka. Acknowledgements: Thanks to the support and advice given by Prof. S. Widanapathirana, Prof. R.D. Sharma, Prof. Premawathi Tekvari, Prof. Manjari Dwivedi, Dr. Usha Singh, Dr. N.C. Arya, Dr. B.M. Nageeb and all other co-researchers, and the financial grant from Link Natural Pharmaceuticals, and National Science Foundation, Sri Lanka, to carry out this research study.

Antidepressant New Herbal Product Development

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Depression related disorders are among the most common psychiatric disorders that affect all age groups of the general population. Currently, the preferred treatment is with pharmacological drugs that have antidepressant or anti-anxiety properties. However, these synthetic antidepressants have numerous and often serious adverse effects, including impaired cognition, ataxia, aggression, sexual dysfunction, tolerance dependence and so on. Withdrawal reactions on termination after long-term administration are also a major limiting factor in the use of these agents. Herbal remedies, for example St. John’s wort (Hypericum perforatum) or Kava has recently gained popularity as an alternative treatment for mild to moderate depression. Excitingly, we have discovered a medicinal plant named ADP, Chinese traditional medicine, used for inflammation and rheumatic conditions. Its extracts showed significantly antidepressant effect, and minor analgesic, tranquilizing actions, simultaneity, without exciting effect. We believe that it could soon become “Chinese St. John’s wort”. Pharmacodynamics-experiment (positive control is fluoxetine and Venlafaxine) showed the curative dosage of ADP for mouse ED50: 4.56 mg/kg (FST); 5.40 mg/kg (TST); rat ED50: 1.85 mg/kg (FST). Acute-toxicity-experiment showed its LD50 values > 500 mg/kg i.g; Long-term-toxicity-experiment showed through 6month SD-rats test and 9month Beagle-dogs test, under 40 mg/kg/d (amount to clinical mimic-dose 80 times) ADP was safety. The safe index of ADP for mouse is LD50/ED50 = 152–162 (TST) (Fluoxetine’s LD50/ED50=62). The in vitro test and the mechanism of action test indicate that ADP obtained through the method for this invention has prominent (re) uptake inhibiting effect on noradrenaline (NA) and/ or 5-hydroxytryptamine (5-HT), and when compared with the extract prepared by using the existing reflux method, it has the advantages of increasing the alkaloid content and the biological activity of the extract. Therefore, ADP may serve as the noradrenaline and/or 5-hydroxytryptamine and/or dopamine (re) uptake inhibitor for development into antidepressant drug, anti-anxiety drug, sedative hypnotic, and anti-senile dementia drug. By now, we have executed 2 applications for China invention patents and authorized by Chinese Patent Bureau (ZL03115911.7; ZL200410084791.7). Meanwhile, we have executed 1 PCT application at 2005, and entered into U.S.A, Japan, Canada, Korea, India, Russia and European Union from 2007(WO2006/058487 A1).

Quality Control and Standardisation of Botanicals – From Cultivation of Medicinal Plants up to its Clinical Application

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In the recent years with ever growing commercialization in the field of herbal medicines, there has been an instant demand for quality control of the drugs used in this system. In the present paper an attempt has been made for a sequential study of the quality control protocols for the herbal medicinal products from selection of medicinal plants, good agricultural practices, cultivation, good field collection practices, source and period of collection, identification and authentication, storage, chemical standardization, assay, good manufacturing practices, pre clinical studies up to clinical approach, with special reference to maintain standardization at all stages. Besides the above protocols, this study deals with approaches towards establishing the quality and safety – starting from preliminary examination of the botanicals, inadvertent con-
tamination/substitution, morpho-anatomical, pharmacognostic, physico-chemical and analytical parameters emphasizing the limits of foreign organic matter, pesticide residue, radioactive and microbial contaminations [1]. Chemical assay and phytochemical screening of different extracts using modern extractors and recent Chromatographic and spectrometric techniques have been described. Different stages, i.e. quality control studies of raw botanicals, methods of processing, finished herbal products, standardization procedures at all stages from birth of the botanicals up to its clinical application will be discussed. Practical experiences for the identification of non-prescription and prescription synthetic chemical medicines (illegal addition) in quite a large number of recent herbal medicinal products will be described in detail. Acknowledgements: This work is due to ZCHRTM & HAAD, for providing facilities. References: [1] Quality Control Methods for medicinal plant materials, WHO (1998; 2007).

S-12  Effect of Poly saccharides on Enteric Mucosal Immune Response in Rats

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The effect of ginseng polysaccharide and Polyporus umbellatus polysaccharide on T-lymphocytes in enteric mucosal lymphocytes in rats, including healthy rats, those with collagen induced arthritis, and with C26 colon carcinoma were explored. For this study peripheral blood mononuclear cells (PBMC), peyer’s patch lymphocyte (PPL), intraepithelial lymphocyte (IEL), and lamina propria lymphocyte (LPL) of SD rats were isolated. These lymphocytes were co-cultured with ginseng polysaccharide and Polyporus umbellatus polysaccharide in different dosages. The TNF-α and IFN-γ in supernatants were measured with ELISA. Ginseng polysaccharide and Polyporus umbellatus polysaccharide can regulate the level of TNF-α and IFN-γ in the supernatant of PBMC and PPL; Polyporus umbellatus polysaccharide can decrease the level of TNF-α and IFN-γ in supernatant of IEL; Ginseng polysaccharide and Polyporus umbellatus polysaccharide can regulate the function of lymphocytes in the enteric mucosal immune system.

S-13  Eliminating Analytical Ambiguity in the Scientific Study, Development and Quality Control of Natural Health Products and Dietary Supplements

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S-14  Traditional Knowledge Guided Research to Identify Legitimate Substitutes for Rare and Unavailable Herbs

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As per the principles and practice of Ayurveda, herbs with similar pharmacological properties can be used as substitutes whenever the original herb is in short supply. There are at least 30 pairs of herbs and substitutes that are mentioned in classical Ayurveda texts [1]. Cypripedium rotundum L. (Cypripedioideae) is claimed to be a legitimate substitute for Aconitum heterophyllum Wall. ex Royce (Ranunculaceae). A. heterophyllum is a rare and expensive Himalayan herb while C. rotundum is a common, tropical, marshy weed. Going by published literature, the two herbs are taxonomically unrelated and dissimilar in major chemicals. However, our preliminary studies indicate that the chromatographic profiles [2] and pharmacological (anti-diarrhoeal) activity are similar in the two drugs making further exploration worthwhile. Research of this kind is essential to identify new substitutes for unavailable herbs and to throw light on the Ayurvedic strategy adopted for selecting substitute drugs. Acknowledgements: Thanks go to Al-Ameen College of Pharmacy for conducting the animal studies. Financial support from the TATA Trusts is gratefully acknowledged. References: [1] Sastri, B (Ed.) (2002) Yogaratnakara. Chaukamba, Sanskrit Sansthan. Varanasi, p.171. [2] Shankar, D. et al. (2007) Curr Sci, 92(11): 1499–1505.

S-15  Metabolomics for Discovery of Novel Medicinal Compounds

March SJ1
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Plant tissues have complex chemical profiles consisting of both primary metabolites required for growth and development and secondary metabolites that enable the plant to sense and adapt to changing conditions. The products of plant secondary metabolism are a rich resource for discovery of new medicines but traditional methods of discovery such as bioassay-guided fractionation are expensive and time-consuming while some plant-based treatments rely on synergy between several compounds for full biological effect. Metabolomics is the study of the whole complement of small com-
pounds in a biological sample and recently, this technique has been used to discover novel, medicinally active phytochemicals in traditional plant-based medicines. The overall objective of the Medicinal Plant Metabolomics research program is to assess the capacity for compound discovery by mass spectrometry and NMR-based metabolomics technologies and to quantitatively compare metabolites specific to individual medicinal plants. An extract of a single leaf of St. John’s wort (Hypericum perforatum L.) has been found to contain more than 4,200 individual compounds. A simple cup of coffee from a commercial retailer can contain between 8,000–10,000 distinct phytochemicals. Efforts to understand this phytochemical complexity and to develop models for study of chemodiversity form the foundation of future research in compound discovery, medicinal plant development and optimized diets.

This presentation will introduce a systemic strategy and relative technologies for the quality evaluation of Traditional Chinese Medicine (TCM), including the identification and differentiation of botanicals and also the quality standard of TCM products. The emphasis will focus on the quality control of manufacture of TCM products, especially to introduce an application of NIRS online analytical technique and quality-based control system into the extraction procedure of TCM. The system hardware was composed of the extraction equipment, the online sample pre-treatment subsystem, the NIRS subsystem, the online NIRS analysis and intelligent control subsystem, and the automatic control subsystem. A diagram of the system is shown in Fig. 1. The whole system includes cooperative working hardware and software components. The extraction process of TCM was analyzed using online NIRS, and the results demonstrated that NIRS was feasible to be applied to online monitoring and controlling in the manufacturing of TCM. Based on the online NIRS analysis technology, the real-time monitoring of the effective components or indicative components in the extraction procedure, the analysis of the extraction ratios, the diagnosis of the extraction procedure, and the real-time feedback control based on the quality status were actualized.

Fig. 1 The system framework of the NIRS analysis and intelligent control system for TCM extraction.
identified by advanced Mosher method. By analyzing the NMR data, diospongin C was found to be an acyclic diarylheptanoid with four hydroxyl groups at C-1, C-3, C-5 and C-7; i.e., 1,7-diphenylheptan-1,3,5,7-tetraol. So there was some difficulty in the decision of its relative and absolute configuration. The relative configuration of diospongin C also can be determined by analysis coupling constants of two protons of C-2, C-4 and C-6 in Newman projections of one corresponding acetone derivative and optimizing dihedral angles [1]. Its absolute stereochemistry was identified by the CD spectrum of its dibenzoate product [2]. All the three compounds were examined the inhibitory activity on osteoclast formation and bone resorption induced by PTH in bone organ culture system. Except for diospongin A, diospongin B and C showed potent inhibition even at a concentration of 20 µM, which demonstrates that the stereochemistry was important to structure-activity relationship of these diarylheptanoids.

![Structure of diospongin A, B and C.](image)

**Fig. 1** Structures of diospongin A, B and C.


**S-19**

Sourcing of Quality Raw Materials for Indian System of Medicine (ISM) and Botanical Drugs

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Globally, there has been an unparalleled growth in the plant-derived medicinally useful formulations, drugs and health care products, with annual growth rates between 10–20% in most of the countries. According to WHO, the international market of herbal products is estimated to be US$ 62 billion which is poised to grow to US$ 5 trillion by the year 2050. This has attracted many large pharmaceutical and consumer product companies worldwide to have herbas/botanicals in their product portfolio. India is no exception to it and has a competitive edge as Indian Traditional drugs/products, have their roots in time tested systems of medicine namely, Ayurveda, Unani and Siddha. Renewed interest in botanical products has resulted into a huge international trade in raw plant material, feeding a range of such industries, including the $20 billion botanical medicine market. Presently between 75 and 85% of the raw materials for the botanical industry are sourced from wild.

Obesity and Type 2 diabetes are considered as global epidemics by the WHO. Aboriginal populations such as the Cree of Eeyou Istchee (James Bay area of northern Quebec) are particularly affected and suffer greater complications, in part because of the cultural inadequacy of modern pharmaceutical therapies. A multidisciplinary team was therefore put together to explore the antidiabetic potential of Boreal forest plants stemming from Cree Traditional Medicine (TM). The team is composed equally of scientists with expertise in botany, phytochemistry, nutrition, pharmacology, biochemistry, toxicology and clinical endocrinology as well as Cree Elders and members of various Cree health-related institutions, notably including the Cree Board of Health and Social Services of James Bay (CBHSSJB). A novel ethnobotanical approach based on diabetes symptoms was used to identify potential antidiabetic plants. A total of 17 species were characterized phytochemically and screened for primary and secondary antidiabetic activity, toxicological potential and mode of action using a comprehensive platform of bioassays. Most promising species were subjected to bioassay-guided fractionation to identify active principles. Bioavailability as well as anti-hyperglycemic and anti-obesity efficacy are then confirmed using in vivo animal models of obesity, insulin resistance or diabetes. Clinical studies are also underway to document the safety and efficacy of selected species using a culturally-adapted, all-inclusive, observational protocol. Finally, our project represents a pilot study for the integration of Cree TM into diabetes care for the CBHSSJB. Funded by the Canadian Institutes of Health Research.

**S-20**

The CIHR Team in Aboriginal Antidiabetic Medicines: A Community-Based Collaborative Approach Uniting Healers and Biomedical Scientists to Validate Cree Traditional Medicine

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Interpreting research on botanical dietary supplements, and also replicating research from other labs to confirm results, is complicated by the dietary supplements themselves, which are complex chemical mixtures with composition that may vary dependent on the source of the raw materials, processing and formulation, and stability of the final product. All pharmacological research requires that the substances being tested be characterized sufficiently so that studies can be interpreted as well as replicated and confirmed by other research groups. The chemical composition of botanical dietary supplements is influenced by a wide variety of factors including identity of the source plant material, geographical origin and environmental factors, methods of harvest and processing, formulation, and age of the processed materials. The influence of these factors is reviewed, recommendations are provided for controlling the effect of each variable, and a means of presenting these research results is presented.
Ayurveda is essentially an authentic practical science and all the fundamental principles ascertain in it have initiated from a philosophical background and passing through the science to accomplish its ultimate goal. The main objective of this research was to test the efficacy of an Ayurvedic botanical formula “Shothahara Compound” through scientific and philosophical approaches considering the Ayurvedic pharmacodynamics. The formula containing six botanicals, Cedrus deodara, Resimus communius, Tinospora cordifolia, Terminalia chebula, Boerhavia diffusa and Zingiber officinale was selected in the form of dried water-soluble extract. The study was specially planned to evaluate Ayurveda principles in the light of scientific testing by the animal and clinical experiments. The assessment of Dipana Pachana activity, Muthrala activity, Amahara effect, Rasayana effect and Shothahara effect were evaluated by using a food consumption test, effect on fecal output, effect of food conversion ratio, body weight changes, diuretic activity, effect on serum food consumption test, effect on fecal output, effect of food conversion ratio, body weight changes, diuretic activity, effect on serum

Proteomic method (two-dimensional electrophoresis and MS/MS) was used in studying the mechanisms of Traditional Chinese Medicines (TCMs) including Gastroderma lucidum, Salvia miltiorrhiza, Panax notoginseng and toad venom. For example, the effects of Salvia miltiorrhiza, a TCM popularly used for treating cardiovascular diseases, on the protein expression profiles of platelets, cardiomyocytes and heart tissues were checked. The results indicated that salvinic acids from Salvia miltiorrhiza could inhibit the aggregation and adhesion of platelets, migration of cardiomyocytes and could protect cardiomyocytes from ischemia-reperfusion injury both in vitro and in vivo. The effects of salvinic acids might be based on regulation of expression of proteins related to calcium ion binding, cell skeleton structure, elimination of reactive oxygen species, response to stress, etc. Furthermore, combined effects of salvinic acids and notoginsenosides, a TCM formula were also studied. The proteomic results showed that, in adjusting the un-normal protein expression profiles caused by ischemia-reperfusion injury back to normal, Fufang had better effect than either salvianolic acids or notoginsenosides. Our results indicated the usefulness of proteomic technology in TCM research.

DNA barcoding has been proposed as a novel and powerful taxonomic tool [1,2]. The universal primer COI has been widely applied in animals, but there is no such universal barcode for plants [3]. In this study, we examined the possibility of utilizing DNA barcode markers to identify labiatae medicinal herbs. First, we compared sequences of eight potential barcodes (Accr, rpoB, rpoC1, ycf1, rbcL, PsbA-trnH, ITS, and matK) among different species of labiatae. Our findings were as follows: (1) PsbA-trnH was amplified much easier than the other seven; (2) PsbA-trnH spacer is one of the most variable non-coding regions of the plastid genome in labiatae; and (3) Different species of labiatae can be differentiated effectively by comparing the PsbA-trnH intergenic region. Comparison of PsbA-trnH intergenic region among 71 species of 30 genus has provided solid and practical evidence for applying DNA barcoding on species identification. In summary, DNA barcoding was proven to be useful in identifying different species of labiatae medicinal herbs. Acknowledgements: Thanks go to the International Cooperation Program of Science and Technology (No. 2007DFA30990) and the Special Founding for Healthy Field (No. 200802043), for supporting the study. References: [1] Schindel DE, Miller SE (2005) Nature, 435: 17. [2] Miller SE (2007) PNAS, 104: 4775–4776. [3] lahaye R, et al. (2008) PNAS, 105: 2923–2928.
Implementation of Sustainability Standards that Contribute to Assurance of Pharmacopoeial Quality of Wild Collected Medicinal Plants

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What will happen? When everyone is excited with the tempo of modernization and globalization of an indigenous medical system, when new findings and inventions are making the headlines, when business in herbal trade is booming, and when patients are converted to believe in the salvation power of herbalism... but botanicals are not properly grown, handled, processed, manufactured and traded? When plant and animal populations in the wild are dwindling down due to over-exploitation, when endangered species are illegally poached for herbal preparations, when botanicals are substituted by threatened taxa, what will happen? When farming of medicinal plants is fragmentary making it difficult to ensure quality consistency, when mercury is fused into a botanical to increase its weight for a higher price, when flour is mixed into an herb to make it twice as large for a better sale, when processing and manufacturing procedures are reduced to save expenses regardless of toxin concentrations, what will happen? When prices of botanicals are fixed and investments of talents and financial inputs cannot be recovered, when regulatory agencies can be bribed, when advertisements merge with con artist, what will happen? The answer, my friend, is glowing in the science, the economics and the politics.

Reference substances are used to calibrate and validate the testing methods that are applied within the framework of quality control throughout all of the stages in the production and manufacture of herbal products. The quality of these reference substances is therefore of prime importance to the quality and associated safety and efficacy of these products. Manufacturers of herbal drugs, and dietary supplements in particular, are now also being confronted with a strong increase in the regulations that apply to the reference substances used to analyze their products. While the legal framework and detailed requirements for evidence of quality are clearly regulated for herbal medicinal products these have not yet been defined to the same extent for dietary supplements. However, as health-promoting functions and effects are being claimed to an increasing extent for such products, we must expect the requirements for evidence of their quality to be tightened up as well. This has already taken place in the USA with the introduction of the cGMP for dietary supplements in June 2007. The presentation will focus on the requirements for the analytical characterization of primary reference substances. The necessity to determine not only organic impurities but also water, residual solvents and inorganic impurities will be illustrated by presenting a number of examples of common compounds such as hypericin, hyperforin, hyperoside, silybin and others and by pointing out the crucial points encountered during the establishment, documentation and maintenance of these reference substances. Alternatives, such as quantitative NMR for content assignment of reference substances will be discussed as well.

The main aim of the Chinese Pharmacopoeia (ChP 2010 version) is to build up a quality controlling module that is in accordance with the characteristics of TCMs and is different from that of chemical medicines. It will change gradually from using single ingredient into using active, multiple ingredients, fingerprint or bio-determination to totally control the quality of TCMs. For the safety control of TCMs, the species of pesticides were determined examining the common compounds such as hypericin, hyperforin, hyperoside, silybin and others and by pointing out the crucial points encountered during the establishment, documentation and maintenance of these reference substances. Alternatives, such as quantitative NMR for content assignment of reference substances will be discussed as well.

Stevia is a generic term for extracts from the herb *Stevia rebaudiana* (Bertoni), while the sweet components are more precisely known as steviol glycosides. Long-standing questions about the specifications or characterization of the materials, safety, and special population effects have previously prevented steviol glycosides from being considered a mainstream natural sweetener. In order to provide the answers as well as bridge to the safety gaps, a strategic step-wise, research program was undertaken. Essential elements of the program included: complete characterization of the ingredient, general and reproductive toxicology, metabolism and pharmacokinetic analysis, clinical research, intake/exposure assessment, assurance of appropriate GMP for support specifications, and stability in food systems. A holistic approach to the communication of technical and scientific supporting data was used to ensure general recognition of safety by qualified individuals (GRAS). Efforts are ongoing to promote consistent quality standards within the industry, and to provide due diligence with respect to safety from the post-marketing perspective.

While in Europe products containing herbal extracts as active ingredients are generally handled under the pharmaceutical law and require a marketing authorization, it seems that so-called Botanicals are handled less strictly in the United States and other countries, where Botanicals are marketed as food supplements. In 2007, the U.S. FDA published the current Good Manufacturing Practice (cGMP) for manufacturing dietary supplements in addition to the Dietary Supplement Health and Education Act of 1994 (DSHEA) [1]. Currently, Europe’s Food Safety Authority (EFSA) is evaluating for food and for supplements submissions for health claims with the intention to legalize claims for risk reduction and for reduction of disease risk [2]. Furthermore the Council of Europe and the European Federation of Associations of Health Product Manufacturers made proposals for quality guides for plant based food supplements [3, 4]. Both the U.S. and E.U. approaches to handle products containing herbal ingredients have proven their suitability but still attitudes to Botanicals are in motion. Tackled together, though the approaches on how to deal with food supplements containing herbal ingredients in the United States and in Europe seem to converge, the question about the future position of Botanicals arises. This talk will shed light on different producer related aspects of quality as this debate will consequently also affect GMP for the manufacturer of herbal extracts. References: [1] U.S. Food and Drug Administration: Fact Sheet on FDA’s Strategy for Dietary Supplements. [2] Regulation (EC) No 1924/2006 on nutrition and health claims made on food. [3] Council of Europe: Guidance on the Quality, Safety and Marketing of Plant-Based Food Supplements, 24.06.2005. [4] European Federation of Associations of Health Product Manufacturers: Quality guide for food supplements, Nov. 2007.
Arsenic is present in the environment in both organic and inorganic forms. While organic arsenicals are generally considered to have very low toxicity, the inorganic species is widely recognized as a carcinogen in addition to causing numerous other adverse health effects following acute or chronic exposure [1, 2]. The tolerance limit for arsenic as a contaminant in natural health products (NHPs) currently recommended by Health Canada’s Natural Health Products Directorate (NHPD) is 0.14 µg/kg body weight/day [3]. However, this limit represents total arsenic and does not distinguish between organic and inorganic arsenical compounds. Consequently, this current limit may be unnecessarily restrictive for the NHP industry as certain products may contain high levels of relatively non-toxic organic arsenic forms, but only minimal amounts of the toxic inorganic arsenic. NHPD investigated this issue in order to determine whether there is substantial scientific evidence to support separate limits for inorganic and organic derivatives of arsenic, and whether suitable analytical methodology exists to distinguish between these forms in finished NHPs. The review involved assessing arsenic toxicity, analytical methodology, and exposure scenarios for natural ingredients used in dietary supplements (e.g. kelp). NHPD recommends maintaining the current tolerance limit of 0.14 µg/kg bw/day for total arsenic in NHPs at the finished product stage. However, if total arsenic content in a particular NHP exceeds the current tolerance limit of 0.14 µg/kg bw/day (taking into account dosage and subpopulation), the applicant may undertake additional arsenic speciation testing to demonstrate that inorganic arsenic consumed by ingesting the product would be < 0.03 µg/kg bw/day and that organic arsenic consumed by ingesting the product would be < 0.20 µg/kg bw/day. Acknowledgements: This research project benefited from scientific expertise within Health Canada Offices and Directorates, the United States Pharmacopeia, and NSF International. References: [1] Environment Canada. 1999. Canadian Environmental Protection Act. List of Toxic Substances, Schedule 1, Item 28. URL: http://canadagazette.gc.ca/partII/2000/20000329/html/sor109-e.html accessed 2008–12–09. [2] ATSDR: Agency for Toxic Substances and Disease Registry. 2007. Toxicological Profile for Arsenic. US Department of Health and Human Services. URL: http://www.atsdr.cdc.gov/toxprofiles/tp2.html, accessed 2008-01-02. [3] Health Canada. 2007. Evidence for Quality of Finished Natural Health Products (Version 2). Natural Health Products Directorate. URL: http://www.hc-sc.gc.ca/dhp-mps/prodnatur/legislation/docs/eq-paq_e.html, accessed 2008–12–09.

The Impact of Global Supply and Trade on Botanical Ingredients and Industry Practices

More than ever, the global botanical industry faces unprecedented challenges with respect to quality standards, intentional adulteration, analytical method development, as well as an array of regulatory issues. Understanding global supply, global trade and consumer demand for botanicals is essential if quality, safety and efficacy are to be respected. This presentation will provide an international perspective of leading issues and their implications for botanical traditional medicines and dietary supplements.

Enforcement of the 2006 Dietary Supplement and Nonprescription Drug Consumer Protection Act (DSNDCPA) began in December 2007. FDA published guidance documents regarding compliance and reporting of “serious” events but there has been no guidance on how “serious” and “non-serious” reports are to be differentiated. The FDA has designed a system to score spontaneously reported adverse incidents involving botanicals containing dietary supplements. Using six common parameters to gauge association including expectedness, temporality, biologic plausibility, de-challenge, re-challenge, and consideration of confounding variables, a standardized scoring system has been developed. The system was successfully piloted with a proprietary blend dietary supplement and provides a means for manufacturers to benchmark their product safety experience. Description and application of the scoring system will be presented along with representative scoring of actual adverse events represented in the new FDA adverse event database.
manufacturers in terms of quality, safety and efficacy of these herbal products will be discussed. A comparison will be made with other concepts existing worldwide, taking into account not only the above mentioned properties, but also aspects such as access to the market, cost price, and prospects for innovation of herbal products.

S-38

FDA's Dietary Supplement Good Manufacturing Practice Regulatory Requirements for Globally Marketed Botanicals
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The Dietary Supplement (DS) CGMPs should help prevent inclusion of the wrong ingredients, too much or too little of a dietary ingredient, contamination (e.g., natural toxins, bacteria, pesticides, glass, and heavy metals such as lead), and improper packaging and labeling. Following DS CGMPs will increase consumers' confidence in the quality of the dietary supplement products that they purchase. The CGMPs apply to all domestic and foreign companies that manufacture, package, label or hold dietary supplements, including those involved with the activities of testing, quality control, packaging and labeling, and distributing them in the U.S. The final DS CGMP rule does not apply to raw ingredient manufacturers, although they will continue to need to meet the food CGMP regulations. This presentation will provide an overview of the key CGMP requirements that foreign suppliers of botanical ingredients and dietary supplements should be aware of.

S-39

Adverse Event Reports Submitted to U.S. Food & Drug Administration Associated with Dietary Supplements
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The Federal Food, Drug, and Cosmetic Act was amended in 2006 to require marketers of dietary supplements and nonprescription drugs to submit to the U.S. Food & Drug Administration (FDA), as of December 22, 2007, all reports of serious adverse events associated with and received by marketers of products in those regulatory categories. The new law established additional responsibilities with regard to follow-up reports and recordkeeping. Adverse event reports submitted to FDA during 2008 by marketers of dietary supplements were obtained from FDA through requests under the Freedom of Information Act. Analysis of these records shows that most reports are submitted by marketers, though reports are also submitted by individual consumers and health care practitioners. There are more reports associated with women than men, and with individuals between the ages of 50 and 79 than with older or younger consumers. FDA's issuance on March 27, 2008 of a warning to advise consumers to refrain from purchasing products sold as Total Body Formula followed the agency's receipt of 25 adverse event reports associated with the products, indicating that the reporting system is functioning as a signal generator that as-}

S-40

Improving the Odds of Developing New Drugs from Botanicals: Botanical Review Team's Perspectives
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There is no doubt that plants and animals have provided humankind with numerous purified small molecule drugs and there is reason to hope that botanical mixtures will have more to give us. Botanical mixtures, are widely used as dietary supplements in the United States or as herbal medicines elsewhere, have, for the most part, not been extensively studied through well-controlled clinical trials to show beneficial effects. We hope this will change and that more botanical derived pure compounds as well as botanical mix-
The idea of combination therapy has been practiced in Traditional Chinese Medicine for thousands of years, and has been gaining ever-increasing acceptance in the world. During the past decade, owing to changes in the types of disease and limitations of Western medicine, the usage of Chinese herbal medicines (CHMs) has expanded globally. CHMs are complex mixtures consisting of thousands of compounds. Getting useful chemical and bioactive information from these highly complicated matrices has long been one of the major challenges to chemists, analysts, biologists and pharmacologists. The speaker, Prof. Li, is the head of Key Laboratory of Modern Chinese Medicine, Ministry of Education, and the director of Department of Pharmacognosy at the China Pharmaceutical University and has been working in the field of CHMs for over 20 years. The most often used instrumental technique, high-performance liquid chromatography (HPLC) remains unchallenged for the analysis of CHMs, because of it is low-cost, readily available and easy of use. This report covers current HPLC-based strategies for the analysis of CHMs, and is divided into three major sections. These are simultaneous qualitative and quantification of various components in CHMs (in vitro), metabolite identification and pharmacokinetic investigation of CHMs' components in biological samples (in vivo), and biomacromolecule (protein and DNA) affinity/LC-MS for screening of multiple bioactive candidates in CHMs. Acknowledgements: Financial support for this research from the National Science Foundation of China (No. 90709020, 30530870) is gratefully acknowledged.
tent of the fruit juice and extract, however revealed that ascorbic acid co-elutes with other compounds of similar spectral behavior. Additionally, the hydrolysable tannins, when evaluated were found to be structurally different from the previously reported structures. The earlier reported antioxidant hydrolysable tannins, emblicains A and B, correspond to beta-glucogallin (1) and mucic acid 1,4-lactone 5-O-gallate (2), respectively. Only trace amounts of free ascorbic acid were detected. β-glucogallin is therefore a more relevant and optimal biomarker in Emblica officinalis extract, than ascorbic acid. References: [1] Ghosal S, et al. (1996) Indian J Chem 35B: 941–948. [2] Pozharitskava ON, et al. (2007) J Sep Sci 30: 1250–1254.

Salvia miltiorrhiza Bunge, named “Dan-Shen” in Chinese as a traditional Chinese medicine, is used for improving body function, as well as for cardiac symptoms treatment for hundreds of years in China. The phenolic acids such as rosmarinic acid (RA) and its derivative lithospermic acid B (LAB) aroused scientists’ interest in the last twenty years because of their notable pharmacological activities [1]. In our present study, abiotic elicitors such as methyl jasmonate (MeJA) and Ag+ were found to enhance the phenolic acids at various levels. Meantime, based on the profiling changes of several related gene transcripts and metabolites (intermediates) accumulations, in response to elicitors, a gene-to-metabolite network for understanding of global responses to abiotic elicitation in S. miltiorrhiza is established (1), and a potential (putative) biosynthesis process form RA to LAB was presumed (2), which prompted the possibility of a key gene-based metabolic engineering for the synthesis of active pharmaceutical compounds in S. miltiorrhiza, and would certainly help us to globally and deeply understand metabolic flux of RA synthesis, both at stressed-elicitation and genetic-regulation levels.


Studies on the Chemical Constituents and Biological Activities of Four Medicinal Plants from Ilex Genus

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There are about 204 plant species of Ilex genus in China, and more than 30 of which are used as traditional Chinese medicine (TCM) or folk medicines to treat various diseases [1]. In order to systematically find out the chemical constituent’s and bioactives of Ilex plants, and lay a foundation of discovering leading compounds, we carried out an investigation on several medicinal plants of Ilex genus. Herein, we report the research results of 4 medicinal plants of which, including Ilex kudingcha, Ilex hainanensis, Ilex pernyi and Ilex asprella. In total, 194 compounds were isolated and identified from the above 4 plant species, 61 of those are new compounds, and 98 of those are triterpenoids or triterpenoid saponins. Also, the biological screening of triterpenoids and triterpenoid saponins that are the primary and typical constituents of Ilex genus, were assayed for their affect on the cell’s absorption of aggregated low density lipoprotein (aggLDL). A cell based-screening model was applied on aggregated LDL induced-lipid deposition in macrophages to test the inhibitory effects of these compounds. The compounds with inhibitory effects on the intracellular accumulation of aggLDL in macrophages could be regarded as having the potential bioactivity of anti-atherosclerosis. The data indicated that 19 compounds have an inhibition effect on aggLDL absorption. Remarkably, kudinoside A, C and IPB-20 show the significant bioactivity, whose inhibition ratio is 81%, 92%, and 85% at a concentration of 0.2 mg/ml respectively. Thus, the three compounds could the potential candidate for the treatment of arteriosclerosis. Acknowledgements: Thank the National Science Foundation of China for financial support (No. 30672608). This work was also supported by the program for Changjiang Scholar and Innovative Team in University (No.985-2-063-112). References: [1] The editor committee for Flora of China of Chinese Academy of Sciences. (1999) Flora of China. Science Press, Beijing, China.
Diet-related chronic diseases such as diabetes, high blood pressure, and colon cancer are growing problems in industrialized countries and obesity is the major cause with 36 million deaths annually in the world. Yacon, Smallanthus sonchifolius, (Poep. et Endl.) H. Robinon, is a root crop and is a rich source of phenolic compounds and dietary oligofructans with low glucose content [2]. These constituents have shown efficacy in the treatment and prevention of diet-related chronic diseases, including gastrointestinal disorders and diabetes. The objective of this study is to develop an integrated system that promotes yacon as a sustainable root crop industry in Mississippi, including root and leaf production, as well as processing yacon into value-added commodities as functional food. Yacon is native to Peruvian Andes and originally grows at elevation 1800-2800 of meters above sea level (masl) [1]. The purpose of our work is to evaluate Yacon growth in Mississippi during the hot and dry summers at elevation of 137.8 masl. Yacon propagules were produced by tissue culture and by stem cuttings. Micropropagated plantlets adapted to soil conditions at an average of 90%. A significant difference on plant height, number of roots, leaf and root biomass was noticed for plants cultivated in pots which were produced by tissue culture. Only plants produced from stem cuttings were planted in the field and during the first growing season the average yield reached 0.755 kg of fresh weight per plant. Preliminary organoleptic evaluation (color, aroma, taste, cleanliness) suggests the cultivated Chinese medicinal plants were of higher quality than commercially available plant material imported from China.
groups were found statistically insignificant. These results confirm the clonal fidelity of tissue culture raised plants of Cannabis sativa and suggest that the biochemical mechanism followed to produce the micropropagated plants does not affect the metabolic content and can be used to produce true-to-type plants of this species for commercial pharmaceutical use. Acknowledgements: The work was supported in part by National Institute of Drug Abuse (NIDA). Contract No. N01DA-0-7707. References: [1] Lata H, et al. (2008) In vitro cellular and developmental biology-Plant (In Press; DOI 10.1007/s11627-008-9167-5). [2] Lata H, et al. (2009) Physiology and Mol Biol of Plants, 15(1): January 2009 (In Press). Variations in Temperature Response of Photosynthesis in Drug and Fiber Type Varieties of Cannabis sativa L. Chandra S1, Lata H1, Khan IA1−2, EISOhy MA1,3 1 National Center for Natural Product Research, School of Pharmacy, The University of Mississippi, University, MS, 38677, USA 2 Department of Pharmacognosy, University of Mississippi, MS, 38677, USA 3 Department of Pharmaceutics, School of Pharmacy, The University of Mississippi, University, MS, 38677, USA The effect of temperature on photosynthetic characteristics of three high yielding drug type (HP Mexican, MX and W1) and three fiber type (Kimpolty, Zolo 11 and Zolo 15) varieties of Cannabis sativa, originally from different agro-climatic zones worldwide were studied. The results clearly indicate that among three drug type clones, high potency Mexican (HP Mex) clone was found to be the most thermotolerant. Optimum temperature for photosynthesis (T\text{opt}) was observed around 30 °C in HP Mex whereas, T\text{opt} was observed in the range of 25 to 30 °C in W1 [1]. A comparatively lower value (25 °C) for T\text{opt} was observed in MX. Among fiber type clones, T\text{opt} was observed around 30 °C in Zolo 11 and Zolo 15 (Ukrainian origin) whereas, in Kimpolty (from Switzerland) it was observed around 25 °C. Differences observed in water use efficiency (WUE) among the clones at lower temperature were less pronounced at higher temperatures. Higher WUE and, lower stomatal conduc- tance and transpiration in HP Mex indicate that this clone may be suitable for the plantation in relatively dry and exposed sites. Both stomatal and mesophyll components seemed to be responsible for the temperature dependence of photosynthesis (Pn) however, their magnitude varied with the clones. A two to five fold increase in dark respiration with an increase in temperature was observed in clones. However, higher increases were associated with clones having higher rate of photosynthesis, indicating an association between photosynthetic and respiratory rates. The results provide a valuable indication regarding clonal variations in temperature de- pendence of Pn in Cannabis sativa and may be used as a tool for ini- tial selection of suitable clones for outdoor cultivation or to provide suitable indoor environment depending upon a particular variety/ clone. Acknowledgements: The work was supported in part by Na- tional Institute of Drug Abuse (NIDA). Contract No. N01DA-0-7707. References: [1] Chandra S, et al. (2008) Physiology and Mol Biol of Plants, 14(4), October 2008 (in press). Molecular Analysis of Genetic Stability of Micropropagated Plants of Cannabis sativa L. using ISSR Markers Lata H1, Chandra S1, Techen N1, Khan IA1−2, EISOhy MA1,3 1 National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, MS, 38677, USA 2 Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, MS, 38677, USA 3 Department of Pharmaceutics, School of Pharmacy, The University of Mississippi, University, MS, 38677, USA An efficient micropropagation protocol was developed and plants of a high THC yielding elite variety (MX-1) of Cannabis sativa were produced using nodal segments containing axillary buds [1]. The genetic stability of the micropropagated plants was evaluated up to thirty passages in culture and hardened in soil for 8 months using the method of Inter Simple Sequence Repeat (ISSR) DNA fingerprinting. ISSR profiles of micropropagated and hardened plantlets were compared with the mother plant grown indoor. A total of 15 ISSR primers resulted in 115 distinct and reproducible bands. All the ISSR profiles from micropropagated plants were monomorphic and similar to the mother plants. No variation was detected within the micropropagated plants. These results suggest that the culture conditions used for shoot proliferation are appropriate for clonal propagation of the elite variety of Cannabis sativa as they do not seem to interfere with the integrity of the regenerated plants. This study is of high significance as these plants are selected to be used in the mass propagation for the production of biomass, as a starting ma- terial for the isolation of THC as a bulk active pharmaceutical. Ac- knowledgements: The work was supported in part by National In- stitute of Drug Abuse (NIDA). Contract No. N01DA-0-7707. References: [1] Lata H, et al. (2008) In vitro Cellular and Developmental Biology-Plant, (In Press; DOI 10.1007/s11627-008-9167-5)
Chemical Characterization and Genomic Profiling of *Achillea biebersteinii* from Various Localities in Central Turkey

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Herbal teas prepared from selected *Achillea* (Asteraceae) species are used in traditional Turkish medicine as diuretic, emmenagogue (menstrual flow stimulant), aid in wound healing, treatments for abdominal pain, and used to counteract diarrhea and flatulence [1]. *Achillea biebersteinii* is locally known as “Aydavana, Sari civanpenceri” in Turkey. The aerial parts of five *Achillea biebersteinii* accessions were collected from different locations in Central Turkey to study the essential oil composition and their genetic fingerprinting. Hydrodistilled essential oils were analyzed by GC/FID and GC/MS techniques. Essential oils from Konya region were rich in 34–37% 1,8-cineole and oil from plants obtained from the Ankara region contained 27% p-cymene as the major constituent. *Achillea* oils were also evaluated for their antimalarial, antimicrobial and antiinflammatory activities. Detailed chemical profile will be presented in this study. An increasing application of DNA fingerprinting is the use of marker assisted breeding and authentication/identification of (plant) species used in pharmacology or in commercial available food products. In this study we also describe the construction of a genomic library from *Achillea biebersteinii* enriched for Short Single Repeat (SSR) microsatellite loci. We have isolated several hundred clones with distinct SSRs fragments and designed oligonucleotides based on the identified sequence. The effectiveness of genotypic markers as possible methods in determining specific chemotypes and authentication of plant species from Turkey and USA was evaluated and discussed in this study. References: [1] Konyalioglu S, Karamenderes C (2005) Journal of Ethnopharmacology, 102: 221–227.

Genomic Profiling of *Cannabis sativa* L.

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*Cannabis sativa* is an interesting crop for several industrial uses. It has been used for fiber (hemp), for medicinal purposes, and as a psychoactive. Although the main psychoactive chemical compound in *Cannabis* is δ9-tetrahydrocannabinol (THC), the plant is known to contain about sixty cannabinoids, however, most of these are not psychoactive. Although the main psychoactive chemical compound has been used for fiber (hemp), for medicinal purposes, and as a psychoactive, *Cannabis* is an interesting crop for several industrial uses.

The medicinal plants of the Araceae family are distributed widely throughout China and more than half of them are medicinal plants, whereas materials of similar morphology and chemical fingerprints are often misidentified. DNA barcoding is a new technique that uses DNA sequences from a small fragment of the genome to identify species. Five specific DNA regions (*psbA-trnH*, *rpoB*, *rpoC1*, *rbcL*, *psba-trnH*) of 95 samples of 34 genera were amplified and sequenced. We found that the *psbA-trnH* is difficult to sequence through PCR product, because this region is A, T rich (70%, averaged). The amplification efficiency of *rbcL*, *matK*, *rpoB*, and *rpoC1* were 87.4%, 94.7%, 98.9%, 100%, respectively. However the *matK* was variable enough to identify species, and the intra-specific divergence was 0.2% (97.5%–99.9%). The results indicate that the *psbA-trnH* is not suitable to identify the medicinal plants of the Araceae family. The *matK* can be used as a barcoding to identify all species of Araceae. Acknowledgements: This work is supported by the International Cooperation Program of Science and Technology (No. 2007DFA30990) and the Special Founding for Healthy Field (No. 200802043) References: [1] Chase MW, et al. (2007) A proposed protocol to barcode all land plants 56(2): 295–299. [2] Kress WJ, et al. (2005) Proceedings of the National Academy of Sciences USA 102: 8369–8374.

Relationship between DNA Barcoding and Chemical Classification of *Salvia* L. Medicinal Herbs

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In China, over 20 Salvia species have been used as Danshen in traditional folk medicine [1]. The rapid and accurate identification of species is critical to Salvia L. medicinal herbs. DNA barcodes and chemical fingerprint are two approaches that have recently garnered much attention [2,3]. Here we compared these two methods for identification of the genus of *Salvia* L. First, we sequenced the nucleotide sequences of the internal transcribed spacer region 2 amplified from 32 medicinal plants belonging to *Salvia* L. and seven other groups of labiatea medicinal plants. By using neighbor joining analyses, phylogenetic trees were mapped by their sequence diversity. Secondly, we tested the water-solution bioactive components (Rosmarinic acid, Lithospermic acid and Salvianalic acid B.) and lipid soluble components (Tanshinone and Cryptotanshinone) of every sample by HPLC. Additionally, we compared the relationship between the sequence of ITS2 and the components of every branch in ITS2, and found a significant correlation between them. By contrast, DNA barcoding was sequencing-based and therefore could provide more accurate and fast results in large-scale studies. This is the first paper to show the relationship between DNA barcoding and chemical components. Acknowledgements: Thanks go to

Using DNA Barcodes to Identify Rosaceae

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DNA barcoding has recently been proposed as a technique that employs a short, standardized gene region to identify species. DNA barcoding is well established in animals because of a widely appropriate sequence for them, the cytochrome oxidase 1 [1], but there is not any universally accepted barcode for plants till now. Therefore, the primary task for barcoding plants is to find more useful barcodes that can identify as many species as possible. Medicinal plants have been used as traditional Chinese drugs for treating diseases, some of them are similar in morphology, and are often misidentified by chemical fingerprints. Rosaceae includes many medicinal plants with similar morphology and are usually hard to be identified. Here, we chose five potential barcodes, Universal Plastid Amplicon (matK, rpoB, rpoC1, rbcL) and the nuclear ribosomal DNA (rDNA) internal transcribed spacer (ITS), to identify species from different genera in Rosaceae. The results suggest that the nuclear ribosomal DNA (rDNA) internal transcribed spacer (ITS) is a candidate to discriminate all of plant species in Rosaceae. Acknowledgements: We thank all my teachers and classmates in our laboratory very much for their help. References: [1] Kress WJ, et al. (2005) PNAS, 102: 8369–8374.

P-12

Authentication of the Medicinal Plants in Fabaceae by DNA Barcoding Technique

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Fabaceae is the third largest family of flowering plants, with a large number of medicinal plants. However, it is arduous to identify some of the species in this family because of morphological similarity and frequent variation. The DNA barcode, a short DNA sequence originating from the genome, was first investigated for the medicinal plants in Fabaceae. Now we have completed 86 species of medicinal plants in Fabaceae including over 30 genera. Through six candidate dates, promising markers, four coding (rpoB, rpoC1, rbcL, matK) and two noncoding (ITS1, ITS2) chloroplast regions, we identified potential barcodes of the medicinal plants in Fabaceae by comparing DNA barcoding sequences. The results indicated, efficiency of amplification for six candidate DNA barcodes range from 100% (ITS2) to 93% (matK). Intraspecific variation and interspecific variation for six chloroplast regions derived Wilcoxon signed rank tests of divergence showed that rpoC1 was the least discriminatory region, while ITS2 was the potential candidate of medicinal plant barcoding in Fabaceae in our study. Overall, our findings showed that DNA barcoding is an efficient and powerful tool for the identification of the medicinal plants in Fabaceae. Acknowledgements: This work is supported by the International Cooperation Program of Science and Technology (No. 2007DFA30990) and the Special Founding for Healthy Field (No. 200802043). References: [1] Gregory TR, (2005), [2] Kress WJ, et al. (2005) PNAS, 102: 8369–8374.

P-13

Genetic and Metabolic Studies of Cannabinoids in Standardized Medicinal Cannabis sativa

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In this research we investigated the biosynthesis and accumulation of cannabinoids during the growth phases of Cannabis sativa leaves and flowers. Flowers from standardized indoor breeding were analyzed for transcription and expression of identified genes [1–5] from the cannabinoid pathway and the accumulation of the cannabinoid metabolites [6]. The correlation between the various measurements should give more information on the regulation of the cannabinoid production process within the plant. Plant samples were taken randomly during standardized cultivation. Every week, for eight weeks in a row, three plants were sampled, and materials were treated for analysis by QRT-PCR, HPLC, and 2D-electrophoresis. With QRT-PCR the transcription of CBDA- (BAF65035), THCA- (BAE48253) and olivetol synthase (BAG14339) genes were quantified against cloned genes. 2D-electrophoresis was used to detect any specific protein expression during the cultivation period. From this ongoing study, we have indicated that the amount of THCA in the leaves stays in certain ranges throughout the sampling period and is not dependent on the vegetative or flowering status of the plant. In contrast, the content of THC in the flowers is depending on the growth period, which is in line with previously reported data on the correlation of trichoma and cannabinoids. The information obtained from this study is used as a profound basis for further genetic and metabolic analysis. References: [1] Kim JS, et al. (2006) Biotechnol Lett. 28(13): 999–1006. [2] Sirirkantasams S, et al. (2005) Plant Cell Physiol. 46(9): 1578–1582. [3] Sirikantarams S, et al. (2004) J Biol Chem. 279(38): 39767–39774. [4] Morimoto S, et al. (1998) Phytochemistry, 49(6): 1525–1529. [5] Taura F, et al. (1996) J Biol Chem. 271(29): 17411–17416. [6] Fellermeier M, et al. (2001) Eur J Biochem. 268(6): 1596–1604.

P-14

Profilling Changes in Gene-to-Metabolite Networks for Rosmarinic Acid and its Derivative Biosynthesis in Salvia miltiorrhiza: Hairy Root Cultures Treated with Elicitors

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Salvia miltiorrhiza Bunge (Dan-shen in Chinese), is a commonly used traditional Chinese medicine for improving body function, as well as for the treatment of cardiac symptoms. The phenolic acids such as rosmarinic acid (RA) and its derivative lithiumperinic acid B (LAB) aroused scientists interest in the last twenty years because of their notable pharmacological activities [1]. As for S. miltiorrhiza, hairy root cultures have been suggested to be more stable and efficient than cell suspension cultures in active constituent accumulation [2]. In our present study, we found that methyl jasmonate (MeA) and Ag+ could greatly enhance the phenolic acids at various levels. Meanwhile, several related gene transcripts and metabolites (intermediates) accumulations involved in RA synthesis pathway (1), in response to elicitors, were determined by real-time quantitative PCR and liquid chromatographic-tandem mass spectrometry, respectively. Therefore, a gene-to-metabolite network for understanding of global responses to abiotic elicitation in S. miltiorrhiza is established, and a potential (putative) biosynthesis process from RA to LAB is presumed (2), which is now under intensive investigation by analysis of differential expression protein and precursor feeding experiment in our laboratory. Acknowledgements: This re-
Identification of Weight Loss Supplement Cha De Bugre
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The use of dietary supplement Cha De Bugre for weight loss/appetite suppressant is getting increasingly popular. The efficacy and safety of these products depends on the quality and accurate identity of raw material. Along with taxonomic evaluation, macroscopic, microscopic and organoleptic assessment is one of the reliable, consistent, competent and cost effective methods in authentication of raw material [1]. In Brazil Cordia salicifolia Cham (Boraginaceae) is commonly referred to as cha de bugre or coffee of the woods. On the other hand Casearia silvestris Sw. (Flacourtiaceae) is also frequently referred to as congestas-de-bugre and is often substituted for Cordia salicifolia due to the resemblance in its common name. In the present study we have provided a detailed monographic account (involving taxonomy, species distribution, macro and micro-morphological evaluation, analysis of powder and shifts) for the two species. We also analyzed commercially available cha de bugre samples. Acknowledgements: This research is funded in part by “Botanical Dietary Supplements: Science-Base for Authentication” funded by Food and Drug Administration grant number FD-U-002071-01. References: [1] Joshi V, Khan I (2006) ed. Khan I, Smillie T, Craker L, Gardner Z, in Proceedings of the Fourth International Conference on Quality and Safety Issues Related to Botanicals, ISHS, Leuven, Belgium, Acta Horticulturae 720. [2] Siqueira V, et al. (2006) Brazilian Archives of Biology and Technology, 49: 215–218.

Authentication of Caralluma adscendens var. fimbriata (Wall.) Gravely & Mayur Joshi VC¹, Rao AS¹, Wang YH¹, Avula B¹, Khan IA¹,²
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² Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, MS 38677, USA

Caralluma is an edible succulent plant used by tribes in India to suppress hunger and enhance endurance [1]. It is a new arrival in the family of succulent plants that are becoming increasingly popular for their appetite suppressant and weight loss properties as well as their ability to lower blood sugar. Accurate identity of the raw material is critically important, to ensure the efficacy and safety of these products. Available herbal monographs lack information on Caralluma. The present study, details the macroscopic and microscopic evaluation of Caralluma adscendens var. fimbriata

Taxonomic Clarification on Turnera diffusa Ward and its Demarcation from “False Damiana” using Fluorescence, Scanning Electron Microscopy, HPTLC and UPLC
Joshi VC¹, Rao AS¹, Wang YH¹, Avula B¹, Khan IA¹,²
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References:

The NC Arboretum Medicinal Germplasm Repository will be a collaborative effort by public and private organizations to advance the conservation, authentication, and cultivation of medicinal plants by collection and long-term storage of germplasm and their associated documentation. Germplasm will include but not be limited to seed, DNA, pollen, and entire plants when applicable. In addition soil samples, voucher specimens, and representative tissue samples for chemical analysis will be collected and stored. Located at the NC Arboretum in Asheville, in situ collection efforts commenced in spring 2008. The mission of the NCAM will include: 1) the long-term conservation of diverse medicinal germplasm through field collection and acquisition; 2) Germination and seed viability testing following pre-established IOSA protocols; 3.) establishing collaborative germplasm-related research projects with regional co-operators; and 4) encouraging the use of the collections and associated information for phytopharmaceutical screening, crop improvement and product development. Comprehensive accession information including passport data, images, site maps, and experimental results will be maintained via an interrelational database. Conservation via seed collection and storage will play a central role in protecting the high levels of genetic diversity available in our ex-situ collection efforts by public and private organizations to advance the conservation and natural product discovery; 2) map conservation effort. The project will: 1) build an International Partnership on Conservation and Natural Product Discovery; 3) establish a microbial library of plant associated microorganisms. As the establishment of *in vitro* germplasm bank progresses, endophytic microbes commonly associated with plants will outgrow the host tissues and allow us to detect and identify them. Some of these organisms are responsible for production of secondary metabolites [2,3]. Clonal propagation by *in vitro* methods will supply the biomass for fractionation and isolation of the active metabolite(s) and future developments. In addition, micropropagation will provide a unique opportunity to identify and evaluate the contribution of plant associated microorganism to the biological properties. References: [1] Dias BF, (1992) Manejo e Conesvaco dos Recursos Naturais Renovaveis. Funatura, Brasilia, DF. Brazil. [2] Lata H, et al. (2006) Plant Cell Tiss Org, 85: 353–359. [3] Strobel G, (2006) Curr Opin Microbiol, 9: 240–244.

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### Table 1

<table>
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<tr>
<th>Isolates</th>
<th>Water extract</th>
<th>Fraction I</th>
<th>Fraction II</th>
<th>Fraction III</th>
<th>Fluconazole</th>
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<td>1250</td>
<td>1250</td>
<td>1250</td>
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<tr>
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<td>156</td>
<td>312</td>
<td>625</td>
<td>1250</td>
<td>757</td>
</tr>
</tbody>
</table>

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Cerrado, Brazilian savanna, covers 2 million km², representing 23% of the land surface of the country. It occupies the central part of Brazil, from the margin of the Amazonian forest to outlying areas in the southern states of Sao Paulo. According to Dias’ [1] estimation, the Cerrado contains 160,000 species of plants, fungi and animals. This proposed research program will expand and upgrade the conservation effort. The project will: 1) build an International Partnership on Conservation and Natural Product Discovery; 2) map and protect the genetic resources by establishing germplasm bank of two endemic families Leguminosae and Combretaceae; 3) search for new pharmaceuticals and agrochemicals to control tropical diseases, and agricultural pests and pathogens, 4) create an Eco-extract-library and *ex situ* collections for future studies; 5) es-

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**Development of the NC Arboretum Medicinal Plant Germplasm Repository for Collaborative Research and Conservation**

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**Antifungal Activity of Stryphnodendron adstringens (Mart.) Extracts**

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**Building Partnership for Drug and Ag Discovery and Conservation of the Natural Resources in Brazil**

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Ecological Suitability of Arctium lappa L. and its Suitable Cultivation Regions in China
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The growth of arctii. In addition a probiotic fungus and a inhibition fungus for metals in soil increased the seed germination rate and the rain vol-

Plants are a potential source of antimicrobial compounds. In this re-
search, a plant from the family Cucurbitaceae was studied. Momordica foetida Schumch. Et Thonn is a climber commonly found in swampy areas in Central and Southern Africa. It has medicinal uses ranging from spiritual and psychiatric conditions to physical diseases. Drinking of aqueous leaf extracts of the plant for the treatment of malaria is reported in East and Central Africa [1,2]. The leaves were extracted using 70% ethanol and partitioned into hex-
ane, chloroform, ethyl acetate, butanol and aqueous then screened for antimicrobial activity against 32 bacterial strains for both standard-
and isolates. Thus, ethyl acetate and chloroform fractions were chosen for further studies due to higher antimicrobial activity with minimum inhibitory concentration (MIC) values for 32 bacterial strains ranging from 0.156 and 2.5 mg mL⁻¹. Active fractions were further purified using chromatographic techniques. A detailed phy-
tochemical investigation resulted into isolation of four curcurbatine triterpenoids and flavonoids compounds from chloroform and eth-
yl acetate fractions respectively. The chemical structures of the iso-
lated compounds were established through UV, IR, ¹H, ¹³C, COSY and 2D NMR spectroscopic data. Antimicrobial investigations were carried out on the isolated compounds against 25 bacterial strains of which 38,7β-dihydroxy-cucurbita-5,23,25-trien-19-β-al followed by Kaempferol-3-0-β-D-glucopyranoside displayed minimum inhibitory concentration (MIC) values for 25 bacterial strains ranging from 7.8 to 250 µg mL⁻¹. Acknowledgement: We are grateful to the National Research Foundation and University of Zululand, South Africa for financial support. References: [1] Hakizamunzi E, et al. (1992) J Ethnopharmacology 36: 143–146. [2] Rwangabo PC, (1993) La medicine traditionnelle au Rwanda. Edition Karthala and ACCT, Paris, France.

Comparative Pharmacognostic Studies on Aloe schweinfurthii and Aloe vera (Aloeaceae) Leaves
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In the modern era, herbs are found to be potential medicine for a variety of diseases. The usage of herbal drugs has increased in both developing and developed counties due their natural origin and minimal side effects. At present, the standardization of herbal drugs and herbal preparations is a priority area for Nigerian government and also Nigerian pharmaceutical industries. The Aloe plant (family, Aloeaceae) has been used all over the world for many years for various medicinal and health purposes. Studies on the macro- and micro-morphology of the leaves of Aloe schweinfurthii Baker and those of Aloe vera (Linn.) Burn. f., (a world acknowledged Aloe spe-
cies), were carried out for comparative identification, authentica-
tion, chemo-microscopy, quantitative microscopy and phytochem-
ical profiles that could be incorporated into their monographs in

The genus Achillea L. of Asteraceae is widely distributed and is re-
presented by 42 species in Turkey. Achillea species comprise an im-

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potent biological resource in folk medicine in the treatment of various diseases. In this study, the aerial parts of four Achillea species collected from different parts of Turkey were investigated for their essential oil composition and biological activity. Essential oils obtained by hydrodistillation were analyzed both by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The main Achillea oil constituents were found as follows: Achillea filipendula: 43.8% santolina alcohol, 14.5% 1,8-cineole and 12.5% cis-chrysanthenyl acetate; Achillea magnifica: 27.5% linalool, 5.8%spathulenol, 5.5% terpinen-4-ol, 4.7% α-terpineol and 4.7% β-éudesmol; Achillea tenuifolia: 12.4% artemisia ketone, 9.9% p-cymene, 7.1% camphor, 5.9% terpinen-4-ol, 4.7% carophyllene oxide and 4.5% α-πiene; A. tomentollum: 9.4% camphor, 7.8% linalool, 7.1% α-terpineol, 5.3% trans-pinocarveol and 4.5% trans-verbenol. Achillea essential oils were investigated for antimalarial, antimicrobial and antifungal activities. Achillea oils showed no antibacterial activity against human pathogenic bacteria up to a concentration of 200 mg/mL. A. tomentollum, A. tenuifolia and A. magnifica demonstrated mild antifungal activity against Cryptococcus neoformans (IC₅₀ = 45, 20 and 15 mg/mL, respectively). A. magnifica and A. filipendula showed strong antimalarial activity against chloroquine sensitive D6 (IC₅₀ = 1.2 and 0.68 mg/mL) and chloroquine resistant W2 (IC₅₀ = 1.1 and 0.9 mg/mL) strains of Plasmodium falciparum without cytotoxicity to mammalian cells. Achillea oils also demonstrated weak non-selective antifungal activity against filamentous fungal plant pathogens Colletotrichum acutatum, C. fragariae, and C. gloeosporioides.


Evaluation of the Angiogenic Activity of Salvia triloba L. Essential Oil

The genus Salvia L. (Lamiaceae) is represented by 89 species, three of which are endemic in Turkey [1]. Most of the Salvia species are used in various preparations and forms including the essential oil, in folk medicine among other uses for their anti-inflammatory, antipyretic, pain relieving and wound healing properties [1,2]. In this study, the herbal parts of S. triloba obtained from a commercial source cultivated in Izmir, Turkey, was investigated both for its (anti-)angiogenic properties and for its essential oil composition.
Concern about genetic pest resistance and poisoning of non-target organisms are spurring the search for “softer” insecticides with greater selectivity and multiple modes of action. Essential oils are blends of secondary metabolites that serve plants as deterrents against insect herbivores, but remain relatively safe and even beneficial to vertebrates [1]. We used serial-time mortality bioassays to screen the essential oils from 54 representative plant species from 30 genera comprising 13 families of gymnosperms and angiosperms for bioactivity to laboratory-cultured azalea lace bugs, *Stephanitis pyrioids* (Scott). The principal developmental stages of bugs exposed to the essential oils were the adults-long-lived individuals that provide parental care to their leaf-infecting brood. Cleverenger-type distillation extracted essential oils from dried plant material and lead components were purified and identified with gas chromatography-mass spectrometry (GC-MS). Oils were mixed with de-ionized water and a non-toxic emulsifier 0.9–9.0% dimethylsulfoxide (DMSO). All oil emulsions and sometimes their fractionated components were topically applied to adult bugs in randomized blocks at concentrations of 0, 650, 1300, 2500, 5000, and 10000 ppm. Overall bug mortality, as well as LD50, LD95 and LD99 values were calculated after 1, 2, 3, 4 and 5 hours of exposure. Mortality data were analyzed using multivariate probes [1] and preliminary data show that 1% emulsions derived from oil of *Pelargonium* (94.5% bug-mortality), *Cinnamomum* (81.8%) were more efficacious than the malathion-DMSO emulsion (66.1%) and are four promising botanical sources from which to isolate compounds useful for developing new biorational crop protectants. Acknowledgements: We thank the many generous colleagues who supplied us with plant material and extracts: Ikhsas A. Khan (USA), K. Husna Can Baser (Turkey), Be- rul Demirci (Turkey), Gulmira Ozek (Turkey), Temek Ozek (Turkey), Aruna Weerasooriya, (USA), Zengping Gao (China), Sui Zhang (China), Jian Zhang (China), Peng Nan (China), Zhijun Liu (USA), Hami- dou Sakhanokho (USA), Cecil Pounders (USA), Sandra Gray (USA), Christine Murphy (USA), Eugene K. Blythe (USA). References: [1] Sampson Bj, et al. (2005) Pest Management Sci., 61: 1122–1128.

**Chemical Composition and Biological Activities of Two Angelica Essential Oils from China**

Wedge DE1, Gao Z2,3, Tabanca N1, Demirci B4, Baser KHC4, Prideon JF, Becnel JF, Sampson BP, Werle CT6

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Roots and rhizomes of *Nopterygium incisum* and *Nopterygium forbesii* (Apiaceae) are popular in China for use as Traditional Chinese Medicines. Qiang huo is the Chinese name for the root of *Nopterygium* species. Historically, *Nopterygium* Radix and Rhizome have been used as diaphoretic, antifebrile and anodyne. In the course of screening for novel naturally occurring biologically active compounds in TCM plants, we distilled essential oils from *Nopterygium incisum* and *Nopterygium forbesii* roots and *N. forbesii* rhizomes. Water distilled essential oils were analyzed by GC-FID and GC-MS and evaluated for antimarialar activity, antimicrobial activity against human pathogenic bacteria and fungi, antifungal activities against plant pathogenic fungi and insecticidal activity. Forty, 68 and 59 constituents were characterized and identified representing 99.8% in *N. incisum* root oil, 91.4% in *N. forbesii* root oil and 96.5% in *N. forbesii* rhizome oil. Major components of *Nopterygium* essential oils were 26.5–42.6% a-pinene, 13.3–28.0% β-pinene and 4.5–8.9% limonene. *Nopterygium* oils showed no antimicrobial activity against human pathogenic bacteria or fungi, nor antimarialar activity against *Plasmodium falciparum*. *Nopterygium* oils demonstrated non-selective antifungal activity against the plant pathogens *Colletotrichum acutatum*, *C. frugareae*, and *C. gloeosporioides*. *Nopterygium forbesii* root oil produced 60% mortality to 1st instar larvae of *Ae. aegypti* at 15.625 ppm. *Nopterygium* oils also showed weak insecticidal activity against *Stephanitis pyrioids*, with 1% concentrations exhibiting 33.33–64.00% mortality. References: [1] Fuqan J, et al. (2007) Journal of Ethnopharmacology, 111: 265–270.
Microbial Metabolites of 7, 8-dimethoxyflavone and 5-methoxyflavone

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In selecting methoxyflavones as potential chemopreventive agents it is important to determine how susceptible they are towards metabolism [1]. Since, microorganisms are predictive models for mammalian drug metabolism we investigated prospectively the microbial metabolism of 7, 8-dimethoxyflavone (1) and 5-methoxyflavone (8) using 40 microorganisms. Transformation of 7, 8-dimethoxyflavone (1) by Mucor rammianus produced five metabolites: 7, 8-dimethoxy-4′-hydroxyflavone (2), 3′, 4′-dihydroxy-7, 8-dimethoxyflavone (3), 7, 3′-dihydroxy-8-methoxyflavone (4), 7, 4′-dihydroxy-8-methoxyflavone (5) and 8-methoxy-7, 3′, 4′-trihydroxyflavone (6) (Table 1). It was however, completely converted to a single metabolite, 7-hydroxy-8-methoxyflavone (7) by Aspergillus flavus. 5-Methoxyflavone (8) when fermented with Beauveria bassiana gave a single product, 5-methoxyflavanone (9). Conversion of 8 with Aspergillus alliaceus yielded the metabolite, 4′-hydroxy-5-methoxyflavone (10). The structures were established by spectroscopic methods. Compound 1 showed moderate susceptibility towards oxidative metabolism [1]. 5-Methoxyflavone which was highly resistant to human microsomal oxidation [1] underwent transformation to metabolites 9 (7.47%) and 10 (71.92%) when fermented with B. bassiana and A. alliaceus respectively.

Table 1

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</tr>
<tr>
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</tbody>
</table>

C-2,3 dihydro

Phytochemical investigation of the whole plant of Gaura biennis led to isolation of eleven flavonol glycosides (1–11). Three of them (1–3) are new compounds and their structures were determined as quercetin 3-O-(2-O-α-rhamnopyranosyl-6-O-E-p-coumaroyl)-β-glucopyranoside (1), quercetin 3-O-[2-O-α-rhamnopyranosyl-6-O-Z-p-coumaroyl]-β-glucopyranoside (2), and kaempferol 3-O-(2-O-α-rhamnopyranosyl-6-O-E-p-coumaroyl)-β-glucopyranoside (3) by spectroscopic interpretations. The known compounds were kaempferol 3-O-glucopyranoside (4), kaempferol 3-O-(2-O-α-rhamnopyranosyl)-β-glucopyranoside (5), kaempferol 3-O-rutinoside (6), quercetin 3-neohesperidoside (7), quercetin 3-rutinoside (8), and kaempferol 3-O-rhamnopyranoside (9) (10). The structures were determined as quercetin 3-O-(2-O-α-rhamnopyranosyl)-β-glucopyranoside (11) by spectroscopic interpretations.

Flavonoid Glycosides from Sutherlandia frutescens
Fu X1, Li XC2, Avula B3, Smillie TJ, Mabusela W4, Sycz J, Johnson Q5, Folk W6, Khan IA7,8
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Sutherlandia frutescens (L.) R. Br. (Fabaceae) is a well-known multipurpose medicinal plant in South Africa that has been widely used as a dietary supplement. Our previous paper has reported the isolation and structure elucidation of four novel cycloartane glycosides from its leaves [1]. Our continuing studies on this medicinally important plant led to the isolation of four new 3-hydroxy-3-methylglutaroyl-containing flavonol glycosides, sutherlandins A–D. Their structures were elucidated by chemical and spectrosopic methods as quercetin 3-O-(β-D-xylopyranosyl)-(1→2)-6-O-(3-hydroxy-3-methylglutaroyl)–β-D-glucopyranoside (1), quercetin 3-O-(β-D-apiofuranosyl)-(1→2)-6-O-(3-hydroxy-3-methylglutaroyl)–β-D-glucopyranoside (2), kaempferol 3-O-(β-D-xylopyranosyl)–β-D-glucopyranoside (3), and kaempferol 3-O-(β-D-apiofuranosyl)-(1→2)-6-O-(3-hydroxy-3-methylglutaroyl)-β-D-glucopyranoside (4). These compounds, along with the previously isolated triterpene glycosides, have been served as chemical markers for commercial products derived from S. frutescens.

Acknowledgements: The authors thank Mr. Frank T. Wiggers for the assistance in obtaining NMR spectra, and Dr. Charles L. Cantrell for the assistance in GC analysis. This work is supported in part by “The International Center for Indigenous Phytotherapy Studies” funded by NCCAM, grant number P50 AT 00264 and the USDA Agricultural Research Service Specific Cooperative Agreement No.58-6408-2-0009. References: [1] Fu X, et al. (2008) Nat Prod, 71: 1749–53.
ple preparation were investigated. This study demonstrated that the NMR-based metabolomics is a useful tool for the characterization, classification and authentication of botanicals. **Acknowledgements:** This work was funded by the FDA/CFSAN grant entitled “Science Based Authentication of Dietary Supplements” Number 2 U01 FD 002071-07. **References:** [1] LindonJC, et al. (2006), Pharm Res, 23(6): 1075–1088. [2] Hollywood K, et al. (2006), Proteomics, 6: 4716–4723.

**P-39**

**Constituents from Sarcotestas of Ginkgo Fruits**

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1 National Center for Natural Products Research, 2 Department of Pharmacognosy, School of Pharmacy, University of Mississippi, MS 38677, USA

Ginkgo tree (*Ginkgo biloba*, Family: Ginkgoaceae) is called as a living fossil, as one of the oldest trees still living on earth. The tree has a high economic value. Numerous ginkgo plantations have been developed over the world because of the increasing demand of ginkgo leaves [1]. Unlike the leaves, the fruits of ginkgo have not been well utilized. A ginkgo fruit consists of a soft and fleshy section (the sarcotesta) and a hard section (the sclerotesta). Previous pharmacological studies have reported that the extract of sarcotestas has various bioactivities including antibacterial, anti-tumor, pesticidal, mutagenic, allergenic, anti-HIV and immunomodulatory properties [2,3]. In the present study, a phytochemical investigation of the constituents of sarcotestas of ginkgo fruits led to isolation and identification of twenty three compounds. Four of them were new (compounds 1–4). The structures of compounds 1–3 are unusual and have not been reported in nature yet. Their structures were elucidated by using spectroscopic, spectrometric and chemical methods. The biosynthesis pathways of compounds 1–3 are also proposed. **Acknowledgements:** The authors would like to thank Dr. Bharathi Avula for recording the mass spectrometric data. This work was funded by the FDA/CFSAN grant entitled “Science Based Authentication of Dietary Supplements” Number 2 U01 FD 002071-07. **References:** [1] van Beek, T. A. (2000) Ginkgo biloba. Harwood Academic, Australia. [2] Duan, K. (2002) Shipin Yu Faqiao Gongye, 28 (8), 57–61. [3] Jaggy, H.; Koch, E. (1997) Pharmazie, 52(10), 735–738.

**P-40**

**Chemical Constituents of Labisia pumila**

Khan IA1,2

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*Labisia pumila* (Blume) Fern.-Vill., a short herbaceous plant belongs to a small genus of the Myrsinaceae family. It grows widely throughout the Malaysian rain forest and is locally known as Kacip Fatimah. The traditional practitioners have used *L. pumila* to maintain a healthy female reproductive system, to cure delayed fertility and to regain body strength. Kacip Fatimah is also used to reduce excessive gas, treat flatulence, dysentery, dysmenorrhoea, gonorrhea and bone sickness [1]. The extract of the plant is also used as a drink to gain energy. There is a remarkable boom in the market for Kacip Fatimah, unfortunately there is no scientific report on its chemical constituents to support these claims. In this study we explored the chemistry of *L. pumila* for the first time. A multi-class of natural products belonging to phenolic compounds containing long chains, glycerogalactolipid, cerebrosides, alpha-tocopherol, sterols and lipids were isolated from the methanolic extract of *L. pumila*. Their structures were determined by chemical and extensive spectroscopic methods including NMR and HRESIMS techniques. **Acknowledgement:** The work was supported by the United States Food and Drug Administration (FDA) Specific Cooperative Agreement No. U01 FD 002071-07. **References:** [1] Effendy AWM, et al. (2006), Journal of Sustainability Science and Management, 1: 40–46.

**P-41**

**Chemical Constituents of Terminalia chebula**

Khan IA1,2

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*Terminalia chebula* Retz., a flowering evergreen tree belongs to the genus *Terminalia* of the Combretaceae family. Its fruit has been traditionally used for household remedy for human ailments. *T. chebul-
la has been extensively used in Ayurveda, Unani and homeopathic medicine. Though it is a rich source of tannins and other phenolic compounds, some triterpenes and/or their glycosides were also reported from T. chebula [1]. For further phytochemical discoveries we investigated this plant and isolated olanolic acid-derived triterpenes. These structures were determined by spectroscopic methods including NMR and HRESIMS techniques.

Acknowledgement: The work was supported by the United States Food and Drug Administration (FDA) Specific Cooperative Agreement No. U01 FD 002071-07. References: [1] Chattopadhyay RR, Battacharyya SK, (2007), Pharmacognosy Reviews, 1: 151–156.

Chemical Constituents from Centella erecta (L.f.) Fern.

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Centella or Indian Pennywort, *Centella asiatica* (L.) Urb. belongs to the family Apiceae. It has been widely cultivated in China, Southeast Asia, India, Sri Lanka and Africa as green vegetable and medicinal herb. It is valued in Indian system of medicine for improving memory and for the treatment of nerve disorders and skin diseases. The plant and its extract were incorporated into the Indian Pharmacopeia for the treatment of inflammation and epidermal wound healing. *C. asiatica* is becoming a popular ingredient in various herbal products. However, *Centella erecta* (L.f.) Fern. is very closely related species to *C. asiatica* that is commonly found in the southern US and is easily confused with each other. Although *C. asiatica* has been thoroughly investigated, no compressive chemical studies were done on *C. erecta* [1,2]. A new triterpene (2α,3β, 4α)-23-(sulphonyl)-2,3-dihydroxys-12-en-28-oic acid O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (1) together with eleven known compounds including asiatic acid (2), madecassic acid (3), asiaticoside (4), madecassoside (5), (2α,3β, 6β)-trihydroxyolean-12-en-28-oic acid O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (6), Betulaside A (7), 3-oxo-α-allyl-9-β-D-glucopyranoside (8), vomifoliol-9-β-D-glucopyranoside (roseoside) (9), 1,8-heptadecadiene-4,6-diyne-3,10-diol (10), (25)-1-O-steroyl-2-O-stearoyl-3-O-[α-D-galacto-pyranosyl-(1″)-β-D-galactopyranosyl] glycerol (11),(25)-1-O-linolenyl-2-O-linolenyl-5-O-[α-D-galactopyranosyl-(1″)-β-D-galactopyranosyl]glycerol (12) (Fig. 1) were isolated from the whole plant of *Centella erecta* and their structures were elucidated using 1H-NMR, 13C-NMR, HSQC, HMBC, COSY and HRMS as well as comparison with reported data. Acknowledgements: This research is funded in part by The United States Department of Agriculture Specific Cooperative Research Agreement Number 58-6408-6-067 and the FDA/CFSAN grant entitled Science Based Authentication of Dietary Supplements Number 2 U01 FD 002071-07. The Authors would like to thank Dr. Vaishali Joshi for authenticating the plant material. References: [1] Mabberley DJ, (1997), The Plant Book: A portable dictionary of the higher plants. Cambridge University Press. [2] Shakir JS, et al. Nat. Prod. Radiance 6(2): p. 158–170, (2007).

P-43

Chemical Fingerprint Analysis of Two Centella Species, Quantification of Triterpenoids and its Glycosides by using HPTLC Method

Table 1 Validation Parameters.

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<tr>
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<th>MA</th>
<th>AS</th>
<th>MS</th>
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<td>200 – 600</td>
<td>100 – 500</td>
<td>100 – 500</td>
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<td>2 Correlation coefficient</td>
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<td>0.998</td>
<td>0.997</td>
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<tr>
<td>3 LOD (ng/spot)</td>
<td>30</td>
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<td>30</td>
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<td>4 LOQ (ng/spot)</td>
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<td>200</td>
<td>100</td>
<td>100</td>
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<td>5 Specificity</td>
<td>Specific</td>
<td>Specific</td>
<td>Specific</td>
<td>Specific</td>
</tr>
<tr>
<td>6 Regression equation</td>
<td>( Y = 94.580 +8.961 ) X</td>
<td>( Y = 61.937 +3.124 ) X</td>
<td>( Y = 22.600 +0.495 ) X</td>
<td>( Y = 12.773 +0.113 ) X</td>
</tr>
<tr>
<td>7 RF</td>
<td>0.72</td>
<td>0.61</td>
<td>0.17</td>
<td>0.11</td>
</tr>
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</table>

Centella asiatica (L.) Urb. (Family Apiceae commonly known as Gotu Kola or Indian Pennywort) has long been used in the Ayurvedic system of medicine for improving memory and for the treatment of a variety of ailments [1]. The triterpenoid compounds purportedly represent the chief pharmacologically active constituents. The triterpenoids, especially asatioside, triterpine triaccharides, are reported as the most active compounds in the plant [2]. A simple and fast method was developed for the quantitative determination of four triterpenes and their glycosides i.e. asiatic acid (AA), madecassic acid (MA), asiaticoside (AS) and madecoside (MS) in...
Centella asiatica and Centella erecta by using high performance thin layer chromatographic method. The separation was achieved with chloroform: methanol: water: 13.0:6.5:0.5 v/v/v on silica gel 60F254 HPTLC plates. Quantitation was performed with densitometry in absorption-reflection mode at 600 nm by scanning the HPTLC plates after a color development by anisaldehyde reagent. The linear regression data for the calibration plots showed a good linear relationship with r = 0.999, 0.998, 0.997 and 0.998 for asiatic acid, madecassic acid, asiaticoside and madecoside, respectively. The established method was validated in terms of LOD and LOQ, linearity. Acknowledgements: This research is funded in part by The United States Department of Agriculture Specific Cooperative Research Agreement Number 58-6408-6-067 and the FDA/CFSAN grant entitled Science Based Authentication of Dietary Supplements Number 2 U01 FD 002071-07. References: [1] Shakir JS, et al. (2007), Nat. Prod. Radiance, 6 (2): 158–170. [2] de Paula Reis, et al. (1996), Revista Brasileira de Farmácia, 77(2): 71–72.

Table 2  Percentage (w/w) of asiatic acid, madecassic acid, asticoside, and madecoside in plant sample.

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<th>MS</th>
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</tr>
<tr>
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<td>0.1</td>
<td>4.5</td>
<td>3.1</td>
</tr>
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</table>

Ludwigia hyssopifolia L. (Synonym Jussiaea hyssopifolia G. Don, Jussiaea linifolia Vahl non Ludwigia linifolia Poir. Family-Onagraceae, Bengali name – Lalbunlonga) is extensively grown in Bangladesh, India and Ceylon. This plant is considered as an astringent, anthelmintic, carminative and diuretic. A decoction of this plant is used for the treatment of diarrhea, dysentery, flatulence, leucorrhoea, spitting of blood, vermifuge and purgative [1]. The leaves are used in poultices for orchitis and glands in the neck. Previous phytochemical investigation of Ludwigia hyssopifolia found piperine as a potential marker compound in addition to the isolation of vitexin, isovitexin, orientin and isoorientin [2]. As a continuation our dietary supplement work we isolated a series of coumarins and triterpenoids from this plant. Compounds 1–4 are known, but this is the first report of their isolation from this plant.


Ludwigia alternifolia L. belongs to the Onagraceae family and is distributed throughout the Northeast, Midwest and Southern US. Shikimic acid (Fig. 1) was first isolated in 1885 by Eijkman from the fruit of the Japanese plant Illicium religiosum Sieb [1]. The elucidation of its structure and the development of the synthetic methods led to a surge of interest in its potential as a marker compound for Ludwigia alternifolia L. Analysis of the results showed that shikimic acid is present in Ludwigia alternifolia L. in high concentrations.

Cissus a genus of approximately 350 species of a woody climber (Family: Vitaceae) includes Cissus quadrangularis Linn (Veldt grape, winged treebine) which is often used as a medicinal plant. Commonly known as “bone setter”, the plant can be found in the warmer regions of India, Ceylon, East Africa, Malaysia and Thailand [1]. C. quadrangularis is used as a common food supplement in southern India, while the stem is traditionally used for the treatment of gastritis, bone fractures, skin infections, constipation, eye diseases, piles, anemia, asthma, irregular menstruation, burns and wounds [2,3]. The fresh stem and leaves of C. quadrangularis is used for the treatment of hemorrhoids, menstrual disorders, scurvy and flatulence [4–8]. Phytochemical analysis of C. quadrangularis resulted in the identification of several classes of compounds e.g., flavonoids, triterpenoids and stilbene derivatives. As part of our continuing program to identify chemical and/or biomarkers of dietary supplements, we isolated four new megastigmane derivatives (1–4), and thirteen known compounds (5–17), trans-3-Oxo-ionol (5), corchoinoside C (6), resveratrol (7), pallidol (8), quadrangularin A (9), quadrangularin B (10), quadrangularin C (11), parthenocissin A (12), quercetin 3-O-α-L-rhamnoso (13), quercetin 3–O–β-D-glucopyranoside (14), isoeugenol (15), (-)-isoraricresinol 3–O–β-D-glucopyranoside (16) and avaculin (17) from Cissus quadrangularis Linn. 


Structure Elucidation and Absolute Configuration of Megastigmane Derivatives from Cissus quadrangularis Linn

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Cissus a genus of approximately 350 species of a woody climber includes Cissus quadrangularis Linn (Veldt grape, winged treebine) which is often used as a medicinal plant. Commonly known as “bone setter”, the plant can be found in the warmer regions of India, Ceylon, East Africa, Malaysia and Thailand [1]. C. quadrangularis is used as a common food supplement in southern India, while the stem is traditionally used for the treatment of gastritis, bone fractures, skin infections, constipation, eye diseases, piles, anemia, asthma, irregular menstruation, burns and wounds [2,3]. The fresh stem and leaves of C. quadrangularis is used for the treatment of hemorrhoids, menstrual disorders, scurvy and flatulence [4–8]. Phytochemical analysis of C. quadrangularis resulted in the identification of several classes of compounds e.g., flavonoids, triterpenoids and stilbene derivatives. As part of our continuing program to identify chemical and/or biomarkers of dietary supplements, we isolated four new megastigmane derivatives (1–4), and thirteen known compounds (5–17), trans-3-Oxo-ionol (5), corchoinoside C (6), resveratrol (7), pallidol (8), quadrangularin A (9), quadrangularin B (10), quadrangularin C (11), parthenocissin A (12), quercetin 3-O-α-L-rhamnoso (13), quercetin 3–O–β-D-glucopyranoside (14), isoeugenol (15), (-)-isoraricresinol 3–O–β-D-glucopyranoside (16) and avaculin (17) from Cissus quadrangularis Linn. 


Psoralens, also known as furanocoumarins and coumarine derivatives, are naturally occurring or synthetic tricyclic aromatic compounds. They reveal interesting photobiological activities such as skin photosensitization, characterized by the onset of erythema followed by dark pigmentation. The related angular isomers, namely angelicin, are also present in plants and have been chemically synthesized [1]. Psoralens are also of interest because they are used as a probe in molecular biology and nucleic acid chemistry [2]. Coumarins can be classified in the latter group [3]. In this paper we discuss the synthesis of psoralens (Scheme 1 and II). Currently there is only one report of antifungal activity reported for angular coumarins [4–5]. As part of our ongoing research program to identify chemical and/or biomarkers of dietary supplements we have synthesized a series of psoralens for biological evolution.

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Synthesis of Psoralens

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Indolizidine, Antinfective and Antiparasitic Compounds from Prosopis glandulosa Torr. var. glandulosa

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A new potent antinfective and antiparasitic 2,3-dihydro-1H-indolizinium chloride, (1), was isolated from Prosopis glandulosa Torr. var. glandulosa. Three additional new (2–4) and one known (5) indolizidines were also isolated, and the dihydrochloride salts of 1–3 (compounds 6, 7 and 8) were prepared. The structures were determined by 1D and 2D NMR and mass spectra. Compound 1 showed potent in vitro antifungal and antibacterial activities against Cryptococcus neoformsans, Aspergillus fumigatus, methicillin-resistant Staphylococcus aureus and Mycobacterium intracellulare. The remarkable fungicidal activity of 1–4 against C. neoformsans and 2, 3, and 5 against A. fumigatus were similar to amphotericin B, but > 2–4-fold more potent than 6–8. Prosopilosidone (1) showed potent in vivo activity at 0.0625 mg/Kg/day/ip for 5 days in a murine model of cryptococcosis by eliminating ~76% of C. neoformsans infection from brain tissue compared to ~83% with amphotericin B at 1.5 mg/Kg/day. Compounds 1 and 4 exhibited potent activity against chloroquine sensitive (D6) and chloroquine resistant (W2) strains of Plasmodium falciparum. Prosopilosidone (1) also showed in vivo antimalarial activity with an ED50 value of ~2 mg/Kg/day/ip against Plasmodium berghei-infected mice after 3 days of treatment.

Astraeus pteridis (Shear) Zeller, which mimics a truffle in its early developmental stage, is an earth-star fungus in the Astraeaceae, (Phylum Basidiomycota, Order Boletales). It is known only from western North America, occurring alone or in groups on the ground in forests of conifers, with which it forms symbiotic, mycorrhizal associations [1]. It is unpalatable because of its leathery texture and powdery spore mass. The related Astraeus hygrometricus (Pers.) Morgan has been used traditionally in Chinese folk medicine as a hemostatic agent [2]. Several triterpenoids have been isolated from A. hygrometricus, but no biological activities have been investigated [3]. Bioassay-guided fractionation of the EtOH extract of the Astraeus pteridis, led to the isolation and identification of three new (3–5) and two known (1, 2) lanostane triterpenes, and phenylalanine betaine. The structures of the isolates were elucidated based on 1D and 2D NMR spectroscopic data, HRESIMS results, and X-ray crystallographic analysis. The antituberculosis activity of the isolates was evaluated. Compounds 5 and 1 showed moderate antituberculosis activity with MIC values of 34.0 and 38.0 µg/mL, respectively.
Postia balsamea (Aphyllophorales, Basidiomycota) is the causal agent of root rot and butt rot in balsam fir (Abies balsamea family Pinaceae). Mechanical or insect caused wounds to the roots or basal areas of trees provide entrances for the fungi. Root rot and butt rot cause considerable losses in softwood production [1]. Our previous studies reported the presence of polyacetylene compounds having phytotoxic activity from Postia balsamea [2]. We report herein on the isolation and characterization of new phenolic compounds methyl 3-(3,4-dihydroxy-phenyl)-2-hydroxypropanoic acid (1), 3-(3,4-dihydroxyphenyl)-2-hydroxypropanoic acid (2), 3-(3,5-dichloro-4-hydroxyphenyl)-2-hydroxypropanoic acid (3) along with two known lanostane-type triterpenes, acetyl eburicoic acid and eburicoic acid from the ethyl acetate extract of the fermentative broth of Postia balsamea. These two triterpenes have previously been isolated [3,4] and found to inhibit the proliferation of human HL-60 myeloid leukemia cells in a dose dependant manner [4].


Chemical Constituents of Postia balsamea

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Free Energy Calculations on the Binding of Natural Latrunculins and Semi-synthetic Derivatives to G-Actin

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Latrunculins are significant biological molecules isolated from Nectomia species, characterized by a macrocyclic lactone ring and a 2-thiazolidinone moiety. In vitro experiments revealed that the latrunculins disrupt actin polymerization. Despite having a wide variety of biological activities, their direct therapeutic use is limited by cytotoxicity. However modified latrunculins show great potential to have a wide range of useful biological activities including related to Alzheimer’s disease [1,2]. We have designed a few synthetically feasible analagies of Latrunculin B with intentions to have compounds with reduced toxicity and better binding. Both naturally available and newly designed molecules were subjected to induced fit docking into G-actin. Molecular dynamics simulations and binding free energy (BFE) calculations of G-actin and the latrunculins were carried out. The docking studies revealed the binding mode of latrunculin B and analogs and were helpful to suggest possible modifications to reduce the toxicity [3]. The BFE calculations agreed well with actin polymerization inhibition data demonstrating that the recently isolated oxalatraculin B binds more weakly than latrunculins A and B to G-actin. The binding of the latrunculins to G-actin and details of the protein-ligand interactions explain the decrease in activity of oxalatraculin B and semi-synthetic analogs, reduced inhibition which should be beneficial for avoiding general toxicity.
Chemical Investigation of Two Species of the Family Cycadaceae

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Cycas is the only genus of the family Cycadaceae, order Cycadales. Chemical investigation of the constituents of the leaves of Cycas revoluta Thunb. and C. circinalis L. afforded the lignan lariresinol (1), the flavane naringenin (2) and 10 biflavonoids (3–12) which are derivatives of amentoflavone (A) and hinokiflavone (B). Five of these compounds were previously isolated [1,2] and seven are reported for the first time in C. revoluta Thunb. and C. circinalis L. The structures of these compounds have been established by detailed analysis of their spectroscopic, mainly 1D and 2D NMR and CD data. The antimicrobial, antimalarial, and antiinflammatory activities were tested. References: [1] Varshney AK, et al. (1973), Indian Journal of Chemistry, 11(12): 1209–1214. [2] Gadek PA, (1982), Phytochemistry, 21(4): 889–890.

Productivity and Biochemical Composition of Peppermint Cultivars

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Peppermint (Mentha × piperita L., Lamiaceae) is widely cultivated for the essential oil used worldwide in the confectionery and pharmaceutical industries. To determine oil characteristics of peppermint plants suitable for cultivation in salt-stress conditions of Egypt, 57 peppermint cultivars, obtained from National Clonal Germplasm Repository, Corvallis, Oregon were grown in a greenhouse at the University of Massachusetts-Amherst during 2007 and 2008 to determine growth characteristics and oil production. The essential oil was extracted from fresh aerial parts of each cultivar using steam distillation for 3 h to extract a pale, yellow colored, aromatic oil. The oils were analyzed by gas chromatography (FID, Cupelcowax 10 capillary column, 30 m × 0.25 mm film). Constituents were identified by co-chromatography with known standards. A high degree of variability among the cultivars for fresh weight and total essential oil was observed with the highest fresh weight per plant was obtained from cultivars labeled PM09 (144.5 g plant⁻¹) and PM01 (138.0 g plant⁻¹). The highest essential oil content was obtained from cultivars labeled MP07 (733.3 µL plant⁻¹) and MP52 (520.0 µL plant⁻¹). Menthone, menthol, and pulgone were the major constituents in all tested oils. The highest menthol content was measured in the oils from cultivars MP12 (85.93%) and MP56 (53.76%).

Essential Oil Components in Plant Organs of Japanese Spicebush

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Japanese spicebush (Lindera obtusiloba Blume, Lauraceae), which grows wild in mountainous areas of Korea, Japan and Northeast China, is known for odd-shaped leaves that are a light green color in the spring, a dark green color in the summer, and a vivid gold color in the fall. In Korea, the plant stem and bark have been used in traditional medicine and as an insect repellent, while spring leaves were used in making cookies and tea. The essential oil was used for lamplight and hair oil. To understand the multiple uses of plant organs, this study examined the essential oil from flowers, leaves, stems, and roots from plants collected in Gyeong-Gi-Do, located in the northern part of South Korea. The essential oils were obtained by steam distillation/extract methodology using a Likens-Nickerson apparatus. Each extracted oil was analyzed by gas chromatography-mass spectrophotometry using an Agilent 6890 N GC connected to a Agilent 5975D (Agilent, U.S.A.). A HP-35NWax polyethylene glycol capillary column (30 m × 0.25 mm) was used and the constituents were identified by comparison of the spectral data with that in the NIST mass spectral library, ver. 2.0 (NIST, U.S.A.). The essential oil content of the plant organs varied with the flower (0.25% F.Wt.) and young stem (0.23% F.Wt.) containing a higher concentration of oil than the leaf (0.08%, F.Wt.), old stem (0.05% F.Wt.) and root (0.05% F.Wt.). Main oil constituents were α-phellandrene and β-phellandrene in flower oil, Caryophyllene in leaf oil, limonene in the stem oil, and camphene in the root oil.

Phytochemical and Biological Investigation of Bluebird Vine (Petrea volubilis)

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A chemical analysis of bluebird vine (Petrea volubilis, Verbenaceae) (additional common names, queen’s wreath and sandpaper vine) cultivated in Egypt as a botanical insecticide, identified the primary constituents as β-amyrin, stigmasterol, β-sitosterol, lupeol, and ursolic acid. The essential oil, extracted from fresh herb by hydrodistillation and analyzed by gas chromatography, had cineole (26.8%).
Polychlorinated biphenyls (PCB) are common environmental contaminants that have been linked to many detrimental health conditions in humans and marine life. These industrially produced compounds are ubiquitously used in capacitors, transformers and frequently as coolants. PCBs were prized for their stability and lack of reactivity; however, these same properties allow PCBs to become persistent organic pollutants (POPs) in many environments. A number of different bioremediation strategies have been proposed, but as yet, no one method has been completely successful for PCB removal in the environment. Studying the microbial communities that survive within the PCB containing sediments may allow a better understanding for the anaerobic dehalogenation of these contaminants. In this study sediment samples were collected from eight locations with varying levels of PCB contaminants. Microbial DNA extractions, followed by PCR amplifications were successfully performed utilizing a previously designed primer set used for amplifying known dechlorinating anaerobes. Restriction length polymorphisms (RFLP) analysis of the constructed clone library has not been clearly established, in part because comfrey PAs are commercially available and the isolation of the individual isomers is difficult. Milligram quantities of PA components from Symphytum are needed for use as analytical standards in quantitating PCB dechlorination in contaminated sediments will provide a better understanding of this process in the environment.

Carbamate compounds are useful pest control agents because they are alternatives to ozone-depleting organochloride pesticides, and because they are active against organophosphate-resistant pests. As a result, the use of carbamate pesticides has increased globally in recent years [1]. Despite this increase in use, there remain few accurate descriptions of the chemical fate of carbame pesticides under environmental conditions. We report on studies on the aquatic chemical fate of three N-methyl carbamate pesticides used extensively in both urban and rural environments: carbofuran, carbaryl, and propoxur (Fig. 1). UV-vis and NMR spectroscopy were utilized to identify and monitor products of decomposition under various conditions. The results from characterisation and kinetics studies, suggest that the degradation rates of these carbamate pesticides are governed by the identity of the substituent group on the benzene ring: carbaryl was found to hydrolyze fastest, followed by propoxur, and finally carbofuran. A mechanism for the pesticide decomposition is postulated and an explanation for the trend is proposed. Future work will investigate the reactivity of degradation products, in particular with water – soluble metals like copper (II), which are themselves components of pesticides. Thus, unexpected environmental coordination and/or organometallic reactions may be revealed in the future. References: [1] Hideyuki K, et al. (2005) Journal of Photochemistry and Photobiology A: Chemistry, 170: 239–245.
Development and validation of a reliable analytical method to analyze complicated natural ingredients derived from popular medicinal plant Aloe vera have been challenging. Fresh Aloe vera consists of three major components: acetylated polysaccharides, glucose, and malic acid, which are markers for good aloe materials. High content of lactic acid and acetic acid indicate bacterial degradation, hydrolysis and thermal degradation of the material. A proton NMR method was developed by Dr. Bernd Diehl at Spectral Service, Köln, Germany, and accepted by IASC as an analytical method to certify aloe based ingredients and finished products. This presentation will report the validation of the quantitative NMR method according to the AOAC guidelines. The validation includes specificity, linearity, accuracy, robustness, repeatability and reproducibility, limit of detection and limit of quantification. Data was collected with two different NMR instruments in two independent NMR labs. This simple and non-destructive $^1$H NMR method was able to quantify the amount of acetylated polysaccharides, glucose, malic acid, lactic acid and acetic acid in Aloe vera powder. Acknowledgements: Support from the International Aloe Science Council (IASC) is gratefully acknowledged.

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Quality Control of Botanicals through Identification and Quantification of Multiple Characteristic Components by Ultra-Fast HPLC-DAD-ELSD and LC-TOF/MS

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Over the last decades, the usage of botanicals for herbal medicines has expanded globally. Safety and efficacy as well as quality control of botanicals-derived products have become important concerns. Addressing these topics usually relies on validated analytical methods, which allows rapid and sensitive identification and quantification of relevant constituents. Botanicals are complex mixtures consisting of thousands of compounds, and getting useful chemical information from these highly complicated matrices has long been one of the major challenges to chemists and analysts. In this report, we introduced two most potential and prospective methods for quality control of botanicals, i.e., ultra-fast HPLC-DAD-ELSD method and ultra-fast HPLC-TOF/MS method. This report includes three important aspects: (i) We applied ultra-fast HPLC system to routine analysis and quality control of botanicals, providing up to 5–20 times faster analysis and 60% higher resolution than conventional HPLC without sacrificing resolution, precision or sensitivity (Fig. 1). (ii) We connected UV/DAD with ELSD for simultaneous determination of various compounds in one run. UV could detect strong UV absorbing compounds such as isoflavonoids, phenolics, and phenicolic acids, while as a complementation role, ELSD could detect non- or poor UV absorbing compounds such as saponins (Fig. 2). (iii) We suggest that TOF-MS provides much higher sensitivity and selectivity, as well as accurate mass measurement. It enables the simultaneous identification and determination of compounds in botanicals even with trace contents. Acknowledgements: Financial support for this research from the National Science Foundation of China (No. 90709020, 30530870) is gratefully acknowledged.

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Qualitative and Quantitative Analysis of Steroidal Alkaloids in Fritillaria Species by Solid-Phase Extraction followed by Rapid Resolution LC/TOF/MS

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Steroidal alkaloids are naturally occurring nitrogen-containing compounds in many edible or medicinal plants, such as potato, tomato, Fritillaria and American hellebore, which possess a variety of toxicological and pharmacological effects on humans. Such biological effects of these compounds create a critical demand for developing a sensitive and selective analytical method to accurately evaluate the presence and content of the major and minor steroidal alkaloids in these plants. In this report, we present a high-selective and sensitive method for rapid analysis of steroidal alkaloids in Fritillaria species, utilizing selective solid-phase extraction and rapid resolution liquid chromatography/time-of-flight mass spectrometry (SPE-RRLC/TOF-MS). The selective solid-phase extraction step was developed using a mixed-mode cation-exchange/reversed-phase cartridge (Oasis MCX). The strong cation exchange capacity of MCX can selectively capture basic analytes and remove acidic components, their separation was achieved by semi-preparative chiral HPLC. These chiral stationary phases consist of cellulose immobilized on silica with carbamates as chiral selectors (Chiralpak IA and IC, Chiral technologies Inc. West Chester PA). The compositions of individual fractions were monitored by GC-MS or LC-MS and the purified components were characterized by mass and NMR spectroscopic studies [3–5]. The isolated PAs will be used for the development of analytical methods that can be applied to develop chemical profiles of different species of Symphytum and to determine alkaloid contents of comfrey preparations sold in the market. References: [1] Botanical Safety Handbook 1997 CRC Press, Fl. [2] Mei N, et al. (2005) British Journal of Cancer 92: 873–875. [3] Logie CG, et al. (1994) Phytochemistry, 37: 43–109. [4] Roeder E, et al. (1990), Phytochemistry, 25: 11–29. [5] Kim NC, et al. (2001) Journal of Natural Products, 64: 251–253.
Ginkgo biloba (ginkgo), used in traditional Chinese medicine for many centuries, is one of the most popular botanical dietary supplements in North America. Commercial ginkgo products are usually standardized to the levels of flavonoids and terpene lactones (ginkgolides A, B, C, J, and bilobalide) based on the biological activities. Flavonoids have strong UV absorption. However, terpene lactones are very inactive to UV, refractive index, and ELSD detections (ginkgolide A, B, C, J, and bilobalide).

Quality of natural health products remains a challenge to regulators, researchers, and manufacturers. Quality parameters include specifications for sanitation, contaminants, and content of natural chemicals. Validated analytical methods and reference materials to ensure the purity and strength of natural health products are essential. Because these products and their ingredients are often complex mixtures they pose analytical challenges, and methods validation may be difficult. In response to concerns about quality, in 2002 the U.S. Congress directed the Office of Dietary Supplements at the National Institutes of Health to accelerate methods validation, and the Analytical Methods and Reference Materials Program (AMRM) was created. The program is stakeholder driven and provides a coordinated approach to validation that facilitates methods validation and production of reference materials. The major accomplishments of the first five years of the AMRM program involve collaborative efforts with FDA, AOAC, and NIST. The program has resulted in 18 collaborative studies of analytical methods. Twelve methods have been approved as Official Methods of Analysis (OMA), and 3 of these are final action OMA. The NIST reference materials project has resulted in the production of 5 suites of standard reference materials, with an additional 12 suites in various stages of completion. The NIST has also created a pilot Laboratory Quality Assurance Program that will assist laboratories to become proficient at analysis. A more detailed account of these accomplishments and an outline of the future scope and direction of the program will be presented.
Chromatographic Method Comparisons for the Determination of Magnoflorine and Triterpene Saponins from Roots of Blue Cohosh (Caulophyllum thalictroides)

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The roots of Caulophyllum thalictroides is traditionally used for the treatment of menstrual difficulties and as an aid in childbirth. C. thalictroides is known to contain saponins which are considered to be responsible for the uterine stimulant effects together with teratogenic alkaloids [1]. A comparison study between HPLC-UV-ELSD, UPLC-UV-ELSD and HPTLC methods was presented for the determination of major alkaloid and triterpene saponins from roots of Caulophyllum thalictroides (blue cohosh) and dietary supplements claiming to contain blue cohosh. The procedure involves the common extraction of the alkaloid and saponins from the plant and dietary samples. By liquid chromatography method with PDA and ELSD, C18 column, mobile phase consisted of solvent A (10 mM ammonium acetate) and solvent B (acetonitrile). Owing to their low UV absorption, the triterpene saponins were detected by evaporative light scattering. Within 35 minutes for HPLC-UV-ELSD, UPLC-UV-ELSD and 15 minutes for HPTLC methods, eight triterpene saponins could be separated, with detection limits of 5 µg/mL for saponins and 0.05 µg/mL for magnoflorine by UPLC-UV method, respectively. The methods were successfully used to analyze different dietary products. For the products containing blue cohosh, there was a significant variability in the amounts of the triterpene saponins. The compounds in plant materials and commercial products of blue cohosh were further confirmed by LC-MS-TOF.

Acknowledgements: This research is funded in part by “Science Based Authentication of Dietary Supplements” Funded by the Food and Drug Administration grant number 2 U01 FD 002071-07. The authors would like to thank Annette Ford, University of Mississippi for extraction of samples. References: [1] Ganzera M, et al. (2003) Phytochem Anal, 14: 1–7.

Quantitative Determination of Pregnenes from Caralluma fimbriata by using HPLC-UV Method and Identification by LC-ESI-TOF

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Caralluma fimbriata, Fam. Asclepiadaceae, is a succulent plant and plants from Caralluma genus occur throughout Africa, and Asia, majority being indigenous to the Indian subcontinent and Arabian peninsula. Recently it has gained popularity as a weight-loss dietary supplement [1]. An HPLC method with UV detection for analysis of five pregnane compounds from Caralluma fimbriata was developed. The simultaneous chromatographic separation of the five compounds was achieved with a Gemini NX reversed phase C18 column, using gradient mobile phase of water and acetonitrile, both containing 0.1% acetic acid, aided with a detection using a PDA detector. This method was applied to the fingerprint identification of three plant materials of C. fimbriata and seven dietary supplements containing C. fimbriata. The five pregnane derivatives, boucheron (1), caraumbelloside I (2), caraumbelloside III (3), caraumbelloside II (4)
Quantitative Determination of Curcuminoids from Roots of Curcuma longa, Curcuma Species and Dietary Supplements by UPLC-UV-MS Method

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The rhizomes of turmeric (Curcuma longa L., Zingiberaceae) play an important role as a coloring agent in foods, cosmetics and textiles [1]. The main yellow bioactive substances in the rhizomes are due to curcumin and two related demethoxy compounds, demethoxycurcumin and bisdemethoxycurcumin. Turmeric has been reported to possess anti-inflammatory, hepatoprotective, antitumour, antiviral activities, anticancer activities and is also used in gastrointestinal and respiratory disorders [2–3]. An HPLC method was developed for the determination of curcuminoids from roots of Curcuma longa L., different species of Curcuma (C. zedoaria, C. phaecaulis, C. wenyujin and C. kwangsiensis) and dietary supplements that claim to contain C. longa. The separation was achieved within 3.5 minutes by using C-18 column material, a water/acetonitrile mobile phase, both containing 0.05% formic acid gradient system and a temperature of 35°C. The method was validated for linearity, repeatability, limits of detection (LOD) and limits of quantification (LOQ). The limits of detection and limits of quantification of curcuminoids were found to be 0.01 µg/mL and 0.035 µg/mL, respectively. The wavelength used for quantification with the diode array detector was 420 nm for curcuminoids and 240 nm for Ar-turmerone. The total content of curcuminoids was found to be in the range from 0.825–35.37% in different species of C. longa and dietary supplements. The curcuminoids were not detected in roots of C. wenyujin and C. kwangsiensis. The developed method is simple, economic, rapid and especially suitable for quality control analysis of curcuminoids.

The roots of *Hydrastis canadensis* (goldenseal) are popular phyto-medicines for the treatment of gastrointestinal disorders and upper respiratory tract infections [1–2]. Simple and fast UPLC-UV-MS methods were developed for the quantification of the major constituents, berberine and hydrastine from roots of *Hydrastis canadensis* L and dietary supplements containing goldenseal and *Echinacea purpurea/goldenseal* combination formulations. The extraction (with acidified water and methanol) and analysis were applied to several other alkaloids including canadine, hydrastinine, palmitate, cotinine, and jatrorrhizine by a UPLC method with PDA and MS, C18 column. The mobile phase consisted of solvent A (50 mM ammonium formate, pH 3.3) and solvent B (acetonitrile with 0.05% formic acid). The developed method was validated for all the parameters tested and successfully applied to the identification of seven alkaloids in plant sample and ten dietary supplements. The plant material and ten dietary supplements were found to contain major alkaloids, hydrastine and berberine. One commercial product also contained palmitate, cotinine and jatrorrhizine, thus indicating that the material was not pure goldenseal. LC-mass spectrometry coupled with electrospray ionization (ESI) method is described for the identification of seven alkaloids in plant sample and dietary supplements. This method involved the use of the [M]+ ions for cotinine, jatrorrhizine, palmitine and berberine, [M+H]+ ions for hydrastine and canadine, [M+H+18]+ ions for hydrastine in the positive ion mode with selective ion recording (SIR).

**Fig. 1** UPLC Chromatograms of a mixture of standards (A), roots of golden-seal (B) and dietary supplements (C–D) at 290 nm. 1 hydrastine, 2 hydrastine, 3 cotinine, 4 jatrorhizine, 5 canadine, 6 palmitine and 7 berberine.


Heavy metals are natural components of the earth’s crust and are widely used in agricultural, manufacturing and food/material processing industries. Some heavy metals such as selenium, iron, copper, chromium and zinc are essential at low concentrations, others such as arsenic, cadmium, lead and mercury are toxic. Determination of 11 metals (including arsenic, chromium, mercury, iron, copper, nickel, zinc, selenium, lead, cadmium and thallium) in botanicals and dietary supplements were carried out by using ICP-MS. Closed vessel microwave digestion of two plant samples and one product assisted by HNO3+HCl (8:2) (Procedure-A), water (Procedure-B), methanol (Procedure-C), HNO3 (Procedure-D), 0.5 M HCl (Procedure-E) and HNO3+6 M HCl (Procedure-F) were used to determine the recovery of 11 metals by ICP-MS. Sample digestion was done in a MARS 5 microwave. Elemental measurements were performed using Agilent 7500 ce CRC-ICP-MS operating in hydrogen mode for Se and Fe, and He mode for As, Cr, Cu, Ni, Cd to remove spectral interferences. The method was validated for linear, repeatability, limits of detection (LOD) and limits of quantification (LOQ). The limits of detection and limits of quantification for these heavy metals were found to be 0.004–0.51 ppb. Digestions A, D and F gave significantly higher recoveries than compared with other digestions. Microwave digestion followed by analysis by ICP-MS has been shown to be a simple, reliable method for the multi-element determination of trace metals in dietary supplements and botanicals. About 12 plant samples and 22 dietary products were analyzed and all were found to contain Fe, Zn, Cu, Cr, and Ni. Four samples for As and one sample for Cr were found to contain elevated concentrations above the recommended limit. Acknowledgements: This research is funded in part by “Science Based Authentication of Dietary Supplements” Funded by the Food and Drug Administration grant number 2 U01 FD 002071-07. References: [1] Dolan SP, et al. (2003). J Agric & Food Chem, 51: 1307–1312.
417.12 were well resolved chromatographically ($t_r = 17.83$ and 20.18 min). These were characterized by losses of 120 and 162 amu upon fragmentation, respectively. The loss of 120 amu is characteristic of C-glycoside flavonoids. Acknowledgements: This research is funded in part by “Science Based Authentication of Dietary Supplements” Funded by the Food and Drug Administration grant number 2 U01 FD 002071-07. The authors would like to thank Annette Ford, University of Mississippi for extraction of samples. References: [1] Prasain JK, et al. (2007), Phytochem. Analysis, 18: 50-59. [2] Lukas SE, et al. (2005), Alcohol Clin Exp Res, 29(5): 756–762.

**Fig. 1** Extraction (A) and Heavy Metal analysis of Botanicals and Dietary Supplements (B) using ICP-MS method.

Red yeast rice is produced by cultivating *Monascus purpureus* on polished rice. China is the world’s largest producer of red yeast rice. Red yeast rice may provide benefits beyond those provided by stat-
Researchers have reported that the benefits seem to exceed those reported with lovastatin alone [1]. Statins are a class of drugs commonly prescribed to decrease cholesterol levels and have recently been shown to also stimulate bone formation. The HPLC and UPLC methods were developed for the quantitative determination of lovastatin in red yeast rice extracts and dietary supplements that claim to contain red yeast rice. The separation was achieved by using C-18 column material, a water/acetonitrile mobile phase, both containing acid gradient system and a temperature of 35°C. The method was validated for linearity, repeatability, limits of detection (LOD) and limits of quantification (LOQ). The LOD and LOQ of lovastatin were found to be 10 & 50 ng/mL by UPLC-UV method and 100 & 250 ng/mL by HPLC-UV method, respectively. The wavelength used for quantification with the diode array detector was 238 nm. The analysis of commercial products showed considerable variation of 0.37 – 5.65 µg of lovastatin/g of red yeast extract. LC-mass spectrometry coupled with electrospray ionization (ESI) interface method is described for the identification of lovastatin in red yeast rice samples. This method involved the use of [M+H]+ ions (m/z = 405.2641) in the positive ion mode with extractive ion monitoring (EIM).Acknowledgements: This research is funded in part by “Science Based Authentication of Dietary Supplements” Funded by the Food and Drug Administration grant number 2 U01 FD 002071-07. References: [1] Lu Z, et al. (2008), Am J Cardiol, 101(12): 1689–1693.

Fig. 1  UPLC-UV and HPLC-UV chromatograms of lovastatin, red yeast rice extract and dietary supplements (P-1 to P-3) at 238 nm.

Nigella sativa Linn. belongs to the Ranunculaceae family and is an indigenous herbaceous plant that is more commonly known as the fennel flower plant. The plant is also known as black cumin (English) and black-caraway (USA). The spicy seeds from this plant have medicinal usage dating back to the ancient Egyptians, Greeks and Romans. In Egypt and the Middle East the black seed oil is popularly used for certain cases of chronic cough and bronchial asthma [1,2]. An HPLC method was developed for the simultaneous determination of nine compounds of Nigella sativa L. The separation was achieved within 23 minutes by using C-18 column material, a water/acetonitrile mobile phase, both containing 0.1% acetic acid gradient system and a temperature of 35°C. The method was validated for linearity, repeatability, limits of detection (LOD) and limits of quantification (LOQ). The LOD and LOQ of nine compounds were found to be in the range from 0.09–10 µg/mL and 0.3–25 µg/mL, respectively. The wavelength used for quantification with the diode array detector was 205 and 260 nm. The seeds of N. sativa and commercial products showed the presence of all nine compounds. LC-mass spectrometry coupled with electrospray ionization (ESI) interface method is described for the identification of compounds in Nigella sativa L samples. This method involved the use of [M+H]+ and [M+Na]+ ions in the positive ion mode with extractive ion monitoring (EIM).Acknowledgements: This research is funded in part by “Science Based Authentication of Dietary Supplements” Funded by the Food and Drug Administration grant number 2 U01 FD 002071-07.
Characterization and Screening of Cycloartane and Flavonoid Glycosides from Stem-Leaves of Sutherlandia frutescens by Using HPLC-UV-ESI-MS and MS-MS Fingerprint Analysis

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Sutherlandia frutescens (L.) R. BR. (Family Fabaceae) is a widely used medicinal plant from South Africa. It is traditionally used for stomach problems, internal cancers, diabetes, inflammatory conditions and recently to improve the overall health in cancer and HIV/AIDS patients [1,2].

LC-ESI-MSD-TOF and ESI-MS-MS analysis were performed on cycloartane and flavonoid glycosides employing two mass spectrometers equipped with ion-trap and TOF analyzers. The data illustrates the ability of the ESI techniques in the identification of cycloartane and flavonoid glycosides, including the nature of parent compound, the number of sugar residues and the type of saccharide moiety. The preliminary analytical results showed that numerous compounds have not been investigated yet. Additionally, screening and structural characterization offered more information about the chemical constitutions of Sutherlandia frutescens. About 55 compounds were screened using the ESI-TOF method, the major base peak ions generated by cycloartane glycosides are m/z 435, 437, and 439 [M+H-sugar-3 H2O]+ and flavonoid glycosides at m/z 287, and 303, respectively. Sutherlandioside B was found to be the major compound among the analyzed glycosides. Fragments detected in the LC-ESI-TOF spectra of plant sample of S. frutescens and the dietary supplement are m/z 653.42 [M+H]+, 491.37 [M+H-sugar]+, 473.36 [M+H-sugar-H2O]+, 455.35 [M+H-sugar-2 H2O]+, 437.34 [M+H-sugar-3 H2O]+. In the MS-MS spectra, fragmentation reactions of the [M+Na]+ were recorded to provide structural information about the glycosyl and aglycone moieties.


Fig. 1 HPLC chromatograms of standard mix, plant sample and dietary supplement at 260 nm (1) magnoflorine, (2) Kaempferol-3-O-β-glucopyranosyl (1→2)-O-β-galactopyranosyl (1→2)-O-glucopyranoside, (3) sieboldianoside A, (4) tauroside H2, (5) tauroside G3, (6) decaisoside D, (7) sapindoside B, (8) thymoquinone, (9) tauroside E.
Quantitative Determination of Cycloartane and Flavonoid Glycosides from *Sutherlandia frutescens* by UPLC-UV, UPLC-ELSD Methods and Confirmation by UPLC-MS

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*Sutherlandia frutescens* (L.) R. BR., Family Fabaceae, is a well-known and widely used medicinal plant from the Western Cape, South Africa [1,2]. Traditionally it has been used as a remedy for stomach problems, internal cancers, diabetes and various inflammatory conditions. Recently, it has been used for the management of HIV/AIDS in patients [1]. This paper describes the analytical method suitable for the determination of four flavonoid glycosides (Sutherlandin A, B, C, D) and four cycloartane glycosides (Sutherlandioside A, B, C, D) from stem-leaves of *Sutherlandia frutescens* (L.) R. BR. A separation by UPLC was achieved by using Acquity shield RP18 column, PDA with ELS detection, and a water/acetonitrile gradient as the mobile phase. The major cycloartane glycoside compound (sutherlandioside B) was detected at a concentration as low as 1.0 µg/mL. The analysis of plant material and products showed considerable variation of 0.6–2.7% for the major compound. This method involved the use of the [M+H]+ and [M+Na]+ ions in the positive ion mode with extractive ion monitoring (EIM). The eight compounds were further confirmed by UPLC-MS method in plant sample and products. In the positive ion mode, the protonated species [M+H]+ at m/z 741.2, 741.2, 725.2, 725.2, 653.4, 651.4, 635.4 and 635.4 and sodiated species [M+Na]+ at m/z 763.2, 763.2, 747.2, 747.2, 747.2,

Fig. 1 UPLC chromatograms of a mixture of standard [Sutherlandin A (1), Sutherlandin B (2), Sutherlandin C (3), Sutherlandin D (4), Sutherlandioside B (5) Sutherlandioside C (6), Sutherlandioside D (7), Sutherlandioside A (8)] (A, C), leaves of *Sutherlandia frutescens* (B, D) by ELSD and UV detection at 260 nm.

Fig. 2 MS spectrum of Sutherlandioside B (peak 31 in Fig. 1). Inset is the structure and its MS fragment pathway.
Turnera diffusa Willd. (Turneraceae), common name damiana, is an aromatic shrub with small yellow flowers. The leaves and sometimes the stems of damiana have medicinal uses. Evaluation of herbal dietary supplements marketed on the internet for recreational use shows that 10% of the most common products were claiming to contain damiana in the product ingredients [1, 2]. An HPLC/UV method permitting the simultaneous determination of 8 compounds isolated from T. diffusa has been developed. A separation was achieved within 45 minutes by using the C-18 material column. The mobile phase was comprised of acetonitrile/methanol (90:10, v/v) containing 0.1% acetic acid and 50 mM ammonium acetate (pH = 4.2) at a flow rate of 1 mL/min and the column temperature was maintained at 30°C. The method was validated for linearity, repeatability, limits of detection (LOD) and limits of quantification (LOQ). The developed method was applied for the quantitative determination of eight compounds [1] for two different species of Turnera and dietary supplements. The separation was achieved by using C-18 column material in both HPLC and UPLC method using a water/acetonitrile mobile phase. For the HPLC method, both solvents contain 0.1% acetic acid and in the UPLC method, both solvents contain 0.05% formic acid. The column temperatures were maintained at room temperature and 35°C for HPLC and UPLC methods, respectively. The methods were validated for linearity, repeatability, limits of detection (LOD) and limits of quantification (LOQ). The LOD and LOQ of fucoxanthin was found to be 50 & 150 ng/mL, 10 & 35 ng/mL and 1 & 3 ng/mL using HPLC-UV, UPLC-UV and UPLC-MS methods, respectively. The wavelength used for quantification with the diode array detector was 449 nm and m/z at 659.4 [M+H]+. LC-mass spectrometry coupled with electrospray ionization (ESI) interface method is described for the identification of compounds in extracts containing fucoxanthin and dietary supplements. This method involved the use of [M+H]+ ions in the positive ion mode with single ion recording (SIR). Acknowledgements: This research is funded in part by “Science Based Authentication of Dietary Supplements” Funded by the Food and Drug Administration grant number 2 U01 FD 002071-07. The authors would like to thank Annette Ford, University of Mississippi for extraction of samples. References: [1] Hayato M, et al. (2007). Journal of Oleo Science, 56: 615–621.

Fig. 1  HPLC-UV chromatograms of a standard mix. (A), extracts of T. diffusa,(B) and dietary supplements (C–D) at wavelength 280 nm (1) and 345 nm (2).
ESI-MS$^n$ as a Tool to Characterize Isoquinoline Alkaloids and Identify Possible Adulterant from Dietary Supplements that Claimed to Contain Goldenseal

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Hydrastis canadensis L., commonly known as goldenseal, is a perennial herb in the buttercup family Ranunculaceae, native to southeastern Canada and the northeastern US, and an economically important North American medicinal plant that has been subject to adulteration in commerce. The phytochemicals of interest in goldenseal are the isoquinoline alkaloids hydrastine, berberine, and canadine. Other compounds of interest are palmatine, coptisine and jatrorrhizine, alkaloids that are found in potential adulterant species but not in goldenseal [1–2]. Isoquinoline alkaloids β-hydrastine, hydrastinine, canadine, berberine, coptisine, jatrorrhizine and palmatine have been characterized by using electrospray ionization multi-stage tandem mass spectrometry (ESI-MS$^n$) coupled with an ion-trap analyzer. Fragments C$_{11}$H$_{12}$NO$_2^+$ are dominant or major products ions in hydrastinine and β-hydrastine, respectively. The C-ring is relatively weak and likely broken in tetrahydrisoquinoline alkaloid canadine. In ESI source, the product ions of canadine are found at m/z 176 corresponding to fragments C$_{10}$H$_{11}$NO$_2$. This fragment bears the core skeleton of dominant ions in hydrastinine. However, for highly unsaturated isoquinoline alkaloids, its skeleton is relatively stable. In this sub-group, the major ions, such as presenting ions at m/z 308, 294 and 292 in palmatine, jatrorrhizine and berberine respectively, may involve the re-arrangement of D-ring. The results of the current study have classified the fragmentation pathway of each sub-group into isoquinoline alkaloids. It can be used to characterize the structures of trace isoquinoline alkaloids in dietary supplements that claimed to contain goldenseal, and will benefit to identify adulterant in dietary supplements.


Fig. 1  HPLC-UV, UPLC-UV and UPLC-MS chromatograms of fucoxanthin standard (A), and extracts (B–C) at 449 nm.
Heimia salicifolia (Lythraceae), also known as sun opener or shrubby yellow crest, is a wild flowering shrub distributed from Mexico, southwestern Texas to northern Argentina. It has been used as antipyretic, emetic, laxative, diuretic and anti-inflammatory and for its wound healing activity in Central and South America. The folkloric reports claimed the plant had psychotomimetic activity [1]. Nine quinolizidine alkaloids and biphenyl quinolizidine lactone alkaloids isolated from H. salicifolia have been structurally characterized by using electrospray ionization multi-stage tandem mass spectrometry (ESI-MS<sup>n</sup>) coupled with an ion-trap analyzer. The fragmentation patterns of these alkaloids are dominated by the existence of bridge between C-2 and C-4, and less affected in accordance with structural variations of substitution at C-2 and C-12. When forming the lactone bridge between C-2 and C-4 over a biphenyl moiety, a neutral loss of 44 Da corresponding to carbon dioxide is easily generated. Moreover, the product ions will further yield fragment ions related to the cleavage of A-ring at C-1/C-2 and C-4/C-5. B ring bearing nitrogen atom has been found as one very easily loss group in the fragmentation pathways of all analyzed quinolizidine alkaloids. The results of this study can benefit the determination of trace quinolizidine alkaloids and biphenyl quinolizidine lactone alkaloids in crude plant extract and also provide background information to aid the structural investigations of related biological studies and forensic science. Acknowledgements: This research was funded in part by “Science Based Authentication of Dietary Supplements” funded by the Food and Drug Administration grant number 2 U01 FD 002071-07. References: [1] Malone MH, et al. (1994), J Ethnopharm, 42: 135–159.


Lipids are important constituents of all living organisms. Galactolipids are a class of acylated membrane lipids with a sugar molecule attached to the third carbon of the glycerol molecule. These compounds are associated primarily with plastid membranes in seed plants [1]. The fruit of Lycium barbarum L. has been widely used in the health food industry because of its possible role in the prevention of chronic disease like age-related macular degeneration. In addition, it may possess antioxidant and antitumor activities, neuroprotective effect, and enhance immunity [2]. An SPE assisted HPLC/ELSD method has been developed for the quantitative determination of galactolipids from Lycium barbarum L. fruits. The separation of six galactolipids and one steroid were achieved by using C-18 column material in HPLC method coupled with an ELS detector. A water/acetonitrile mobile phase, both containing 0.1% acetic acid, was selected for the outlined method. The column temperature was maintained at 25 °C. The method was validated for logarthmic linearity, repeatability, limits of detection (LOD) and limits of quantification (LOQ). The LOD and LOQ of galactolipids were found to be in the range from 10–20 µg/mL and 20–50 µg/mL, respectively. The structures of six galactolipids and one steroid were further characterized by ESI-MS/MS method. Ion-trap tandem mass spectrometry coupled with electrospray ionization (ESI) interface method is described for the identification of compounds in L. barbarum. The developed HPLC-ELSD method has been successfully applied for determination of target analytes in different populations of same species. Acknowledgements: This research is funded in part by “Science Based Authentication of Dietary Supplements” funded by the Food and Drug Administration grant number 2 U01 FD 002071-07. References: [1] Guella G, et al. (2003), Rapid Commun Mass Spectrom, 17: 1982–1994. [2] Inbaraj BS, et al. (2008) J Pharm Biomed Anal, 47: 812–818.
Isolation and Qualitative Characterization of Antidepressant Marsiline by Liquid Chromatography Tandem Mass Spectrometry [LC-MS/MS] from <i>Marsilea quadrifolia</i> L. 

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Anxiety, depression and mental health problems constitute the second most common chronic condition in clinical practice. Various types of herbal medicines are being used as anxiolytic drugs, which necessitates the development of newer and more effective antidepressants from traditional medicinal plants whose psychotherapeutic potential needs to be assessed in a variety of animal models [1, 2]. The main objective of this work was to develop a simple, sensitive, rapid and reliable liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the simultaneous identification of Marsiline (Fig. 1), a major central nervous system active principal, that has been found to be responsible for sedative and anticonvulsant activity in <i>Marsilea</i> sp. [1, 2]. The LC-MS/MS system (API 2000) with triple quadrupole tandem mass spectrometer (AB Sciex Instruments, Foster, Canada) was used for qualitative determination of Marsiline from methanolic extract. The most active ingredient Marsiline was extracted by simple liquid-liquid extraction with organic solvent (benzene: n-hexane 1:1 v/v). The protonated analyte was...
quantitated by positive ionization in multiple reactions monitoring with a mass spectrometer. The MRM transition of 815–225 m/z was used to measure Marsline. Data acquisition was performed with Analyst 1.4.1. software. This method will be further applied to quantify Marsline content from other Marselia sp. Acknowledgements: The authors are indebted to the University Grants Commission (UGC), New Delhi, India for their financial support in the form of a Major Research Project. We express our thanks and gratitude to Bioequivalence Study Centre, Jadavpur University, Kolkata–700 032, West Bengal, India for providing us the necessary instrumental facility (LC-ESI-MS/MS). References: [1] Bhattachisra SK, et al. (2007) Journal of Herbal Medicine and Toxicology, 1(1): 15–20. [2] Chatterjee A, et al. (1963) Journal of Experimental Medical Sciences, 7: 53–67.

In Vivo and In Vitro Evidence for Genotoxicity of Acrylamide

Acrylamide is a chemical intermediate used in a variety of laboratory and commercial products including soil-conditioning agents, dyes, pigments, and in the treatment of drinking water. Acrylamide also finds its way into the human diet when amino acids and sugars present in food are heated at high temperature during food processing. Earlier studies have demonstrated that chronic acrylamide treatment produced tumors in rats and mice; yet, the mechanism of acrylamide carcinogenicity remains unresolved. The aim of the present study was to investigate the biologic consequences of acrylamide exposure both in vitro and in vivo animal models. Animals were subjected to bone marrow micronucleus assays, chromosomal analysis, and flow cytometry analysis. Significant increases of chromosomal aberrations, in a dose dependent manner, were observed in human leukocytic culture and bone marrow cells of mice. There was also an increase in micronucleus frequency in bone marrow cells of mice. Flow cytometry analysis showed a reduced DNA content in liver cells of treated mice indicating acrylamide clastogenicity. Although acrylamide is a common laboratory reagent, its role as an environment contaminant will only be resolved with further investigations of its detrimental effects.

Localisation of NAD+ Synthesis Enzymes in the Pathogenic Yeast Candida glabrata

Candida glabrata is an opportunistic yeast pathogen of humans and accounts for approximately 4% of all catheter associated urinary tract infections. It is normally controlled by the body’s immune system and the body’s bacteria flora, but can cause serious mucosal and systemic infections. C. glabrata is a nicotinamide adenine dinucleotide (NAD+) auxotroph, which depends on the environmental supply of NAD+ precursors using nicotinamide riboside (NR), nicotinamide acid (NA), and nicotinamide (NAM) as NAD+ precursors. These precursors are used in a functional Preiss-Handler pathway to produce NAD+. We focused on the location of enzymes used in the Preiss-Handler pathway of C. glabrata under conditions replete for NAD+ precursors and under extreme conditions such as NAD+ precursor starvation. The C-terminus of the Npt1, Nqs1, Nrk1 and Pnc1 was tagged with GFP to identify the location of the enzymes in the yeast before and after starvation of NA and NR. Under the fluorescent microscope, localization of enzymes was found in the cytoplasm before and after starvation. Therefore, within the limits of our assay, we conclude that localization of the Preiss-Handler pathway enzymes in C. glabrata is unaffected by environmental conditions. We intend to confirm and extend these results by exploring the subcellular localization of pathway enzymes using different tags for localization.

Vaccine Created to Defend Against Staphylococcus aureus Biofilms in Cases of Severe Osteomyelitis

Methicillin-resistant Staphylococcus aureus (MRSA) is a bacterium that causes infections to be especially difficult to treat. S. aureus has become a particularly significant problem in hospitals, where they often grow as biofilms and are currently the largest contributor to nosocomial infections. In previous experimentation, multiple strains of immunogens were found to be upregulated in biofilm growth. In this experiment, the antigens to four of these immunogens were grown in culture and combined to create a vaccine. This vaccine was administered to New Zealand white rabbits that were later infected with S. aureus tibial osteomyelitis. The initial vaccine was given on day zero of the experiment. The rabbit titer was boosted with a second injection after ten days. The animals were challenged after ten days with MRSA introduced to the left tibia. Responses to vancomycin were evaluated by examining osteomyelitic infection in the rabbit tibias. The combination of vaccine and vancomycin treatment significantly lowered levels of biofilm infection. From these results, we postulate that the vaccine was able to prevent the formation of the biofilm and vancomycin was able to destroy the remaining bacteria. From the positive results of this experiment, we plan on expanding this study to mouse models.

Determining the Sensitivity of Gustatory Neurons in the Maxillary Styloconic Sensilla of Gypsy Moth Larvae

Gypsy moth larvae, Lymantria dispar (L.), are highly polyphagous and display a wide host preference, feeding on the foliage of many species, but favoring leaves of deciduous hardwood trees, such as oak, maple, and sweet gum. Gypsy moth larvae are major pest defoliators in the United States and destroy millions of acres of trees annually. These lepidopteran insects possess gustatory sensory organs located on the maxillae, namely the medial and lateral galeal styloconic sensilla, which play an important role in host-plant selection. Using a single cell electrophysiological recording method, this study characterized the sensitivity of the receptor cells housed within each sensillum of gypsy moth larvae when exposed to a panel of selected phytochemicals by performing dose response experiments. Electrophysiological tip recordings from these sensilla revealed that medial styloconic sensilla responded to the alkaloids, strychnine and atropine, while lateral styloconic sensilla responded to aristolochic acid and atropine. In general, these different taste cells exhibited characteristic temporal firing patterns. Thus, this study provides correlative insights into the feeding behavior and taste physiology of this larval insect. It also provides a gateway to use other alkaloids in temporal and dose-response experiments as a possible means of biocontrol.
It is widely accepted that recognition of exposed glycans on the cell surface of potential pathogens by host humoral or cell-associated lectins is a key component of the innate immune response of vertebrates and invertebrates. However, the protozoan parasite Perkinus marinus causes “Derma” disease in the eastern oyster Crassostrea virginica, and is responsible for catastrophic damage to shellfisheries in North America. Until recently, the parasite’s mechanism(s) for entry into the hemocyte had remained obscure. The recent results suggest identification and characterization of oyster hemocytes a galectin (CvGal) with a unique carbohydrate-recognizing domain (CRD) organization that, unlike most mammalian galectins with four CRDs that contain seven of the nine amino acid residues specificities, we initiated the recombinant expression of the CvGal components, and to a variety of potential microbial pathogens, phytoplankton lectins, recognizes exogenous carbohydrate ligands [1]. CvGal binds to a variety of potential microbial pathogens, phytoplankton components, and Perkinsus trophozoites, suggesting that it functions as a hemocyte surface receptor for this parasite, and facilitates its entry into the host cells. Unlike all galectins known so far, CvGal displays four CRDs that contain seven of the nine amino acid residues that bind ligand in the bovine galectin-1. Because the CvGal CRDs are similar, but not identical to each other, their carbohydrate specificities may be also different. To characterize their carbohydrate specificity, we initiated the recombinant expression of the CvGal CRDs, individually and as combinations of 2 and 3 CRDs to enable the rigorous analysis of their binding specificity and affinity. We developed expression constructs into a pET expression vector for the expression, purification, and characterization of each recombinant CRD are underway. Acknowledgements: Thanks go to the Center of Marine Biotechnology, University of Maryland, and to Dr. Geraldo Vasta and Dr. Rifts Ahmed, and Tyson Wendland. (Supported by Marine Biotechnology, University of Maryland, and to Dr. Geraldo)

Fig. 1 Effect of BC on GATA2 (panel A) and endothelin-1 (panel B) mRNA expression in medaka embryo. Fertilized medaka eggs on 3-day post fertilization were exposed to 10 µg/ml BC for 0, 0.25, 0.5, 1, 2, 4, 6, and 8 h, and the extracted mRNA was used for semi-quantitative reverse transcriptase polymerase chain reaction (rtPCR). Lowercase a indicates that the values are significantly different (p < 0.05, n = 4) after 0.25 h of BC treatment.

Blue cohosh (Caulophyllum thalictroides) (BC) is a perennial herb used by Native American Indian women to induce labor and for the treatment of other uterine complications. Several studies indicated that BC was not absolutely safe for the fetus and able to induce perinatal stroke and ischemia in newborn babies [1]. A recent chemical analysis identified 15 alkaloid-triterpene compounds present in BC [2] and some of them are potential teratogens. We used Japanese medaka (Oryzias latipes) embryo-larval development as our experimental model to verify the teratogenic potency of BC during embryogenesis. We observed that BC was able to induce cardiovascular defects in medaka embryo during development; however, total protein, RNA and several transcription factor mRNAs (emx2, en2, iro2, otx2, shh1, wnt1 and zic5) which were expressed in central nervous system (CNS) of medaka embryo during embryogenesis remained unaltered. Further, we have used subtractive hybridization technique to identify BC-sensitive genes in medaka embryogenesis. We have observed that transcription factor GATA2 was over expressed by BC and in situ hybridization analysis indicated that GATA2 over expression was occurred in CNS. Analysis by semi-quantitative reverse transcriptase polymerase chain reaction (rt-PCR) indicated that GATA2 mRNA expression was very rapid (significantly increased within 15 min of BC exposure). We predict that teratogenic effects of BC are due to over expression of GATA2 gene that can induce the expression of endothelin-1 mRNA in the cerebral microvessels and peripheral vessels, and thus cause dysfunction of cerebrovascular and cardiovascular system of Japanese Medaka during development.

The drugs approved by FDA for the treatment of alcoholism are not recommended for the women in pregnancy [1]. Therefore, a drug
with anticraving property as well as non-toxic to fetus is required for the treatment of Fetal Alcohol Spectrum Disorder (FASD), a neurobehavioral disorder observed in the babies of alcoholic mothers who consumed alcohol during pregnancy. We have evaluated the potency of Radix puerariae (RP), the root extracts of a wild leguminous creeper kudzu (Pueraria montana), as an alternative natural medicine to prevent FASD using Japanese medaka (Oryzias latipes) embryo-larval development as the model. Previously, we have observed that ethanol was able to induce skeletal dysmorphogenesis in medaka by reducing skeletal growth in a dose-dependent manner [2]. In this experiment we have used RP and puerarin (Sigma-Aldrich) as preventive agents of ethanol-induced skeletal dysmorphogenesis. Medaka RP was collected from the Lafayette County of Oxford and HPLC analysis indicated that puerarin is the major isoflavone present in the methanolic extract of RP. Fertilized medaka eggs in standard laboratory conditions (16 L: 8D, 25 °C) were exposed to RP extract (0–1.5 mg/ml) for 6 day post fertilization (dpf) and then maintained in 48 well tissue culture plate in hatching solution (one embryo/ml/well). Embryo mortality was observed on 10 dpf. In separate experiments embryos were exposed to RP (0–0.5 mg/ml), Puerarin (0.25–1 mM) with or without ethanol (300 mM) for 2 dpf and then transferred to hatching solution. The calculated IC50 of RP as determined on 10 dpf is 785.3 ± 2.66 µg/ml (n = 5). Hatched embryos on 10 dpf were used for morphometric analysis of skeletal features including the skeleton, cranium, jaw, ethmoid and hypophyseal plate. It was observed that ethanol was able to reduce the growth of all the four skeletal features; however, RP or puerarin alone had no effect. When the embryos were treated together with ethanol and RP or puerarin, ethanol-induced skeletal growth reductions were attenuated specifically by puerarin. It is therefore concluded that puerarin, the major flavonoid present in RP, has the potency to prevent ethanol-induced teratogenesis during development and can be used as an alternative natural medicine for the prevention of FASD or other alcohol related disorders. Acknowledgements: This work is supported in part by the United States Department of Agriculture, Agricultural Research Specific Cooperative Agreement No 58-6408-2-0009, National Center for Natural Product Research, School of Pharmacy, University of Mississippi, National Institute of Alcohol Abuse and Alcoholism (1RO1 AA016915) and from The Center of Research Excellence in Natural products Neurosciences (P20RR021929). References: [1] Williams SH, (2005), Amm Fm Phys, 72: 1775–1780. [2] Wang, et al. (2006), Birth Def Res 77B: 29–39.

Inhibition of Cytochrome P450 by Constituents from Blue Cohosh (Caulophyllum thalictroides) 
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Blue cohosh, Caulophyllum thalictroides is a popular herb that is extensively used for women's health. Alkaloids and saponins are considered to be responsible for its pharmacological effects. In this study the effects of methanolic extract of the roots of blue cohosh, alkaloidal fraction and isolated constituents on major drug metabolizing cytochrome P450 (CYP450) enzymes were evaluated. Methanolic extract did not show any effect but the alkaloidal fraction showed a strong inhibition of CYP 2C19, 3A4, 2D6, and 1A2 (> 80% inhibition at 100 µg/ml) with IC50 values in the range of 2–20 µg/ml. Among the constituents, caulophyllumine B (a piperidine type alkaloid), O-acetylbaptifolin, anagyrine, and lupanine (lysine derived alkaloids) inhibited these enzymes to various extents (IC50 2.5–50 µM). N-methylcytisine weakly inhibited CYP3A4 (32% inhibition at 100 µM). A more pronounced inhibitory effect on all the four enzymes was observed by an equimolar mixture of alkaloids. Among the saponins, caulosides C and D inhibited CYP3A4 at the highest test concentration of 100 µM (43% and 35% inhibition, respectively). Other enzymes were not affected. This in vitro study indicates the possibility of drug-drug interactions. The dietary supplements containing blue cohosh may pose a risk if taken with other drugs or herbs, metabolism of which involves CYP450 enzymes. Acknowledgements: FDA grant no. FD-U-002071-07 and USDA, Agriculture Research Service Specific Cooperative Agreement no 58-6408-2-0009 are acknowledged for partial support of this work.

The current study evaluates the preformulation characteristics of THC-Serine, a novel prodrug of the poorly water soluble compound Delta-9-Tetrahydrocannabinol (THC). Aqueous solubility and stability and solubility in different surfactants and 2-hydroxypropyl-β-cyclodextrin (HPβCD) were studied. The LogP and pKa were calculated using computer modeling. Chemical, thermal and enzymatic stability of the prodrug was assessed at different pH (25 °C), elevated temperature (120 °C) and in human saliva, respectively. THC-Serine demonstrated pH dependent solubility. Highest solubility was observed at pH 2.0 (92-fold greater than THC). Solubility of the prodrug in Tween® 80 was 320-fold higher (256.65 ± 20.52 µg/ml) than THC. With increasing concentrations of HPβCD solubility of THC-Serine was also observed to increase. Log P and pKa of THC-Serine were 3.18 and 7.05, respectively. Prodrug was most stable at pH 2.0, with a degradation rate constant of 3.17 × 10^-1 h^-1. Almost 80% of the prodrug remained intact after heating at 120 °C for 8 minutes. The degradation rate constant in saliva was found to be 11.52 × 10^-2 h^-1. The above results indicate that THC-Serine is a lead candidate for transmucosal THC delivery and warrants further investigation. Acknowledgements: This work was supported by Grant Number P20RR021929 from the National Center for Research Resources (NIH/NCCR).

Preformulation Characterization of a Novel Delta-9-Tetrahydrocannabinol Amino Acid Prodrug 
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Δ9-Tetrahydrocannabinol (THC, Fig. 1) is the primary active ingredient of the plant Cannabis sativa (marijuana) and is responsible for the majority of the pharmacological effects. While THC in marijuana is mainly known for its abuse potential, it also exhibits the therapeutic effects in the treatment of nausea and vomiting during cancer chemotherapy. The only dosage form currently approved by FDA is an oral, soft gelatin capsule (Marinol®). This dosage form is expensive, resulting in inconsistent pharmacological effects and pharmacokinetic profiles. Hence, prodrugs of THC are synthesized for the delivery by transbuccal route. The objective is to enhance the thermal stability and permeation properties of the hemisuccinate ester prodrug of Δ9-tetrahydrocannabinol (THC-HS) by complexation with a nonrandom methylated beta cyclodextrin (RAMEB). An inclusion complex of THC-HS/RAMEB was prepared by freeze-drying THC-HS and cyclodextrin (1:2 and 1:10 ratios). Stability was evaluated at 4°C, 25°C and 40°C in open and closed vials over a period of 1 month. Diffusion of THC-HS from THC-HS/RAMEB com-
plex, across porcine buccal mucosa, was studied at 37 °C, using side-by-side diffusion cells. The degradation rate was higher in open vials as compared to closed vials. The permeability of THC-HS/RAMEB (1:2) freeze-dried complex was increased four-fold and that of the 1:10 complex increased two-fold compared to the permeability of the THC-HS alone. The inclusion complex of THC-HS/RAMEB significantly enhances the thermal stability and permeation properties of THC-HS.

We previously reported that this extract is a potent activator of THP-1 monocytes in vitro and that oral consumption enhanced several immunological functions in mice [1]. In this study we further characterized Immulina by determining that Braun type lipoproteins are responsible for a major portion of the in vitro monocyte activation exhibited by this material. In order to understand the effect of Immulina on the human immune system, a pilot study was conducted on ten healthy individuals who supplemented their diet with Immulina (400 mg/day) for seven days. Blood was drawn from the participating individuals at two time points: before and after seven days of Immulina supplementation. Changes in mononuclear and polymorphonuclear phagocytosis were determined in heparinized whole blood as well as the cytotoxicity exhibited by natural killer (NK) and lymphokine activated killer cells. We observed statistically significant increases both in tumor cell killing by NK cells (p = 0.0019) and in phagocytosis by blood mononuclear cells (p = 0.0124) after Immulina supplementation. Acknowledgements: This research was partly funded by a USDA, Agricultural Research Service Specific Cooperative Agreement No. 58-6408-7-012. References: [1] Pugh ND, et al. (2008), Int Immunopharmacology, 8: 1023–1032.


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Variability of In Vitro Macrophage Activation by Commercially Diverse Bulk Echinacea Plant Material is Due Predominantly to Bacterial Lipoproteins and Lipopolysaccharides

We previously reported that the majority of in vitro monocyte/macrophage activation exhibited by extracts of Echinacea and other botanicals depends on bacterial lipopolysaccharides and Braun type bacterial lipoproteins [1]. We determined the contribution made by these bacterial components to the overall immune enhancing activity detected in E. purpurea and E. angustifolia from bulk root and aerial material obtained from six major growers/suppliers in North America. Substantial variation in activity (up to 200-fold) was observed in extracts of these materials when tested in two monocyte/macrophage cell lines. The majority of activity was negated by treatment with agents that target bacterial lipoproteins (lipoprotein lipase) and lipopolysaccharides (polymyxin B). Experiments comparing the activity of freeze dried, freshly harvested Echinacea plants with those harvested and dried using various commercially relevant conditions, suggest that post-harvesting procedures do not substantially contribute to the variation observed in the commercial material. Acknowledgements: This research was partially funded by grants from the National Institutes of Health R01 AT002360 (NCAAM) to DSP and the USDA, Agricultural Research Service Specific Cooperative Agreement No.58-6408-7-012. References: [1] Pugh ND, et al. (2008), Int Immunopharmacology, 8: 1023–1032.

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Enhancement of Natural Killer Cell Activity and Phagocytosis in Healthy Subjects by Immulina, a Spirulina Extract Enriched for Braun Type Lipoproteins

We determined that oral consumption enhanced several immunological functions in mice [1]. In this study we further characterized Immulina by determining that Braun type lipoproteins are responsible for a major portion of the in vitro monocyte activation exhibited by this material. In order to understand the effect of Immulina on the human immune system, a pilot study was conducted on ten healthy individuals who supplemented their diet with Immulina (400 mg/day) for seven days. Blood was drawn from the participating individuals at two time points: before and after seven days of Immulina supplementation. Changes in mononuclear and polymorphonuclear phagocytosis were determined in heparinized whole blood as well as the cytotoxicity exhibited by natural killer (NK) and lymphokine activated killer cells. We observed statistically significant increases both in tumor cell killing by NK cells (p = 0.0019) and in phagocytosis by blood mononuclear cells (p = 0.0124) after Immulina supplementation. Acknowledgements: This research was partly funded by a USDA, Agricultural Research Service Specific Cooperative Agreement No. 58-6408-7-012. Immulina capsules were supplied by Scandinavian Clinical Nutrition Denmark A/S, Greve, Denmark. References: [1] Balachandran P, et al. (2006), Int Immunopharmacology, 6: 1808–1814.

P.94

Can Green Tea Extract Become a Cause of Acute Pancreatitis?

Acute pancreatitis is a local inflammatory process that could occur due to multiple causes. This condition is diagnosed by elevated plasma amylase. In mice there is only one predominant model of acute pancreatitis, in which hyper-stimulatory doses of cholecystokinin or its analog caerulein are administered [1]. Nothing is known about herbs and botanicals for their potential to cause acute pancreatitis. We report a suspected potential of green tea extract to cause acute pancreatitis in mice. Balb/C mice 20–25 g was administered by oral gavage 200 ul of commercially available green tea extract. After 18 hours blood samples were taken and were analyzed for plasma chemistry profile and complete blood picture. Mice that were given green tea extract showed elevated plasma amylase (mean = 1428 ± 546.27 U/L) whereas in the normal mice the mean was 58.0 ± 0.4 U/L. In addition, slight elevation of plasma Alanine Aminotransferase (ALT) was observed (mean 127 ± 79.45 U/L) com-
pared to normal controls (30 U/L). The Blood Urea Nitrogen (BUN) values were also raised (81 ± 51.0 mg/dl) compared to normal control (21 U/L). Green tea administered mice showed hyperactivity or restlessness compared to normal controls. The blood picture showed slight elevation of granulocytes (ranging from 26.8 to 83.2% Mean54%) as compared to normal that range between 8 to 48%. Plasma amylase elevation is a good indicator of acute pancreatitis. An increase in BUN and BUN: Creatinine (CRE) ratio is one of the manifestations of dehydration. In our study, plasma amylase was remarkably increased in mice administered green tea. The caffeine in the green tea extract may have caused dehydration due to increased urination hence increasing BUN and BUN: CRE ratio. We conclude that green tea extract in the doses administered in this study could lead to acute pancreatitis. Further studies are needed to confirm these results along with histopathology of treated pancreas. References: [1] Lampel M, Kern HF, (1977), Virchows Arch A Pathol Anat Histol, 373(2): 97–117.

Allicin Bioavailability from Alliinase-Inhibited Garlic
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Allyl thiosulfimates (75% allicin) are responsible for most of the known health benefits of crushed raw garlic. Absent in garlic cloves, they are rapidly produced from alliin when endogenous alliinase is activated by crushing the cloves. The alliinase-dependent production of allyl thiosulfimates (hereafter called allicin) is known to be completely inhibited by heat and acid (pH ≤3.5) in vitro, bringing into question any allicin-related health benefits of cooked garlic or garlic powder supplements not protected from gastric acid. Indeed, most supplement brands have been shown to produce little allicin under USP/NF-defined simulated gastrointestinal conditions. To determine if allicin production in the human body might be different from in vitro predictions, a method for measuring allicin bioavailability was developed (break AUC of its main metabolite, allyl sulfide) and applied to heat-inactivated and acid-inactivated garlic. Allicin bioavailability from the alliin of boiled garlic was found to be 18% (14–25%), much higher than expected, with a similar result for garlic powder consumed in 1 N HCl (pH 0.6). When garlic powder was consumed in capsules with a low protein meal (expected gastric pH ≤3), 34% of the alliin was converted to allicin, indicating that the local pH is increased by the dissolving capsule. When pure alliin was consumed, only 4% of it was converted to allicin, probably by intestinal bacteria. The substantial difference in allicin bioavailability between heat- or acid-inactivated garlic (18%) and pure alliin (4%) indicates that the body has the ability to partially reconvert inactive alliinase. This work has important implications: (1) the health benefits of raw garlic can be obtained with cooked garlic, if consumed in larger amounts, as is often the case, and (2) allicin bioavailability from garlic powder supplements may be considerably higher than predicted in vitro, depending on how they are made and consumed.

Antimicrobial Activity of Marrubium vulgare L. (Lamiaceae) Extract on MRSA
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Many plants possess potent antimicrobial agents and provide effective remedies for skin conditions. Infusions of the aerial parts of Marrubium vulgare (white horehound) are used in the south Italian pharmacopoeia as a rinse for skin rashes and wounds [1]. Staphylococcus aureus, a common cause of skin infections, has been shown to be remarkably sensitive to the ethanolic extract of this plant. Further, numerous lipophilic fractions exhibit dose-dependent toxicity towards MCF-7 and PC-3 cells, with IC50 values as low as 20 µg/mL. The major cytotoxic compound gives 50% inhibition growth of MCF-7 cells at 100 µg/mL. Bioassay-guided fractionation by reverse phase C18 open column chromatography, followed by reverse phase C18 HPLC, afforded the major cytotoxic component, a twenty-carbon terpenoid, along with an inactive twenty-carbon compound. The major cytotoxic compound gives 73% inhibition growth of MCF-7 cells at 100 µg/mL. The structure has been characterized by NMR spectroscopy and ESI-MS, and these results will be presented.

Antitumor Activity of Arnoglossum atriplicifolium
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Western North Carolina is home to one of the most diverse collections of botanical species in the temperate world. The region is also an extensive repository of herbal natural healing knowledge, developed through the centuries by Native American and European settlers, regional plant species with documented medicinal properties number in the hundreds. These factors combine to present urgent need for Western North Carolina to use cutting edge technology to identify, validate, protect, and use its matchless natural resources in innovative, sustainable, and productive ways including careful bioexploration. We have recently launched a targeted screening program for identifying plants indigenous to Western North Carolina with potential antitumor activity. Initial screening against the MCF-7 breast tumor cell line identified an extract of Arnoglossum atriplicifolium (aerial parts) as having cytotoxic activity. Combined CH2Cl2 extractions of the acidified crude organic extract showed dose-dependent toxicity towards MCF-7 cells, with IC50 around 100 µg/mL. Bioassy-guided fractionation by reverse phase C18 open column chromatography, followed by reverse phase C18 HPLC, afforded the major cytotoxic component, a twenty-carbon terpenoid, along with an inactive twenty-carbon compound. The major cytotoxic compound gives 73% inhibition growth of MCF-7 cells at 100 µg/mL. The structure has been characterized by NMR spectroscopy and ESI-MS, and these results will be presented.

Acknowledgements: We thank the Western Carolina University SURF Program for summer support for T.J.W. We thank Wake Forest University Health Sciences Virus and Vector Core Laboratory for assay work.

Muscadine (Vitis rotundifolia) is a native and valuable fruit crop in Southeastern US. Today muscadine products are commercially available as nutraceuticals. Major concerns in nutraceuticals are product quality and their effects on human health. This study was conducted to evaluate muscadine nutraceutical powder derived from pomace (cv. Noble) for biomarker compounds and their effects on colon cancer cell lines. The powder was extracted after acid hydrolysis. The extract (CE) was further fractionated to obtain flavonoid and anthocyanin fractions (FAF). Total phenolic (TP) and flavonoid (TF) contents, and individual biomarker compounds in each fraction were analyzed using colorimetric assays and HPLC-PDA, respectively. The TP and TF contents in the fractions were higher compared to those of CE. The main polyphenol present in CE was ellagic acid, not resveratrol as in table grapes. The major anthocyanins present were 3,5-diglucosidic anthocyanins in contrast to monoglucosidic anthocyanins present in table grapes. The effects of CE and FAF were tested in two colon cancer cell lines, HT-29 and Caco-2, for their antioxidant activity. Data obtained from the MTT assay indicated that L-asparaginase was characterized by size exclusion chromatography, PAGE and 2-D PAGE. The antitumor and growth inhibition effect of L-asparaginase was characterized by size exclusion chromatography, PAGE and 2-D PAGE. The purified enzyme is a homodimer, with a molecular mass of 72 ± 0.5 kDa, and pI value of the enzyme was around 5.1. The enzyme was purified from the crude extract of the fruits of *Withania somnifera* L., a popular medicinal plant. L-asparaginase was purified from the crude extract of the fruits of *Withania somnifera* L. up to 95% through column chromatography. The purified L-asparaginase was characterized by size exclusion chromatography, PAGE and 2-D PAGE. The antitumor and growth inhibition effect of the L-asparaginase was assessed using MTT colorimetric dye reduction method. The purified enzyme is a homodimer, with a molecular mass of 72 ± 0.5 kDa, and pI value of the enzyme was around 5.1. It is the first report for plant L-asparaginase with antitumor activity. Data obtained from the MTT assay indicated that L-asparaginase significantly (P < 0.05) reduced the viability of lymphocyte cells in a dose-dependent manner, showing a LD50 value of 1.45 ± 0.05 IU/ml. *Withania somnifera* L. proved to be an effective and a novel source of L-asparaginase, furthermore it shows lot of similarity with bacterial L-asparaginases which have already been commercialized for the treatment of acute lymphoblastic leukemia. 

**References:**


By injecting 0.1 ml carrageenan suspension (1%) subcutaneously into the sub-plantar side of hind paw [6]. Paw volume was measured by dislocation of the water column in a plethysmometer. Methanolic seed extract showed significant (P < 0.01) anti-inflammatory activity at 500 mg/kg dose. **References:**

analgesic and anti-inflammatory activities at various doses (100, 250 & 500 mg/kg) and compared with Diclofenac sodium (7 mg/kg) taken as standard. The extract showed maximum effect at 500 mg/kg. The statistical analysis of all the results was carried out using one-way ANOVA followed by Dunnet’s t-test and all the results obtained in the study were compared with the vehicle control group. P values < 0.001 were considered statistically significant. Acknowledgement: The authors are highly thankful to Director, I.P.S., Kurukshetra University, Kurukshetra for carrying out research activities. References: [1] Joshi SG, Medicinal Plants, Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, 2003, 157–158. [2] Nadkarni AK, Nadkarni KM, Indian Materia Medica, Vol. I, Popular Prakashan, Bombay, 2000, 403–404. [3] The Wealth of India: A dictionary of raw materials & Industrial products, 1st supplement series (Raw Materials), Vol. 2: Cl-Cy, NISCAIR, CSIR, New Delhi, 2004 255.

P-103 Evaluation of Ethanolic Extract of Withania somnifera on Haemopoietic Deficiency Anemia in Albino Rats
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Medicinal plants are believed to be useful in strengthening the haematopoietic and immune system. Our objective was to investigate ethanolic extract of the root part of Withania somnifera (WS) on hematological parameters as well as serum iron and serum protein in iron deficiency anaemia induced using haloperidol and observe the morphological changes in red blood cells. The animals were divided into five groups. Group I acted as control, group II was haloperidol control (0.2 mg/kg body weight i.p.), group III was treated with ethanolic extract alone (200 mg/kg body weight i.p.), group IV and V were given HP and ethanolic extract at the doses of 100 and 200 mg/kg body weight i.p., respectively [1]. Effect of haloperidol on group II showed significant (P < 0.05) decrease in blood parameters, serum iron and serum protein, as compared to control animals group I. Comparison of group II with group III, IV and V exhibited significant (P < 0.05) increase in hematological parameters, serum iron and serum protein after four days and after recovery period study (on 19th day). This effect may be due to presence of iron in extract (947 µg in 50 ml) estimated quantitatively by spectrophotometric method. Effect of ethanolic extract of Withania somnifera on morphology of blood cells was observed. It accelerated the oxygen carrying capacity of red blood cells and showed increased number of RBCs with normal counts and normocytic shape. We conclude that WS exhibited potent haematopoietic activity against haloperidol induced iron deficiency anaemia [2]. Acknowledgement: Thanks go to the University Grant Commission, (UGC-SRF Fellowships) New Delhi for financial support. References: [1] Ziauddin, Met al (1996). J. of Ethnopharmac., p.50: 69–76. [2] Wasti, A., Ghani, R., Manji, M.A. and Siddiqui, N.A., (2004) Pak. J. Med. Sci., Vol. 20 (3), p.197–200.

P-104 Anti-carcinoma Activity of Polyphenolic Extract of Withania somnifera
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Dietary polyphenol antioxidants are known to decrease the risk of many diseases such as cancer and cardiovascular diseases [1]. In this study polyphenolic extract (PPE) of leaves of Withania somnifera was evaluated for anticancer activity in vivo. Murine Ehrlich ascites carcinoma (EAC) model was used to assess PPE antitumor activity in vivo [2]. PPE cytotoxicity was determined in vitro in U-937 monocytoid leukemia and K-562 erythro leukemia cell lines. The total phenolics content was quantified by the Folin-Ciocalteau method [3]. Results of in vivo study showed a significant decrease in tumor volume, viable tumor cell count and a significant increase of life span in the PPE treated group compared to untreated one: the life span of PPE treated animals increased by 53.41% (50 mg PPE/kg) and 73.95% (100 mg PPE/kg). PPE (5, 10 and 20 µg/ml) effectively inhibits in vitro proliferation of U-937 and K-562 cell lines. The in vitro and in vivo anti-tumor activity of PPE from Withania somnifera could be due to rich polyphenols and flavonoids [4].Acknowledgements: All India Council of Technical Education (AICTE), Government of India, New Delhi, India is greatly acknowledged. References: [1] Kuroda Y, Hara Y, (1999), Mutation Research, 436: 69–97. [2] Clarkson BD, Burchenal JH, (1965), Progress in Clinical Cancer, 1: 625–629. [3] Chang CC, et al. (2002), Journal of Food Drug Analysis, 10: 178–182. [4] Singh RP, Singh RP, (1987), Journal of Indian Chemical Society, 715–756.

P-105 Obesity Associated Dementia Among Elderly – Role of a Plant Based Formulation
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The main object of the present study was to evaluate association between obesity and dementia in elderly people and its prevention and management by a herbal formulation. Under this clinical trial 80 men and women (aged 61 to 79 years,) underwent a detailed health evaluation, showing a high BMI with a major complaint of loss of memory and deterioration of other cognitive functions were treated with a novel herbal formulation containing hydro-alcoholic extract of Dioscorea bulbifera, Salacia oblonga and Hippophae rhamnoides in effective doses. Normal 58 aged (31 male and 27 female, BMI 18–25) with normal cognitive functions, and 57 (33 male and 24 female) underweight aged (BMI < 18 with poor mental abilities) were also treated with test formulation. The test drug exerted beneficial effects on BMI, mental functions particularly on memory and attention span, and attentional marker CR including Homocysteine, plasma leptin and adiponectin levels during six months of study period. It is concluded that test formulation enhanced the satiety, decreased appetite and fat absorption through regulation of 5-HT, leptin and adiponectin receptors involved in the onset of obesity. Thus, by regulating adipokines, memory, attention span and other cognitive impairments significantly improved among obese elderly demented subjects. Improvement in mental performance was also noticed in normal as well as under weighted aged also following test formulation treatment. Pre-clinical toxicity studies revealed that drug is safe and can be given for longer time.

P-106 Neurupsycho-Cardiologic Risks Associated with Menopausal Women – Benefits of a Plant Based Formulation
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The anatomic, physiologic alterations in the ovary that eventually result in diminished estrogen production begin several years before permanent cessation of menstruation among the women. The relationship between menopause and cardiovascular risk is established and it is well documented that estrogen depletion is responsible for cardiovascular risk. A double blind placebo controlled study was carried out with the object to minimize the neuro-psycho-cardiologic risks associated with menopausal women by a
Ginkgo biloba L. (Ginkgoaceae) usage has recently gained interest among herbalists and modern medical practitioners because of its unique pharmacological actions that are attributed to active substances such as flavonoids and terpenoids [1]. It is commonly prescribed for improvement of cerebral circulation, memory improvement and antioxidant activity. Epileptics have a greater chance of prescribed for improvement of cerebral circulation, memory improvement and antioxidant activity. Epileptics have a greater chance of employing Ginkgo biloba. Clinical trials have shown that Ginkgo biloba may be used as an alternative remedy in specific conditions such as oxidative stress and memory impairment [2]. This study aims to evaluate the pharmacokinetic interaction between the aqueous extract of Ginkgo biloba and carbamazepine. Two groups of animals, each containing 6 animals were used. The Group 1 and Group 2 received pretreatment with two different doses of extract for 7 days and on day 8 the extract was co-administered with carbamazepine. The Group 3 (control) received carbamazepine alone on day 8. The blood samples were collected for 24 hours. Samples were analyzed by HPLC and pharmacokinetic parameters were calculated. Analysis of the data reveals that there was very significant decrease (p < 0.05) in the Cmax (4.32 ± 0.24 µg/ml), AUCtotal (20.31 ± 1.41 µg/ml h) and AUC0 to t (18.31 ± 1.06 µg/ml h) of Group 1 when compared to Cmax (6.76 ± 0.40 µg/ml), AUCtotal (36.79 ± 1.57 µg/ml h) and AUC0 to t (34.81 ± 1.23 µg/ml h) of control. There was also a significant decrease in the Cmax (3.51 ± 0.28 µg/ml), AUCtotal (11.60 ± 0.93 µg/ml h), AUC0 to t (10.33 ± 0.82 µg/ml h) and MRT (3.53 ± 0.24 h) of Group 2 when compared to Cmax (6.76 ± 0.40 µg/ml), AUCtotal (36.79 ± 1.57 µg/ml h), AUC0 to t (34.81 ± 1.23 µg/ml h) and MRT (5.59 ± 0.24 h) of control. There was no significant difference in the t1/2 of the drug in both study groups when compared with control. This data suggest that Ginkgo biloba reduced the bioavailability and increased the rate of elimination of carbamazepine which confirms that there is significant herb-drug interaction between the two.

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Anticancer and Antimalarial Dihydroartemisinin Dimer Oximes

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Pregnane Derivatives from Hoodia gordonii

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Hoodia gordonii (Fam. Asclepiadaceae) is a succulent plant indigenous to South Africa, Botswana and Namibia. Hoodia has gained wide popularity as one of the most sought after dietary supplements for its appetite suppressant activity. P57AS3, the reported active constituent from H. gordonii, is claimed to induce increased ATP synthesis in the hypothalamic neurons, thereby reducing appetite by giving out false satiety signals to the appetite center. In our previous phytochemical studies, we had reported isolation of several oxypregnane glycosides and calogenin bisdesmosides, including P57AS3. Here, we report isolation and characterization of nine pregnane glycosides, including two novel abeo-sterol aldehyde glycosides, (1), and (2). This is a first report of abeo-sterols from Hoodia spp. The chemical structures of the glycosides were established by chemical degradation studies and extensive spectroscopic techniques that included one-dimensional and two-dimensional NMR.

Acknowledgements: Part of the research was funded by “Botanical dietary supplements: Science-Base for Authentication" of US Food and Drug Administration Grant No FD-U-002 071. The authors would like to thank Missouri Botanical Garden, USA for authentic plant material and Vaishali Joshi for plant identification. Authors also thank Bharathi Avula for her kind help in acquiring the mass data. Y.J.S. is thankful to NCNPR for graduate research assistant-ship.
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