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/ncnpr 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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Roy Upton
Executive Director, American Herbal Pharmacopoeia

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Research Scientist, NCNPR, The University of Mississippi

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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 Boyleau, Ph.D.
Regulatory and Scientific Affairs, Cargill

Josef Brinckmann
Traditional Medicinals

Paula Brown, M.Sc., MCIC
British Columbia Institute of Technology

Paul Pui-Hay But, Ph.D.
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Shi-lin Chen, Ph.D.
Institute of Medicinal Plant Research, China

Wan-sheng Chen, Ph.D.
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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 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 botanically authenticated and chemically and biologically standardized extract. It was ascertained 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 subjected 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 Prempro 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.

In order of appearance

**Keynote Speaker**

Trials and Tribulations of Planning and Conducting a Phase 2 Clinical Trial with Black Cohosh and Red Clover

Farnsworth NR

1 UIC/NIH Center for Botanical Dietary Supplements Research, College of Pharmacy, University of Illinois at Chicago

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 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 botanically authenticated and chemically and biologically standardized extract. It was ascertained 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 subjected 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 Prempro 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.

**Oral Presentations – In order of appearance**

Botanicals and NMR-based Metabolomics: A Perfect Holistic Match

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An estimated 40,000–70,000 plant species are used as medicines worldwide. Some of these are extensively studied and their use is supported by clear clinical evidence. But in fact most have not been studied in any detail, and little is known about their activity, mode of action and possible active compounds. This poses two major problems, one is the acceptance of botanicals that are not yet evidence-based, the other is quality control. In fact acceptance is hampered by the fact that no methods for proper quality control are available if no active compound(s) is/are known. With globalization the use of botanicals is clearly increasing. Pharmacognosy has thus a major task in developing medicinal plants into evidence-based medicines. This will include both mentioned aspects: evidence for activity and quality control. In the past decades drug development has gone from in-vivo testing into molecular based assays (High Throughput Screening, HTS) for finding new leads. Certainly by HTS one may find active compounds in medicinal plants, but synergy and pro-drugs will certainly not be found in such an approach. We pharmacognosists should thus rethink our approaches for proving activity of medicinal plants. This is where systems biology and metabolomics do offer interesting options. It means going back to in-vivo pharmacology in combination with the “-omics” technologies to measure the response of a test organism on treatment with the medicinal plant, and metabolomics to phytochemically characterize the medicinal plant. By chemometric methods, such as multivariate analysis, links can be made between compounds present in the plant and activities observed in the model organism. That means that not only active compounds, but also synergy and pro-drugs can be found. This approach will also be the basis for quality control. By using metabolomics in combination with multivariate analysis one can define the required profile for activity. Particularly NMR-based metabolomics has a great potential for both quality control and identification of compounds related to activity.

**Evaluation of the Anti-inflammatory Potential of Chinese Botanicals**

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Traditional Chinese medicine (TCM) has been using 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 quinolinone alkaloids, like 1-methyl-2-nonyl-4(1H)-quinolinone, 1-methyl-2-(6Z)-6-undecenyl-4(1H)-quinolinone, 1-methyl-2-(4Z,7Z)-4,7-tridecadienyl-4(1H)-quinolinone, evo-carpine and 1-methyl-2-(6Z,9Z)-6-pentadeca-
dieneyl-(4(1H)-quinolinone, have been isolated which showed strong inhibitory activity on leukotriene biosynthesis in human polymorphonuclear granulocytes [1]. They showed no cytotoxic activity and might bind to the lipid binding site of 5-LOX [2]. Moreover they were very effective against mycobacteria [3]. From Centu-
peda minima (L.) A. Braun & Asch. (Asteraceae) a series of sesquiter-
pene lactones, like 6-O-methylacyrpllenolin, 6-O-angeloylpllenolin and 6-O-tigloyl-pllenolin, have been isolated, which exhibited strong inhibitory properties on inductive nitric oxide synthase (iNOS) in RAW 264.7 macrophages [4]. Extracts from Chinese herbs have shown inhibitory properties on expression of NF-κB1 in THP-1 cells [5]. Overexpression of this transcription factor is associated with inflammatory diseases, like rheumatoid arthritis, atheroscle-

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, including our own, has "devolved," or disappeared when its practitioners die without teaching the knowl-
edge to the next generation. The implications of this for natural products research and development and safe and proper use of new plant species as dietary supplements will be discussed. Herbs used by traditional peoples have been subjected to many genera-
tions, even centuries of trial and error experimentation, and there is much that these people can teach us about their efficacy and use. Ethnobotanical knowledge can be of great value in addressing con-
temporary issues in supplement and drug development, public health and sustainable resource use and conservation. However, in seeking to fulfill this potential, scientists find themselves in a race against time, with both habitats being destroyed and indigenous knowledge about the uses of the plants and their environment rapid-
lly being lost. There are ways to reduce this destruction of humanity’s collective wisdom before it is too late.

To date more than 170,000 natural compounds [1,2] are published. The main part of these compounds belongs to secondary metabo-
lites, which provide living systems with their characteristic fea-
tures mandatory for surviving. They contain an inherently large-
scale of structural diversity. About 40% of the chemical scaffolds of published natural products (NPs) are unique and have not been synthesized by any chemist [3]. Accordingly, a large number of drug leads and hits are conserved in the inexhaustible pool of NPs pre-
screened by evolution. But how to dig out and to recognize the re-
spective drug leads is a challenging task. Although a random selec-
tion of plant materials seems not to be a very efficient strategy for the discovery of new biologically active compounds, many today well-known natural drug leads are based on a serendipitous find-
ing. An example of a successful random study will be presented from our laboratory, which has recently resulted in the identifica-
tion of isogentisin, a secondary metabolite of Gentiana lutea L., as a novel compound for the prevention of smoking-caused endothelial injury [4]. A more rationalized access to bioactive compounds is of-
fered by in silico tools e.g. pharmacophore-based virtual screening, docking experiments and the parallel screening concept. Screening of compounds against a set of models representing a large number of targets aims to predict the pharmacological profiles of these molecules including desirable activities and undesirable effects. In this presentation an example of an application employing a virtual paral-
lel screening approach with a collection of 2208 in-house gener-
ated pharmacophore models on constituents of the aerial parts of the medicinal plant Ruta graveolens L. will be illustrated [5]. Refer-

Plants are still an important resource for the discovery of new drugs, such as new antimalarial agents. In search for novel antima-
larial compounds, we focussed on neocryptolepine (5-methyl-5H-
indolo[2,3-b]quinoline), 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. The evaluation included cytotoxicity (MRCS cells), inhibition of β-hematin formation and DNA-interac-
tions (DNA/methyl green assay). Introduction of aminoalkylamino chains increased the antimalarial activity of the neocryptolepine core substantially. The most active compounds showed antimal-
arial activities in the nM range. Nevertheless, some compounds that were selected for in vivo evaluation in infected mice were not sufficiently active, or toxic to the animals. A different approach to develop antimalarial drugs from nature is the standardisation of plant extracts with a proven efficacy used in traditional medicine. Nauclea pobe
guinii (Rubiaceae) is a tree from which the bark is widely used in African traditional medicine against malaria-like symptoms. Alkaloids such as the major compound strictosamide are expected to be responsible for the activity. An HPLC method was developed and validated for the quantification of strictosamide in an 80% EOH extract of the stem bark of N. pobe
guinii. This extract, containing 5.6% (w/w) strictosamide, was evaluated in vivo in the Plasmodium berghei mouse model in a suppressive treatment regimen. It was orally dosed (PO) at 300 mg/kg 2 ×/day during 5 consecutive days. Another group was treated intraperitoneally (IP) at 50 mg/kg using the same dosing regimen. Treatment with the crude extract, either after oral or intraperitoneal dosing, resulted in moderate depression of parasitaemia during dosing, however quickly followed by a full relapse (mean survival time = about 13 days). At termination of the experiment at day 21, a single survivor in the PO group was apparently cured (no parasitaemia), the single survivor in the IP group showed high parasitaemia and was in a moribund state. It can be concluded that the crude extract of N. pobe
guinii has slight antimalarial potential when administered orally in a suppressive dosing regimen of 2 × 5 days at 300 mg/kg. Longer treatment may be necessary.
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 oleagum 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 acetylsalicylic acids (ABAs). Using LPS as an activator of human monocytes, we found that ABAs inhibit NF-κB signaling. We identified specific inhibitory effects on iκB 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 oleagum 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.

S-9

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 complement (C3, C4) levels in infants and young children. Animal experiments with albino rats showed its highest anti-inflammatory activity under 40 mg/kg/d (amount to clinical mimic-dose 80 times) ADP served as the noradrenaline and/or 5-hydroxytryptamine and/or dopaminergic (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, Korea, India, Russia and European Union from 2007(WO2006/058487 A1).

S-10

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 dose of ADP for mouse ED50: 4.56 mg/kg (FST); 3.49 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 reflex 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 dopaminergic (re) uptake inhibitor for development into antidepressant, 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).

S-11

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-
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 C66 colon carcinoma were explored. For this study peripheral blood mononuclear cells (PBMC), peyer’s patch lymphocytes (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.

**Effect of Polysaccharides on Enteric Mucosal Immune Response in Rats**

**S-12**

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Recent surveys have shown that up to 71% of Canadians consume natural health products (NHPs) either on a daily or seasonal basis [1] and 73% of American adults surveyed reported taking some type of non-prescription vitamin, dietary supplement or mineral supplements over the course of a year [2]. The prominence of these products in the health care setting is further buoyed by the current deluge of adverse drug effects in conventional medicines. Yet 48% of Americans surveyed believe supplements are not advertising for use only. Unauthorized distribution is strictly prohibited.

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

**S-13**

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**Metabolomics for Discovery of Novel Medicinal Compounds**

**S-15**

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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.

**Quality Evaluation and Quality Control of Botanicals and Traditional Chinese Medicine**

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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.

**HPTLC for Quality Control of Traditional Chinese Medicines: Identification and Detection of Adulteration**

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For cGMP compliance of dietary supplements and quality control of herbal medicinal products, proper identification of herbal raw material is of great importance. In this respect Traditional Chinese Medicines (TCM) can present challenging tasks because pharmacopeial drug monographs may include multiple species and often don’t provide sufficient analytical methods. High Performance Thin-Layer Chromatography (HPTLC) is a very suitable tool for direct comparison of fingerprints from multiple samples side by side and allows determining similarities and differences of related species. Using “BEIMU” (Fritillaria spp.) and “CANGZHU” (Atractylodes spp.) as examples, the development and use of validated methods for this purpose is illustrated. The traditional approach of associating the quality of an herbal medicine with the quantity of a marker becomes questionable, if the product contains more than one plant material. CANGZHU XUANGLIAN SAN a TCM for veterinary use contains Coptis rhizome, Aucklandia root, and Atractylodes root but the Chinese Veterinary Pharmacopoeia only relies on identification and quantitation of berberin as principal marker. Berberin is present in Coptis only. This creates the possibility for adulterated products, missing either of the other two plants to enter the market. We propose an HPTLC method that allows a more complete monitoring of quality by ensuring the presence of all species in the appropriate quantity.

**Relative and Absolute Structures of Diospongin A, B and C**

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While screening 60 extracts for their stimulatory activity on proliferation of osteoblast-like cell line and on inhibition of osteoclastic formation, the water extract of Dioscorea spongiosa displayed the strongest stimulation on osteoblastic proliferation and strong inhibition on osteoclastic formation. This water extract was separated using bioassay-guiding fractionation and three new diarylheptanoids were isolated and purified. The structures of three new diarylheptanoids were elucidated by analysis of NMR, IR spectra and high resolution FAB-MS. The relative stereochemistry of diospongin A and B was determined by ROESY spectra and coupling constants in 1H-NMR spectra and their absolute structures were...
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 acetonide derivative and optimizing dihedral angles [1]. Its absolute stereochemistry was identified by the CD spectrum of its dibenzoat 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.

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 an 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. Due to the increasing public demand for quality botanical products, some companies are now making efforts to acquire at least a portion of their raw material from sustainable and ethical sources, but most invest little in this side of their business. The existing industry practice often promotes poor management of species and few benefits for the collectors and cultivators, and many companies remain distant and unaware of the conditions under which raw materials are sourced. However, there also exist opportunities to create change in this sector. The source and quality of raw materials, storage, post-harvest handling play a pivotal role in guaranteeing the quality and stability of ISM & botanical preparations. In India, in addition to the promotion of cultivation of medicinal and aromatic plants (MAPs) by certain government departments and R&D institutes, of late some private herbal drug industries have also started sourcing their requirement of herbal raw material from cultivated sources. The cultivation of MAPs, on the other hand, would not only lead to better control over quality of the end products but will also reduce anthropogenic stress on wild stands. The presentation will illustrate the efforts being made in India in general and at the Indian Institute of Integrative Medicine (IIIM – CSIR) in particular for the sourcing and sustainable supply of raw materials for ISM & Botanical industry.

S-20

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

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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 anti-diabetic 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 anti-diabetic 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-21

Understanding Botanical Dietary Supplements: The Research Need for Well-Characterized Test Materials – Research Grade Botanicals

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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 an essentially 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 total cholesterol and high-density cholesterol, adaptogenic activity, carrageenan induced hind paw edema in rats and capillary permeability in mice. Charles foster strain albino rats and mice in either sex, bred in animal house of Institute of Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurveda University, Jamnagar, India – India, were used for animal trials. Patients suffering from different types of oedema were subjected for clinical study. The data generated from the studies clearly indicated that the subjective Ayurveda basic principles can be tested more efficiently and interpreted logically using modern scientific parameters and results can be expressed objectively to open discussions in the scientific forums for the advancement of science.

Proteomic method (two-dimensional electrophoresis and MS/MS) was used in studying the mechanisms of Traditional Chinese Medicines (TCMs) including Ganoderma 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 salvianolic 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 salvianolic 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 salvianolic 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.

The drug and pharmaceutical industry is one of the most rapidly growing and R&D intensive industries in the world. The search for new therapeutic agents and drugs from natural sources, such as plants, received a boost in the recent past due to increased awareness of side effects and toxicity associated with the allopathic drugs, coupled with the belief that botanicals products are green and more acceptable to humans. India, being the fertile ground of several medicinal systems, has given birth to a multitude of medicinal practices, some of them have survived with intact traditional knowledge. The rich Indian heritage associated with prevailing healing practices led to the identification of several medicinal plants and formulations that were traditionally used for curative purpose. Botanicals, as a source of small molecules with a view to identify new therapeutic agents, remains as one of the major developmental as well as academic activities pursued by several institutes and universities in the post independent era in India. However, the resurgence of natural products in the last decade has also forced the participation of private industry in this race. Though Indian contribution in the area of therapeutics agents, may it be a single molecule or standardized botanical preparations, have been far and few, yet some of the leads generated have been noticed globally and developed into useful products. The present review will cover some of the past and recent efforts made by various agencies in the development of new leads or therapeutics in the Indian context. It will also include the research and development work being carried out at the Indian Institute of Integrative Medicine at Jammu.

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, rpb1, rpoC1, ycf5, 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] Iahay 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...?

But PPS, #1 Show PC2, Ling KH3, Chan PWH4

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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 fumed 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 particularly, 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 pesticides residues according to the actual utility of chemical pesticides. This residue determination is required in more and more monographs within the Chinese material medica. The pesticides residue limits have been established in the ChP (2010 version). Other pollutants, such as heavy metals, sulphur dioxide, etc., were determined, controlled, and their acceptable limits established in the ChP (2010 version). The efficacy control of TCMs, TLC-bioautography and bio-activity determination techniques were used to establish the quality of TCMs. These results may reflect the true quality more directly and precisely than using a single ingredient. For well-controlled quality TCMs, DNA molecular marking and fingerprint techniques were adopted by ChP. DNA molecular marking technique was also used in Chinese medicinal monographs to define their species which can not be identified by microscopic, chemical or chromatographic methods, especially in multi-origin CMMs. Fingerprinting techniques were used to control the uniformity and stability of TCMs in order to reflect the integrity of the herbs and their complex ingredients.

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 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: Guideline 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.

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 to 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.
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 benefitted from scientific expertise within Health Canada Offices and Directorates, the United States Pharmacopeia, and NSF International.


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.

Within the group of industrially prepared herbal or botanical products there is a large variation worldwide with regard to the properties and the legal status of these products. Some herbal products are close to or are medicines, while others are close to or even identical to foods such as dietary supplements, functional foods, novel foods, etc. and still others are considered as cosmetics or medical devices. Therefore it is not surprising that recently appropriate regulatory actions have been undertaken to regulate and harmonize the legal status of these various groups of plant preparations throughout Western countries. The European Union (EU) has recently considered herbal products in several legislative texts. Medicinal use has been harmonized for herbal medicinal products (HMP) with regard to well-established (WE) and traditional (T) uses through Directives 2004/27/EC and 2004/24/EC amending Directive 2001/83/EC. Use of herbal preparations in unit dose form under food law is covered in the Food Supplements Directive (FSD) 2002/66/EC. Regulations on nutrition and health claims and the addition of vitamins and minerals and certain other substances to foods have been adopted on December 12, 2006. (Council Regulations (EC) n°1924/2006 and 1925/2006). Nevertheless, the distinction between traditional herbal medicinal products and food supplements containing herbal products without nutritional value but having physiological effects remains vague and controversial. In this presentation the implementation of the current European regulations at the level of the EU Member State authorities and
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 these 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 with men, and with individuals between the ages of 50 and 79 than with older or younger consumers. Most reports are submitted and subsequently approved. Although the reasons for this are no doubt different in different cases, several common issues related to quality control and trial designs, among others, have been observed by the Botanical Review Team. A discussion of these issues could shed light on the seemingly low percentage of botanical INDs entering late-stage drug development. We would love to see more botanicals being further developed as new drugs with more success.

**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 human-kind 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 mixtures will be developed as drugs. The publication of FDA’s “Guidance for Industry-Botanical Drug Products” (drafted in 2000 and finalized in 2004) paved the regulatory pathway for developing botanical mixtures as new drugs. The first botanical drug (Veregen®, derived from green tea) approval through investigational new drug (IND) and new drug application (NDA) processes in 2006 shows that well defined botanical mixtures can be approved as new drugs with demonstration of safety and efficacy through well-controlled clinical trials. Since the publication of the guidance, there has been a growing interest in botanical drug development judged by the increasing numbers of botanical INDs and pre-IND consultations, with a cumulative total of over 350 and growing. Few of the botanical INDs with phase 1 and/or 2 clinical trials have, to date, advanced into late-phase clinical trials. So far, the Veregen® NDA remains the only one submitted and subsequently approved. Although the reasons for this are no doubt different in different cases, several common issues related to quality control and trial designs, among others, have been observed by the Botanical Review Team. A discussion of these issues could shed light on the seemingly low percentage of botanical INDs entering late-stage drug development. We would love to see more botanicals being further developed as new drugs with more success.
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 Medicines, China Pharmaceutical University, Ministry of Education; IMM, CAMS & PUMC, 100050, Beijing, China.

Natural products play a dominant role in the discovery of leads for the development of drugs for the treatment of human diseases. In China, much of nature sources remain to be explored, particularly the toxic plants, that no doubt host novel, bioactive chemotypes that await discovery. There are more than 900 species of toxic plants in our country. The bioactivities of extracts of over 150 toxic plants were investigated in our group. It was found that more than 20 toxic plants showed vasodilator activities and anti-tumor activities, of which 7 toxic plants were further studied by bioassay-guided technique. From the five toxic plants, more than 250 compounds were isolated, including 7 toxic plants were investigated in our group. It was found that more than 80 novel compounds, of which more than 50 compounds exhibited significant bioactivities to different targets. It lays a foundation for the study of innovative drugs and the elucidation of bioactive substances from toxic plants.

The fruit extract of Emblica officinalis Gaertn. (Euphorbiaceae), commonly known in India as amla (Indian gooseberry), has been popularized as a dietary supplement in the United States and elsewhere, with its antioxidant benefits being attributed to a high content of ascorbic acid. The presence of ascorbic acid in the extract was questioned by earlier researchers, and hydrolysable tannins, emblicains A and B were identified [1] and structurally defined [2]. Our investigations on the emblicains and ascorbic acid con-
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 S-O-gallate (2), respectively. Only trace amounts of free ascorbic acid were detected. Beta-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. Acknowledgements: This research was financially supported by National Natural Science Foundation of China (20572130, 30600807). References: [1] Liu AH, et al. (2006) J Pharm Biomed Anal, 41: 48–56.

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 may show potential bioactivity of anti-atherosclerosis. The data indicated that 19 compounds have an inhibition effect on aggLDL absorption. Remarkably, kudinoside A, C and IP8-20 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.
Effects of Nitrogen on the Yield and Quality of Selected Chinese Medicinal Plants of the Lamiaceae Family

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With many of the practicing acupuncturists in the United States prescribing herbal formulas, the demand for Chinese medicinal plants has been increasing. In the past several years, however, quality concerns have been raised about medicinal plants imported from China. To assure the safe and efficacious care for patients, practitioners need good quality plant material produced under controlled and documented conditions in accordance with good agricultural practices. The objective of this research was to determine whether quality plant material of selected species of Chinese medicinal plants could be cultivated in the northeastern United States and whether such cultivation was economically feasible. For these reasons, Agastache rugosa (Fisch. & C.A. Mey.) Kuntz, Leonurus heterophyllus Sweet, L. sibiricus L., and Schizonepeta tenuifolia Briq. were field grown in a randomized complete block design using 0, 100, and 200 kg ha⁻¹ of nitrogen supplied as soybean meal. The nitrogen treatments resulted in dose-related increases in yield in all species. 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.

In vitro Monocytone Activity of Echinacea purpurea: Endophytic Bacteria is Affected by the Host’s Genetic Diversity and Harvest Timing

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Our previous report demonstrated that the majority of in vitro monocytone/macrophage activation exhibited by extracts of Echinacea and other immune enhancing botanicals depends on bacterial lipopolysaccharides and Braun type bacterial lipoproteins (1). We later showed that the activity of diverse commercial Echinacea bulk material varied substantially (up to 200-fold), and that the majority of this activity was also due to these two bacterial components (2). The objective of this study was to determine the contribution of host plant genetics and time of harvest as factors influencing the variation of E. purpurea root and leaf activity. The immune enhancing activity of the aerial part was substantially higher when harvested during the onset of leaf/stem senescence and was the only harvest time where significant differences were observed. There was less variation in root activity due to harvest time and genotypic diversity. Although these two factors may have contributed to the large variation in immune enhancing activity previously observed in bulk E. purpurea material obtained from different suppliers in North America, other environmental and agronomic factors may have a greater influence. Acknowledgements: This research was partially funded by grants from the National Institute for Health R01 AT002360 (NCAAM) and by the USDA, Agricultural Research Service Specific Cooperative Agreement No. 58-6408-7-012. References: [1] Pugh ND, et al. (2008) Immuno Pharmacol 8: 1023–1032. [2] Tamta H, et al. (2008) J. Agric. Food Chem. 56 (22): 10552–10556.

Assessment of Cannabinoids Content in Micropropagated Plants of Cannabis sativa L. and Their Comparison with Vegetatively Propagated Plants and Mother Plant at Different Stages of Growth

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True-to-type clonal fidelity is one of the most important prerequi-sites for rapid multiplication of plant species. However, there is always a concern of potential differences due to mutation and their effect on the chemical constituents of in vitro propagated (IVP) and vegetatively propagated (VP) plants from same source (MP). Clonal fidelity was tested among the three groups of plants (MP-indoor, IVP and VP). After the plants were well established in the soil [1,2] samples from all three groups of plants, were periodically analyzed for their cannabinoids content to determine if differences in secondary metabolites exist within and among these groups of plants. The content of six major cannabinoids: D9-THC, THCV, CBD, CBC, CBG and CBN were identified and analyzed using gas chromatogra-phy/flame ionization detection (GC/FID). In general, THC content in all groups increased with plant age up to a highest level during bud-ding stage where THC content reached a plateau. These results before the plants were harvested. The pattern of changes occurred in the concentration of other cannabinoids content relative to the plants age and has followed a similar trend in all groups. Minor differences observed in cannabinoids concentrations within and among the
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). **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, ElSohy MA1,3**

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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<sub>opt</sub>) was observed around 30°C in HP Mex whereas, T<sub>opt</sub> was observed in the range of 25 to 30°C in W1 [1]. A comparatively lower value (25°C) for T<sub>opt</sub> was observed in MX. Among fiber type clones, T<sub>opt</sub> 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 dependence of Pn in *Cannabis sativa* and may be used as a tool for initial 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 National Institute of Drug Abuse (NIDA), Contract No. N01DA-0-7707. **References:** [1] Lata H and colleagues (2008) Physiology and Mol Biol of Plants, 14(4), October 2008 (in press).**

**P-6**

**Molecular Analysis of Genetic Stability of Micropropagated Plants of *Cannabis sativa* L. using ISSR Markers**

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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 *C. 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 material for the isolation of THC as a bulk active pharmaceutical. **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)
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 “Ayardana, Sari civanpercemi” 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 antimarial, antimicrobial and antifungal 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 pharmacognosy 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 genetic 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 “minor” cannabinoids are produced in trace amounts. Short Single Repeat (SSR) Microsatellite loci are highly informative genetic markers useful for population genetic studies, linkage mapping and parentage determination. Methods to identify novel microsatellite loci commonly use subtractive hybridization to enrich small-insert genomic libraries for repeat sequences. We have developed a method that allows highly efficient ligation to genomic DNA and improves recovery of sequences after subtractive hybridization to biotinylated oligos. The method improves current repeat-enrichment strategies, resulting in representative small-insert libraries with a very high proportion of positive clones. The effectiveness of genetic marker associated to determining three different chemotypes in *Cannabis* was evaluated and discussed, as possible method in marker-assisted breeding of *Cannabis* in the pharmaceutical field.

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* 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 labiatae 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 Salvianal 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 that allows highly efficient ligation to genomic DNA and improves recovery of sequences after subtractive hybridization to biotinylated oligos. The method improves current repeat-enrichment strategies, resulting in representative small-insert libraries with a very high proportion of positive clones. The effectiveness of 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...

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.

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 plant in Fabaceae. Now we have completed 86 species of medicinal plants in Fabaceae including over 30 genera. Through 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), Nature, 434: 1067. [2] Kress WJ, Erickson DL. (2007) Plos One, 2: 508. [3] Kress WJ, Erickson DL. (2008) Proceedings of the National Academy of Sciences, USA, 105: 2761–2762. [4] Frézel L, Leblois R (2008) Infection, Genetics and Evolution, 8: 727–736. [5] Yao H, et al. (2008), Planta Medica (Accepted). [6] Lahaye R, et al. (2007) PNAS, 1–6.

Genetic and Metabolic Studies of Cannabinoids in Standardized Medicinal Cannabis sativa Muntendam R1, Erkelens T2, Kayser O1
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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-(BFA65035), 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 THCA 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] Sirikantarams 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.

Profiling 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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“Damiana” is used traditionally as stimulant, aphrodisiac, nerve tonic, diuretic, laxative, and for kidney, menstrual and pregnancy disorders [1]. The ancient Mayans used it to treat giddiness and loss of balance [2] while the Mexican Indians made a beverage for its reputed aphrodisiac properties [3]. Though “damiana” has a long history of usage, confusion over its precise identity and nomenclature still exists. According to British Herbal Pharmacopoeia (1996) “Damiana folium” consists of dried leaves of Turnera diffusa Willd. Ex Schults. var. aphrodisica and related species. Beside “false damiana” are often used as substitutes for damiana. The name “false damiana” is referred to both T. ulmifolia (Turneraceae) as well as for Aploppus discoides DC (Asteraceae) [4]. We observed that existing studies were not opportune and dependable in providing the exact identity of T. diffusa and discriminating it from the known “false damiana” species. In the present study we have provided taxonomic account on Turnera diffusa and furnished easy and reliable method to authenticate T. diffusa and to detect its possible substitute’s using morphological and micro-morphological characteristics, with the aid of light, fluorescent and scanning electron microscopy. For the first time HPTLC, and UPLC comparative analysis was financially supported by National Natural Science Foundation of China (20572130, 30600807). References: [1] Liu AH, et al. (2006) J Pharm Biomed Anal, 41: 48–56. [2] Chen H, et al. (1999) J Ind Microbio Biotechnol, 22: 133–138.

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 depend 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 cagunias-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

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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

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along with details on its distribution and nomenclature. 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. We would like to thank Dr. Aparna Waive and Dr. Gaikwari, from Hi-Tech Bio India, for providing authenticated plant material. References: [1] Kurian R, et al. (2007) Appetite 48: 338–344.

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

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The NC Arboretum Medicinal Germplasm Facility 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 ISOA 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 extraordinarily rich bioregion. The collections will be suitable for a wide variety of research purposes including but not limited to analysis of metabolites of interest for pharmaceutical purposes, cultivar breeding studies, and genetic population analysis.

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

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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 ext situ collections for future studies; 5) es-

Table 1 Minimal inhibitory concentration (MIC) µg/mL of water extract and fractions of S. adstringens against two strains of Trichophyton rubrum. (Standard Deviation 6.78).

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Water extract</th>
<th>Fraction I</th>
<th>Fraction II</th>
<th>Fraction III</th>
<th>Fluconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYA-3108</td>
<td>156</td>
<td>1250</td>
<td>1250</td>
<td>1250</td>
<td>75</td>
</tr>
<tr>
<td>TruMDR2</td>
<td>156</td>
<td>312</td>
<td>625</td>
<td>1250</td>
<td>7575</td>
</tr>
</tbody>
</table>

Antifungal Activity of Stryphnodendron adstringens (Mart.) Extracts

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Stryphnodendron adstringens (Mart.) Coville, is a medicinal plant that belongs to Mimosoideae. It is also known as barbatimão. Its aqueous extract has anti-inflammatory and antimicrobial properties [1]. This study was conducted to evaluate the phenols and tannins content and the antifungal activity of the aqueous extract against Trichophyton rubrum. A clinical isolate of T. rubrum (ATCC-MYA3108) was obtained from a patient admitted to the University Hospital of Ribeirão Preto University, SP, Brazil. The mutant strain TruMDR2 was obtained from the disruption of the TruMDR2 gene from isolate MYA3108. Phenol concentrations were determined by colorimetric method and the antifungal potential was determined in bioassays measuring the minimal inhibitory concentration (MIC). The antifungal activity of the extracts was confirmed against T. rubrum. The aqueous extract S. adstringens contains phens and tannins and showed a minimal inhibitory concentration (MIC) of 156 µg/mL for both isolates of T. rubrum (Table 1), as compared to fluconazole at 75 µg/mL. The fractions were less active than the whole extract suggesting that the activity is related to possible interactions of compounds not due to a specific metabolite, as mentioned by Bezerra et al. [2]. References: [1] Souza C, Felfili J, (2006) Acta Botanica Brasílica, 20: 135–142. [2] Bezerra JCB, et al. (2002) Fitoterapia, 73: 428–430.
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 species), were carried out for comparative identification, authentication, chemo-microscopy, quantitative microscopy and phytochemical profiles that could be incorporated into their monographs in the proposed Nigerian Herbal Pharmacopoeia (NHP). The results showed that both Aloe species possessed many similarities in epidermal characteristics with the ranunculaceous stomata that is more abundant in A. schweinfurthii. The TS of A. vera is clearly distinguished from A. schweinfurthii with the presence of calcium oxalate and raphides. Physical evaluation points out that the total ash value of the dried leaf, acid insoluble ash, water soluble ash, water soluble extractive and alcohol soluble extractive values of A. schweinfurthii are greater than that of A. vera. General phytochemical analysis of the methanolic extracts of both Aloe species revealed similarities in the presence of free and combined anthraquinones, starch, flavonoids, steroidal and phenolic compounds.

Plants are a potential source of antimicrobial compounds. In this research, a plant from the family Cucurbitaceae was studied. Momordica foetida Schum. 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 hexane, 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 phytochemical investigation resulted into isolation of four curcurbitane triterpenoids and flavonoids compounds from chloroform and ethyl acetate fractions respectively. The chemical structures of the isolated compounds were established through UV, IR, MS, 1H, 13C, COSY and 2D NMR spectroscopic data. Antimicrobial investigations were carried out on the isolated compounds against 25 bacterial strains of which \(38.7\%\)-dihydroxy-cucurbita-5,23,25-trien-19-al followed by Kaempferol-3-\(\beta\)-D-glucopyranoside displayed minimum inhibitory concentration (MIC) values for 25 bacterial strains ranging from 7.8 to 250 \(\mu\)g mL\(^{-1}\). Acknowledgement: We are grateful to the National Research Foundation and University of Zululand, South Africa for financial support. References: [1] Hakizimungu E, et al. (1992) J Ethnopharmacology 36: 143–146. [2] Rwangabo PC, (1993) La medicine traditionnelle au Rwanda. Edition Karthala and ACCT, Paris, France.
The essential oil was obtained by hydrodistillation, which was analyzed both by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Main constituents were identified as 1,8-cineole (44%), camphor (12%), α-pinene (6%), β-pinene (6%), camphene (5%), and myrcene (3%). Using the in vivo CAM (Chorio Allantoic Membrane) assay the Salvia essential oil and its main constituents (0.5–100 μg/pellet) as well as in vitro cytotoxicity (MTT), cell migration and tube formation tests (HUV-EC-C cell lines) of the essential oil (0.01–200 μM) in comparison with standards such as suramin, thalidomide, cortisone were investigated for their antiangiogenic properties. As a result, S. triloba essential oil showed in both tests antiangiogenic activity in a dose dependent manner. Acknowledgements: TUBITAK SBAG–1075282 (3756) for financial support. References: [1] Demirci B, et al. (2005) Pharmaceutical Biology 43: 666–671. [2] Kintzios SE (2000) Sage: The Genus Salvia. Series No. 14, Medicinal & Aromatic Plants. Abington, Gordon and Breach, Harwood Academic Publishers.

Insecticidal Activities and Composition of Essential Oils from the Medicinal Plant Garden at the National Center for Natural Products Research

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Plant-derived natural products are used world wide as biologically active pharmaceuticals and agrochemicals. Because of the necessity of finding safer insecticides in combination with the need of preventing environmental degradation and pollution, there is increasing interest in the use of plants as insecticides and insect feeding deterrents. In screening for new natural product-based insecticides, 12 different plant essential oils were tested for larvicidal activity against Aedes aegypti and insecticidal activity against azalea lace bugs, Stephanitis pyrioides. Study samples were obtained from the cultivated collection at the Medicinal Plant Garden at the NCNPR. Harvested samples were air-dried and processed to preserve volatile oils. All samples were subjected to water distillation using Clevenger-type apparatus to obtain essential oils. Twelve essential oils belonging to six families were analyzed by gas chromatography and gas chromatography-mass spectrometry techniques. Hydrocarbons and oxygenated derivatives of terpenoids, aldehydes, and phenylpropanoids comprised the volatile compounds in these essential oils. Artemisia annua essential oil resulted in 100% mortality at 30 ppm to 1st instar larvae of Ae. aegypti. Twelve oils tested at 1% concentrations exhibited 21–86% mortality against S. pyrioides. Detailed insecticidal results will be presented.
Bioactivity of 54 Essential Oil Extracts Topically Applied to Adult Azalea Lacebugs Stephanitis pyrioides (Scott) [Tingidae: Hemiptera]: A Rapid Bio-Pesticide Discovery Program

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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 occurring in 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 pyrioides (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-infeasting brood. Cleverly 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% dimethylosulfoxide (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 10,000 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 probits [1] and preliminary data show that 1% emulsions derived from oil of Pelerigonium (94.5% bug-mortality), Cinnamomum (91.4%), Hedychium (85.9%) and Tagetes (81.8%) were more efficacious than the malathion-DMSO emulsions (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 plant material and extracts: Zhe D. Khan (USA), K. Hussain Can Basier (Turkey), Reuter Demirci (Turkey), Gulmira Ozek (Turkey), Aruna Weerasooriya (USA), Zengping Gao (China), Sui Zhang (China), Angelica pubescens (Dahurian Angelica), A. dahurica, A. pubescens (Umbelliferae) were fragmented and hydrodistilled to obtain the volatile compounds, and were then identified using gas chromatography and gas chromatography-mass spectrometry. Main Angelica oil constituents were found as follows: A. dahurica: 46.3% a-pinene, 9.3% sabinen, 5.5% myrcene, 5.2% do-decanol and 4.9% terpinen-4-ol and A. pubescens: 37.6% a-pinene, 11.6% p-cymene, 8.7% limonene and 6.7% cryptone. Angelica essential oils were examined for antimarial, antimicrobial, antifungal and insecticidal activity. Antifungal activity of the essential oils from both Angelica species was non-selective at inhibiting growth and development of reproductive stroma of the plant pathogens Colletotrichum acutatum and Phytophthora cinnamomi. Angelica pubescens oil resulted in 40% mortality at 62.5 ppm to 1st instar larvae of Aedes aegypti at 24 h. Angelica dahurica oil at 1% concentration exhibited an 86.67% mortality in laboratory bioassays with azalea lace bugs, Stephanitis pyrioides, in comparison with A. pubescens oil at 44.0%. References: [1] The Pharmacopoeia Commission of P.R. China (2005) The Pharmacopoeia of P. R. China, 1: 69 and 185. [2] Wang YS (1983) The Pharmacology and Application of Chinese Medicine, People’s Medical Publishing House, Beijing.

The Chemical Composition and Biological Activities of Notopterygium incisum and Notopterygium forbesii Essential Oils from China

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Roots and rhizomes of Notopterygium incisum and Notopterygium forbesii (Apiaceae) are popular in China for use as Traditional Chinese Medicines. Qiang huo is the Chinese name for the root of Notopterygium species. Historically, Notopterygium 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 N. incisum and N. forbesii rhizomes. Water distilled essential oils were analyzed by GC-FID and GC-MS and evaluated for antimalarial 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 Notopterygium essential oils were 26.5–42.6% a-pinene, 13.3–28.0% β-pinene and 4.5–8.9% limonene. Notopterygium oils showed no antimicrobial activity against human pathogenic bacteria or fungi, nor antimalarial activity against Plasmodium falciparum. Notopterygium oils demonstrated non-selective antifungal activity against the plant pathogens Colletotrichum acutatum, C. frugareae, and C. gloeosporioides. Notopterygium forbesii root oil produced 60% mortality to 1st instar larvae of Ae. Aegypti at 15.625 ppm. Notopterygium oils also showed weak insecticidal activity against Stephanitis pyrioides, with 1% concentrations exhibiting 33.33–64.00% mortality. References: [1] Fuquan 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′-dihydroxyflavone (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.

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Flavonol Glycosides from the Flowering Plant Gaura biennis

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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-glucuronide (9) (Table 1).

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C-2,3 dihydro

Flavonoid Glycosides from Sutherlandia frutescens

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Sutherlandia frutescens (L.) R. Br (Fabaceae) is a well-known multi-purpose 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, along with the previously isolated triterpene glycosides, have been elucidated by chemical and spectroscopic methods as quercetin 3-O-β-D-xlylopyranosyl[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-xlylopyranosyl[1→2]-[6-O-(3-hydroxy-3-methylglutaroyl)]-β-D-glucopyranoside (3), 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 5U1AT003264 and the USDA Agricultural Research Service Specific Cooperative Agreement No.58-6408-2-0009. References: [1] Fu X, et al. (2008) J Nat Prod, 71: 1749–53.


Scutellaria lateriflora L. (skullcap) is native to North America, but now widely cultivated in Europe and other areas of the world. It has been used for over two hundred years as an effective therapy for anxiety, nervous tension, and convulsions [1]. In America, skullcap is regulated as a dietary supplement and has been classified as an “Herb of Undefined Safety” by the FDA. Despite its extensive use, little data exist regarding the chemical constituents of Scutellaria lateriflora. To provide the scientific support for the uses of this plant, a systematical chemical study has been conducted. Two new dihydropyranocoumarins, named scuteflorins A and B, together with the known compounds, decursin, chrysin, oxoroxin A, wogonin, 5,7-dihydroxy-2’-8-dimethoxyflavone, dihydrochrysin, dihydroxoryxin A, lupenol, 3x,24-dihydroxy-olean-12-en-28-oic acid, 3β,19α-dihydroxy-urs-12-en-28-oic acid, ursolic acid, β-sitosterol, daucosterol, palmitic acid, a mixture of arachidic acid, behenic acid and lignoceric acid in a ratio of 2:1:0.3, and a mixture of 1-triactanol and 1-dotriactanol in a ratio of 2:1, were isolated from the aerial parts of this plant. Their structures were established by means of extensive 1D and 2D NMR spectra as well as HRMS data. The absolute configuration of dihydropyranocoumarins was determined by a comparison of the experimental and theoretical CD spectra. All the compounds except for wogonin and chrysin are reported for the first time from this plant. Acknowledgement: This work is funded in part by the Food Drug Administration contract “Botanical Dietary Supplement: Science-Base for Authentication” FD-U-002071-07. Authors are thankful to Dr. Vaishali Joshi for the authentication of plant material. References: [1] Foster S, (1996), The Business of Herbs, May/June, p.14–16.
New Terpenoids from *Pfaffia paniculata* Kuntze

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*Pfaffia* (Amaranthaceae) has around ninety species in Central and South American, of which *Pfaffia paniculata* Kuntze (commonly called suma), is the most employed species in commercial preparations in Brazil as “Brazilian ginseng” and has been commonly used for three centuries for the same indications as American and Asian ginseng [1,2]. It is also known as “Para Toda” which means “for all things” since the root of this plant has been used by native Brazilians as a tonic, aphrodisiac, and as a remedy for many types of illnesses, such as diabetes, ulcers, cancer etc [3]. Phytosterols (mainly β-ecdysone), pfaffic acid (hexacyclic nortriterpene) and their glycosides, named pfaffosides A–F (saponins), have been reported from *P. paniculata* [4–7]. The saponins have demonstrated the ability to inhibit the growth of cultured tumor cell melanomas in vitro [6,7]. These saponins and pfaffic acid derivatives were patented as anti-tumor compounds in several Japanese patents in the mid-1980s [9,10]. In the present study, a detailed phytochemical investigation of *P. paniculata* was carried out. Two new nortriterpenoids pfaffine A and B, one monoterpene glycoside pfaffine C, along with the known compounds, ecdysone, 20-hydroxyecdysone, pterosterone, rapisterone, pfaffic acid, pfameric acid, mesembryanthemoidigenic acid, Calenduloside E 6′-methyl ester, oleanolic acid 28-O-β-D-glucopyranoside were isolated from the roots of this plant. Their structures were determined through the extensive analysis of 1D- (1H, 13C, DEPT) and 2D-NMR (COSY, HSQC, HMBC, NOESY) spectra, as well as chemical methods. **Acknowledgement:** This work is funded in part by the Food Drug Administration contract “Botanical Dietary Supplement: Science-Base for Authentication” FD-U-002071-07. Authors are thankful to Dr. Vaishali Joshi for the authentication of plant material. **References:** [1] Vasconcelos JMO (1982), Estudo taxonomico sobre Amaranthaceae no RS, Brasil. Porto Alegre, 151 p. [2] Taniguchi SF, et al. (1997), Phytother. Res., 11: 568–571. [3] Oliveira F, (1986), Revista Brasileira de Farmacognosia, 1: 86–92. [4] Wakunaga Pharmaceutical Co., Ltd., Japan (1984), Jpn. Kokai Tokkyo Koho., 5 pp. [5] Takemoto T, et al. (1983), Tetrahedron Letters, 24, 1057–60. [6] Nishimoto N, et al. (1984), Phytochemistry, 23: 139–42. [7] Nakai S, et al. (1984), Phytochemistry 23: 1703–1705. [8] Oshima M, Gu Y, (2003), Journal of Reproduction and Development, 49: 175–180. [9] Takemoto T, Odajima T, (1984), Jpn. Kokai Tokkyo Koho., 7 pp. [10] Takemoto T, Odajima T, (1984), Jpn. Kokai Tokkyo Koho., 11 pp.

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Application of NMR-Based Metabolomics in Assessment of Botanicals

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Metabolomics is increasingly being used in a broad range of sciences including systems biology, drug discovery, molecular and cell biology and other medical and agricultural sciences [1,2]. The metabolomic analyses of *Hoodia* (*Hoodia gordonii*), *Maca* (*Lepidium meyenii* Walp.) and *Ginkgo* (*Ginkgo biloba*), as well as their products, were performed using 1H-NMR spectroscopy and multivariate statistical analysis. The different extraction conditions for sam-

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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 [4-7]. The work was supported by the United States Food and Drug Administration (FDA) Specific Cooperative Agreement No. U01 FD 002071-07. References: [1] Lindon JC, et al. (2006), Pharm Res, 23(6): 1075–1088. [2] Hollywood K, et al. (2006), Proteomics, 6: 4716–4723.

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, dysmenorrhea, 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.

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. chebula...
C. asiatica 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 olaanic acid-derived triterpenes. Their 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 Apiaceae. It has been widely cultivated in China, Southeast Asia, India, Sri Lanka and Africa as a 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-dihydroxyurs-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), Betuluboside A (7), 3-oxo-a-angeloyl-9-β-D-glucopyranoside, (8), vomifoliol-9-β-D-glucopyranoside (roseoside) (9), 1,8-heptadecadiene-4,6-diyne-3,10-diol (10), (2S)-1-0-stearoyl-2-O-stearoyl-3-0-[α-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl] glycerol (11)/(2S)-1-0-linolenyl-2-O-linolenyl-3-0-[α-D-galactopyranosyl-(1→6)-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. Acknowledgement:

Table 1  Validation Parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AA</th>
<th>MA</th>
<th>AS</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Linearly range (ng/spot)</td>
<td>200–600</td>
<td>200–600</td>
<td>100–500</td>
<td>100–500</td>
</tr>
<tr>
<td>2 Correlation coefficient</td>
<td>0.999</td>
<td>0.998</td>
<td>0.997</td>
<td>0.998</td>
</tr>
<tr>
<td>4 LOD (ng/spot)</td>
<td>30</td>
<td>60</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>5 LOQ (ng/spot)</td>
<td>180</td>
<td>200</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>6 Specificity (ng/spot)</td>
<td>Specific</td>
<td>Specific</td>
<td>Specific</td>
<td>Specific</td>
</tr>
<tr>
<td>7 Regression equation</td>
<td>$Y = 94.580 + 8.961X$</td>
<td>$Y = 61.937 + 3.124X$</td>
<td>$Y = 22.600 + 0.495X$</td>
<td>$Y = 12.773 + 0.113X$</td>
</tr>
<tr>
<td>8 Rf</td>
<td>0.72</td>
<td>0.61</td>
<td>0.17</td>
<td>0.11</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>AA</th>
<th>MA</th>
<th>AS</th>
<th>MS</th>
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</thead>
<tbody>
<tr>
<td>C. asiatica</td>
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<td>0.2</td>
<td>3.6</td>
<td>2.0</td>
</tr>
<tr>
<td>C. erecta</td>
<td>0.1</td>
<td>0.1</td>
<td>4.5</td>
<td>3.1</td>
</tr>
</tbody>
</table>

**Coumarins and Triterpenoids from Ludwigia hyssopifolia L.**

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*Ludwigia hyssopifolia* Linn. (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 of 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.


**Shikimic Acid as a Marker Compound from Ludwigia alternifolia L.**

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*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 elucida-

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**Fig. 1**: (A) *Centella asiatica*, (B) Standard mix, (C) *Centella erecta*.
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 I 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 identity chemical and/or biomarkers of dietary supplements we have synthesized a series of psoralens for biological evaluation.

Cissus a genus of approximately 350 species of a woody climber (Family: Vitaceae) includes Cissus quadrangularis (Veldt grape, winged treebine) which is often used as a medicinal plant. A genus of approximately 350 species of a woody climber Cissus (Family: Vitaceae) includes C. quadrangularis (Linn) which is often used as a medicinal plant. C. quadrangularis (Veldt grape, winged treebine) which is often used as a medicinal plant. Com.

A new potent antifungal 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 and 7) 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 neoforms, Aspergillus fumigatus, methicillin-resistant Staphylococcus aureus, and methicillin-sensitive Staphylococcus aureus, and Markable fungicidal activity of 1 was tested against S. aureus, methicillin-resistant C. neoformans, Aspergillus fumigatus potent in vitro. Compounds 1, 2, and 3, were found to be more active than amphotericin B against chloroquine sensitive (D6) and chloroquine resistant (W2) antimalarial activity with an ED50 value of ~2 mg/Kg/day/ip vivo. Compounds 1, 2, and 3 were also found to be more active than amphotericin B against C. neoformans, A. fumigatus with ~76% of ~76% of C. neoformans activity at 0.0625 mg/Kg/day/ip for 5 days in a murine model of infection.


P-48

**Indolizidine, Antifungal and Antiparasitic Compounds from Prosopis glandulosa Torr. var. glandulosa**

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A new potent antifungal 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 and 7) 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 neoforms, Aspergillus fumigatus, methicillin-resistant Staphylococcus aureus and Mycobacterium intracellulare. The remarkable fungicidal activity of 1–4 against C. neoforms and 2, 3, and 5 against A. fumigatus were similar to amphotericin B, but > 2–4-fold more potent than 6–8. Prospolisinone (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. neoforms 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. Prospolisinone (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.
Chemical Constituents of Postia balsamea
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2 National Center for Natural Products Research,
Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, MS 38677


Biosynthesis of Salvinorin A: Overexpression and Biochemical Characterization of Carboxy Methyltransferase from EST of Salvia divinorum Glands
Kutrzeba LM1, Zjawiony JK1,2, Koo HY1, McDowell E1,2
1 Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677, USA
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Abuse of unregulated substances by young adults has been a great concern of the US and international community. The active component of Salvia divinorum, salvinorin A (1) has a potent affinity to kappa opioid receptor in CNS. We studied the biosynthesis of a diterpenoid through the isolation of RNA and construction of cDNA library. Sequencing of the genetic material resulted in building an EST library containing all genes involved in biosynthetic assembly of 1. We then cloned and overexpressed carboxy methyltransferase (CMT) gene in Escherichia coli to determine the substrate for the enzyme, and biochemically characterize it. We have employed 14C-SAM, and five different substrates to test for the CMT activity in the cell free assay. We observed methylation of C-18 carboxylic group in divinatorin A, divinatorin C and hardwickiic acid, but not in highly oxygenated substrates like salvinorin A and B acids. This strongly suggests that CMT is substrate specific and that it is involved in the early stage of the pathway. Methyl esters of those substrates were independently synthesized to determine the products of the enzymatic reaction. Future work will involve purification of the enzyme and determination of KM and KCAT.

Free Energy Calculations on the Binding of Natural Latrunculins and Semi-synthetic Derivatives to G-Actin
Doro PR1, Odde S1, Hamann MT2,3, Doerksen RJ1,3
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2 Department of Pharmacognosy, School of Pharmacy, University of Mississippi, MS 38677
3 National Center for Natural Products Research, School of Pharmacy, University of Mississippi, MS 38677

Latrunculins are significant biological molecules isolated from Neogombata 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 analogs 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 oxalatrunculin 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 oxalatrunculin B and semi-synthetic analogs, reduced inhibition which should be beneficial for avoiding general toxicity.
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. cirrhosa L. afforded the lignan lariciresinol (1), the flavanone 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. cirrhosa 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 antileishmanial 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.

Peppermint (Mentha x piperita L., Lamiaceae) is widely cultivated for the essential oil used worldwide in the confectionary 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, Capilcowax 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 PM01 (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 pulegone 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%).

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 and the seed 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-INNOWax 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.
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 decomposition in humans and marine life. These industrially produced compounds, 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.

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 carbamate 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 characterization 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.

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Fig. 1

From HPLC to ultra-fast HPLC, the analytical time was greatly reduced to 1/4


**P-60**

**1H-NMR Method Validation for Quantitative Analysis of Aloe Products**

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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: acetyle 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 1H NMR method was able to quantify the amount of acetyle 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.

**P-61**

**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, phthalides, and phenolic 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.

**P-62**

**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


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and neutral compounds in the plant extract, thereby reducing the matrix effect and improving the MS detection sensitivity. The sample recoveries on Oasis MCX cartridges were found to be > 80%. The analysis of steroidal alkaloids was carried out by RRLC/TOF-MS. The use of RRLC can shorten analytical time and improve chromatographic resolution, and TOF-MS provides abundant structure information by accurate mass measurements for each molecular ion and fragment ions at different fragmentor voltage. As a result, the SPE-RRLC/TOF-MS was successfully used for simultaneous determination of 26 steroidal alkaloids in different *Fritillaria* species in a single run within 18 min (Fig. 1), which is 5-times faster than conventional HPLC/TOF-MS method [1].

**Acknowledgements:** Financial support for this research from the cultivation fund of the key scientific and technical innovation project, Ministry of Education of China (No. 2004–295).


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**Determination of Terpene Lactones in Ginkgo Biloba Using Liquid Chromatography-Electrospray Tandem Mass Spectrometry**

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**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 and difficult HPLC separation to eliminate interferences and to resolve all analytes even though their concentrations are high in ginkgo extracts. In this study, we developed and validated a sensitive, accurate and reliable assay method for determination of terpene lactones in ginkgo products using HPLC-electrospray tandem mass spectrometry (LCMS/MS) technique, which minimized the requirements of major sample cleanup and chromatographic resolution. The validation of the method showed that the analyte recoveries are in the range of 90–110%, and the relative standard deviations are less than 10% for all five analytes, ginkgolide A, B, C, J and bilobalide. References: [1] Yongkai S, et al. (2005) J Mass Spectrom, 40: 373–379.
The roots of *Cauphyllum 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 *Cauphyllum 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-ESLD and HPTLC 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-MSD-TOF.

**Fig. 1** HPLC (A, B) and UPLC (C, D) chromatograms of a mixture of standard (A, C), and roots of blue cohosh (B, D).

**Fig. 2** Comparison of blue cohosh with dietary products by HPTLC method. Tracks: 1–3, 7, 8; dietary supplements, 5, standard mix-8; 4 & 6, roots of blue cohosh under visible light (Saponins (A)) and at 366 nm (Magnoflorine) (B).

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and caraumbellogenin (S) have been quantitatively identified in the plant extracts. The limit of detection (LOD), and limit of quantitation (LOQ) were in the range from 1–5 µg/mL, and 3–15 µg/mL for compounds 1–5, respectively. This method also provides a distinction between the chromatographic profiles of Caralluma, Hoodia, and Opuntia spp., and thus can be aptly employed to distinguish between these plant materials or the botanical products thereof. In the ES positive ion mode, the [M+Na]+ ions at m/z 373.23, 679.33, 841.41, 517.27 and 355.22 were observed for compounds 1–5.


The rhizomes of turmeric (Curcuma longa L., Zingiberaceae) play an important role as a coloring agent in foods, cosmetics and textiles [1]. The 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. phaeacaulis, 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. 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 Dr. Aruna Weerasooriya, University of Mississippi for providing the plant samples and Annette Ford, University of Mississippi for extraction of samples. References: [1] Sekar N, (2004), Colourage, 51: 59–60. [2] Ammon HTP, Wahl MA, (1991), Planta Med, 57: 1–7. [3] Radha KM, et al. (2006), Life Sci, 78: 2081–2087.
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-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, palmatine, cotisine, 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 palmatine, cotisine 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+H]⁺ ions for cotisine, jatrorrhizine, palmatine and berberine, [M+H]+ ions for hydrastine and canadine, [M+H+18]+ ions for hydrastinine in the positive ion mode with selective ion recording (SIR).

**Fig. 1** UPLC Chromatograms of a mixture of standards (A), roots of goldenseal (B) and dietary supplements (C–D) at 290 nm. 1 hydrastine, 2 hydrastine, 3 cotisine, 4 jatrorrhizine, 5 canadine, 6 palmatine 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 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 hydrodynamic mode for Se and Fe, and He mode for As, Cr, Cu, Ni, Cd to remove spectral interferences. The method was validated for linear- ity, 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. Acknow- ledgegements: This research is funded in part by “Science Based Authe- ntitication 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.

Radix of *Pueraria* spp. is a popular traditional Chinese medicine. Kudzu has been traditionally used in China to treat diabetes, alco- holism, gastroenteritis (inflamed stomach or intestine), and has shown to have cardiovascular, neurological, anti-oxidant properties [1,2]. Kudzu (*Pueraria lobata*, Family Fabaceae) is a rich source of isoflavones and isoflavone glycosides, which include puerarin, daidzin, genistin, genistein, daidzein, and daidzein-4', 7-digluco- side. Puerarin and daidzin were the major isoflavone glucosides in kudzu root in comparison with kudzu leaf. LC-MS-TOF and MS-MS tools have been employed for profiling and characterization of isoflavones and isoflavone glycosides including distinction between flavonoid O- and C-glycosides. The mass spectrum of O-gly- coside is generally characterized by the presence of an abundant fragment ion resulting from (terminal) glycosyl cleavage and the aglycone moiety of C-glycoside was not produced. Thus puerarin ([m/z = 416.10]) and daidzin ([m/z = 416.10]) are readily distinguished. These two glucosides with [M-H]+ at m/z 415.10 and [M+H]+ at m/z...
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.

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-

Fig. 1 Base peak chromatograms of leaves and roots of Pueraria lobata at positive and negative mode of ionization.
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.

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**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).

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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-MS-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.

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 653.4 and sodiated species [M+Na]+ at \(m/z\) 763.2, 763.2, 747.2, 747.2, 747.2, 747.2, 725.2 and 653.4.

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 ELS 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 [3-8] 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 by 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 fucxanthin and dietary supplements. This method involved the use of [M+H]+ ions in the positive ion mode with single ion recording (SIR).

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**References:**


**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 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-MSn) coupled with an ion-trap analyzer. Fragments C11H12NO2+ are dominant or major products ions in hydrastinine and β-hydrastine, respectively. The C-ring is relative 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 C10H16NO2+. 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.

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Fig. 1  HPLC-UV, UPLC-UV and UPLC-MS chromatograms of fucoxanthin standard (A), and extracts (B–C) at 449 nm.

Fig. 1  Fragmentation Pattern Proposed for M+ Ions of Palmatine.
Heimia salicifolia (Lythraceae), also known as sun opener or shrub-by 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.

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^n) 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 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] 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 logarithmic 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 Marsilea quadrifolia 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 Marsilea 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

Fig. 1 HPLC-ELSD chromatograms of standards (A) and extracts of L. barbarum L. (B–C), and typical ESI-MS/MS spectra of analytes (D–F).
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 vivo and in vitro 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.

**P-82**

In Vivo and In Vitro Evidence for Genotoxicity of Acrylamide

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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 vivo and in vitro 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.

**P-83**

Localization of NAD⁺ Synthase Enzymes in the Pathogenic Yeast Candida glabrata

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*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), nicotinic 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-terminal of the Npt1, Qns1, 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.

**P-84**

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

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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.

**P-85**

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

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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 larvae insect. It also provides a gateway to use other alkaloids in temporal and dose-response experiments as a possible means of biocontrol.
Carbohydrate Specificity of the Oyster (Crassostrea virginica) Galectin CvGal: Recombinant Expression and Characterization of Selected Carbohydrate Recognition Domains

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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 Perkinsus marinus causes “Dermo” 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 in oyster hemocytes a galectin (CvGal) with a unique carbohydrate-recognition domain (CRD) organization that, unlike most mammalian galectins, recognizes exogenous carbohydrate ligands [1]. CvGal binds to a variety of potential microbial pathogens, pythplankton 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 specificities, 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 rigorous analysis of their binding specificity and affinity. We indicated that functions 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 Vasta GR, (2007), J Immunol, 179: 3086–3098.

Teratogenic Effects of Blue Cohosh is Mediated through GATA2 Signaling Pathway

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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, iro3, 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 transcription 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.

Puerarin Attenuates Ethanol Toxicity in Medaka (Oryzias Latipes) Embryos During Development

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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 fetuses is required for the treatment of Fetal Alcohol Spectrum Disorder (FASD), a neurobehavioural 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. Medicinal RP was collected from 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 days 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 hypophysial plate. It was observed that ethanol was able to reduce the growth of all major skeletal features, however, RP or puerarin alone has no effect. When the embryos were treated together with ethanol and RP or puerarin, ethanol-induced skeletal growth reductions were attenuated significantly 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 58-6048-2-0009, 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 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.

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 pipericdine type alkaloid), O-acetylbaptifolin, anagyrine, and lupanine (lysin derived alkaloids) inhibited these enzymes to various extents (IC50 2.5–50 μM). N-methylcysteine 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. 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 × 10−3 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 × 10−3 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/NCRRR).
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.


Fig. 1 Effect of temperature on stability of THC-HS: RAMEB complex.

Fig. 2 Effect of RAMEB on permeability of THC-HS.

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 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.

Immulina is a commercial extract of *Spirulina* (Arthrospira) platensis that is standardized by biological activity. 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 = 0.0024) after Immulina supplementation. Acknowledgements: This research was partially funded by a USDA, Agricultural Research Service Specific Cooperative Agreement No. 58-6408-012. 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.

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 μl. of commercially available green tea extract. After 18 hours blood samples were taken and were analyzed for plasma cholesterol 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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The southern Appalachians are home to an extraordinary variety of plant species, many of which have been used medicinally by local populations. The vast majority of these species have not been studied for their antitumor activity, constituting a significant bio-exploration opportunity. We have recently begun 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. 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. 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.

Antitumor Activity of *Arnoglossum atriplicifolium*
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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* (whole plant) 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. 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. 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.

Anti-Biofilm 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 generated increasing concern among health care professionals due to the prevalence of drug resistant strains. Identification of novel antibiotics and anti-biofilm agents for methicillin-resistant *S. aureus* (MRSA) is important to healthcare on a global scale. The aim of this study was to evaluate extracts from *Marrubium vulgare* for in vitro inhibition of planktonic growth, biofilm formation and adherence in MRSA. A broth microtiter dilution method was employed to determine the MIC after 18 hours growth using an optical density (OD600 nm) reading using a MRSA isolate (ATCC 33593). The impact of extracts of both biofilm formation and adherence was tested by growing biofilms for 40 hours, then fixing and staining with crystal violet. After washing, 10% Tween 80 was added and OD570 readings were taken. A crude ethanolic extract of the roots was the most effective at inhibiting both biofilm formation (IC50 = 32 µg/ml) and adherence (IC50 = 8 µg/ml). A significant dose-dependent response for the inhibition of both biofilm formation and adherence was evident. Acknowledgements: This work was funded by NIH/NCCAM F32AT005040 (PI: C.L. Quave).

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 settler practitioners, 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* (whole plant) as having cytotoxic activity. Numerous lipophilic fractions exhibit dose-dependent toxicity towards MCF-7 and PC-3 cells, with IC50 values as low as 20 µg/mL. The results of bioassay-guided fractionation by reverse phase C18 open column chromatography followed by reverse phase C18 HPLC will be presented as will data demonstrating that many of the frac-
Biomarker Compounds in Muscadine and their Effects on Colon Cancer Cells

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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 anthocyanin present was 3,5-diglucosidic anthocyanin 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 HCT-15, for cytotoxicity and cell cycle arrest. Cell proliferation assays and flow cytometry data showed that FAF decreased viable cell proliferation in both cell lines, and evidence of G1 arrest as compared to CE. These results indicate the bioactivity of fractions rich in flavonoids and anthocyanins may be higher than that of CE in inhibiting colon cancer cell growth.

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Withania somnifera L. has been traditionally used as a sedative and hypnotic. Withania somnifera L. is reported to have anti-carcinogenic effects in animal and cell cultures by decreasing the expression of nuclear factor-kappaB, suppressing intercellular tumor necrosis factor, and potentiating apoptotic signalling in cancerous cell lines [1]. The present study was carried out on the purification, characterization and in vitro cytotoxicity of L-asparaginase from 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 pl 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:

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Antioxidant, Analgesic and Anti-inflammatory Activities of Santalum album Linn.

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Santalum album Linn. [Family: Santalaceae] is commonly known as White sandalwood (English), Safed Chandan (Hindi) and Srigandha (Sanskrit). It is found wildly and cultivated in southern states of India. Traditionally, this plant is used in headache, fever and inflammation. The wood oil is used as diuretic, stimulant and disinfective. Sandalwood contains a volatile oil 2.5–6%. The main constituents of volatile oil are santalol, isovaleric aldehyde, santanone, santalone and tannic acid. Based upon its traditional use and chemical constituents, the wood of the plant was selected and evaluated for antioxidant, analgesic and anti-inflammatory activities. The methanolic wood extract was screened for antioxidant and free radical scavenging effects at various doses (100–500 µg/ml) by different specific in vitro methods and compared with L-Ascorbic acid and BHA. It was found that extract showed maximum antioxidant effect at 500 µg/ml. The methanolic extract of wood was also screened for induced 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.

References:
analytic 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 2004, 403. [3] The Wealth of India: A dictionary of raw materials & Industrial products, 1st supplement series (Raw Materials), Vol. 2: CI-Cy, NISCAIR, CSIR, New Delhi, 2004 255.

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Evaluation of Ethanolic Extract of Withania somnifera on Haloperidol Induced Iron 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]. Acknowledgements: Thanks go to the University Grant Commission, (UGC-SRF Fellowships) New Delhi for financial support. References: [1] Ziauddin, Met al (1996) J. of Ethnopharmacol. p. 50: 69–76. [2] Wasti, A., Chani, R., Manji, M.A. and Siddiqui, N.A., (2004) Pak. J. Med. Sci., Vol. 20 (3), p. 197–200.

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Anti-carcinoma Activity of Polyphenolic Extract of Ichnocarpus frutescens

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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 Ichnocarpus frutescens was evaluated for antitumor 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 erythroleukemia cell lines. The total phenolics content was quantified by the Folin–Ciocalteu 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. In the in vitro and in vivo anti-tumor activity of PPE from Ichnocarpus frutescens 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, (1985), 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.

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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, inflammatory marker CRP 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 weight aged also following test formulation treatment. Pre-clinical toxicity studies revealed that drug is safe and can be given for longer time.

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Neuropsycho-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
Pharmacokinetic Interaction of Ginkgo Biloba with Carbamazepine

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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 oxidative stress and memory impairment. G. biloba can 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 AUCO-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 AUCO-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), AUCO-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), AUCO-0-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.

References:

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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 calogemn 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.

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