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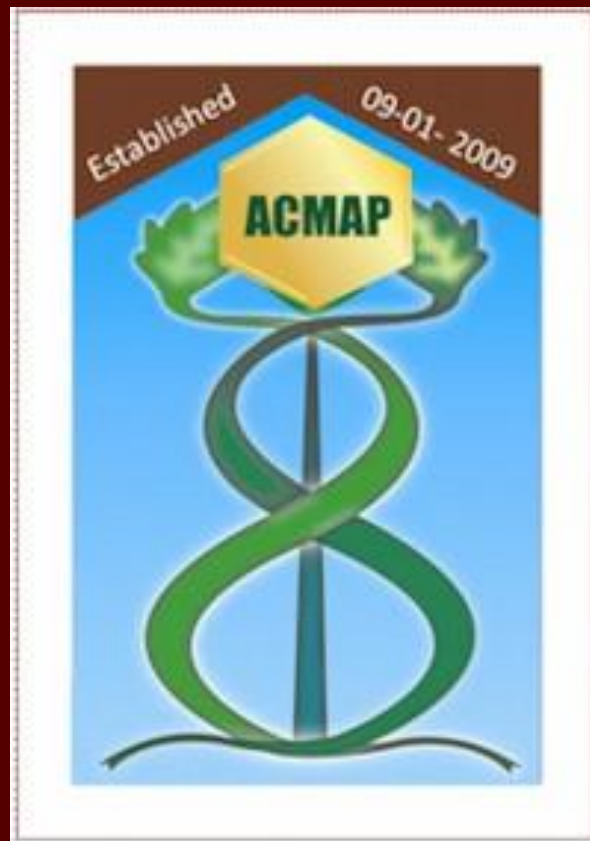
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**2<sup>ND</sup> ANNUAL CONFERENCE**  
**AMERICAN COUNCIL**  
**FOR**  
**MEDICINALLY ACTIVE PLANTS**



**July 17-20, 2011**  
**Holiday Inn – Downtown**  
**Huntsville, Alabama, USA**

## The Role of Natural Products in Cancer Chemoprevention

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Through serendipity or epidemiological observations, many dietary constituents are considered active as cancer preventive agents. Using a battery of *in vitro* assays to monitor activity, we have discovered a large variety of potential natural product chemopreventive agents, some of which have shown promise for clinical trials. One of our most notable discoveries is the structurally simple stilbene known as resveratrol. This common constituent of grapes and grape products was originally reported by us to mediate anti-inflammatory and cancer chemopreventive activity. Stimulated by this report, resveratrol is now the subject of nearly 4,000 manuscripts, and it has been entered into clinical trials for the prevention of colon cancer. More recently, we have focused on the discovery of marine microorganism-based cancer chemopreventive agents, and several interesting leads have emerged. Clearly, chemoprevention is a viable strategy in the fight against cancer. The current armamentarium of agents has resulted largely from epidemiological observations, off-shoots of cancer therapeutic agents, or agents that were used for other therapeutic indications. With concerted effort involving a range of expertise, new natural product chemopreventive agents with clinical potential are being uncovered using a systematic approach of drug discovery. (Supported by program project P01 CA48112 awarded by the National Cancer Institute)

### BIOACTIVES- HUMAN HEALTH BENEFITS

#### Immune enhancing action of plant endophytic bacterial components from *Echinacea* and other botanicals

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We have identified a melanin fraction within *Echinacea* and other plants traditionally used to enhance immune function that exhibits potent innate immune enhancing activity. Further analysis of this fraction indicated that the activity of this fraction was due to trace levels (<0.01%) of bacterial Braun-type lipoproteins, structures unique to prokaryotes. These Braun-type lipoproteins together with bacterial lipopolysaccharides (LPS) were responsible for 85 to 98% of the *in vitro* macrophage activation exhibited by extracts of *Echinacea* and other immune enhancing botanicals. Furthermore, our data indicates that endophytic bacteria are the principal source of these components. Both freshly harvested and dried *Echinacea* plant material that exhibits high activity contains total bacterial loads of  $> 10^8$  /g and our studies indicate that the total amount of endophytic bacteria within a plant is a major factor contributing to its immune enhancing activity. Our long term hypothesis is that components of these bacterial endophytes together with other plant derived compounds contribute to the clinically relevant immunomodulatory properties of *Echinacea* and other immune enhancing botanicals.

#### Inhibition of Essential Peptidyl-tRNA Hydrolase Activity by Tropical Plant Extracts

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Peptidyl-tRNA Hydrolase (Pth) is a highly conserved, essential enzyme in bacteria. It removes the peptide portion from peptidyl-tRNA. Build-up of peptidyl-tRNAs is toxic and defects in Pth function result in cell death. Herein we use *in vitro* activity of recombinant *E. coli* Pth to screen tropical plant extracts for inhibition. Multiple extracts were found to have inhibitory activity with some exhibiting different inhibitory effects depending on extraction conditions. The inhibitory compounds in these extracts may serve as lead compounds for development of novel antibacterials. Further, binding of inhibitors will be mapped to Pth using two-dimensional heteronuclear NMR spectroscopy.

### **Chemodietary agents augmenting chemosensitization of pancreatic cancer**

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Prognosis for patients diagnosed with pancreatic cancer (PaCa) remains dismal due to acquisition of de novo and acquired resistance to chemotherapy by tumor cells. This limits sensitivity to chemotherapy in the clinical management of PaCa patients. Within goal of our research strategy we report the benefit of incorporating “chemodietary agents” harboring pleiotropic effect as a rational basis to sensitize PaCa cells to chemotherapy. To this end, as “proof of principle”, we report the effect of some selective food bioactive compounds - Genistein, 3,3’ Diindolylmethane (DIM), Thymoquinone (TQ) and a novel analog of curcumin-CDF (with superior bioavailability than curcumin) in sensitizing pancreatic tumor cells to suboptimal concentrations of multiple chemotherapeutic agents resulting in significant reduction of viable cells ( $p < 0.01$ ) in a synergistic manner. Mechanistically, our findings affirm enhanced apoptosis of tumor cells thru the down-regulation of constitutive, as well as drug induced activation of NF-(B and several downstream anti-apoptotic genes. Of greater relevance, using orthotopic mouse model of pancreatic cancer, we found significant reduction in tumor size ( $p < 0.01$ ) and locoregional lymph node metastasis when the investigative agents was given in combination compared to monotherapy. As further corollary, novel analogs of some of these agents have been synthesized for evaluation in PaCa therapy.

### **Characterization of potential antidiabetic mechanisms of serviceberry (*Amelanchier alnifolia*).**

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Several plant-based remedies offer cost-effective management of diabetes. Serviceberry (*Amelanchier alnifolia*), found in Browning, MT, has been traditionally used by American Indians in the management of type 2 diabetes. The goal of this study was to validate and identify potential antidiabetic mechanisms associated with serviceberry. Aqueous and ethyl acetate extracts of serviceberry leaves, branches, and leaves with berries were tested for potential antidiabetic activities. Previously we have shown that serviceberry extracts activate AMPK, increasing glucose uptake in skeletal muscle cells and suppressing hepatic glucose production. In this study, we demonstrate that serviceberry extracts inhibit intestinal alpha glucosidase activity *in vitro*, at doses comparable to acarbose, an alpha-glucosidase inhibitor and antidiabetic drug. To further validate these findings, serviceberry extracts (50 mg/kg or 100 mg/kg) were administered orally, 60 min before an oral gavage of starch (3 g/kg) or sucrose (4g/kg) to high-fat fed obese, insulin resistant mice. Animals administered serviceberry extracts (50 and 100 mg/kg) showed a significantly lower AUC<sub>GLUCOSE</sub> versus control animals administered saline. The lower AUC<sub>GLUCOSE</sub> of serviceberry was comparable to acarbose. These studies indicate that serviceberry extracts can delay the absorption of carbohydrates, contributing to the antidiabetic properties associated with serviceberry.

## **I. BIOACTIVES- HUMAN HEALTH BENEFITS**

### Anti-cancer and Immune-modulatory mechanisms of *Scutellaria flavonoids*

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We have been studying the efficacy of a leaf extract of *Scutellaria ocmulgee* (SocL) and constituent flavonoid wogonin against malignant gliomas and breast carcinomas. SocL extract delayed the growth of F98 glioma in F344 rats, both in intracranial and subcutaneous tumor models, which was associated with an inhibition of Akt, GSK-3 $\alpha/\beta$  and NF- $\kappa$ B and NF- $\kappa$ B phosphorylation. *In vitro* Akt kinase assay demonstrated that the SocL extract or constituent flavonoid wogonin could indeed bind to Akt and inhibit its kinase activity. These studies provided the first *in vivo* evidence and mechanistic support for anti-glioma activity of *Scutellaria* flavonoids. Malignant tumors have been known to suppress immune responses via secretion of TGF- $\beta$  as well as via induction/expansion of intra-tumoral regulatory T (Treg) cells. We have observed that the relative frequency of intra-tumoral Treg cells were significantly lower in animals treated with *Scutellaria* flavonoids compared to the control group while the frequency of total intra-tumoral T cells remained unchanged. Moreover, *Scutellaria* flavonoids significantly inhibited the secretion of TGF-beta by malignant tumors *in vitro* as well as *in vivo*, suggesting that *Scutellaria* flavonoids may enhance anti-tumor immune responses via inhibition of TGF-beta and Treg activity. These results have tremendous implications in potential usage of *Scutellaria* in adjuvant therapy for malignant tumors.

### Asiatic acid is an effective alternative agent against malignant gliomas

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Malignant gliomas are one of the most devastating and incurable tumors. The poor prognosis is due to characteristics of glioma cells to proliferate uncontrollably, sustain excessive angiogenesis and invade aggressively. Additional/alternative strategies are warranted to control this deadly malignancy. In this regard, inhibiting growth and progression of gliomas through non-toxic and effective medicinal plants could be a useful strategy. One such agent suited ideally against advance gliomas is Asiatic acid (AsA), the active constituent in *Centella asiatica*. Our completed studies showed that AsA treatment results in rapid activation of apoptotic machinery leading to apoptotic death in glioma cells. AsA treatment also inhibited the migratory and invasive potential of glioma cells. Mechanistic studies revealed that AsA induces ER (endoplasmic reticulum) stress releasing excessive Ca<sup>++</sup> in the cytoplasm, which was mainly responsible for AsA's inhibitory effects on cell survival and migration/invasion. *In vivo*, AsA feeding (30 mg/kg body weight) for 5 weeks decreased the U87 xenografts volume by 54% (p $\leq$ 0.05) and strongly inhibited tumor vasculature without causing any adverse effects to animals. AsA also inhibited the angiogenesis in several *in vitro* assays. Together, these results suggest that AsA could be an effective agent against malignant glioma and recommend its clinical use.

### Chemopreventive effect of stilbenoids and blueberries on the formation of aberrant crypt foci in colon carcinogenesis model in rats

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Stilbenes are phytochemicals that naturally occur in a wide variety of plants, such as grapes and blueberries. Resveratrol and pterostilbene are well known stilbenes that exhibit antioxidant, anti-inflammation, and anticancer activities. Experiments were carried out to study the inhibitory effect against the formation of azoxymethane (AOM)-induced colonic aberrant crypt foci (ACF), preneoplastic lesions, in male F344 rats. Rats were treated with AOM twice at 6 and 7 weeks of age, and fed either control AIN-76A, whole blueberry powder, blueberry skins from pressed blueberries, pterostilbene, resveratrol, *trans*-3,5,4'-trimethoxystilbene (t-TMS), or *cis*-3,5,4'-trimethoxystilbene (c-TMS) diets for 8 weeks. Dietary administration of stilbenes and blueberries, especially pterostilbene and t-TMS, significantly reduced the number of ACF, cell proliferation, and inflammation in the colon, as shown with immunohistochemical analysis of proliferating cell nuclear antigen, inducible nitric oxide synthase, cyclooxygenase-2, cyclin D1,  $\beta$ -catenin, and phospho-p65. Pterostilbene and t-TMS were found at higher levels in the serum ( $43.1 \pm 12.9$  and  $65.1 \pm 13.1$  ng/mL, respectively) compared to those of resveratrol and c-TMS ( $25.5 \pm 5.0$  and  $14.8 \pm 4.7$  ng/mL, respectively), which could explain the higher activity of pterostilbene and t-TMS. In conclusion, stilbenes, especially pterostilbene and t-TMS, are potent chemopreventive agents for the prevention of colon cancer.

## II. BIOACTIVES- ANIMAL HEALTH BENEFITS

### Goat & Sheep Parasite Management

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The most significant health concern affecting goat production in the world is internal parasitism. Goat producers should take a multi-pronged approach to parasite control: (1) Employ husbandry and feeding practices that minimize parasite infection; (2) Use novel de-worming practices that maximize endectocide efficacy and minimize parasite resistance; (3) Identify and remove animals from pasture that do not appear to be resistant to internal parasites. Culling these animals, while keeping and breeding goats that appear to perform better in the face of parasites, will enhance a flocks' overall production on a farm where parasites are a major problem; (4) Ensure proper nutrition to help sheep naturally ward off internal parasites and recover from parasite-induced damage after de-worming. Providing good forages and meeting protein, energy, mineral and vitamin requirements are critical to maximizing flock or individual goat production and reducing dependence on de-worming agents; (5) Rotate pastures, allowing goats access to condensed tannin and/or protein rich forages; use proper pasture fertilization techniques, and properly administer effective anthelmintics in order to maximize herd production while minimizing the harmful effects of internal parasites.

### Plant secondary compounds in forages and animal self-medication

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Although forages are rich sources of primary (nutrients) and secondary (potential pharmaceuticals) compounds (PSC), increasingly recognized as important to health, welfare, and nutrition of livestock, grazing animals do not always have access to bioactive forage with the needed PSC. For instance, at appropriate concentrations, condensed tannins can reduce internal parasites, alleviate bloat, and improve nutrient utilization in ruminants; phenolic compounds can negatively impact the viability of *Escherichia coli* O157:H7, and alkaloids, terpenes, and saponins have anti-parasitic properties. Saponins and tannins inhibit methane and ammonia production in the rumen, thus improving nutrient utilization and animal growth while reducing greenhouse gas emissions. Saponins and tannins also improve fatty acid profiles, flavor, and oxidative stability in meat and milk products. The ability of herbivores to self-select these medicinal plant compounds emerges from the inherent adaptive nature of behavioral responses in living organisms. Observational and controlled studies suggest self-medicative behaviors occur not only in wild but also in domestic herbivores. Self-medicative behaviors may allow individuals to seek medicinal plant products when infected by parasites, even at times when the farm manager is unaware of the existence of parasitism in the flock. Thus, behavior of self-medicating animals may lead us to novel PSC that can improve livestock health without affecting productivity.

**The anthelmintic effect of polyphenols on a *Caenorhabditis elegans* model, and their possible common role against both cancer and parasitic nematodes.**

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Parasitic nematodes are a major problem for humans and livestock. Currently, we lack natural alternatives for failing anthelmintics. While most research on the natural control of nematodes involves condensed tannins, little is known on the anthelmintic effect of other polyphenolics. We used a *C. elegans* model to screen plant extracts rich in tannins and antioxidant activity and found that extracts containing both condensed and hydrolyzable tannins were more toxic to *C. elegans* than extracts that contained only condensed tannins, indicating stronger activity for hydrolyzable tannins. All extracts had high antioxidant activity and, because flavonoids can inhibit a multi-drug resistant (MDR) mechanism found in both parasitic nematodes and cancer cells, we hypothesize that, although their effect was not seen in vitro, they could impair the nematode MDR, a potential target for flavonoid and tannins to potentiate currently-used anthelmintic drugs in vivo. Although our results indicate a direct anthelmintic effect of tannins on nematodes, both tannins and flavonoids are potent antioxidants that can have indirect beneficial effects boosting immune system and curbing inflammation in human and animals. Thus, our results suggest that plants rich in condensed and hydrolyzable tannins, and possibly flavonoids, can be valuable natural additions to anthelmintic compounds.

### **Immunomodulatory Properties of Noni (*Morinda citrifolia*) in broiler chickens**

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Noni (*Morinda citrifolia*) is a popular medicinal plant of family Rubiaceae. Its fruit is rich in various phytochemicals and polysaccharides. Anecdotal evidence indicates that it is used in folk medicine to promote health and prevent disease. The purpose of this study was to examine the immunomodulatory properties of Noni fruit in the broiler chicken. In two experiments, different concentrations of dietary Noni were fed to day-old broiler chickens for 3 to 6 weeks. Gut tissue and blood were sampled to determine the expression of selected genes, concentrations of immunoglobulins and  $\alpha$ 1-acid glycoprotein. The results showed that dietary Noni at 6% increased the expression of Toll-like receptor (TLR)-4 and TLR-5, a chemokine, interleukin (IL)-8, IL-12, and decreased the expression of IL-6 and TLR-7. At 4%, dietary Noni stimulated the expression of TLR-3. Increased expression of TLR-3, TLR-4 and TLR-5 indicate antiviral and antibacterial activities in chickens fed Noni fruit that are further enhanced by increased expression of IL-12 and IL-8. Decreased expression of IL-6 indicates anti-inflammatory inducing properties of Noni. However, Noni did not have effects on serum and gut immunoglobulins or  $\alpha$ 1-acid glycoprotein concentrations. This study suggests immunomodulatory properties of dietary Noni in chicken via modulating genes expression in gut tissue.

### **Investigation of the sparing effects of American skullcap, *Scutellaria lateriflora* on aflatoxin-contaminated feed in broiler chickens**

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Poultry are very sensitive to the toxicity caused by aflatoxin (AF) contaminated feed. American skullcap could reduce oxidative damage and inflammation caused by AF. The objective of this research was to determine if skullcap reduces damage caused by AF fed to chickens. Each treatment group was composed of 3 cages, each containing 12 chicks. Three different groups of chicken were given feed amended with a range of doses (50, 250 and 1250 mg/kg, BW) of dried,

powdered skullcap for 42 days; 3 treatment groups consumed feed amended with the same doses of skullcap for 1 week, followed by five weeks of feed amended with the same doses of skullcap and 1.4 PPM AF. Negative and positive control groups received basal diet and basal diet amended with AF, respectively. Results showed that the highest dose of skullcap (1250 mg/kg BW) reduced ( $p < 0.05$ ) body weight after 42 days of exposure. Whereas exposure to AF alone for 42 days resulted in liver damage characterized by elevated serum levels of liver enzyme glutamate dehydrogenase (GLDH) [ $9.8 \text{ IU/L} \pm 1.99$ ] that was not observed in chicks consuming AF and the highest dose of skullcap ( $4.16 \pm 0.73$ ). The highest dose of skullcap partially protected chickens from AF-induced increased liver weight; and as the dose of skullcap increased, there was a linear ( $R^2 = 0.85$ ) decrease in the AF-induced hepatic lipidosis. The protective effect of skullcap on AF-induced liver damage could be due in part to skullcap's flavonoids decreasing AF metabolism to its toxic form by the liver.

## SESSION B:

### I. TRADITIONAL SCHOOLS OF NATURAL MEDICINE AND HERBAL PRODUCTS

#### Where do we stand now? The rise and fall of traditional herbal movements in America

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American history gives many examples of specialized schools of traditional herbalism. Early on, robust herbal traditions thrived by folk influences of the Native American and early American settler. As knowledge became more specialized, America saw the emergence of more formalized systems of herbalism through the thomsonian, eclectic, and physiomedical movements. We will explore the rise and fall of these herbal movements in America and address the current status of traditional herbalism in this country with a special emphasis on education and the transmission of traditional knowledge. This timeline is paralleled by the transition from home remedies to commercial and patent remedies to the advent of pharmaceutical drugs.

#### Creating mind-body medicine through Ayurveda

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Ayurveda is a holistic science that emphasizes disease prevention and aims at maintaining harmony of the body, mind and soul. At Spa Moksha at The Center for Mind-Body Medicine, we utilize Ayurvedic Herbal Therapies to bring a state of health where every aspect of one's being works in harmony with all its other aspects. Ayurvedic Mind-Body Rejuvenation Therapies are designed to facilitate the removal of deep rooted stresses and toxins in the physiology. Many of these therapies utilize oils and herbs to effectively remove toxin build up from the body. In collaboration with the U.A.B. School of Medicine we are conducting studies to see the effects of these therapies along with oral herbal formulations on cancer patients undergoing chemotherapy.

#### Traditional Medicines with special reference to plants used in Unani Medicines for improving human health

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Ever since the incorporation of traditional medicine in its program in 1976, the World Health Organization (WHO) has gone a long way in creating worldwide interest in the promotion and development of different systems of traditional medicine and their best utilization in health care services. The ninth and tenth centuries have been designated "The



Golden Age of Arabic Medicine". The spread of the Arabic language had a whole some influence on science and particularly medicine.

Unani system of Medicine, as the very name suggests originated in Greece and is based on the teaching of Hippocrates( 460-377 B.C.), Dioscorides (1<sup>st</sup>Century A.D.), Galen (131-210 A.D.), Rhazes (850-925 A.D.), Geber (702-765 A.D.), Avicenna (980-1037 A.D.), Al Beruni (925-1050 A.D.), and many others. Galen, the pioneer of experimental medicine, wrote 256 treatises, most of which were medical in nature, advised to his readers, "In order to know drugs inspect them not once or twice but frequently for though twins look alike to strangers, they are easily distinguished by friends", in one of his famous books "On Anatomical Preparations" – a standard medical text for some 1400 years. Some important medicinal plants, used in Unani Medicine specially having a controversial identity, are discussed in detail.

### **Collaborating with practitioners, herb schools, and growers to establish local sources of Chinese medicinal herbs**

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Almost all of the herbs used in Traditional Chinese Medicine (TCM) in the U.S. are imported. Increasing concerns over the quality and safety of these imported herbs, rising transportation costs, and the growing 'buy local' movement, have fueled demand for domestically produced Chinese medicinal herbs. In response, a diverse group of people in western North Carolina, representing community colleges, cooperative extension, herbalists, a Chinese medicinal herb school, and a TCM distributor, came together to start a Chinese medicinal herb growing, marketing, and research project. We are currently working with ten popular TCM herbs: Anemarrhena, Angelica, Astragalus, Chrysanthemum, Lycium, Paeonia, Pinella, Polygonum, Schizandra, and Scutellaria. Different members of the project are producing plants in tissue culture, growing them on a research station and six on-farm sites, doing analytical testing, providing organoleptic testing, preparing vouchers, and conducting workshops for growers, herbalists, buyers, and consumers. It is expected that this effort will develop into a long-term project to develop this industry in our region.

## **SESSION B**

### **BIOTECHNOLOGY**

#### **Advancing Drug Development from Medicinal Plants using Transcriptomics and Metabolomics**

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Medicinal plants produce a wealth of pharmaceutical compounds such as digitoxin and vincristine. Unfortunately, the specialized metabolic pathways leading to such compounds remain poorly understood and progress in elucidating and manipulating these taxonomically-restricted metabolic pathways has been correspondingly slow. Development of "omics"-level resources for medicinal plants has been limited, which has meant that research in medicinal species has not benefited to the same extent from the genomics revolution, as has research in model plants and agronomic crop species. With the combined use of state-of-the-art sequencing technologies, metabolomics capabilities, and bioinformatics, we are developing an unrestricted, public resource to address this growing gap in our knowledge base of species-specific plant metabolism. This resource, which includes a publicly-accessible metabolomics and transcriptomics database for 14 widely used medicinal plant species, is designed to accelerate the identification and functional analysis of genes involved in natural product biosynthesis. Progress towards the discovery of the pathways and genes responsible for biosynthesis of key pharmaceuticals and their diversification will be discussed.

#### **Ten years of *Scutellaria* research at Fort Valley State University**

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Skullcap is a North American plant (genus *Scutellaria*, Family Lamiaceae). Skullcaps are used in alternative medicine as anti-inflammatory, antispasmodic, emmenagogue, nervine, sedative and strong tonic. We report our research on various aspects of *Scutellaria* biology that evolved over the past ten years on germplasm collection, screening of flavonoids, bioactivity and development of micropropagation protocols for rapid multiplication and conservation. Many skullcaps have showy, beautiful blooms with great potential as ornamental plants. This study addresses concerns to pay closer attention to issues of sustainable and ethical harvests, as the majority of plants entering the trade were still being sourced from the wild. There is very little scientific evidence for the anti-cancer activities of *Scutellaria* species. We have been working on the bioactive flavonoids extracted from *Scutellaria* for their antitumor properties. Leaf and stem extracts of *Scutellaria*, although known to be substantially rich in flavonoid content and other active ingredients have rarely been studied for their potential bio-medical activities. Select extracts showed consistent, dose-dependent, anti-proliferative and pro-apoptotic activities against various malignant cell lines. We will take examples of studies being performed on extracts of *Scutellaria* for examining their efficacy and mechanisms of action against cancer. This presentation highlights our cumulative research accomplishments.

### Differential elicitation of stilbenoids in hairy root cultures of muscadine grape and peanut

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Stilbenoids are polyphenolic compounds with important antioxidant and anti-inflammatory properties. These natural products are synthesized by species from unrelated plant families like the Vitaceae and Fabaceae. In order to study the biosynthesis of stilbenoids, hairy root cultures of muscadine grape (*Vitis rotundifolia*) and peanut (*Arachis hypogaea*) were established by *Agrobacterium rhizogenes*-mediated transformation. Selected hairy root lines were treated with different elicitors including methyl jasmonate, hydrogen peroxide, sodium acetate and trichloroethylene. Phenolic compounds were extracted from the tissue and medium with ethyl acetate and analyzed by HPLC. Different group of stilbenoids were produced in hairy root cultures of peanut and muscadine grape. Resveratrol and several prenylated stilbenoids were induced in peanut hairy roots. In muscadine grape hairy roots, the main induced stilbenoids were resveratrol, piceid and viniferins. Interestingly, in peanut most of the stilbenoids were secreted whereas in muscadine grape, a large percentage of these compounds were retained in the tissue. Overall, among the different elicitor treatments, an earlier accumulation of stilbenoids was observed upon methyl jasmonate treatment.

### *In planta* production of the high-potency resveratrol analogue pterostilbene via co-expression of a stilbene synthase and an O-methyltransferase

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Resveratrol and related stilbenes are thought to play important roles in defense responses in several plant species, and have also generated considerable interest as nutraceuticals due to their diverse health-promoting properties. Pterostilbene, a 3,5-dimethylether derivative of resveratrol, possesses properties similar to its parent compound, and also exhibits higher fungicidal activity *in vitro* and superior pharmacokinetic properties in studies involving animal models. Using a previously characterized O-methyltransferase sequence from sorghum in conjunction with a stilbene synthase sequence from peanut, pterostilbene-accumulating Arabidopsis and tobacco stable transformants were generated using the CaMV 35S promoter to direct the expression of both transgenes. A concomitant reduction in floral pigmentation was observed in tobacco, and subsequent HPLC analyses revealed a significant reduction in the levels of dihydroquercetin-derived flavonoids and phenylpropanoid-conjugated polyamines in these events. These results demonstrate the utility of the present strategy for *in planta* pterostilbene production, and also underscore the need for the development of additional approaches aimed towards minimizing potential reductions in key phenylpropanoid-derived metabolites.

## GRADUATE STUDENT AND POSTDOCTORAL FELLOWS ORAL PRESENTATIONS

### Cultivar identification and characterization of genetic diversity in *Psidium guajava* using simple sequence repeat markers

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Guava (*Psidium guajava* L.) is one of the most nutritious fruits that have gained food value importance in recent years. Accurate identification of cultivars and assessment of diversity is imperative for germplasm management and genetic improvement of guava. In this study, 19 simple sequence repeats (SSRs) were used to characterize the genetic relationships among 24 guava accessions obtained from the Pacific Basin Tropical Plant Germplasm Resource Center, Hilo, HI. Microsatellite genotyping was performed on a CEQ-8000 DNA analyzer using WellRED fluorescent-labeled primers and capillary electrophoresis. Based on SSR fingerprints, all 24 accessions of guava could be unambiguously differentiated. A total of 152 alleles were detected among these 24 accessions ranging from a minimum of 2 to a maximum of 15 alleles. Expected heterozygosity for the individual loci ranged from 0.28 to 0.89 with an average of 0.69. The mean observed heterozygosity was 0.19, indicating a high rate of selfing among guava cultivars. Multivariate analysis showed clustering of the 24 guava accessions into well-defined groups that reflected their geographical origins. Results of this research demonstrated that these SSRs are valuable in identifying individual guava cultivars and assessment of their diversity. These potential applications are significant for developing improved quality guava cultivars.

### Applications of genetic engineering in *Vitis* improvement

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Grape and its products are an excellent source of health-beneficial compounds that have the potential to reduce incidence of debilitating medical conditions including cancer and cardiovascular diseases. Conventional breeding has limited applications in developing improved varieties because of extreme heterozygosity of the *Vitis* genome, which is fostered by inbreeding depression. Genetic engineering has emerged as an alternative to breeding for introduction of single traits, such as disease resistance to elite varieties without changing desirable characteristics. Several *Vitis* cisgenic sequences, i.e., genes derived from the grape genome itself, encoding pathogenesis-related (PR) proteins were identified and transferred to binary vectors under the control of a CAMV35S-derived bidirectional duplex promoter complex. Grapevine embryogenic cultures were transformed with *Agrobacterium* to regenerate cisgenic plants. Genetically engineered grapevines constitutively expressing cisgenic proteins exhibited broad-spectrum fungal disease resistance in greenhouse and field tests, while berries showed improved rot resistance in postharvest storage tests. The use of cisgenic sequences for development of disease-resistant grapevines is the first step toward development of genetically modified grapevines with purely *Vitis* DNA sequences, thereby decreasing environmental concerns regarding use of transgenes for grapevine improvement. This technology is currently being extended to incorporate other desirable traits such as cold hardiness in *V. rotundifolia*.

### Use of Standard Area Diagrams to Improve Assessment of Scab on Fruit of Pecan: A Nutraceutical Tree Nut

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Pecan (*Carya illinoensis*) contains high levels of antioxidants, particularly tocopherol and other phytochemicals, that are currently being studied for treatment of cardiovascular diseases, obesity, diabetes, and nervous system disorders. The southeastern US supplies approximately 30% of the world's pecans. Pecan scab (*Fusicladium effusum*) causes significant

economic losses and reduces nutmeat composition. Accurate and precise disease assessments are required for developing effective scab control measures, but visual assessment, which is widely used, results in estimates of disease severity which are often inaccurate and imprecise. This study produced a method that improves visual assessments for pecan scab. Twenty-six raters estimated scab severity on 2 sets of 40 images of pecan fruit valves, either with or without the aid of a standard area diagram (SAD) set. Rater precision improved using the SADs ( $r^2=0.44-0.94$  and  $r^2=0.84-0.95$ , without and with SADs, respectively). Agreement (measured with Lin's Concordance Correlation Coefficient [ $\rho_c$ ]) improved using the SAD set ( $\rho_c=0.28-0.95$  and  $\rho_c=0.68-0.96$  without and with SADs, respectively). A better rater estimate of fruit scab severity will improve the ability to choose the best disease management options for enhancing profitability and increasing the nutraceutical value of pecan nutmeats.

### **Extraction and Preliminary HPLC Analysis of Therapeutic Flavonoids in Two Medicinal *Scutellaria* species.**

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Medicinal uses of *Scutellaria* species (Family Lamiaceae) have been documented in indigenous medical systems worldwide. Many of these secondary metabolites have been shown to possess anti-oxidant, anti-inflammatory, anti-tumor and several others therapeutic properties. Plant material for this experiment was raised, maintained and harvested just before blooming stage from the greenhouse in Fort valley State University. Extraction of dried and powdered *Scutellaria* samples done using ASE 200 at pressure 1000 psi, Temp 40 ° C with solvent MeOH: H<sub>2</sub>O (80:20) and subsequently dried by vacuum centrifuge. The resulting extracts were analyzed by high performance liquid chromatography. Mobile phase consisted of acetonitrile (Solvent 1) and 0.005% phosphoric acid in H<sub>2</sub>O (Solvent 2). HPLC analysis of the crude methanolic extract of *Scutellaria barbata* and *Scutellaria racemosa* was performed to characterize major flavonoids – baicalin, baicalein, luteolin, wogonin, and chrysin. 6-Hydroxyflavone was used as an internal standard.

### **POSTER ABSTRACTS**

#### **P-1: Identifying New Biological Targets Through Reverse Docking Library Screening**

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Advances in pharmacognosy have allowed the identification and physicochemical characterization of large numbers of phytochemicals that are of pharmacological interest. Typically, whole extracts are screened for activity using arrays of biological assays and active compounds isolated and their structures determined by spectroscopy. Drawing from the observation that many compounds from natural products sources interact with multiple targets, structures with known activity may be screened computationally for potential secondary targets. In reverse docking, collections of proteins established as known biological or drug targets, and with structures determined from X-ray crystallography are prepared for ligand-receptor docking. The utility of reverse docking is twofold: 1) to probe for targets in cases where biological activity has been determined by assay but the specific target has not yet been identified and 2) where secondary targets are identified, additional biological testing is indicated. In this work, twelve quinoline alkaloids from *Stauranthus perforatus* recently shown in the literature as having cytotoxic activity were screened for additional activity with the Potential Drug Target Database (PDTD) of 1207 known drug targets. The most frequently occurring targets for this set of structures include human HIV-1 protease and essential proteins in several pathogenic bacteria in the *Pseudomonas*, *Streptococcus* and *Klebsiella* genera.

#### **P-2: Antimicrobial activity of sea buckthorn oil against *Helicobacter pylori***

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Sea buckthorn (*Hippophae rhamnoides* L.) oil has been used for centuries in the traditional medicine of Tibet, Russia, and other Central Asian countries for the treatment of ulcers. *Helicobacter pylori*, a rod shaped, gram-negative, microaerophilic bacterium observed in the gastrointestinal tract of about 50% of the population over 60, is considered a primary cause of stomach ulcers and is known to play a significant role in other gastroduodenal diseases. In this study, the effect of sea buckthorn fruit pulp oil (secured from the Altay region of Russia) on *H. pylori* was studied by placing discs saturated with a 50 % mixture of oil and DMSO on agar plates that had been inoculated with cultures of the bacterium. Observations of the cultures after incubating at 37°C for 48 h showed only limited inhibition of the *H. pylori* growth, bringing into question the traditional uses of sea buckthorn oil in Russia and other regions for treatment of stomach ulcer. Perhaps the oil is more effective against the bacterium within the human gut or different physiological mechanisms of the oil promote ulcer healing.

### P-3: Trypanosome Cysteine Protease Inhibition by (-)-Bornyl Esters

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Human African trypanosomiasis, like other parasitic diseases, remains a major health concern in sub-Saharan Africa with an estimated 60 million people at risk of infection. Over the last decade, parasitic proteases have been the subject of extensive investigations to identify novel antitrypanosomal agents due to the enormous roles they play in the parasites. The inhibitory activity of hydroxycinnamic esters of borneol isolated from *Verbescina turbacensis* Kunth were tested against rhodesain, the major cathepsin L-like protease of *Trypanosoma brucei rhodesiense*. The three borneol esters were found to have micromolar  $IC_{50}$  against the cysteine protease. Molecular docking analysis to probe the inhibitory interactions of the esters was also carried out.

### P-4: Inhibition of tumor cell growth by *Hibiscus sabdariffa* anthocyanins

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Hibiscus extracts are used in traditional African and Chinese medicine. Previous investigators have demonstrated medicinal properties of select *Hibiscus* extracts, however information is very limited about variations among *Hibiscus sabdariffa* accessions. Accessions of *Hibiscus sabdariffa* can be grouped by calyx color into green, pink, red, and dark red. We previously examined the effects of crude extracts of *Hibiscus sabdariffa* accessions on tumor cell proliferation and on proliferation and migration of vascular smooth cells and reported that the dark red and pink extracts were the most active. Here we examine effects of an anthocyanin enriched fraction from the dark red extract on proliferation of tumor cells *in vitro*. Three rapidly dividing tumors cell lines (U-87 MG glioblastoma, PanC-1 pancreatic cancer and CH157-MN meningioma) were exposed to concentrations ranging from 0.0 to 2.55 mg/ml of anthocyanin fraction. Tumor cell proliferation, evaluated by MTT cell proliferation assay, showed a dose dependant decrease in cell proliferation by day 3 of treatment for all cell lines, with significant inhibition of the PanC-1 cells starting at 1 mg/ml and of U-87 MG and CH157-MN cells at 2.55 mg/ml.

### P-5: $\pi$ - $\pi$ Molecular Interactions between Intercalating Antitumor Agents and Caffeine

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Many anti-tumor drugs function by intercalating into DNA and forming  $\pi$ - $\pi$   $\square$ molecular complexes with DNA and other aromatic structures such as the xanthine alkaloid caffeine. Caffeine also has the ability to intercalate into DNA and form  $\pi$ - $\pi$   $\square$ molecular complexes with other planar alkaloids and anti-tumor drugs. The presence of caffeine could interfere with

the intercalating anti-tumor drug by forming  $\pi$ - $\pi$  molecular bonds with the drug, thereby blocking the planar aromatic drugs from intercalating into the DNA and ultimately lowering the toxicity of the drug to the cancer cells. By testing the cytotoxicity of the several known DNA intercalators on MCF-7 breast cancer cells, the  $IC_{50}$  values of these compounds were determined. An appropriate concentration of caffeine was determined by the same process and was then used in conjunction with the drugs to determine whether or not there was attenuation in the cytotoxicity of the drugs due to the presence of caffeine. Computational molecular modeling studies involving several intercalating anti-tumor drugs with caffeine were also performed using the density functional theory (DFT) and the recently developed MO6 functional. For each drug, the two molecules (drug and caffeine) were docked in many different orientations to discover the most probable configuration of the two interacting molecules.

**P-6: Antibacterial activity of mycelial culture fluid from submerged cultivation of Shiitake (*Lentinula edodes*) mycelia *in vitro*.**

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Organic farming is the fastest growing segment of agriculture in the US and plant disease management in such sustainable crop production systems is an important challenge for growers. Of the many tactics considered, biological control remains the requisite strategy to mitigate specific devastating pest problems. This study was conducted to determine the antimicrobial potential of *Lentinula edodes* mycelial culture fluid as a biopesticide for managing bacterial diseases. Fifteen shiitake strains obtained from American Type Culture Collection and maintained in the laboratory on the YVMSA media, were grown in liquid culture media under submerged condition. Culture fluid harvested after 30 days of fermentation at 25°C and 250 rpm, was filtered (0.45 $\mu$ m) and tested *in vitro* on overnight cultures of *Erwinia amylovora* and *Ralstonia solanacearum* causal organisms of fire blight of apple and pear and bacterial wilt of tomato. High and low controls were 100 ppm streptomycin and bacteria alone, respectively. *L. edodes* culture fluid from all fifteen strains inhibited the growth of *Erwinia amylovora* and the inhibition varied from 66.5 to 100% compared to low control.

**P-7: Chemoinformatic characterization of combinatorial libraries and natural products databases for drug discovery**

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A comprehensive chemoinformatic analysis of the chemical space of several commercial and public natural products databases is presented. Natural products libraries were compared to compound collections from different sources including combinatorial libraries, drugs, and small-molecules frequently used in high-throughput screening. The compound databases were compared in terms of physicochemical properties, scaffolds, and fingerprints using well-established methods. The approach enabled a comprehensive analysis of property space coverage, degree of overlap between collections, scaffold and structural diversity and overall structural novelty. The results of this study further emphasize the importance of computer-guided discovery of natural products as lead compounds for drug discovery.

**P- 8: . Antimicrobial Effects of Selected Herbs on the Viability of *Escherichia coli*, *Staphylococcus epidermis* and *Bacillus subtilis***

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Sweet basil (*Ocimum basilicum*), red clover (*Trifolium pratense*) and purple coneflower (*Echinacea purpuria*) are well known for their antimicrobial properties, but their relative efficacy against common food-borne pathogens is not known. In this study, the antimicrobial potential of these plant species against *E.coli*, *Bacillus subtilis* or *Staphylococcus aureus* was determined by growing them in Hoagland media containing either .01M or .002M concentrations of potassium nitrate for a

duration of two weeks. Control plants were grown in water. All plants were defoliated and their leaves were dried. The leaves were then extracted in 70% ethanol. The extracts were filtered using both filter paper and Millipore filters. Ethanolic extracts from the plants were diluted to 55% with nutrient broth and inoculated with .1ml of a 48-hour culture of either *E.coli*, *Bacillus subtilis* or *Staphylococcus aureus*. The growth of these organism in the extract/ nutrient broth mixture was monitored with a spectrophotometer over a 24 hour period. Spectrophotometric analysis revealed a reduction in bacterial growth when compared to control. However, there was no difference in the inhibition of bacteria with increasing concentrations of potassium nitrate in the Hoagland's medium. Extracts from the leaves of the basil had greater effect when compared with Echinacea and red clover. The research was conducted at Fisk University with funds provided by a Presidential Award by Fisk University

**P-9: Exploring the potential of hyoscyamine 6 $\beta$ -hydroxylase (*h6h*) gene polymorphism on tropane alkaloids in the plant black henbane (*Hyoscyamus niger* L.)**

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Exclusively derived from plant sources, the tropane alkaloids (TAs) hyoscyamine and scopolamine are essential pharmaceutical compounds used worldwide. Using black henbane (*Hyoscyamus niger* L.) as a model to explore the potential for marker assisted selection breeding, hybrid and F<sub>2</sub> populations from 13 accessions were developed and compared with the parental chemotypes. After LLE from root and leaf samples, scopolamine and hyoscyamine content were quantified by HPLC analysis. Primers were designed to amplify the putrescine N-methyltransferase (*pmt*), tropinone reductase 1 (*tr1*), and hyoscyamine 6 $\beta$ -hydroxylase (*h6h*) genes of the TA biosynthetic pathway. Two alleles for the *h6h* gene locus were identified. Genotype by chemotype comparison of a small number of F<sub>2</sub> samples from a monohybrid cross showed a trend of greater activity associated with the 690 bp amplicon allele (*b*) over the expected 550 bp amplicon allele (*a*) Increased levels of TAs in root tissue of the *bb* genotype were accompanied with reduced scopolamine in leaf tissue. Research currently underway involves development of an optimized SPE method followed by UPLC-MSMS detection and quantification of the TAs. These methods, along with genotype identification of 50 F<sub>2</sub> root and leaf samples, are being examined for heritability, chemotype, and significance of *h6h* polymorphism for breeding practices.

**P-10: Anti-inflammatory, antioxidant and anticancer activities of medicinal herbs and the impact of growth conditions**

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Herbs have been long valued for their culinary attributes as flavorings, seasonings and scents. A number of species have been used as traditional medicines for thousands of years. We here review the antioxidant, anti-inflammatory and anticancer activities of some herb species that we have recently evaluated. The anticancer activity was assessed under in vitro conditions using a SW-480 colon cancer cell line. Anti-inflammatory activities were evaluated using cyclooxygenase (Cox-1 and Cox-2) bioassays. Results showed that extracts from five species caused significant inhibition to cancer cell growth in a dose-dependent pattern with sage extract exhibiting the strongest anticancer activities. Herbs also significantly decreased cyclooxygenase activities, and some extracts showed stronger inhibition of Cox-2 than of Cox-1, suggesting that they may be useful as anti-inflammatory agents with fewer side effects than regular non-steroidal drugs. The biochemical and biological activities of these herb species can be significantly impacted by growth conditions. Many herbs grown in the greenhouse showed significantly higher total polyphenolic content and antioxidant capacity than those grown in the field.

**P-11: The GIBEX Bioassays to Assess Biological Activities of African Medicinal Plants**

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The Global Institute for BioExploration (GIBEX) is a global research and development network that promotes ethical,

natural product-based pharmacological bioexploration. The GIBEX program has developed simple bioassays to assess the biological activity of plants. The objective of this research is to assess the biological activities of selected African natural plant products using the GIBEX bioassays. Selected African plant products were used in this study including the spices *Piper guineense* and *Piper nigrum*, and the herbal teas *Cymbopogon citratus*, *Aspalathus linearis* and *Lippia multiflora*. The bioassays included antimicrobial, antifungal, glycosidase and glycosidase inhibiting activities roundworm lethality assay. The *Piper* species and the herbal teas *L. multiflora* and *C. citratus* showed almost no activities against fungi, while *A. linearis* was the only product to show activity. All species exhibited low antimicrobial activities, while lemongrass showed not activity at all. The *Piper* species were most active against round worms killing more than 50% of them while the herbal teas showed almost now activity. All the herbal teas presented glycosidase activity, the spices a low activity while none of them showed glycosidase inhibiting activities.

### **Challenges in Developing Traditional Herbals as Medicines: Standardization, Quality Control/Quality Assurance (QC/QA), Regulatory and Commercialization Issues**

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Herbals/botanical products have been used for centuries worldwide, in various cultures, to treat the sickness, diseases, and different ailments; however, there have been very little scientific efforts to validate their efficacy and mechanism of action. It is often overlooked that about 25% of the prescribed drugs in use today originate from natural sources. Some examples include: Reserpine from *Rauwolfia serpentine* for psychotic symptoms, Digitalis from Foxglove for Arrhythmia, Morphine from *Papaver somniferum* "opium" as analgesic, Quinine from *Cinchona officinalis* for Malaria, Artemisinin from *Artemisia annua* for Malaria, Vincristin, Vinblastin from *Periwinkle* and Taxol<sup>®</sup> from *Taxus brevifolia* "yew bark" for cancer treatment, and many more have been isolated from several other medicinal plants. These pure single products are used in the advanced/western countries, whereas the tribes, developing and underdeveloped countries still use "Herbal Plants" as medicines for their treatment.

With the advancement and better living standard, increase in life expectancy, certain diseases are getting resistance to the existing pharmaceuticals and the baby boomers are turning to the alternatives or natural herbal remedies for healthy living. As these products are designated "Food Supplements," the regulatory requirements of agencies like US-Food and Drug Administration (US-FDA) are different.

Examples from the author's laboratory work in developing new "Herbal Products Therapy" for lowering cholesterol, reducing obesity and finding treatment for Sickle Cell Disease (SCD) have clearly shown, through the application of the modern advance technologies such as High Performance Liquid Chromatography (HPLC), Liquid Chromatography – Mass Spectrometry (LC-MS), that many such marketed product ingredients do not contain what is printed on the label. From the analysis of the marketed herbal product, it is clear that the Quality Control/Quality Assurance (QC/QA) is very important in the standardization of the herbals for the product to have any efficacy for the treatment of a disease. The role of non-bioactive molecules in the herbal extracts show the synergistic effect necessary to avoid any toxicity of the active molecules in the product.

## **SESSION A**

### **ETHNOBOTANY AND BOTANICAL SOURCING**

#### **Utilizing national and state germplasm repositories as a valuable tool for collaborative botanical research**

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Both national and state germplasm collections are optimal choices for a wide variety of botanical and microbial research ranging from cultivar breeding, chemical analysis of metabolites of interest for new product development, drug discovery, endophyte isolation, phylogenetic studies, and conservation. This presentation will summarize how researchers can collaborate to utilize regional and national germplasm repositories for high-quality replicable botanical sourcing. With new GMP regulations mandating stringent botanical sourcing protocols including source of origin, voucher specimen preparation, and long-term sample storage, collaboration with facilities specializing in these areas is strongly encouraged. Examples of current collaborative research projects will be discussed along with illustrations of plant exploration, field collection methods, laboratory sample and extract processing, seed and control-pollinated cage propagation, endophyte isolation, and facilities utilized for seed cleaning, testing and storage.

### **Cultural and Historical Considerations in Ethnobotanical Sourcing; World Politics, Pop Culture, Business Exploitation, and the Ultimate Discretion of the Eye of the Beholder.**

M. Hernandez. Appalachian Center for Ethnobotanical Studies, Frostburg State University, Frostburg, MD. **Contact:** Mimi Hernandez; [mhernandez@frostburg.edu](mailto:mhernandez@frostburg.edu)

The sourcing of medicinal plants from nature has been subject to the cultural context of world politics, pop culture, business exploitation, and the ultimate discretion of the eye of the beholder. Elucidating these connections we begin by exploring the world politics of quinine discovery and its procurement from the bark of not just any Cinchona tree. We then will consider the implications of cultural appreciation and the consumer eye of the beholder on the markets that shaped the intentional quest for the esthetically appropriate Ginseng root. Overharvested Goldenseal then reveals public misconceptions as we trace the source of its pop culture use in masking drug screening tests. And finally we examine the revolutionary effects of the Wild Yam whose root activities are distinct from its drug activities. All this amidst a background setting of America's historical transition from home remedies to commercial remedies to an industry based primarily on the crude drugs of Appalachia and on to an industry practice of constituent isolation subject to standardization beginning with the 1907 Standardization Pure food and drug act.

### **Putting the "Ethno" back in Ethnobotany**

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In 1999, the Economist printed an article declaring the ethnobotanical approach to drug discovery as officially dead with the demise of Shaman Pharmaceuticals. Years of research and investments had resulted in very few actual pharmaceutical products and the promise of potential cures from the rain forest appeared to be a bust, overshadowed by new chemical processes that rendered the search for novel compounds cumbersome at best. But was this approach misguided from the onset? Ethnobotany still has much to offer, but not necessarily as a means to discover novel molecular compounds for drug research. This talk will explore what ethnobotany truly represents and how that could change the nature of future research efforts.

### **How to Qualify and Approve Botanical Raw Material Suppliers Under the New GMP's**

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This presentation will address the recently updated requirements for purchasing botanical raw materials under the new GMP (Good Manufacturing Practice) regulations and how they have changed purchasing decisions and market trends. Additional topics addressed will include how the new regulations are affecting both small and large-scale growers in addition to impacts this is having on the market. The presenter has a long history in the botanical industry as a small and large scale buyer and will share his experiences with the audience. The presentations format will encourage discussion and hands-on interaction with the audience with questions encouraged. The organoleptic concept for plant species

identification required by new GMP regulations will be introduced with botanical samples provided for audience participation.

## BREEDING AND CROP IMPROVEMENT

### Molecular Breeding in Medicinally Active Plants: Bitter Melon as a Model

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Global market of herbal medicines is currently US\$ 60-80 billion and expected to grow to US \$5 trillion in 2050. Natural products including their derivatives and analogs contribute to 53% of drugs in clinical use, with natural products derived from 50,000 different medicinal plant species sharing about 18% of the total. American consumers pay \$8 billion annually for prescribed drugs of higher plant origin. During 1992-2003, US imported 51,200 tons of pharmaceutical plants valued at \$1.4 billion and exported 13,050 tons, valued at \$1.05 billion. These statistics, however, excludes fruit, vegetable and spice crops that contain phytomedicines and consumed as functional foods. Albeit huge importance of these medicinally active plants as a source of herbal drugs, dietary supplements and functional foods, no serious efforts have been made in their genetic improvement. Employment of genomics and biotechnological strategies can compliment traditional breeding efforts in these plants leading to improvement in content of phytomedicines. A road map for this purpose will be depicted with bitter melon (*Momordica charantia*) as the model plant with emphasis on genetic diversity evaluation, association mapping, genetic map construction, gene and QTL mapping and development of genomics resources with ultimate goal of molecular breeding for fruit traits and phytomedicines.

### Selection of Nutraceutical Roselle (*Hibiscus sabdariffa*) Accessions for Cultivation and Niche Market Production in USA

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Roselle (*Hibiscus Sabdraiffa*) is a favorable specialty crop demanded highly in the international commerce due to its demonstrated nutritional and medicinal properties. The roselle leaves and fruits (calyces) are rich in total polyphenols and antioxidants, and possess anti-microbial and anti-cancer functions. The calyces are commonly used in teas, jams, and jellies. However, roselle is a non-native species to the United States. This project focuses on introduction and selection of various roselle accessions from various regions around the world for cultivation and niche market production in the USA. We have cultivated 23 roselle accessions including Cuba(2 accessions), Georgia(1), Ghana(2), India(1), Jamaica(1), Liberia(1), Malaysia(1), Nigeria(2), Poland(1), Senegal(1), South Africa(3), Sudan(3), Taiwan(1), Thailand(1), and Zambia(2), in Baton Rouge, Louisiana, a subtropical area in the USA. Data collections include botanical characteristics (herbarium), phenology, growth and development, foliage and fruit production, nutrient analysis, and phytochemistry. All individual accessions studied proved to be similar in species, but exceptionally diverse in representation. All the accessions show potential for foliage production. Several accessions including Jamaica, Nigeria, Senegal, and South Africa have demonstrated potential for calyx production which has been promoted for niche market production to Louisiana growers.

### Genetic resource management of medicinal plants under Indian Council of Agricultural Research

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India is blessed with two mega centers of biodiversity in plant species (Northeastern Himalayan region and Western Ghats in Karnataka). Among 250,000 species of higher plants on earth, 49,219 species are reported in India, of which

7,500 species are medicinal plants (15.9%), 800 species are commercially used (1.7%), 120 species are used in large volumes (0.25%) and about 70 species have been investigated for large scale domestic cultivation (0.1%). Under ICAR, DMAPR with its outreach program, AICRP-MAP having 22 research centers is engaged in research on PGR management by survey, collection, characterization, conservation and utilization. At present, 3000 accessions of 30 species are maintained in field gene banks. Utilizing PGR, 43 cultivars of 25 species were released for cultivation. To deal the emerging IPR issues DUS descriptors have been prepared for *Plantago ovata* and *Withania somnifera*. A few more are in their final stages. Eleven elite germplasm lines of seven species with special characters have been registered. Efforts are in progress to strengthen PGR utilization with pre-breeding and molecular characterization.

## PRODUCTION AND POST HARVEST SYSTEMS

### Research and extension efforts to support North Carolina farmers in the production and marketing of medicinal herbs

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For hundreds of years, North Carolina has had the reputation of being a source for high-quality, wild-harvested medicinal herbs. In recent years, conservation concerns and demand for cultivated herbs has resulted in wide-spread interest in building an expanded agricultural industry around medicinal herbs. A series of research and extension projects have been conducted to help farmers produce, process, and market medicinal herbs. Initially, farmers across the state were recruited to grow a wide variety of sun and shade requiring herbs as requested by cooperating buyers. We found that NC farmers could grow the quality and quantity of herbs required, but there were issues with pricing, testing, and processing that inhibited more agricultural production. Over the next few years a community college opened a natural products laboratory to provide testing services and offered classes on good manufacturing processes, we helped farmers build herb dryers and washers, enterprise budgets were created, and a shared-use kitchen incubator started a natural products processing facility. Now a new cooperative effort, involving a regional economic development partnership, a community college, two universities, and a business incubator, are working together to create a system of resources to support the growing, manufacturing, and marketing of medicinal herb products.

### Effect of nitrogen levels and source on yield and oxalic content of Purslane (*Portulaca oleracea* L.

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Purslane (*Portulaca oleracea* L.) is an herbaceous plant with excellent nutritional and medicinal properties. It is rich in essential fatty acids (alpha linolenic acid), antioxidants (ascorbic acid, alpha-tocopherol, beta-carotene, glutathione), minerals (calcium, magnesium, potassium) and mucilage (polysaccharides). Humans have used it as food, medicine and feed for centuries. Purslane accumulates oxalate to levels that can be toxic to humans and domestic animals, preventing a wider use. To evaluate the effect of nitrogen on purslane leaf oxalate content and herbage yield, plots, subplots and sub-subplots of cultivar Goldberg were subjected to four levels (0, 28, 56, 112 kg/ha) of nitrogen, three nitrate:ammonium ratios (1/2:1/2, 1/3:2/3, 0:1) and four harvests (31, 39, 47, and 55 days after planting (DAP)), respectively. Treatments were arranged in a split-split-plot design with four replications. Increasing N level from 0 to 56 kg/ha significantly increased both, leaf oxalate content and herbage yield, whereas increasing N level from 26 to 112 kg/ha increased leaf oxalate content only. Leaf oxalate content was significantly reduced when ammonium was increased from 50% and 67% to 100% in the ratio. Leaf oxalate content significantly decreased at every subsequent harvest while herbage yield significantly increased at every harvest up to 47 DAP and then decreased at 55 DAP.

### Leaf Damage and Photosynthetic Rate on leaves of *Hibiscus sabdariffa* Infested by *Popillia japonica*

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Feeding by adult Japanese beetle results in significant removal of leaf area; however, the remaining leaf area may continue to provide significant photosynthetic activity following control measures. Accurate assessment of the extent of damage to leaf area was determined for a new host crop (*Hibiscus sabdariffa*) red and green sorrel to determine physiological effects of sorrel leaves partially consumed by the Japanese beetle. Current digital and neural-network software could provide precise measurement of leaf area consumed by the insect pest. Japanese beetle preferred red sorrel to green one as the leaf area remaining after insect damage was significantly greater (5.37 cm<sup>2</sup>) in green sorrel than for red sorrel (4.98 cm<sup>2</sup>). Extent of tissue damage within the 6 cm<sup>2</sup> area measured for photosynthetic activity differed significantly ( $P < 0.0341$ ) from the actual tissue that remained after herbivory. The measurements from the MATLAB were on the average  $\leq 1\%$  lower than those from the LICOR-3100 leaf area meter. Photosynthetic rate ( $\mu\text{mole CO}_2 \text{ 6 cm}^{-2}$ ) measured by the LICOR-6400 showed the green sorrel (26.17  $\mu\text{mole CO}_2 \text{ 6 cm}^{-2}$ ) was significantly higher than the red sorrel 22.49  $\mu\text{mole CO}_2 \text{ 6 cm}^{-2}$ . However, there was no significant difference between red and green sorrel for total photosynthesis. Highly significant differences were observed among three levels of damage based on measurements taken by LICOR-3100 and MATLAB.

### Effects of Nitrogen Fertilizer Levels on Growth and Phytochemical Content of Basil (*Ocimum tenuiflorum*) Grown in North Alabama

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*Ocimum tenuiflorum*, a popular herb rich in antioxidants is known for its medicinal properties. Lack of cultivation techniques and effects of agronomic inputs on phytochemical content of basil are limiting its potential for commercial cultivation as a medicinal herb in the US. A field trial was conducted to determine effects of four levels of N (0 control, 75, 150 and 225 kg/ha) on crop growth, biomass, and leaf and stem essential oil content and its composition using *Ocimum* accession PI 652056. The experiment design was a randomized complete block with 4 replications. Eugenol with 47.9% of total essential oil was the major constituent at 30 days after transplanting (DATP) in the control treatment (0 kg/ha) compared to 40.7% at 60 DATP in treatments receiving 150 kg of N/ha. The relative percentage oil content decreased with plant maturity in control plots, whereas it increased between 30 and 60 DATP in treatments that received N. Linalool and trans- $\beta$ -guaiene were predominantly high at the time of 30 DATP; whereas, eugenol, methyl chavicol and 1,8-cineole were the major constituents at 60 DATP. Of the 28 essential oil components identified, eugenol, (trans)- $\beta$ -guaiene, methyl chavicol, 1,8-cineole and linalool were most abundant at all harvest stages.

## SESSION B

### PHYTOCHEMISTRY

#### Natural Products as antiprotozoal leads - In-vitro/in-silico studies and a new network initiative: "ResNetNPND"

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Certain sesquiterpene lactones (STLs) from plants of the Asteraceae family show considerable antitrypanosomal and antileishmanial activity. Selected Asteraceae species of European and African origin were screened for such activity and their active constituents isolated. Structure-activity data were acquired for about 60 different STLs that were used to construct quantitative structure-activity models (QSAR). Such models, based on a wide range of molecular descriptors, can serve to explain the differential activity and selectivity within this compound class and to predict the activity of further, yet untested compounds. Compounds from various other classes of natural products (NPs), e.g. a cembrane diterpenoid, several flavonoids and lignans as well as chromene derivatives active against protozoa were also found. Molecular modelling and QSAR studies can serve to evaluate these compounds' potential role as lead structures. Molecular Modelling and Chemoinformatics tools are being applied to establish large databases of natural products with antiprotozoal and other activities. Such databases can be screened virtually for potential lead structures against parasite-specific biomolecular targets. Molecular models of parasite proteins (X-ray structures or such obtained by comparative protein modelling) are also used in virtual screening/docking. Besides results of these studies, Research Network Natural Products against Neglected Diseases (ResNetNPND) a multinational research initiative will also be discussed.

## Total Polyphenols, Antioxidant Content, and Chemical Profiling in Leaves of Roselle (*Hibiscus Sabdariffa*) Accessions

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A field trial was conducted during the 2009 growing season in Baton Rouge, LA for 22 roselle (*Hibiscus Sabdariffa*) accessions with seed sources originated from Cuba(2 accessions), Georgia(1), Ghana(2), India(1), Jamaica(1), Malaysia(1), Nigeria(2), Poland(1), Senegal(1), South Africa(3), Sudan(3), Taiwan(1), Thailand(1), and Zambia(2). Seeds of these accessions were planted in April and mature leaves were collected in September and oven dried for chemical analysis. The total polyphenols and antioxidant activities in leaves of the 22 accessions were quantified using a UV-Vis spectrometric method and their chemical profiles were analyzed using LC/UV/MS. The total polyphenol content ranged from 1.2-1.8% (by wt) and total antioxidant content ranged from 1.9-5.1% (by wt) in leaf samples from the 22 accessions. Based on the UV/MS data and authenticated standards, a total of 10 polyphenols/flavonoids were identified that include gallic acid, chlorogenic acid and the isomers, quercetin, and kaemferol and the glucosides and rutosides in the leaf extracts. The profiles of leaf samples from the 22 accessions were compared using the developed method and the results indicate that, except the India and Thailand accessions, the profiles of all other accessions were very similar. The results may serve as the baseline quality control information for future analysis of nutritional compounds and their values in selected roselle accessions.

## Prenylated Isoflavonoids from *Rhynchosia edulis*

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Parasitic diseases remain a major health concern in the tropics and in our continued effort to characterize structural leads for the development of antitrypanosomal agents. Four new prenylated isoflavones were isolated by bioassay-guided fractionation of the dichloromethane bark extract of *Rhynchosia edulis*. Five previously described compounds, scandenol, ulexin B, cajanone, cajanin, and cyclochandalone, were also isolated. These isoflavonoids showed weak inhibitory activity towards rhodesain, the major cathepsin-L like protease in *Trypanosoma brucei*. They also have weak antiproliferative activity towards MCF-7 cells. Molecular modeling of these compounds suggests that their structural analogues could have higher potency against rhodesain.

## Linking Education, Research and Development: Quality and Safety of Selected NonTimber Forest Products from Liberia

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Non-timber forest products (NTFPs) are becoming increasingly important for income generation. The lack of information on the chemistry and quality can sometimes limit their commercialization. Also the lack of information on their safety can generate concerns at the time of their commercialization in international markets. The objectives of this work are to evaluate the quality, chemistry and safety of new Liberian NTFPs to develop initial standards of quality. Three plant products harvested from the Guinea forest (northern Liberia) were selected for this study, the spice *Piper guineense*, bitter kola (*Garcinia kola*) and wolof (*Beilschmiedia mannii*). Round worms were used as model organisms to assess the potential toxicity of these new products. The results suggest they all have similar lower toxicities when compared with similar products used as spices, foods or medicinal plants. The levels of aflatoxins, that can present problems in the production of spices, were low in *P. guineense*. *P. guineense* was characterized by a unique composition of essential oils. Wolof originally cited as a spice was found to be rich in carbohydrates and devoid of spiciness, which suggested its

use as a food. Bitter kola showed significant levels of antioxidants.

## SESSION B

### CONTROLLED ENVIRONMENTAL HORTICULTURE AND PROPAGATION

#### Thinking Inside the Box: Manipulation of Plant Behavior within Closed Environmental Systems.

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Aside from the rapid and mass clonal plant propagation avenues available from using tissue culture the *in vitro* environment also offers the possibility to obtain a number of phytochemical products from plant cultures. Over the past 30 years the expressions of numerous commercial phytochemicals have been attained. Most research interest has dealt with procurement of individual phytochemicals, usually through callus or cell suspensions. However, the commercialization of employing the sterile environment to procure secondary metabolites has yet to be widely used. Closing the cost gap between productions of secondary metabolites *in vitro* with field grown crop sources often remains elusive. As an alternative avenue, whole plantlet culture has been shown to readily express secondary metabolites *in vitro*. Since these processes can be manipulated by nutritional and environmental means, a resultant recognizable intact product is obvious (i.e., the whole plant containing phytochemicals). Whole plantlets capable of producing edible products offers a number of interesting possibilities which are not available from cell and organ cultures. However, the control of the sterile environment is difficult and much research is required to exploit whole plantlet culture adequately. A discussion of various techniques to control this environment is provided as well as suggestions as to future products that can be obtained from whole plantlets.

#### Sustainable Production of high-artemisinin *Artemisia annua*, a Medicinal Plant with Anti-malarial and other Pharmacological Activities

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*Artemisia annua* L., also known as Sweet Annie, annual wormwood, or qing hao in Chinese, has long been recognized for its medicinal properties. This critical medicinal plant is the only commercial source of artemisinin, mostly known for its remarkable effects against multi-drug resistant malaria. Globally, an estimated 300 million cases and almost 1 million deaths are attributed to malaria annually. In addition, *A. annua* produces bioactive metabolites with anti-cancer, anti-inflammatory, and anti-parasitic activities. The leaves, as the source of artemisinin, must be supplied through crop production. With demand growing, satisfying the need for artemisinin will require improved plant material containing consistently high artemisinin. Unfortunately, seed production usually produces plants with variable artemisinin content. A strategy for varietal improvement of *A. annua*, which includes selection of genotypes with high artemisinin content and desirable agronomic traits are described here. Further, tissue culture propagation methods have been developed, which when integrated with selection have the advantage of producing genetically uniform plants with consistently high artemisinin content. These combined techniques have the potential to provide a crop with homogeneous biomass and artemisinin concentration that could translate into more efficient production of artemisinin and lower costs of the final product:artemisinin-based combination therapies.

#### Nutrient media formulation for primary metabolism: Turmeric as a model plant

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Turmeric (*Curcuma longa* L.) synthesizes a wide range of bioactive phenolics in subterranean rhizomes. A whole plant model in defined tissue culture media has been constructed in 2 liter vessels. Phenolic chemistry in concentrations that exceed field plants was assayed by antioxidant activity. Standard MS medium (high N) reduced antioxidant activity and

low-N media reduced biomass. Biomass is more related to sucrose (carbon) than inorganic nutrients. Three different d-optimal multi-factor experiments are presented to show how lowered-N environments were tested with minimizing loss to biomass. Over the 35-day time course, the 1<sup>st</sup> experiment simultaneously altered plant density, media volume, sucrose, potassium, nitrate and ammonium in small vessels, whereas the 2<sup>nd</sup> experiment had removed 75% of the ammonium, and altered plant density, potassium, nitrate, phosphorus, calcium and magnesium, in small vessels. The third experiment involves a fed-batch process where biomass was generated over 180-days using the full design of the second experiment in large enough quantities to analytically determine a full profile of secondary metabolites, is ongoing, and biomass data up to day-120 will be presented. In conclusion, d-optimality drastically reduced the number of experimental units in 5-factor designs that allowed quadratic lines and second order interactions to be significantly resolved.

### Vernalization and Forced Growth of *Veratrum californicum*

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*Veratrum californicum* Durand (corn lily) is native to alpine regions in the western USA. Corn lily has long been used in herbal medicine and has potential pharmaceutical uses. To develop the vernalization protocol for its cultivation, field collected bulbs were stored at 10 °C for 2 weeks and subsequently at 5 °C for 1 to 6 months in pots filled with Fafard 3B media. Each month, twenty bulbs were grown using ebb-and-flood irrigation in a greenhouse and harvested at two stages (leaf emergence and expansion) for mineral nutrient analysis; another ten bulbs were placed in a growth room (25 °C, 185 ± 6 μmols·m<sup>-2</sup>·s<sup>-1</sup>) and net photosynthetic rates (NPR) were measured. Vernalization significantly affected time to leaf emergence ( $p < 0.0001$ ) and expansion ( $p < 0.0001$ ). As bulb time in cold storage increased, days to leaf emergence and expansion decreased. Optimal vernalization was 5.7 months. Plants emerged at low temperature in the dark after being stored for 6.5 - 7.5 months. Net photosynthetic rates were different among vernalization treatments ( $p < 0.0001$ ). Plants with short vernalization (1 month) had low NPR 1.1 ± 0.1 μmols·m<sup>-2</sup>·s<sup>-1</sup>; medium vernalization period (2 - 3 months) had greater NPR (2.2 ± 0.2 μmols·m<sup>-2</sup>·s<sup>-1</sup>), and the optimal (4 - 6 month) treatment had highest NPR (5.0 ± 0.1 μmols·m<sup>-2</sup>·s<sup>-1</sup>). Longer bulb storage resulted in taller plants ( $p < 0.001$ ) that produced more, large leaves ( $p < 0.001$ ). In conclusion, vernalization of at least 4 months is necessary to force quality corn lily growth.

### Patent perspectives for phytopharmaceuticals

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From the plant to the patient, patents play an important role in protecting in the industrial world for protecting materials so that marketing approval can be obtained through a regulatory agency. Patents are defined by explaining the components of patent application, the process of obtaining a patent, and how to enforce an issued patent. Utility patents for plants and plant patents are described as well as an additional protection scheme, the Plant Variety Protection Act.

### Standardization & Quality Control of Medicinal & Aromatic Plants & Products

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Medicinal and aromatic plants (MAPs) are used in the traditional healthcare system since ancient times in all major civilizations around the world. The important cause is that medicinal plant products provide rational means for the treatment of many diseases that are obstinate and incurable in other system of medicine. In the recent years with ever

growing commercialization in the field of herbal medicines, there has been an instant demand for quality control (Q.C.) of the drugs used in this system. For this standardization is usually recommended as a solution to the problem. In the present paper an attempt has been made for a sequential study of the Q.C. of MAPs and Products (HMP) starting from Selection of Medicinal Plants; Good Agricultural Practices; Cultivation; Good Field Collection Practices; Source and Period of Collection; Identification; Storage; Chemical Standardization; Assay; Good Manufacturing Practices; maintaining standardization at every stage. Modern techniques used for quality & safety and over an above discussed in detail the challenges of standardization with special reference to marker compounds in plant species and their fingerprinting. Practical experiences of laboratory work on inadvertent contamination & substitution and intentional adulteration with pharmaceutical drugs in herbal plants & products have been discussed in detail.

### **A New Paradigm in Goldenseal Product Standardization**

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Raw material from goldenseal (*Hydrastis canadensis* L.), is currently judged based on the quantification of alkaloids, primarily berberine and hydrastine. Accordingly, consumer products are standardized to specified levels of these alkaloids with product potency perceived to be proportional to alkaloid content, i.e. more is better. However, a wide range of bioassays strongly suggests that simple measurement of alkaloid content alone is not sufficient to accurately predict bioactivity of the final product because products containing equal amounts of alkaloids exhibited different results in the same assay. In some assays, an inverse relationship between alkaloid content and bioactivity was observed depending on which plant part(s) the alkaloid was extracted from. The data also indicated that a particular formulation which works well in one assay may not be ideal for another. These disparities were found to be due to the presence of multiple compounds, some with no bioactivity on their own, which either synergize with berberine to increase its effectiveness, act through different pathways, and/or mitigate undesirable effects of berberine. Therefore, the current industry method of standardizing goldenseal products guarantees the level of certain chemical markers in the product but fails to consider the contribution of these other compounds to the overall bioactivity of the product thereby rendering reliable clinical study outcomes impossible and leading to inevitable consumer disappointment in the products they purchase. The development of research grade goldenseal products which combine quantitative chemical analysis with extensive bioassays to index the bioactivity of the product to that of a berberine reference standard will be discussed.

### **Standardization for Quality Control: The Industry Perspective**

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We will walk through the various protocols utilized in the Medicinal Herb or Dietary Supplement Industry developed to better 'standardize' the quality control aspect of the products coming into the market. I will show several different approaches from different organizations and companies depending on their requirements and the level of the market they address. Then we will walk through the SIDI (Standardized Information on Dietary Ingredients) protocol which was developed through a SIDI Working Group. The Working Group was a joint trade association effort with participants representing both ingredient suppliers and finished product manufacturers from the American Herbal Products Association (AHPA), the Consumer Healthcare Products Association (CHPA), the Council for Responsible Nutrition (CRN) and the Natural Products Association.

### **POSTER ABSTRACTS**

**P-12: Synergistic effect of cyclodextrin and methyl jasmonate on production of resveratrol and its prenylated analogues arachidin-1 and arachidin-3 in hairy root cultures of peanut.**



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Stilbenoids are polyphenolic compounds present in selected plant species. Among these compounds, resveratrol has been extensively studied for its biological activities impacting human health. In addition to resveratrol, species like peanut produces resveratrol analogues which may harbor similar or even enhanced bioactivities when compared to resveratrol. Of increasing interest are the prenylated stilbenoids such as arachidin-1 which has shown stronger antioxidant activity than resveratrol. Because these prenylated compounds are not commercially available, limited studies have focused on elucidating their biological properties. In order to establish a sustainable bioproduction system for prenylated stilbenoids, we established hairy root cultures of peanut and studied the effect of cyclodextrin and methyl jasmonate on production of resveratrol and two prenylated analogues arachidin-1 and arachidin-3. Selected peanut hairy root cultures were treated with either cyclodextrin alone or in combination with methyl jasmonate in 50 ml and 1 liter cultures. After different periods of elicitation, stilbenoids were extracted with ethyl acetate from the tissue and culture medium and then analyzed by HPLC. Cyclodextrin alone induced the production of the three stilbenoids, whereas the combination of cyclodextrin and methyl jasmonate resulted on a much higher induction of these compounds which were mostly present in the culture medium.

#### **P-13: Elemental analysis of fruiting bodies of shiitake, oyster and button mushrooms.**

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Shiitake mushroom is high in protein, dietary fiber and has been implicated in reduction of cholesterol, cancer, and infections. Four Strains of *L. edodes*, LE 054, LE 6, LE 015, and LE 013 obtained from American Type Culture Collection were grown on Potato Dextrose Agar for 21 days. The resulting mycelia were harvested, freeze-dried, acid digested in a microwave digester and elemental analysis of the digested samples were carried out using Inductively coupled plasma-optical emission (ICP-OES) spectrometry. Mycelial heavy metal accumulation of Pb, As, Co, Ni, Cd, Cu, Zn, Mn, Fe, Pb, As, and Co as well as other micro and macro elements Mg, Al, P, Ca, Na, and K were determined. Also, the fruiting bodies of three types of mushrooms: shiitake, button and oyster were comparatively analyzed for elemental content. Shiitake caps and stems were analyzed separately to decipher any differences in the accumulation. The stems of shiitake showed higher heavy metal content while caps had higher micro and macro elements. The highest accumulation of copper was observed in button followed by shiitake and then oyster mushroom. Button had more sodium than shiitake and oyster mushroom. The button and oyster had similar but higher iron content than shiitake mushroom.

#### **P-14: Sequencing *Lentinula edodes* for characterization of nutritional and medicinal properties.**

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Two strains of *Lentinula edodes* namely LE 015 and LE6 were chosen among the other strains that were obtained from the ATCC (American Type Culture Collection). They were proven to obtain the fastest mycelial growth under the following two extreme temperatures; LE 015 grew best at 25°C while LE 6, a summer tolerant strain, grew best at 30°C. For this endeavor, the DNA was extracted and amplified using both *L. edodes* specific primers as well as universal primers. The sequences of three strains LE 217, GL 51 and Cro4 were picked from the NCBI because of their specificity to *L. edodes* and for their maximum length of sequences to design specific primers of entire 18S length. The consensus was obtained using the ClustalW2 and specific primers were designed using primer designing tool which is the primer blast. The designed primers LE1F, LE1R, LE2F, LE2R, LE5F, LE5R, LE7F, LE7R, and LE8F, LE8R and the universal primers NS3/4 and NS5/6 were also used. Both specific and universal primers were used to conduct the PCR. The PCR was conducted at 60°C for specific primers, 52°C for NS3/4 and 56°C for NS5/6. The PCR gave successful amplification with all the

primers for both of the strains. Primers were designed to identify the strains specific to *L. edodes* before using it for 454 sequencing. The sequences obtained showed higher score of similarity for each of the strains. However, there was a variation in the similarity between the two strains LE 6 and LE015.

#### **P-15: Plant Characteristics that Alleviate Human Stress**

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Stress is serious human problem in the world, frequently leading to a sense of hopelessness and exhaustion as the body attempts to cope by engaging the sympathetic nervous system and secreting hormones that affect cardiovascular and other body systems. Continued stress can increase vulnerability to diseases and initiate changes in the brain that lead to depression. Horticulture therapy, the utilization of plants and horticultural activities to relieve stress, has been used to improve social, educational, and psychological perspectives of individuals suffering from stress. Yet, no studies have investigated the physical characteristics of plants, such as color, aroma, shape, and size of foliage and/or flowers, that produce the most positive effects on patients. In this study, major plant characteristics, including size, color, shape, and aroma related to plant foliage and flowers, favored by individuals were evaluated to gain some preliminary information about the interaction of plant traits and people. A total of 302, non-stressed volunteers, recruited from the student body following approval by the University Human Subjects Committee, did self-evaluations of favorable plant traits in two studies, using live plants and computer generated images. Results demonstrated preferences for large and flowering plants and simple, large, and blue colored flowers.

#### **P-16: Traditional Uses and Chemical Components of commercial samples of *Ilex guayusa*.**

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*Ilex guayusa* (Aquifoliaceae) is a native plant from the Amazon rainforest and it is naturally occurring in South America in Colombia, Ecuador, Peru and Brazil. It is a shrub or small tree of about 20 meters high. It is locally known as guayusa or huayosa. Jivaro Indians use guayusa in religious ceremonies for purification before important ceremonies or tribal conferences. The infusions of high concentrated leaves of guayusa are used to prepare stimulant beverages. It is also known as the "watchman's plant", because even when you are sleeping you are aware of what is happening around you. In popular medicine, guayusa is used mainly as antidiabetic, antiseptic, digestive, hallucinogenic and tonic. The objective of this work was to study the chemical components and biological activity of commercial samples of *I. guayusa* leaves. Percentage of total mineral, total phenols and antioxidant activity were determined. Results were compared with *I. paraguayensis*, which is used to prepare the popular beverage "mate" in South America. Guayusa tea product consisted of the whole leaves (99%) indicating absence of foreign materials. Guayusa contain higher levels of total minerals (9.3 %) than yerba mate (5.3%), while the antioxidant activity and total phenols were lower than yerba mate.

#### **P-17: Comparative analysis of wild and *ex situ* cultivated *Harpagophytum procumbens* shoots and tubers from eastern Botswana**

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*Harpagophytum procumbens* (Burch.)DC. Ex Meisn(Pedaliaceae) commonly known as Kalahari devil's claw is one of the most harvested plants native to Southern Africa. In Botswana the plant is overharvested for its clinical role in the treatment of heart disease, degenerative rheumatoid arthritis, kidney inflammation and tendonitis. Globally, *H.procumbens* can bring

annual economic returns in magnitudes of 30million Euro. Despite these extensive encouraging therapeutic and economic benefits, not a lot has been done in Botswana to conserve this near threatened species in *ex situ* botanical gardens. The IUCN\* red data list criteria in South Africa assessed the conservation status of *H.procumbens* as the least concern even though this plant is assessed as Near threatened in Botswana. In this study, *H.procumbens* was removed from the wild and cultivated *ex situ* in a newly established botanical garden. Analysis of the tubers of *ex situ* cultivated plant material showed presence of iridoids harpagosides comparable with those found in the wild plants. The leaves were also found to contain these iridoids and therefore could potentially provide an alternative and more sustainable source of therapeutically active compounds.

**P-18: Comparison of anti-malaria agent artemisin and essential oil composition in *Artemisia annua***

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The chief ingredient in the forefront of treatment for malaria in Africa is artemisinin, a compound from a medicinal plant called *Artemisia annua*. *Artemisia* is an indigenous herb of the northern parts of China; it has been used for over 2000 years in traditional Chinese medicine for chills, fever, and malaria. The plant grows in many countries, and it can grow under an array of circumstances. However, artemisinin yields fluctuate significantly. Artemisinin drugs are currently being utilized profoundly in South-East Asia and increasingly in Africa. The reason for this experiment is because quinine and replacement drugs have lost their remedial effects due to the disease's growth of resistance. However, derivatives of artemisinin combined with other drugs have shown efficiency in multi-resistant malaria. We are comparing the artemisinin contents with developing advanced lines. The Artemisinin contents differed by being grown in different years ranging from 2008-2010, and as a result, plants grown in greenhouse contained more artemisinin than the field grown ones. Essential oils were also compared with the advanced each *Artemisia* lines using hydro-distillation. Essential oils obtained from the Rutgers breeding lines are rich in camphor content from 38.2 to 51.6%, this is not the typical profile of a commercial *Artemisia*, where the oil extracted from the Seeds of Change did not have any determinable trace level.

**P-19: Analysis of phenolic compounds in purple rice**

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The purple rice variety Blanca Isabel, created at LSU AgCenter, is deemed of having an increased level of antioxidants and possesses many of the characteristics of long grain rice. HPLC analysis of anthocyanins in the grains showed high levels of cyanidin-3-O-glucoside and peonidin-3-O-glucoside; dephinidin-3-O-glucoside and malvidin-3-O-glucoside were not detected. Catechin and epicatechin were also found in the grains, as well as resveratrol. This is the first study to show resveratrol in purple rice. These phenolic compounds, which have been shown in several studies to possess diverse health-beneficial effects, add value to this new rice variety.

**P-20: Chromatographic quantification of medicinal compounds in flowering and nonflowering wild-harvested *Actaea racemosa* L.**

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Black cohosh (*Actaea racemosa* L.) is a perennial herb native to deciduous woodlands in Eastern North America used traditionally as a specific remedy for musculoskeletal pain and to support uterine function. Today, extracts of black cohosh are marketed primarily for use in relieving hot flashes with a majority of the raw material supplying this global market being harvested from naturally occurring wild populations in Appalachian woodlands. In Maryland and throughout the Appalachians, a vegetatively similar but threatened (NC S1, IL S1, KY S5, MD S2, NC S4, PA S3, WV S3) species (*A. podocarpa* DC.) is concurrent and threatened by accidental collection due to confusion with *A. racemosa*. This research addresses whether harvesting only flowering individuals could allow a wild harvester to increase the yield of medicinal

compounds per harvested plant creating an economic incentive to simultaneously insure correct identification and protect threatened species. In August 2011, 40 plants were harvested from naturally occurring populations in western Maryland. Plants which had produced a flowering raceme failed to differ in rhizome mass from those which had not ( $p = 0.429$ ). These rhizomes were extracted and quantified using UPLC with MS for actein, 23-epi-26 deoxyactein, isoferulic acid, caffeic acid and kaempferol concentrations.

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