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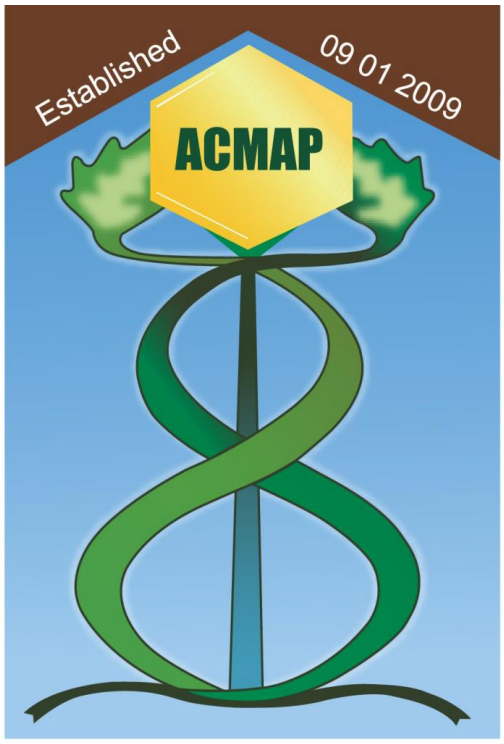

PLENARY SPEAKER ABSTRACTS

PL-1. Targeting Inflammatory Pathways by Agents Derived from Mother Nature for Prevention and Treatment of Cancer

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Abstract: Chronic infections, obesity, alcohol, tobacco, radiation, environmental pollutants, and high-calorie diet have been recognized as major risk factors for the most common types of cancer. All these risk factors are linked to cancer through inflammation. While acute inflammation that persists for short-term mediates host defense against infections, chronic inflammation that lasts for long-term can predispose the host to various chronic illnesses, including cancer. Linkage between cancer and inflammation is indicated by numerous lines of evidence; first, transcription factors NF-κB and STAT3, two major pathways for inflammation, are activated by most cancer risk factors; second, an inflammatory condition precedes most cancers; third, NF-κB and STAT3 are constitutively active in most cancers; fourth, hypoxia and acidic conditions found in solid tumors activate NF-κB; fifth, chemotherapeutic agents and gamma irradiation activate NF-κB and lead to chemoresistance and radioresistance; sixth, most gene products linked to inflammation, survival, proliferation, invasion, angiogenesis, and metastasis are regulated by NF-κB and STAT3; seventh, suppression of NF-κB and STAT3 inhibits the proliferation and invasion of tumors; and eighth, most chemopreventive agents mediate their effects through inhibition of NF-κB and STAT3 activation pathways. Thus suppression of these proinflammatory pathways may provide opportunities for both prevention and treatment of cancer. We will discuss the potential of nutraceuticals derived from spices and from traditional Indian medicine in suppression of inflammatory pathways and their role in prevention and therapy of cancer.

PL-2. Dereplication strategy for the identification of bioactive compounds and their role in reducing risk from cancer

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Abstract: In natural product research, the isolation of bioactive compounds from complex natural extracts represents critical and challenging steps for de novo identification and bioactivity assessment. To obtain pure natural compounds in milligram quantities, the chromatographic steps are generally laborious and time-consuming. The main limiting factor in natural products research is repetitive isolation of known or even readily available natural products. Dereplication has been used in recent years to isolate and identify particular targeted or non-targeted compounds from natural products. Our group has isolated various monoterpenes and triterpenoids and identified by spectroscopic methods including LC-MS, GC-MS, Flash chromatography-Mass spectrometer and LC-NMR. Several cohort and case control studies have also demonstrated the association of diet with the incidence of prostate cancer. Molecules that suppress androgen activity have important roles in the prevention and treatment of prostate cancer. In this direction, citrus derived obacunone and obacunone glucoside (OG) caused dose-dependent inhibition of proliferation of human prostate cancer cells. Biochemical analysis of treated cells suggests that inhibition may be due to induction of programmed cell death, as indicated by the activity of key enzymes, and protein levels in cells treated with limonoids. Finally, immunoblotting results indicate that obacunone and OG cause cytotoxicity to cells by activating intrinsic apoptosis, suppressing inflammation, and downregulating AR and PSA. All these events were mediated through negative regulation of the cell-signaling Akt pathway. In addition, kumquat volatile constituents also inhibited

proliferation of androgen-dependent human prostate cancer cells through induction of apoptosis and inhibition of inflammation. Results of the current study suggest that the volatile principles of kumquats have great potential for the prevention of cancer. This project is based upon work supported by USDA-NIFA No. 2010-34402-20875, "Designing Foods for Health," through the Vegetable & Fruit Improvement Center and State funding 2013-121277 VFIC-TX State appropriation.

ORAL SESSION ABSTRACTS

Oral 1. Epigenetic potential of dietary stilbenes in prostate cancer

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Abstract: Dietary stilbenes (resveratrol and its potent analogs) are phenolic compounds with anti-inflammatory, anti-oxidant and anti-cancer activity. Importantly, these compounds can modulate epigenetics and influence cancer susceptibility and progression. Prostate cancer is the most often diagnosed cancer and the second leading cause of cancer deaths in males in the US. Epidemiological studies suggest association between diet and lower risk of prostate cancer. Epigenetic mechanisms implicated in prostate cancer include gene silencing via action of co-repressor complexes (i.e. deacetylation) and changes in microRNA profiles. In this presentation, I will provide compelling evidence on epigenetic mechanisms of resveratrol and its analogs through regulation of the chromatin modifier metastasis-associated protein 1 (MTA1), MTA1-associated network and certain oncomiRs and highlight the anticancer effects of these compounds in vitro and in preclinical models of prostate cancer. It is our hope that resveratrol's analogs with better bioavailability, thereby conferring superior pharmacological potency and greater anticancer effects through epigenetic mechanisms, may become strong candidates for clinical development.

Oral 2. Further studies on pterostilbene as PPARα ligand

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Abstract: We previously demonstrated pterostilbene is an agonist for the nuclear transcription factor peroxisome proliferator activated receptor alpha isoform (PPARα), and showed that PPARα activation by pterostilbene was higher than that of ciprofibrate, a clinically used hypolipidemic drug, at 100 μM. In the present study, we investigated PPARα activation by pterostilbene together with compounds known to occur in blueberries (resveratrol, anthocyanins, catechins) and fibrate drugs (as positive controls) at concentrations ranging from 3.12 to 50.00 μM, in rat hepatoma cell line H4IIEC3. Pterostilbene exhibited a dosedependent activation of PPARα, similar to those of the fibrate drugs ciprofibrate and fenofibrate. In the presence of chenodeoxycholic acid, a specific inhibitor of PPARα, pterostilbene showed dose-dependent decreases in luciferase response paralleling those of Wy-1463, a synthetic PPARα ligand. Docking studies were performed and provided validation of the results obtained in the *in vitro* assays. We further explored whether pterostilbene

upregulates PPARα gene expression, and found pterostilbene significantly and dose-dependently increased PPARα gene expression (at 10, 20 and 50 μM), and exhibited greater induction than 100 and 200 μM of fenofibrate, in H4IIEC3 cells. Correlation of the results with, and insight into, previously observed up-regulation of hepatic PPARα mRNA expression in hamsters fed diet supplemented with 2% ethanolic extract of blueberry skins will be presented.

Oral 3. Selective separation of polyphenolic compounds from grape pomace and their antioxidant activities

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Abstract: During wine and grape juice making from grapes, high quantities of waste products including grape pulp, seeds and skins remain unused. These residues play the major role in the waste management issue for both ecological and environmental reasons. However, these waste products in the food processing industry, so called grape pomace, is a good and cheap source of high quality polyphenolic compounds. Due to the important role of polyphenols in preventing obesity, coronary heart disease, cancer, the utilization of grape pomace by effective and economical extraction and purification methods are essential. In this work, a combined extraction and purification process was developed to separate the polyphenols from grape pomace in high quantities. A purification method based on magnetic polymeric particles with hydrogen bonding affinity ligands was developed and their adsorption behavior for grape pomace polyphenols was investigated. Polyphenolic compounds were extracted from grape pomace with sonication-assisted solvent extraction by using ethanol: water solution (30:70; v/v). The purification of crude grape pomace extract were performed with magnetic particles grafted with polyethylene glycol 600 (PEG-600). The total polyphenol content prior to and after the purification step was determined by the Folin-Ciocalteu colorimetric method and expressed as gallic acid equivalents (GAE) in mg/ml of bulk solution or mg/g dry adsorbent. The results indicated that the magnetic particles grafted with polyethylene glycol 600 (PEG-600) could separate polyphenols up to 38 mg/g dry adsorbent, whereas the commercially available polymeric macroporous resin, XAD-4 could only separate up to 7 mg/g dry adsorbent. Furthermore, it is likely that the polyphenols separated via magnetic particles through hydrogen bonding would have better antioxidant ability compared to those separated via XAD-4 resins due to their high content of hydroxyl groups. The utilization of magnetic particles with affinity ligands can provide rapid and efficient recovery of polyphenols from grape pomace.

Oral 4. Genomic approaches to understand the enhancement of phenolic compounds and other phytonutrients in plants grown under organic nitrogen regime

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Abstract: In a previous study, it was shown that tomatoes fertilized with organic nitrogen sources had higher phytonutrient content than those fertilized with conventional nitrogen sources. In particular, lycopene, ascorbate, soluble solids, and antioxidant contents were found to be higher in ripe fruit tissue from plants fertilized with organic nitrogen. We employed a transcriptomic approach testing the hypothesis that the genomic response of a tomato genotype differs when fertilized with organic vs. conventional nitrogen. Fruit and leaf samples from the plants grown in the previous study were used to generate quantitative transcriptome data. Alignment of the transcriptome data to the tomato genome revealed that the quantity and amplitude of genetic expression differs according to the specific treatment, thereby implying the two soil management regimes elicit differential genomic responses from a single

genotype. Transcriptome data analysis revealed a high degree of variation between the different treatments. Relative gene expression ratios were calculated and compared between the treatments. 3,034 of the 109,262 leaf tissue and 2,324 of 97,738 fruit tissue sequences were significantly over or under expressed between the organic and conventional treatments. BLAST and Gene Ontology analysis was performed and the annotated sequences underwent enzyme mapping to identify associated biochemical pathways. The analysis revealed metabolic pathway genes associated with phytonutrients, such as ascorbate and lycopene, were preferentially upregulated in the organic treatment. This observation leads to the hypothesis that different varieties of tomato will show differential genetic responses leading to the identity of genotypes amenable to production under organic practices.

Oral 5: *Centella asiatica* phenolics - Modulation of the brain metabolome

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Abstract: *Centella asiatica* (CA), also known as gotu kola, has received considerable attention as a medicinal herb that exhibits neuroprotective properties, with possible applicability to the management and treatment of age-related cognitive impairments and Alzheimer's disease. We report on our comparative metabolomics studies designed to elucidate the mechanisms with which extracts of CA leaves may modulate brain function in healthy young and aged wild type mice showing behavioral and gene expression changes in response to CA extracts. We used untargeted metabolomics workflows based on high-resolution mass spectrometry coupled to UPLC for metabolite detection and quantification.
For the characterization of the phenolic compounds in extracts of CA leaves we utilized advanced tandem mass spectrometry with ion mobility spectrometry. Identification of metabolites was based on experimental accurate mass and fragment ions and comparison with entries from our in house compound library containing >600 metabolites. We detected and quantified more than 300 metabolites and lipids in the hippocampal extracts. Initial data analyses involved multivariate and univariate methods. Metabolites were found in the following functional groups/pathways: purine metabolism/DNA synthesis; amino acid utilization/protein synthesis and turnover, energy utilization/mitochondrial function, polyamine metabolism/cell proliferation, glutathione synthesis/oxidative stress response, and several lipid classes. Our data indicate that, in particular, multiple lipid species showed altered levels in the CA-treated mice, suggesting that CA phenolics may affect lipid metabolism.

Oral 6. Rapid growth of high quality goldenseal plants in controlled environment growth chambers

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Abstract: Goldenseal, *Hydrastis canadensis* (L.), is a slow growing perennial forb, native to the Eastern United States, and listed by CITES due to habitat loss and destructive harvesting for alkaloids found in roots and rhizomes. Since it takes 4 to 6 years to reach a harvestable size, controlled environments have been tested to determine if cultivation could be shortened without a loss in product quality. Two year old seedlings of goldenseal were grown in climate controlled growth chambers with two media, each with and without perlite supplements, at 3 CO2 concentrations (380, 880, 1380 ppm) for 15 weeks. Fresh mass was harvested, dried, extracted and measured for berberine and hydrastine concentration, and the bioactivity

(compared with extracts of forest cultivated goldenseal). Concentrations of both alkaloids, and the bioactivity of plant extracts were near identical when chamber grown and forest field plants were compared. Plants in forest field cultivation double in mass every 2 to 3 years. During 15 weeks in the chamber plants doubled in mass, and with a coarse medium they more than tripled in mass (this was reduced by additional perlite). Most of the plant mass (about 75%) was in the roots and rhizomes. Plants with the greatest root mass produced the lowest concentrations of hydrastine and berberine. Leaf growth was reduced by the additional perlite, but the concentration of hydrastine was increased by additional perlite. Roots and rhizomes accumulate more alkaloids than leaves, and root mass was more important than concentration in determining yield. Therefore, plants with the greatest amount of alkaloid came from the coarse mix without perlite. Using controlled growth environments it was possible to accomplish several years of growth in a few months and generate plants with greater or equal quality to forest cultivation.

Oral 7. Crop improvement in Stevia (*Stevia rebaudiana* Beroni) through mutation breeding

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Abstract: Developing mutants of *Stevia* with higher content of steviol glycosides is the primary aim of plant breeders concerned with the improvement and utilization of the source of natural sweeteners. Manipulation of ploidy is a valuable tool to improve agronomic yield and quality. Different Stevia mutants were developed in this regard using Colchicine treatments (0.25%, 0.50%, 0.75%, 1.0%, 1.50%, and 2.5 %) and each were tested for better growth, yield and content of active components. Morphological measurements like plant height, leaf length, width, thickness, stomatal number per unit area and number of inflorescences per plant were recorded. Ploidy level was identified by Flow cytometry analysis. The steviol glycoside content in the leaves was determined by HPLC. Analysis of variance revealed highly significant differences among the plants and the tetraploids and mixaploid plants showed better morphological characters, stevioside and rebaudioside content compared to control. Thus induction of tetraploidy in Stevia confirmed the effectiveness of colchicines as a polyploidizing agent.

Oral 8. Effects of various level of coal-bed methane water on dill (*Anethum graveolens* L.*)* and its essential oil

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Abstract: Pumping out water from coal seams decreases the pressure in the seam which in turn releases trapped methane. This is the most common and economic way of extracting methane. The water being pumped out is known as coal bed methane water (CBMW) which is high in sodium and other salts. US in past 25 years has seen 16 folds increase in production of coal bed methane gas and trillions of cubic meter of this gas are yet to be extracted. CBMW lacks sustainable disposal and there are very few studies investigating effect of this water on plants, their secondary metabolites and even soil. Hence, the main objective of this study was to elucidate the effect of this water on soil and on essential oil yield and composition of dill (*Anethum graveolens)*. This crop was grown in green house and was subjected to different level of CBMW treatment, namely, 0% CBMW (tap water only), 25% CBMW (25%CBMW plus 75% tap water), 50% CBMW (50% CBMW and 50% tap water), 75% CBMW (75% CBMW plus 25% tap water)and 100% CBMW(only CBMW). After the study soil, plant weight, plant

height, essential oil yield and composition were statistically analyzed. The major oil constituents limonene and α-phellandrene were not affected by the treatments. With increasing CBMW levels, the concentration of dill ether increased, whereas the concentration of carvone decreased. In soil, bicarbonate and sodium levels showed significant increase with increasing level of treatment, however soil pH and cation exchange capacity were not much affected. The results demonstrated that CBMW could be used for irrigation of essential oil crops when diluted with high-quality water.

Oral 9. Comparison of Photosynthesis between Treatment and Non-treatment of Lime Bordeaux Mixture in 3 Year Old Root in Panax ginseng C. A. Meyer

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Abstract: Korean ginseng has been used for thousands of years as an important medicinal plant. Lime-Bordeaux mixture (LBM) was made with copper sulfate and quicklime, which was sprayed instead of pesticides in ginseng field. Net photosynthesis (PN) was compared between Treatment and Non-treatment of LBM in 3 Year Old Ginseng. PN in control plot recorded 2.94 μmol (CO2) m-2s-1 at the first day of experiment, which was similar until the last day of experiment. However, The PN in LBM recorded 2.23 μmol (CO2) m-2s-1, which was lower than that in control plot. Over the course of the experiment, The PN in LBM gradually increased up to 3.21 μmol (CO2) m-2s-1 and finally, it was similar with that in control plot at 7th day as a 3.20 μmol (CO2) m-2s-1). SPAD of ginseng leaf in control plot was 35 levels at the first day, which was higher than that in LBM treatment. In contrast, SPAD in LBM plot demonstrated lower than that in the control plot after spraying LBM. However, over time, SPAD in LBM plot had a slow increase and was recovered to the fourth day after spraying LBM. It continued to have a similar level between two of them at 7th day. Underground growth characteristics and root yield between the treatment of LBM and agrochemicals showed no significant differences statistically. Root yields per 3.3 m2 were 1.06 kg and 1.18 kg in the treatment of LBM and agrochemicals, respectively.

Oral 10. Medicinal plants used for human and animal health in a rural Guatemalan community

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Abstract: The purpose of this project was to investigate the use of medicinal plants in a smallholder, primarily Ladino community located on the pacific coast of Guatemala. In this smallholder community households are primarily subsistence oriented and rely on a number of livelihood activities such as animal husbandry, fishing, and participation in the local tourism economy. In rural Guatemala, medicinal plants often form part of a pluralistic medical system where home remedies are used in combination with the biomedical system. In the study community, households engage in a pluralistic medical system that includes the use of medicinal plants as home remedies for common ailments for both humans and animals. The most culturally salient treatments for common ailments such as the common cold, ear ache, gastritis, and intestinal parasites for humans and chickens were investigated along with the importance of each treatment to overall economic structure of the household. It was found that for the majority of households, regular use of medicinal plant treatments have the potential to maintain good overall health of household members and their animals. Thus, reducing the number of visits to biomedical practitioners and veterinarians, and the need to purchase overthe counter medication.

Oral 11. Dominican explanations and herbal treatments of fright, a Caribbean

ethnopsychiatric condition

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Abstract: "Fright" (in English) or *sésisma*, in French Creole, is a Caribbean folk syndrome, of persistent distress. Caribbean people theorize that an overload of stressful emotions (fear, panic, anguish or worry) causes a cold humoral state in which blood coagulates causing prolonged distress and increased risks of other humorally cold illnesses. Ethnobotanical and epidemiological data come from freelist tasks with nearly all adults (N=112) of an eastern village in Dominica, and analyzed for salience. Local explanations and treatments of fright were collected using a village survey on medicinal plant recognition and use (N=106), participant-observation, informal key informant interviews and a village health survey. Three herbs are salient fright treatments: *Gossypium barbadense, Lippia micromera, and, Plectranthus [Coleus] amboinicus*. Twenty-seven percent of village adults had medicated themselves for fright, and the probability of having (and medicating for) fright increases with every year of age. While sufferers are often uncomfortable recalling personal fright experiences, reporting use of medicinal plants is less problematic. Inquiry on fright phytotherapies serves as a proxy measurement for fright occurrence. Cross-cultural and ethnopharmacology literature on the medicinal plants suggests probable efficacy in accord with Dominican ethnomedical notions of fright. Further, the cultural salience and beliefs about these medicines may give these medicines extra psychoneuroimmune (i.e. mind-body) benefits, or placebo-like effects, for this stress-related disorder.

Oral 13. Metabolic biology of bioactive medicinal foods from American Indian food systems

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Abstract: Changes in dietary patterns and calorie dense nutritional composition in the past century have significantly contributed in the rise of type 2 diabetes (T2D) globally. The prevalence of T2D is highest among the American-Indian populations as evolutionary makeup of these populations has been affected globally with rapidly changing food habits and lifestyles. The diabetes epidemic and associated cardiovascular risks are major threat and causes significant morbidity in Native populations. Traditional food systems have the potential to offer protection against T2D in these communities since over centuries these provided balanced nutritional composition with rich bioactive phytochemicals profile. The evolution and domestication of crops in these traditional communities with specific climate and soil niche has sound scientific foundation and needs special attention to develop diets for cost effective management of T2D. Previous and current research in our laboratory has shown potential health benefits of the traditional Native American three sisters' crops (corn, bean and pumpkin) with relevance to T2D and hypertension management. High inhibition of enzymes related to glucose metabolism and hypertension was observed in traditional pumpkin and bean varieties. Similar functional benefits were observed in indigenous grains from Peruvian Andean region. Rich functional phenolic profile in indigenous grains offers significant promise to incorporate as dietary strategies for management of T2D and hypertension. Based on this scientific rationale it is feasible to design research program on traditional food and health to offer better preventative and disease management strategies to combat T2D in Indigenous communities.

Oral 14. Systems analysis of spatial and temporal control of metabolism in medicinal plants

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Abstract: Plants produce a vast array of specialized metabolites (often called "secondary" metabolites or "natural products") that are responsible for determining to a large extent the chemical diversity that exists within the plant kingdom. Such compounds mediate specific interactions between individual plants/plant species and their environment, such as by playing critical roles in plant defense, in pollinator attraction and seed dispersal, among other functions. What effect specific compounds have on the physiology of the plant and on its interactions with the environment pose significant questions in plant biology. How the production of such compounds is regulated is now being addressed using "omics" technologies, such as proteomics, metabolomics and large scale gene expression profiling (transcriptomics). In addition, tools that can determine fine-scale resolution of biochemical processes within plant tissues, such as laser microdissection and imaging mass spectrometry, now open up new avenues to understand how metabolism is structured and regulated within plant tissues. Examples of how such tools have led to an increased understanding of the regulation of the accumulation of specialized metabolites that are of interest to humans due to their medicinal or other important bioactivities will be discussed. Implications for plant breeding and/or engineering to enhance plant properties will also be presented.

Oral 15. Teasing out the unique biochemistry in specialized cell types that produce natural products in medicinal plants

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Abstract: Plants accumulate a diverse array of metabolites, which is enabled by the occurrence of secretory structures that first emerged in early vascular plants. I will provide an account of our current understanding of how these structures and the biochemical machinery contained therein evolved. The ability to sequester secondary (or specialized) metabolites (also commonly referred to as natural products) and defense proteins in secretory structures was a critical adaptation that shaped plant-herbivore and plant-pathogen interactions. Single cell technologies, which have played a fundamental role in furthering our knowledge of specialized metabolism in plant secretory structures, with important implications for the medicinal plant research community, will be highlighted as well.

Oral 16: Product specificity changes in mutants of a model monoterpene synthase

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Abstract: Terpene synthases catalyze complex, chain length-specific cyclization reactions that often constitute the first committed step in the biosynthesis of structurally diverse terpenoids. (4S)-Limonene synthase from *mentha spicata* is a model enzyme for evaluating and understanding the reactions leading to the cyclization cascade from geranyl pyrophosphate (GPP) to various products in monoterpene synthases. The product profiles obtained after alanine scanning mutagenesis of 48 residues in closest proximity to the substrate indicated which residues were most likely to be directly involved in catalysis. Site saturation mutagenesis at selected locations was employed to evaluate potential roles of specific residues in stabilizing carbocation intermediates and/or serving as catalytic base in the final deprotonation reaction. The broader implications of our findings for understanding monoterpene synthase reaction catalysis and termination chemistry will be discussed.

Oral 17: Characterization of unknown plastid targeted genes involved in production of metabolic compounds in medicinally active plants

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Abstract: Plants have been used as medicine throughout human history. However, many of

the medicinally relevant compounds are synthesized via pathways that are only partially characterized. In order to maximize the efficient production of these valuable compounds in their native species or in a heterologous plant species, elucidation of the genes involved in these pathways is key. For many of these pathways, key steps are known or suspected to occur within the highly reductive environment of the plastid. Precursors such as terpenes and aromatic amino acids are also produced in plastids, giving this organelle an unparalleled degree of control over biochemical flux. Production of the antimalarial drug quinine (*Cinchona pubescens*), the neurotransmitter antagonist atropine (*Datura innoxia*), and the anticancer drug paclitaxel (*Taxus x media*) are known to be largely plastid-synthesized. We are characterizing the plastid-targeted proteomes of these species using the UCLUST algorithm to elucidate common and unique genes from each species. We analyzed RNAseq data using a custom assembly and analysis workflow. The resulting assemblies were used to derive peptide sequences that were further analyzed using TargetP to identify plastid targeted genes. Comparative analysis of predicted plastid targeted proteomes was performed using a recently published method (Schaeffer et al., 2014). This analysis is expected to yield candidates for genes involved in respective medicinal compound biosynthesis, and will facilitate the unraveling of the biochemical pathways involved in the synthesis of these drugs.

Oral 18. Transcriptomic and targeted metabolomics approaches to elucidate the biosynthesis of prenylated stilbenoids: Cannabinoid receptor modulators from peanut

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Abstract: Peanut (*Arachis hypogaea*) produces stilbenoids upon abiotic and biotic stress. Interestingly, in peanut the majority of these polyphenols are prenylated. Among them, arachidin-1 and arachidin-3 are potentially beneficial to human health due to their antiinflammatory and antioxidant properties. In addition, we demonstrated that these prenylated stilbenoids have the ability to modulate the cannabinoid receptors and thereby may have applications in the control of obesity and prevention and treatment of neurodegenerative diseases and cancer (Xenobiotica 2012, 42:139-156). In order to identify the genes involved in the biosynthesis of prenylated stilbenoids, we developed a bioproduction system using hairy root cultures that was able to yield very high and sustainable levels of diverse types of nonprenylated and prenylated stilbenoids. To elucidate the biosynthetic pathways of these compounds, the transcriptome of the elicited hairy roots was sequenced. Candidate genes involved in the biosynthesis of these compounds were selected and are being characterized. These integrated metabolomics and transcriptomics approaches will advance our understanding of the mechanisms that affect that the production and accumulation of these health-related compounds in this economically important crop and lead to metabolic engineering strategies to introduce bioactive compounds into other crops.

Oral 19. Mineral nutrition of *Curcuma longa* L. in bioreactors affects subsequent development of curcuminoids following transfer to the greenhouse

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Abstract: Multifactor design of experiments, with D-optimal response surface methods established how *in vitro* minerals concentrations, P, Ca, Mg, and KNO3, and plant density effected curcuminoids concentrations in plants after 6 months of greenhouse growth, with both high and low-input fertilizer treatments of *Curcuma longa* L. Curcuminoids content in dry rhizomes was analyzed by HPLC-MS. In the high fertilizer treatment, the *in vitro* interaction of

P with Ca and Mg concentrations was important to curcumin and demethoxycurcumin (DMC) concentrations in the rhizomes regardless of plant density. The optimal medium for curcumin and DMC was 1.25 mM P, 3 mM Ca, and 1.5 mM Mg, 60 mM KNO3 with 18 or 11 buds/vessel respectively. Reducing KNO3 concentration to 20 mM at 1.5 mM Mg maximized BDMC concentration with 9 buds/vessel and 9 mM Ca. The best induction media under the low-input fertilizer treatments had similar optimal set points for all three curcuminoids; the concentrations were maximized with 6 buds/vessel, 6.25 mM P, 3 mM Ca, 1.5 mM Mg, and 60 mM KNO3. In the high fertilizer treatments, each compound had distinct mineral uptake efficiency, but in the nutrient limited treatments all curcuminoids had the same mineral concentrations and plant density requirements. The multifactor design sufficiently identified the interactive effects among varied minerals on curcuminoids concentrations with the stringent error controls applied during application of treatments in micro-rhizome culture system.

Oral 20. A standardized extract of the edible fruits of Phyllanthus emblica (Amla) – The next generation heart health product

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Abstract: A standardized extract of the edible fruits of Phyllanthus emblica, popularly known as Amla with a history of medicinal use for thousands of years, has been a subject of several clinical studies in type 2 diabetics as well as pre-diabetics at NIMS in Hyderabad, India and OSU in Columbus, OH, and an ischemia-reperfusion study in rats at University of Connecticut. This presentation is a summary of these studies. A highly standardized extract of Phyllanthus emblica, containing polyphenolics Emblicanin-A, Emblicanin-B, Punigluconin and Pedunculagin as bioactives, provides a very comprehensive heart health coverage. It improves endothelial function (US 8,962,576), increases nitric oxide and glutathione levels, reduces LDL, triglycerides, the inflammation biomarker, hsCRP, and HbA1c, and increases HDL, both in type 2 diabetics as well as pre-diabetics, although the effects are less significant in pre-diabetics. It improves blood flow by inhibiting platelet aggregation (US 6,290,996) and is a pro-oxidation-free super antioxidant (US 6,362,167) with a very high ORACFN value of 47,000 μ moles TE/g. It reduces the damage to heart muscle during ischemia in rats and mitigates the cardiovascular problems caused by cold stress and mental stress in healthy volunteers.

Oral 22. Concept of a field portable test for marijuana in breath

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Abstract: The Hill lab at Washington State University is developing a field portable device for the detection of marijuana in human breath. This approach is based on three separate proven concepts that will be integrated into a novel device for the rapid detection of marijuana and other drugs in human breath. This three-pronged approach includes capture, desorption, and detection. The initial market for the device will be the Drug Recognition Expert (DREs), police officers who are responsible for enforcing impaired driving laws with "drugged drivers" – persons whose impairment stems from the use of illicit or prescription drugs. There are 3000+ such specially-trained officers in over 30 US states. The many states with medicinal marijuana (over 20) and bordering jurisdictions already experience significant cannabis impairment, and with WA, CO, OR, AL and Washington DC having passed recreational marijuana statutes (and California schedule to vote on the matter in 2016) the market for these devices is growing exponentially. This presentation will describe the basic chemical

concepts of the breathalyzer and provide some preliminary data. Studies with human breath are underway but have not been completed so no data from human breath will be presented.

Oral 23. Tissue culture technology: Potential tool for conservation, cultivation and commercial production of medicinal plants

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Abstract: Medicinal plants are considered to be an important source of life-saving drugs for majority of the world's population. Recently, awareness in the study of medicinal plants and utilization of herbal medicine in primary healthcare systems have increased worldwide, and caused high demand for medicinal plant products. High demand for certain important medicinal plants caused over exploitation and total destruction of medicinal plant habitats. Therefore, conservation, cultivation and commercial production of high quality medicinal plants have become a matter of urgency for the future generation. Application of biotechnological tools may speed-up and enhance the efficiency in this process than the conventional methods. Plant tissue culture studies were carried out for the conservation, cultivation and commercial production of medicinal plant resources and efficient production of secondary metabolites. Micropropagation methods for several medicinal plants were developed and optimized for large-scale production. These micropropagation methods enabled much more efficient in conservation and propagation of medicinal plants when compared to the conventional methods. Micropropagated medicinal plants showed uniform growth in the field and they constituted relatively high alkaloid contents compared to the plants grown from seedling. In vitro methods showed to be a better method for conservation of medicinal plant resources. Plant tissue culture technology proved to be a potential tool for conservation, cultivation and commercial production of medicinal plants.

Oral 24. Characterization of polymorphisms for genetic tracking in *Cannabis* sp. for regulation and enforcement

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Abstract: Commercially valuable *Cannabis* species include C. sativa, C. indica, and C. ruderalis. Genetic diversity and selection in Cannabis sp. have resulted in an array of biochemical profiles in modern hybrids - thought to confer specific therapeutic properties. Generally, strains grown for medicinal or recreational purposes derive from indica-dominant (ID) or sativa-dominant (SD) genetic backgrounds. ID and SD strains correlate to discrete chemotypes and are known to develop variable profiles of phytocannabinoids. ID strains typically contain increased levels of the medicinally-active cannabidiol (CBD), cannabichromene (CBC), cannabinol (CBN), and cannabigerol (CBG), while SD strains are enriched in cannabidivarin (CBV), tetrahydrocannabivarin (THV), tetrahydrocannabinol (THC). Recent pedigree-based information has allowed correlations of genetic backgrounds with these unique biochemical profiles, yet DNA sequence-based correlations remain unclear. To address this knowledge gap, genome-wide characterization of sequence variants (SNPs, MNPs, indels) between a modern SD-strain parent ChemDawg (CMD) and haploid ID-strain parent Purple Kush (PRK) was performed. Initial results reveal over 37,000 sequence variants between the two genomes. These results provide a foundation to tag and track individual Cannabis genotypes in the current legal environment in the USA. This information may also clarify the BD/BT alloenzyme model of CBD-THC interconversion described in prior work. This data provides an opportunity for targeted improvement of Cannabis germplasm toward accumulation of specific metabolites with medicinal or industrial utility.

Oral 25. Forest Medicinal Plant Species for Potential Forest Farming in the Southeastern United States

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Abstract: Among non-timber forest products, medicinal plants are probably the most lucrative and perhaps the least understood forest resources. Forest farming is a holistic land-use approach that can enable woodland owners to diversify income opportunities, improve management of forest resources, and increase biological diversity. The forests in southeastern US are considered to be the most diverse in the country. Yet, there have been limited studies on the potential for growing shade tolerant plants as understory crops in southeastern forests. There is a renewed interest in forest farming as an enterprise either via wild simulation or cultivation of native forest botanicals in the woods. The USDA listed nearly 50 forest medicinal plant species with potential for commercial use for treating common colds to complex chronic illnesses such as cancers and diabetes. While American ginseng (*Panax quinquefolius*), goldenseal (*Hydrastis canadensis*), and black cohosh (*Actea racemosa* L.) among a few others, are well known and perhaps well exploited, the following medicinal plant species have potential for commercial production in Alabama in particular and the southeastern US in general: vanilla leaf (*Carphephorus odoratissimus),* fringe tree (*Chionanthus virginicus),* roundleaf sundew (*Drosera rotundifolia),* evening trumpet flower *(Gelsemium sempervirens),* Canadian licorice root *(Ligusticum canadense),* queen's delight *(Stillingia sylvatica),* yellowroot *(Xanthorhiza simplicissima),* true unicorn root *(Aletris farinosa)* and Hercules' Club *(Zanthoxylum clava-herculis).* Thus, Alabama with diverse forest types across the state offers much potential for woodland owners to generate forest-based income in the short term from forest farming with a wide variety of medicinal plant species while waiting for returns from timber in the long term.

Oral 26. Dietary modulation of gut microbiota, a key player under the scenes that might help to reduce risk factors for cardiovascular disease

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Abstract: Bioactive compounds in the traditional cereal wheat (*Triticum* spp.) and the pseudocereal quinoa (*Chenopodium quinoa*) include polyphenols and fiber that can reach the colon and provide benefits to reduce risk factors that cause chronic diseases. The obese diabetic (db/db) (obese control) mice and the lean counterparts (lean group) fed with a standard diet were compared to a db/db group fed with an isocaloric Std. diet supplemented with quinoa (quinoa group) of whole wheat (wheat group) for 8 weeks (n=5). Results showed that body weight gain was prevented in quinoa group during the first 5 weeks (~ 84.87 ± 1.46% of obese control), though at the end of the study body weights were similar among the obese control, and the wheat and quinoa groups. Both, the wheat and quinoa diets promoted changes in relative abundances of fecal bacteria at the phylum levels. The wheat-induced changes in bacteria phylum resembled those reported in obese subjects under caloric restriction diet. The changes in fecal bacteria in wheat and quinoa groups were accompanied with downregulation of mRNA levels of markers of cell stress and inflammation in colonic mucosal cells. The protective effects of colonic mucosal cells might be due to the polyphenols present in quinoa and wheat that also downregulated pro-inflammatory markers in colonic epithelial Caco-2 cells in vitro. Changes in intestinal inflammatory markers seem to modulate host responses regarding plasma and oxidative stress and metabolic disorders associated with lipid profiles, which might result in protection of whole grains consumption against events

that lead to inflammatory-related chronic diseases.

Oral 27. A randomized, double-blind, placebo controlled, parallel-group study to evaluate the effect of a standardized extract of *Terminalia chebula*, a chromium complex and their combination on joint health in subjects with moderate osteoarthritis

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Abstract: Treatment with an aqueous extract of *Terminalia chebula* 500 mg and 250 mg BID, a chromium 400 mcg (as chromium emblicate) OD, and a combination of *Terminalia.chebula* 500 mg BID and chromium 400 mcg (as chromium emblicate) OD for a period of 12 weeks in moderate osteoarthritis patients have shown a significant reduction in modified WOMAC score, Kneeswelling index, and VAS – Pain, Stiffness and Disability, when compared to baseline and placebo. Both Terminalia chebula and chromium emblicate, when given individually have shown reductions in the efficacy variables, but the predicted synergism of their combination was not observed. Further, *Terminalia chebula* 500mg group produced better reduction in outcome parameters both statistically and clinically as compared to the other treatment groups .The number of rescue medication tablets (Acetaminophen 650mg) was minimally used by *Terminalia chebula* 500 mg group as compared to the other groups. The safety laboratory parameters were within normal limits at the end of study. All the study medications were well tolerated and no serious adverse events were observed and none of the patients have discontinued the study due to any adverse event. It was interesting to note that *T. chebula* extract was devoid of significant GI side effects which are normally observed in patients routinely taking NSAIDs for symptomatic relief in OA.

Oral 28. Transverse Thin Cell Layer Culture to Assist Micropropagation and Genetic Transformation of *Scutellaria*

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Abstract: *Scutellaria* genus has close to 400 species and many of them are medicinally important. We are conducting research on *Scutellaria ocmulgee* that is threatened in distribution but highly medicinal. We have been conducting research on developing micropropagation protocols to assist ex situ conservation as well as multiplication of existing stock. We present our research on the shoot bud induction, multiplication, rooting and acclimatization process using transverse thin cell layer (tTCL) explants. We also establish that it could be suitable technology for threatened and endangered plants as the process involves use of minimal plant material to initiate cultures. We further tested the application of leaf tTCL explants in *Agrobacterium tumefaciens* mediated gene transfer. Introduction of GFP reporter gene was used as the primary tool to screen putative transformed explants. Successful transformation resulted in transgenic plants after regeneration and further molecular characterization was carried out by PCR, RT-PCR and Southern blotting.

Oral 29. Understanding *Cannabis* titration through edibles and cannabinoid effects on different diseases

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Abstract: Too much or too little, is there such a thing? Or is there an exact dosage of cannabis required to effectively treat a disease? When discussing cannabis in the edible form, there truly is the "right dose". Not only is consuming the correct dosage important, especially in edible form, the proper phenotype or variety of cannabis being used to treat the

ailment or disease is just as crucial. To cover a variety of diseases that cannabis treats one will need to understand the varieties of cannabinoids that make up the *Cannabis* plant. Each particular strain or phenotype will also be important when trying to treat each patient. It is important to understand the chemistry that takes place in all forms and stages of the cannabis plant. For example, in the beginning stages, cannabis will have a cannabinoid called Cannabigerol Acid, CBGA, which is the precursor for all cannabinoids in the *Cannabis* plant. This cannabinoid is what will give each plant its unique expression of its cannabinoid makeup. Through this, CBGA, will be transformed into THCA, CBDA, or CBCA, etc. through enzymatic reactions. These forms are all in their acid form. For the medicinal effects, along with the psychedelic effects cannabis provides, most of these cannabinoids will need to be decarboxylased. Decarboxylation takes place by heating the molecule to a specific heat. With edibles, it's unique because the decarboxylation process takes place during most of the heating process that naturally comes with cooking. There are different methods of edible ingestion including sublingual and regular digestive consumption. Understanding how much to consume is very important in getting the correct dosage. I have a 6 year old epileptic patient that started out using 3 mg per kg of body weight. The patient then stepped up the dosage of 5 mg per kg of body weight. Then stepped up the dosage 1 mg every 4-5 days until the seizures stopped. With this particular patient, he seems to stay between 10 mg-15 mg per kg of his body weight. There are other patients that are getting the effects up to 20 mg per kg of body weight. So it is very important to understand how to start at a low base, and titrate your way up to the proper edible dosage. It has been noticed that by consuming an excess of cannabis in the edible form actually starts becoming less effective to treat the seizures. There is proper dosing, as previously mentioned for seizures, as there are for PTSD, OCD, and several auto immune disorders which will be further discussed.

Oral 30. Results from auditing medical cannabis operations in the United States

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Abstract: Regulation is becoming mandatory in states that allow medical cannabis. The producers, manufacturers, dispensaries, and laboratories involved in this industry can operate legally in their states but function without much regulation or oversight. Due to increasing concerns over the need to standardize medicinal cannabis preparations, the American Herbal Product Association (AHPA) has created industry guidelines on manufacturing, producing, dispensing, and laboratory operation standards. Additionally, the American Herbal Pharmacopeia (AHP) completed the Cannabis monograph, a guide for the standardization of cannabis. The work of AHPA and AHP laid the foundation for a certification body called Patient Focused Certification (PFC) a project of Americans for Safe Access. AHPA and AHP guidelines are being incorporated into state level regulations as mandatory product safety standards in new state programs. PFC launched in early 2014 with facilities in several states having successfully completed the auditing process. Over a dozen operations have been certified in over 8 states. Results from over a year of auditing of medical cannabis facilities will be discussed, including data on corrective actions with research on the impact of such regulations on patients, facilities, government, universities, and neighborhoods.

POSTERS ABSTRACTS

P1. Generation of feruloylated oligosaccharides from rice bran fiber for functional analysis

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Abstract: Whole-grain rice products can contribute diverse functional components to the diet to promote improved gastrointestinal health in humans. We are isolating feruloylated arabinoxylan-oligosaccharides (FAXOs) from rice bran fiber to evaluate *in vitro* their bioactivity in modulating innate immune responses in human colonocytes. A better understanding of the function, bioavailability, and interactions of rice bran components in colon functioning will determine their dietary utility and identify effective processing technologies for formulating safe, high quality rice products enriched with these components in a sustainable manner. Our current objectives are to 1) compare yields of FAXO fractions following dilute acid hydrolysis and enzyme treatments, 2) evaluate profiles by hydrophilic interaction liquid chromatography (HILIC) separation chemistry, and 3) determine ability of FAXOs to modulate tight-junction protein levels in T84 colonocytes. Results from these objectives will be presented.

P2. Antiviral effects of selected stilbenoids isolated from peanut hairy root cultures

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Abstract: Rotavirus (RV) is the second leading cause of vaccine preventable disease specific death in infants and children under five years of age worldwide, according to the World Health Organization. While two live attenuated vaccines are available, factors including cost and logistics prevent many from receiving the vaccine. Additionally, the vaccine only reduces the viral load, lessening symptoms but not preventing the virus from spreading. Therefore, the search for a cost-effective, shelf-stable therapeutic could be important in reducing morbidity and mortality of the virus, especially in developing countries. The purpose of this study was to investigate the merit of stilbenoids, extracted and highly purified from peanut *(A. hypogaea)* hairy root cultures, as therapeutic agents to treat diarrheal symptoms of RV infections. Different concentrations of stilbenoids were combined with RV and added to HT29.f8 cells. At 24 hours post infection, the cells and supernatants were collected. Using the supernatants, viral titers were calculated with both focus forming and plaque forming assay. The results obtained from both assays consistently demonstrated a significant decrease in RV titers with two of the four stilbenoids tested, *trans*-arachidin-1 and *trans*-arachidin-3. Western blot analyses performed on the infected cell lysates complemented the titers and implied a decrease in viral replication. Additionally, slot blot data on MA104 and HT29.f8 cells revealed the presence of specific receptors which may be involved in the antiviral mechanism. These data reveal stilbenoids have antiviral properties that may prove to be effective as medicinal treatments for RV infections.

P3. Extraction phytochemical analysis and antioxidant activity of *Vernonia amygdalina* L.

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Abstract: Genus *Vernonia* has about 1000 species of shrubs spread over Asia and West African countries. *Vernonia amygdalina*, generally known as bitter leaf plant belongs to the Asteraceae family. It is a multipurpose medicinal plant which is also used as a vegetable in Africa. Bitter leaf preparation is a cure for diabetics, loss of memory, prostate cancer, insomnia, galactagogue, loss of appetite, dysentery and can combat breast cancer. Proximate analysis and antioxidant activity of *Vernonia amygdalina* leaves were studied. Using methanol as a solvent, the phytocompounds were extracted in a Soxhlet apparatus. The extract was concentrated and analyzed for moisture, ash and ascorbic acid content. An

attempt was also made to quantify coumarin present in *Vernonia amygdalina* using HPLC. Antioxidants act by several mechanisms and a single assay cannot capture the different modes of action of an antioxidant. Free radicals may be either oxygen derived or nitrogen derived molecules. Thus, more than one type of antioxidant assay were conducted. Assays like DPPH, nitric oxide scavenging assay, Reducing power assay, Superoxide anion scavenging assay was carried out. Results revealed that *Vernonia amygdalina* leaf is a very good source of phytochemicals and antioxidant activity underlining its importance and nutraceutical value.

P4. Detection and elimination of Tobacco Streak Virus in basil (*Ocimum* spp)

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Abstract: Basil (*Ocimum* spp.) is the most economically important herb in the US and commonly used for culinary purposes. Several elite germplasm have been developed for unique aroma and flavor and also for resistance to important pathogens such as basil downy mildew and Fusarium wilt. Tobacco streak virus (TSV) is a common viral disease transmitted by thrips and affects a wide range of crops. Once found, this virus can spread rapidly into germplasm and breeding collections. In some cases, infected plants can exhibit necrosis, malformation of leaves and flowers but in other cases no symptoms of the disease may be visible. The objective of this study is to determine an effective method for detection and elimination of TSV from basil plants including a downy mildew resistant sterile hybrid F1 and a Fusarium wilt resistant inbred line. Meristems, approximately 0.2-0.8 mm, consisting of a meristematic dome plus a few leaf primordia, were aseptically excised from an actively growing plant, established on Murashige and Skoog tissue culture medium supplemented with different concentrations (0-0.5mM) of benzyl adenine (BA) and (0-0.05 mM) naphthaleneacetic acid (NAA). Greenhouse and in vitro regenerated plants were then tested for the presence of the virus by enzyme-linked immunosorbent assay (ELISA) and by RT-PCR using specific TSV coat protein primers. This is the first report of TSV in basil plants and the use of *in vitro* meristem tip culture as an effective method for TSV virus elimination in basil plants.

P5. Influence of soil flooding in reducing the density of *Cylindrocarpon destructans* in Ginseng crops

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Abstract: This experiment was performed in order to control root rot due to *Cylindrocarpon destructans*, which decreases the quantity and quality of ginseng and causes the damage of repeated cultivation, by soil flooding. Rice straw was added to the soil where ginseng was harvested and was flooded at 10℃, 20℃ and 30℃ for 60 days. There was no density change at 10℃., and the density was slightly decreased at 20℃. However, it sharply decreased from the initial treatment density of 5.41logCFU/g to 2.92logCFU/g. *C. destructans* mycelia and microconidia lysis was found after 30 days at 30℃. Chlamydosphore lysis was also found after 60 days. So as to actually apply this to the ginseng field, we examined the temperature of soil at a depth of 20cm in the field where rice was grown after harvesting ginseng and field flooded with rice straw. The soil in the field where rice was grown was at approximately 20℃ during the summer, from July through August. However, the soil flooded was at 30℃ or over. Therefore, it shows that flooding with an organic matter such as rice straw was more effective

in reducing the density of *C. destructans* than growing rice in paddy fields where ginseng had been harvested.

P6. The science of medicinal & aromatic plants: Recent trends

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Abstract: A survey of the scientific literature on medicinal and aromatic plants over the past 15 years was used to determine the direction of on-going research in these plant materials. Information was collected from three databases (Google Scholar, CAB Abstracts, and Agricola), using keywords related to plant species, bioactivity, and cultivation. Tabulated data clearly illustrate trends within the science connected to medicinal and aromatic species. This review magnifies the efforts and perspectives of researchers actively exploring current and new plant materials and plant constituents to improve the knowledge base on medicinal and aromatic species. Developmental trends illustrate changes in emphasis as the need for more information on particular species or subjects arise.

P7. "I don't have prostate:" Salient Herbal Medicines Used in the Treatment and Prevention of Prostatitis in Dominica, West Indies.

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Abstract: Male reproductive health has been understudied, particularly within an anthropological framework. This research aims to understand prostatitis from an emic perspective while uncovering the salient medicinal plants that males use to treat and prevent the disease. The research site is a remote village in the Commonwealth of Dominica, a small Windward Island in the Caribbean, where residents have only recently, within the past five years, gained some awareness of the prostate gland, prostatitis and prostate cancer. It is common to hear men say, "I don't have prostate." Men do not question the existence of their prostate, rather, they are reporting themselves as disease-free. According to villagers, prostate trouble develops from untreated kidney stones and inflammation caused by dirty blood. One of the most common symptoms of this condition is abdominal pain which is accompanied by difficult and painful urination. Mean salience values calculated from freelist data reveal *Laportea aestuans, Physalis cordata, Momordica charantia, Chaptalia nutans*, and *Phyllanthus* spp. as the most popular remedies. These medicinal plants alleviate inflammation and purify the blood, while *Physalis cordata* and *Phyllanthus* spp. can also rid the body of kidney stones. They are used cross-culturally for similar purposes and possess bioactive compounds that make them effective treatment and prevention options.

P8. Diterpene Synthases and Cytochrome P450-Dependent Monooxygenases with Putative Roles in the Biosynthesis of Triptolide in *Tripterygium regelii*

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Abstract: Root extracts from members of the genus *Tripterygium* are used in traditional Chinese medicine for the treatments of fever, chills and edema. The diterpene triepoxide triptolide has been characterized as the active principle associated with the medicinally desirable activities. Numerous pre-clinical studies have provided evidence for the utility of triptolide for treating autoimmune diseases and various cancers. Clinical trials to assess its efficacy are now underway in several countries, including the United States. Despite its therapeutic potential, larger scale-clinical trials with triptolide have been hampered by low

quantities occurring in *Tripterygium* roots and unsustainable harvesting methods. Production of triptolide in plant tissue culture or a microbial host are valid alternatives. However, to enable such modes of production, the genes involved in triptolide biosynthesis need to be identified and characterized. We developed an inducible adventitious root culture system to upregulate triptolide production. Based on metabolite and transcriptome analyses, we identified candidate genes for four diterpene synthases and eight cytochrome P450-dependent monooxygenases. These genes have been expressed in heterologous hosts (*Escherichia coli*, yeast and *Nicotiana benthamiana*) and the functions of recombinant proteins characterized. An outline of the triptolide biosynthetic pathway, which takes into account the structures of potential intermediates accumulating in root cultures, will be discussed.

P9. Effects of various level of coal-bed methane water on dill (*Anethum graveolens* L.*)* and its essential oil

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Abstract: Pumping out water from coal seams decreases the pressure in the seam which in turn release trapped methane, this is the most common and economic way of extracting methane. The water being pumped out is known as coal bed methane water (CBMW) which is high in sodium and other salts. US in past 25 years has seen 16 folds increase in production of coal bed methane gas and trillions of cubic meter of this gas are yet to be extracted. CBMW lacks sustainable disposal and there are very few studies investigating effect of this water on plants, their secondary metabolites and even soil. Hence, the main objective of this study was to elucidate the effect of this water on soil and on essential oil yield and composition of dill (*Anethum graveolens)*. This crop was grown in green house and was subjected to different level of CBMW treatment, namely, 0% CBMW (tap water only), 25% CBMW (25%CBMW plus 75% tap water), 50% CBMW (50% CBMW and 50% tap water), 75% CBMW (75% CBMW plus 25% tap water) and 100% CBMW (only CBMW). After the study soil, plant weight, plant height, essential oil yield and composition were statistically analyzed. The major oil constituents limonene and α-phellandrene were not affected by the treatments. With increasing CBMW levels, the concentration of dill ether increased, whereas the concentration of carvone decreased. In soil, bicarbonate and sodium levels showed significant increase with increasing level of treatment, however soil pH and cation exchange capacity were not much affected. The results demonstrated that CBMW could be used for irrigation of essential oil crops when diluted with high-quality water.

P10. Effect of different season extension methods on the yield and quality of herbs bioactives

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Abstract: Season extension methods are often used to alter the micro climate of intended crops. The experiment were conducted in 2014 (March-December) in Sheridan, WY. The three types of season extension methods included (i). Low tunnel (Lt), (ii). High tunnel (Ht) and (iii). Low tunnel within high tunnel (LtHt). The herbs of studied were spearmint, oregano, thyme and rosemary. Herbs were harvested twice: late summer and mid-winter. Significant differences were observed in plant biomass and oil yields as a result of the treatments. Plant biomass and oil yield was highest in low tunnel within high tunnel (LtHt) for all crops studied. Simultaneously, lower yield in plant biomass and oil were observed for low tunnel harvests. Higher average temperature inside low tunnel within high tunnel (LtHt) played major role in increasing the yield and altering bioactives present in oil. These Season extension methods had significant effect on essential oil content and anti-oxidant capacity of the crops studied

and hence, demonstrated the effect on the quantity and quality of the herbs produce.

P11. Transient production of influenza NP vaccine antigen in *N. benthamiana* – enhancing yields and delivery

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Abstract: This research began with a long-term goal of developing a "universal" vaccine for influenza using plant-based bioproduction in *Nicotiana benthamiana*. By utilizing the influenza nucleoprotein (NP) epitopes, which have been found to be highly-conserved, the expectation was that the vaccine would provide immunity against many otherwise-divergent strains of influenza. The proposed approach was to use the plant lectin, RTB, to facilitate NP antigen delivery and immune presentation. However, a problem arose quickly in that our transient expression system in *N. benthamiana* yielded very poor expression of the NP-RTB fusions. This led to an adoption of a short-term goal to find ways to increase yields of the NP-RTB fusions. To this end, a number of experiments were carried out to either increase yields or better understand the construct's limitations. Such attempts included: expression of smaller regions of the NP with retention of the immunogenic epitopes, co-expression of the fusion alongside P19 to prevent gene silencing, analysis of the mRNA and protein levels throughout expression, optimization of the Agro-mediated infiltration methods, codon optimization of the construct for expression in *N. benthamiana*, and mRNA stabilization. Research progress, as well as the conclusion, will be presented in this poster. From the results of this research, we plan to produce sufficient NP:RTB protein to support mouse vaccination trials.

P12. Characterization of inducible and secreted metabolites from hairy root cultures of *Scutellaria lateriflora*

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Abstract: American skullcap (*Scutellaria lateriflora*), a perennial herb native to North America, contains specialized metabolites that have shown various biological activities, including anticancer and anti-inflammatory properties. In particular, these activities have been associated with the flavones wogonin and baicalein produced by this species. Previously, we developed hairy root cultures of *S. lateriflora* via *Agrobacterium rhizogenes*-mediated genetic transformation and showed that hairy root culture line 4 accumulates the wogonin, baicalein, scutellarein and their respective glucuronides in the root tissue. In the current study, we focused on characterizing the inducible and secreted metabolites produced by this hairy root culture. Line 4 root cultures were co-treated with the elicitors methyl jasmonate and cyclodextrin and a time course study was conducted. HPLC analyses of the ethyl acetate extracts from the culture medium revealed that several compounds were induced in the elicited cultures. Interestingly, none of these inducible compounds represent the known flavones that have been described in this species and may represent novel bioactive compounds in *S. lateriflora*.

P13. Annatto hairy roots: A sustainable source of bioactive compounds with applications in human health

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Abstract: Annatto (*Bixa orellana*) is a tropical plant native to South America. It is currently

used as a traditional medicine to treat multiple diseases including skin infections, respiratory problems and malaria. Extracts from leaves, seeds, fruits and roots of this plant have shown to possess biological activities *in vitro*, however the bioactive constituents of these extracts have not been elucidated. Furthermore, the seeds of this plant are the source for the natural pigment "annatto dye" used in the food industry. Previously, we established hairy root cultures of annatto to produce and identify potential bioactive compounds from this species. This work led to the characterization of candidate chemicals with anti-malarial activity *in vitro*, including ishwarane, tocotrienol and stigmasterol (Molecules 2014, 19:756-766). To increase the levels of these bioactive compounds, the hairy root cultures of annatto were treated with different combination of elicitors for different periods. HPLC analyses showed that several compounds were induced and excreted into the culture medium. Further characterization of these compounds is underway. We propose that hairy root cultures of annatto can be developed as a sustainable source for the production and discovery of bioactive compounds with distinct bioactivities.

P14. Enhanced and sustainable production of stilbenoids in hairy root cultures of muscadine grape co-treated with methyl jasmonate and cyclodextrin

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Abstract: Muscadine grape (*Vitis rotundifolia*) has been shown to produce monomeric and oligomeric stilbenoids including resveratrol, piceid, picetannol and several types of viniferins. These compounds are important because of their antioxidant and anti-inflammatory properties and potential beneficial effects in human health. However, sustainable means to produce these natural products are not available. Furthermore, the different factors that regulate the biosynthesis and accumulation of stilbenoids in muscadine grape have not been elucidated. To this end, hairy roots of *V. rotundifolia* were established as a bioproduction system for stilbenoids. Elicitation with methyl jasmonate alone or in combination with cyclodextrin for 24, 48, 72 and 96 hours was studied as an approach to induce the biosynthesis of stilbenoids. The stilbenoids were extracted from the root tissue and culture medium and analyzed by HPLC. The combined methyl jasmonate and cyclodextrin treatment led to the highest levels of stilbenoids, in particular resveratrol and piceatannol, in the medium of the hairy root cultures. In addition, the accumulation of these stilbenoids in the culture medium was maintained at very high levels over time. This research was supported by Student Undergraduate Research Fund (SURF) from the Arkansas Department of Higher Education.

P15. Anti-oxidative effects of natural extracts of traditional medicinal plants: *Acalypha indica, Lippia multiflora, Cyclopia maculata and Aspalathus linearis*

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Abstract: *Acalypha indica* L. (*Euphobiaceae*) is native to South Africa and Indian Ocean Islands. *Lippia multiflora* Moldenke (*Verbenaceae*) is distributed in Africa, Central and South America. Leaves from both plants are used to treat asthma, bronchitis, gastrointestinal disorders and skin infections. *Cyclopia maculata* (Andrews) Kies and *Aspalathus linearis* (Burm. f.) R. Dahlgren (*Fabaceae*) are endemic to South Africa and have been used by indigenous cultures to treat digestive problems, headaches and nervous tension. Antioxidants are beneficial for our body because they help to reduce the effects of oxidative stress. Oxidative stress is indicated by an accumulation of free radicals generated by external factors (radiation, smoking), but also internally during metabolism by electron leakage in mitochondria. There is accumulating evidence that free radicals are one of the major

underlying cause for many degenerative diseases such as cardiovascular, Alzheimer's disease, diabetes and cancer. Many beneficial properties of natural products are associated with their total phenolic content. The objective of this study was to investigate the antioxidant activity and total phenolic in commercial samples of *A. indica, L. multiflora, C. maculata and .A linearis.* Dry plant leaves were grinded and dissolved in DMSO. Antioxidant *a*ctivity and total phenolics content were quantified using the ABTS assay or Folin-Ciocalteu method, respectively. Our results show that *L. multiflora* and *A. linearis* have a highest total phenolic content which correlated to high antioxidant activity. Plant extracts did not show any toxic effects in mammalian monocytes up to high concentration (100 μg/ml) as indicated by the MTT-method. In conclusion, all plant extracts showed high antioxidant activity and non-toxic effects making them promising candidates against degenerative diseases. This research is part of educational experiences in an urban community college setting, enabling students to acquire the critical thinking and research skills necessary to pursue a baccalaureate degree in science-related disciplines.

P16. Comprehensive metabolomics analysis of the whisk fern, *Psilotum nudum* (L.) P. Beauv.

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Abstract: *Psilotum nudum* (*Psilotaceae*) is a seedless, rootless, leafless, fern-like vascular plant found in tropical and subtropical climates across the Asia-Pacific region and the Americas, with isolated occurrences in Southwestern Europe. The simple architecture of *Psilotum* resembles that of the earliest vascular plants. Although the direct descendance from early ancestors is disputed, it is still a viable model system to better understand the evolution of chemical diversity in plants. We therefore used a suite of analytical platforms to obtain broad profiles of metabolites occurring in the *Psilotum* rhizome (underground stem), stem and spore-producing synangium at various developmental stages. The results of these analyses are discussed in the context of the known chemical constituents of extant seedless vascular plants.

P17. Assessment of fatty acid profile and quality of *Santalum album* seed oil

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Abstract: Sandal wood (*Santalum album*) is a commercially and culturally important aromatic tree indigenous to India belonging to the family Santalaceae. These trees are harvested for their valuable heartwood only after 15 to 20 years, while the seeds can become a potential source of additional income which is not exploited currently. *S. album* annually bears a drupe with a hard shelled seed and each tree can produce 1-2 kg of seeds which has 50 – 60% of a fixed oil. The pale yellow fixed oil is characterized by the presence of a high percentage of an unusual acetylenic fatty acid known as trans-xymenynic acid. Studies have also shown that a highly purified xymenynic acid increases cellular detoxification, anti-oxidation capacity. It leads to a strengthening of the extracellular matrix (ECM), increases dermal strength and improves skin elasticity. As global demand for novel cosmetic agents is ever increasing, sandal wood seed oil could enter the market as a cosmetic ingredient that could also act as a vehicle for other oil-soluble agents. Thus a study was conducted on *S.album* seeds to assess its quality and fatty acid profile.

P18. Micropropagation of *Emblica officinalis*- A medicinal Plant

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Abstract: *Emblica officinalis* is a medicinally important perennial tree species cultivated for its fruits. A high efficient microprpagation protocol for this species is necessary for the crop improvement, mass plant production and germplasm conservation. The aim of the current study was to develop a protocol for high efficient plant regeneration through in vitro methods. Shoot tip, leaf, stem node and cotyledon explants were used as explants for the study. Sterilized tissue explants were inoculated onto two groups of MS culture medium: one group supplemented with different concentrations of 2,4-D (0-10 mg/l) combined with 1 mg/l BA and another group supplemented with different concentrations of BA (0-10 mg/l). After 3-4 weeks in culture under 16 h light, organogenic or embryogenic callus regeneration, direct and indirect somatic embryo regeneration, and plantlet regeneration were occurred in relation with different concentrations of 2,4-D and BA in the culture media. MS culture media with 3 mg/l 2,4-D and 1 mg/l BA, and MS media with 1 mg/l BA were optimized for somatic embryo regeneration and plant regeneration respectively. Somatic embryo proliferation, maturation, germination and plantlet growth occurred in hormone-free MS media. Adventitious root induction occurred in half MS media with 1 mg/l IBA. In vitro photoautotrophic culture system prior to greenhouse *ex vitro* hardening supported 100% plant survival. Acclimatized plants were planted in the field. An efficient micropropagation protocol for *Emblica officinalis* was developed through this study.

P19. Optimization of in vitro protocol for mass plant production of Ziziphus through micropropagation

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Abstract: The genus *Ziziphus* belongs to the botanical family Rhamnaceae has about 40 species. Most of the species have medicinal values. Among all the species, *Ziziphus jujube* from China, *Z. mauritiana* from India and *Z. spinachristi* form the Middle East are the cultivated species for food, medicine and fodder. Propagation of these three species are through seeds. Genetic variations noticed in the seedling populations in these species due to their heterozygous nature. Conservation, mass clonal propagation, germplasm exchange and crop improvement in *Ziziphus* require micropropagation technology. This study was aimed at developing a micropropagation technology for three species namely, *Z. jujuba*, *Z. mauritiana* and *Z. spinachristi*. Leaf disc of 5 mm2 with central vein, stem nodal segments and shoot tip explants isolated from the sterilized shoots under the laminar hood were used for the regeneration experiments. MS culture media with different plant growth regulators (PGRs) BA and 2,4-D alone or in combination were used for the experimental study. Cultures were maintained under 16h photoperiod of 1000 lux light intensity at 25 ± 2° C. Three types of regenerations: 1. adventitious shoot regeneration from stem nodal segments, 2. axillary shoot multiplication from shoot tip explants and 3. somatic embryogenesis from leaf disc explants were obtained and protocols common to all the three species were standardized. Large number of plants were produced through tissue culture methods and planted in the field for further evaluation. Somatic embryogenic cultures were conserved under in vitro for the future plant productions.

P21. Development of T-type hedge cultivation method for newly bred cultivars of *Lycium chinense* Mill.

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Abstract: Two newly bred cultivars ‘Cheong-un’ and ‘Jang-myeong’ in *Lycium chinense* Mill. had been developed by backcrossing local cultivars and radiation induced mutation. These new cultivars have some good characteristics including anthracnose and Eriophyidae mite resistance, high betaine content and fresh fruit yield. Their vegetative growths were also much stronger than local cultivars. There are two types of cultivation methods for new cultivars that have been tested through years including I-type tree cultivation and T-type hedge cultivation. Among them T-type hedge cultivation method could increase annual yield by 10% compared with I-type cultivation method. Additionally, we tested the effect of T-type hedge pruning methods on cultivation. By leaving 30-50% of the previous year’s withes on stem we could shorten flowering date about 14 days compared with non-leaving pruning method. Additionally, 30% withes treatment resulted in significantly more fruit setting numbers by 1,588 fruits per tree (a 26% higher yield compared to traditional pruning method). This also resulted in 28% higher dried fruit yield of 355 kg per 10 acres.

P22. Influence of steam and chemical sterilization on growth of ginseng seedling

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Abstract: It is well known that the quality of ginseng seedlings is closely related to the yield and quality of ginseng harvested. Due to the concern of replant failure and difficulty of finding a new farmland for cultivation of ginseng seedlings, the new method for cultivation of ginseng seedlings in the fixed production facilities such as greenhouses has been attempting to overcome these problems. This study was carried out to develop the reuse method of ginseng seedling medium for safe production of ginseng seedlings and saving cost. The leaf length, leaf diameter, root length and root diameter of ginseng seedling at the medium sterilized by steam or dazomet were superior to control group. Moreover, disease incidences of dampingoff and root rot were decreased at sterilized medium than control, and the densities of fungus and *pythium* sp. after cultivation of ginseng seedling at the medium sterilized by steam or dazomet were 0.3±0.2 × 103CFU/g medium, 1.6±0.1 × 103CFU/g medium and 0.7± 0.1 × 103CFU/g medium, 2.4±0.3 × 103CFU/g medium, respectively. These results suggested that sterilization of medium using by steam or chemical could be used as proper method for reuse of ginseng seedling medium.

P23. Plant Regeneration and multiplication from leaf segment and floral bud culture in *Artemisia annua*

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Abstract: *Artemisia annua*, known as *Quinghaosu* in traditional Chinese medicine, has been used for more than two thousand years as a medicinal plant to fight fever and malaria. This common herb is now employed worldwide to effectively treat malaria typically in combination with other antimalarial drugs. *Artemisia annua* produces a major bioactive compound called *artemisinin*, which is then extracted and further developed. Culture of *Artemisia annua* from the leaf segment achieved efficient plant regeneration and multiplication via organogenesis. The result will be able to facilitate large-scale micro-propagation of superior lines and allow multiplication of certain individuals selected for high artemisinin content. Through manipulating the balance of auxin and cytokinin, aged leaf segments as well as floral buds produced shoot formation and multiplication in *A. annua*. Murashige and Skoog media supplemented with isolated cytokinins or in several combinations with NAA yielded proliferating shoot cultures. The highest number of adventitious shoots were obtained on MS media supplemented with 2-4mg/L of Zeatin, while Kinetin and BAP did not result in shoot formation. The highest callus

induction was obtained on media supplemented with 2mg/L 2,4 D. The results show that the determining factor for efficient shoot proliferation is the combination of 2 mg/L BAP and 0.5 mg/L NAA. The comparison of artemisinin content between mother plants and tissue culture derived plants showed similar results.

P24. Yield and composition of ground caraway seed oil fractions obtained at different time points during the hydrodistillation

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Abstract: A distillation study was conducted to elucidate the effect of various distillation times (2, 7, 15, 30, 45, 75, 105, 135, and a Control) on essential oil fractions yield and composition of ground caraway seed. The effect of distillation time on oil yield (g oil/200 g seed), the concentrations (%) of myrcene, limonene, trans-carveol, carvone, and β-caryophyllene, and carvone to limonene ratio was determined using a one-way analysis of variance. Most of the essential oil was eluted in the first 30 min. The low-boiling constituents limonene and myrcene were eluted early, resulting in their higher concentrations in the fractions obtained early in the distillation process. Limonene (24-77% of the total oil) and carvone (20-74% of the total oil) were the main constituents of caraway seed oil. The study found that distillation time can be used to obtain caraway seed oil fractions with differential chemical profile. The resulting caraway seed essential oil fractions may have different industrial and research applications.

P25. Occurrence of calcium oxalate crystals in wild annual herbal plants in Saudi Arabia

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Abstract: Because of the importance of medicinal plants in the pharmaceutical industry and their uses in folk medicine at the present days, and certain herbal wild plants described for patients in randomized ways. Due to the presence of some harmful substances in the plants, which when used in random quantities by patients may cause disease and other complications for patients. One of these harmful substances knowns as crystals which resulting from the increase of calcium and oxalates in the cells and tissues of the plant, these secretory materials remain in the plant parts, like roots, stems, leaves, floral parts, fruit and seeds. The transverse sections of stems and roots of 60 wild herbal plants were studied using light microscopy. The results of study show the presence or absence of calcium oxalate crystals in the plants studied and knowing which plant parts contain the crystals. As well as, considering their presence in the plant parts denote the increase of harmful oxalates and calcium, warning of their use in randomized ways.

P26. Antibacterial and antioxidant activities of Miswak *Salvadora persica* L. root grown in Saudi Arabia

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Abstract: *Salvadora persica* L. commonly known as *Miswak* and have immense medicinal value as anti-microbial and in prevention of tooth decay. Therefore, the present work aimed to determine the total phenolic content and total flavonoids in two Miswak extracts obtained from arak roots collected from two different localities in Saudi Arabia. They were extracted with aqueous ethanol (80%) and used to estimate in vitro their antioxidative abilities. The new findings showed that the two tested extracts contained significantly different amounts of both total phenolic content and total flavonoids. According to the increase of total phenolic contents and total flavonoids obtained from the two extracts, Miswak collected from the southern region

was found to contain more contents than those collected from the middle region. The results of antioxidant activities of Miswak root extract obtained by using different in vitro methods were varied depending on the technique used. According to the malondialdehyde (MDA) method, hydrogen peroxide (H2O2) scavenging ability and 1,1-diphenyl-2-picrylhydrazyl (DPPH) methods, the two Miswak extracts exhibited to have very high antioxidant activities. Mostly, the values of antioxidant activities of Southern region have been shown to be always the highest.

P27. Recent Research, Production, and Traditional Uses of Major Medicinal Plants in Korea

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Abstract: In recent years, interest in traditional medicine has grown due to its long standing as an effective medication with fewer side effects, and the possibility of identifying promising new components. Throughout East Asian history, several crude drugs were established from various types of plants, but Korea independently developed medicine with its specific native plants. The nation of Korea is a small territory in comparison, but its plant life is abundant, encompassing more than 5,500 plant resources. Traditionally, Korean people have used their native plants as medicinal herbs for maintaining health and treating illnesses. Currently, there are approximately 50 kinds of medicinal plants that are in large scale production in Korea. One major research goal in this area is to breed high quality varieties of plants through the development of environmentally friendly cultivation techniques, in order to produce a supply of superior medicinal crops. This poster presents a general introduction regarding the production and traditional uses of Korean medicinal plants. Relevant Korean medicinal plants that will be introduced and discussed are as follows; Astragalus commonly known as milkvetch, Schizandra *(Schizandra chinensis)*, *Dioscorea batatas*, Safflower *(Carthamus tinctorius)*, two *Atractylodes spp.*, Chinese Foxglove *(Rehmannia glutinosa)* which is a perennial herb native to Korea and China, the Korean Milk Thistle (*Silybum marianym*), one of the most significant medicinal plants and also the Peony Root and *Angelica gigas* which are widely used for crude drugs in Korea, China, and Japan.