The Oxford International Conference on the Science of Botanicals is an annual meeting to discuss approaches for post market surveillance, risk and safety assessment, quality control and adverse event reporting (AER) for botanical dietary supplements (BDS) and natural products as well as regulatory aspects with perspectives from government, manufacturers and trade associations.

Celebrating 25 Years of DSHEA

The upcoming year's meeting will celebrate the 25th Anniversary of DSHEA (The Dietary Supplement Health and Education Act of 1994) by reviewing the history, confronting the issues, and discussing future aspects.

Poster Abstracts
PA1

Determining Endogenous Thresholds for Athletically Banned Substances

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Dietary supplements are a multibillion dollar industry. Key stakeholders in this industry include companies that produce sports supplements formulated for athletes. Certain substances are prohibited or banned from sport by organizations including the World Anti-Doping Agency (WADA), National Football League (NFL) and Major League Baseball (MLB). Chemicals/substances are placed on the WADA list when they meet at least two of the following criteria: 1) the substance has the potential to enhance performance, 2) the substance has an adverse effect risk, and 3) the substance violates the spirit of sport. By prohibiting these chemicals, their acceptability criterion in a finished product dietary supplement is represented by the absence of the substance at the analytically feasible detection threshold. Importantly, there are instances where prohibited substances are endogenous in plants or animals, and the endogenous nature of these substances should be accounted for when setting thresholds. Recently, an athletically banned substance, Boldione, was reviewed for its endogenous potential. Boldione is currently listed by WADA as an endogenous anabolic androgenic steroid (AAS) when administered exogenously. Although Boldione is not a phytochemical constituent of botanicals, literature is available describing biotransformation of the phytosterol, \(\beta\)-sitosterol, to Boldione in the presence of mycobacterium. Given that Boldione can be produced endogenously in plants, an endogenous threshold of 0.02 µg per serving was established using endogenous Boldione levels in human urine (1.33 ng/ml x 50 ml urine) multiplied by the maximum excretion rate of Boldione in urine (34.45%). Typical performance enhancing use levels of AAS are roughly 25 mg per day with dosage levels as low as 1.14 mg per day. Additionally, a maximum allowable level of 0.3 mg per day was derived from a subchronic oral study wherein rats were administered the AAS androstenedione. These values are orders of magnitude higher than the Boldione per serving endogenous threshold. Thus, derivation of a risk-based acceptability criteria threshold for Boldione does not violate anti-doping requirements and the endogenous nature of the athletically banned substance, Boldione, should be accounted for when setting an acceptability criteria threshold.

PA2

Isolation of three new flavones from Callistemon lanceolatus and assessment of their biological activity: In vivo hypoglycemic effect and in vitro PPAR-\(\gamma\) transactivation activity.

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\textit{Callistemon lanceolatus} DC (Myrtaceae) is commonly known as ‘red bottle brush’ plant. The hypoglycaemic activity of the ethanolic extractof the leaves of \textit{Callistemon lanceolatus} as well as its petroleum ether and chloroform fractions were studied in streptozotocin induced diabetic rats for 30 days. The effect on biochemical parameters \textit{viz} lipid peroxidation, reduced glutathione levels, activity of antioxidant enzymes etc were also assessed to evaluate their activity in controlling diabetes related metabolic conditions. The results indicate that the chloroform fraction at 150 mg/kg b.w. significantly lowers blood glucose level with insignificant ulceration compared to the standard drug glibenclamide (3 mg/kg b.w.). Also, there was reduction in lipid peroxidation level and reduced glutathione levels and elevation in the activity of antioxidant enzymes.

The phytochemical investigation of the active chloroform fraction led to the isolation of three new flavones with significant hypoglycemic effect in streptozotocin induced diabetic rats in addition to two phenolic esters which have been
characterized using chemical and spectroscopic methods. One of the flavones besides exhibiting significant \textit{in vivo} blood glucose lowering effect and without causing any toxic effect on the pancreas and liver also showed a higher glide score in comparison to the reference molecule (rosiglitazone) against PPAR-\(\gamma\) target in molecular docking studies. Moreover it also exhibited moderate \textit{in vitro} PPAR-\(\gamma\) transactivation activity in comparison to the standard drugs, rosiglitazone and pioglitazone.

The results of this study validates the traditional use of the leaves of \textit{Callistemon lanceolatus} in the treatment of diabetes in folk medicine.

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PA3
\textbf{Rapid Screening and Quantitative Analysis of Amines/Alkaloids from Weight Loss and Pre-Workout Supplements using UHPLC-QToF}

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The use of supplements for weight loss and in sports as pre-workout products is wide spread. Many of these supplements were found to contain active components, which were not claimed on the labels. A validated ultra-high performance liquid chromatography high-resolution mass spectrometry quadrupole time-of-flight (UHPLC-QToF-MS) method was developed for the simultaneous analysis of 111 stimulants, \textit{anorectics} and other active components including phenethylamines, amphetamines, sibutramine or yohimbine. This method involves the detection of [M+H]\(^+\) ions and the separation was achieved using a C\textsubscript{18} column, water/acetonitrile gradient as the mobile phase. The method was validated for linearity, repeatability, accuracy, stability, system suitability, limits of quantification (LOQ) and limits of detection (LOD). The limits of detection were in the range from 0.001-0.5 \(\mu\)g/mL. The validated method was applied to the analysis of twenty-seven weight loss and pre-workout dietary supplements. Two-thirds of the supplements contained compounds that were not listed on the label. These include several phenethylamines (PEA) such as demelverine, hordenine, \(N, N\)-dimethyl-phenethylamine, synephrine, \(N\)-methyl-b-phenethylamine, and methylsynephrine. In addition, the PEA mimics such as dimethylamylamine, dimethylbutylamine other stimulants including fursultiamine, evodiamine, phenibut and theophylline were also observed. One or more of the ingredients listed on the labels were not detected in forty-four percent of the products analyzed. Positive identification was based on retention time, accurate mass and fragment ions in comparison with the respective reference standards. Development of such methods are anticipated to be of aid to regulatory agencies for the identification of xenobiotics commonly found in many dietary supplements.

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The Power of Hyphenated Chromatography - Time of Flight Mass Spectrometry for Unequivocal Identification of Spirostanes in Bodybuilding Dietary Supplements

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In an attempt to assess the ‘naturalness’ of a group of steroid-like compounds called laxogens, UHPLC-QToF method was developed. Several dietary supplements claim to contain 5a-hydroxy laxogenin, which is a derivative of a naturally occurring spirostane-type steroid, laxogenin. Although laxogenin has been isolated from the rhizomes of Smilax sieboldii, 5a-hydroxy laxogenin has not been isolated or reported from any natural sources. These laxogenins have untested anabolic properties. Due to the low UV absorbance of the spirostanes, a mass spectrometric method in positive ion mode was developed for unambiguous identification of laxogenin and closely related compounds. To show the utility of developed method, nine dietary supplements labeled as contain 5a-hydroxy laxogenin or laxogenin as 5a-hydroxy laxogenin were analyzed as a proof-of-concept. Four supplements did not contain any 5a-hydroxy laxogenin, whereas in the remaining five samples, spirostane-type contaminants were identified along with labeled 5a-hydroxy laxogenin. The identity of some of these contaminants was established based on reference standards along with mass fragmentation patterns. One of the unlabeled contaminants was identified as the phytosteroid saponin, diosgenin, a common starting precursor of several steroidal drugs. Several synthetic derivatives of diosgenin were identified in eight products. These findings indicate that the labeled 5a-hydroxy laxogenin along with other spirostanes found in supplements are synthetic and signify a lack of quality controls. Additionally, an unlabeled, anabolic androgenic steroid, arimistane, an aromatase inhibitor, was also identified in one product. Laxogenin, was not detected in any of the samples analyzed during this investigation.

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Quality Integrity of Dietary Supplements that Have Been Implicated in Liver Injury

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Herbal dietary supplements (HDS) are widely used, especially for weight loss, bodybuilding, bones/joint implications, sexual enhancement, general health, and miscellaneous. There is growing evidence to show that some HDS are capable of causing liver injury. 272 products categorized as weight loss (36 products), bodybuilding (46 products), general health (53 products), GI symptoms (22 products), energy boosters (5 products), sexual enhancers (4 products) and miscellaneous (106 products) were analyzed for the presence of labeled ingredients and hepatotoxins including anabolic steroids, pyrrolizidine alkaloids, aflatoxins and pharmaceuticals using ultra-high performance liquid chromatography-QToF-MS in full scan and targeted MS/MS modes with accurate mass measurement. The compounds were identified in both positive and negative ion modes of mass spectrometry. Of the 46 bodybuilding products tested, 37 products contained inaccurate labels; 11 of these contained labeled and unlabeled steroids; 16 of these contained steroids that were different to those indicated on the packaging; Overall, 21 different steroids were identified; 10 of these were controlled under US Drug Enforcement Administration (DEA) Controlled Substances Schedule III. These controlled substances...
have been added to the World Anti-Doping Agency (WADA) list of prohibited substances in sport. 51% of the products analyzed showed for the label inaccuracy. In addition, the development of accurate mass time-of-flight mass spectrometer has enabled the calculation of an empirical formula from the molecular ion and utilizing high-resolution to reduce the number of interfering isobaric compounds. However, the molecular ion present in LC-MS analyses with its mass and elemental formula can be used to search a database of metabolites such as the ForTox database provided by Agilent Technologies, ChemSpider (www.chemspider.com), PubMed and customized In-House developed database. In addition, the development of accurate mass time-of-flight mass spectrometers has enabled the calculation of an empirical formula from the molecular ion. Compound structures were confirmed with authentic standards, and further verified by MS-MS fragmentation patterns.

This research is supported by “Albert Einstein Healthcare Network/National Institutes of Health” award number #3U01DK083027-0952. Supplements were obtained from a repository of products that have been implicated in liver injury.

PA6
Chemical Profiling of Chemical Constituents from aerial parts of Epimedium grandiflorum using UHPLC-QToF-MS

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Epimedium, also known as barrenwort, is a genus of flowering plants in the family Berberidaceae and used traditionally as a tonic, aphrodisiac and antirheumatic in East Asian countries. Prenylflavonoid is a major group of active constituents present in Epimediums. According to Chinese pharmacopoeia [1], the dried aerial parts of Epimedium brevicornum Maxim., Epimedium sagittatum Maxim., Epimedium pubescens Maxim., Epimedium wushanense T.S. Ying, and Epimedium koreanum Nakai contain the highest concentrations of flavonoids. UHPLC coupled with QToF is used for the chemical profiling of 35 components from Epimedium grandiflorum and dietary supplements claiming to contain Epimedium. This method involved the use of [M+H]+ and [M+Na]+ ions in the positive ion mode and [M-H]- and [M-COOH]- ions in the negative ion mode with extractive ion monitoring (EIM). The molecular formula determination and tentative identification of compounds was based on accurate mass measurement and fragments using MS-MS accurate mass spectra with collision induced dissociation (CID) of precursor ions. Based on the MS analysis, prenylflavonoids were the dominant compounds and are mostly present as glycosides containing one or more sugar units. Cleavage of the glycosidic linkage led to elimination of the sugar residue, that is, 162 amu (glucose), 146 amu (rhamnose) and 132 amu (xylose). Epimedin A, epimedin B, epimedin C, icarin were found to be major compounds. The ESI-HRMS of epimedin A, B, C, icarin, gave molecular ion peaks at m/z 839.2960, 809.2858, 823.3010, 677.2442 for [M+H]+. The key fragments for epimedin A were 531.1857 [M+H-Glc-Rha]+, 369.1319 [M+H-2Glc-Rha]+ and 313.0696 [M+H-2Glc-Rha-prenyl moiety]+ corresponding to the loss of two glucoses, one rhamnose and prenyl moieties, the similar fragments were observed for other analytes.


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PA7
Liquid Chromatography -Tandem Mass Spectrometry vs. Ion Chromatography -Tandem Mass Spectrometry for Glyphosate Residues Analysis in Botanical Matrices

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Analysis of polar pesticides such as glyphosate and its metabolites is very problematic due to the physiochemical properties of the molecules. The retention of anionic pesticides using reverse phase chromatography is difficult without derivatization and is not robust enough to implement in a QC laboratory. Although many methods utilizing different instruments and chromatographic approaches are now available, their robustness and performance when analyzing glyphosate residues in difficult matrices is questionable.

Two methods for the analysis of polar pesticides in botanical dietary ingredients were developed and validated. The LC-MS/MS method utilizing polyvinyl alcohol with quaternary ammonium groups column was evaluated against an IC-MS/MS method separating the analytes on a polymer based column. Glyphosate, AMPA, MPPA and glufosinate were either extracted from botanical matrices according to the QuPPe method (LC-MS/MS approach) or extracted in water/cold methanol (IC-MS/MS method). The analytes were identified and quantitated by Thermo Altis mass spectrometer with the use of isotope-labeled internal standards.

Both methods delivered satisfactory results, although the IC-MS/MS approach showed better robustness when analyzing complex botanical matrices. Established limits of detection and quantitation using both methods for glyphosate in the matrix were below regulatory limits in the EU.

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PA8
Microalgae for Supplements and Food Ingredients

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Microalgae is a generic term that refers to a broad category of photosynthetic microorganisms including mainly Spirulina and Chlorella and sometimes including other cyanobacteria. The worldwide market for microalgae products is valued at over $600 million US dollars and is expected to increase to over a billion US dollars by 2024. Products are marked as a “Super Food” for “energizing B vitamins, vitamin K, magnesium, copper, zinc, selenium, iron, omega-3 fatty acids EP and DHA, and protein”. The market is partially driven by the potential for microalgae use in biofuels and metal chelators as well as a nutrient-rich relatively inexpensive food ingredient. Reported adverse effects include allergic reactions, diarrhea, nausea and vomiting. Microalgae are popular ingredients in green smoothie mixes, specialty bars, snacks, and as a natural food dye. Under some production conditions, microalgae can be contaminated with other cyanobacteria species or can be induced to produce hepatotoxins and neurotoxins. Our objective was to determine whether Spirulina supplements and ingredients can be contaminated with the naturally occurring neurotoxic non-protein amino acids, N-β-methylamino-L-alanine (BMAA) and 2,4-diaminobutyric acid (DAB). BMAA and DAB were detected in all spirulina raw materials tested, including ingredients from Hawaii, China, Japan and the United States, at an average of 47 ng/g and 6.8 µg/g respectively. BMAA and DAB were also found in about 10% of the finished products tested, indicating that an individual could consume an average of 3.4 µg/day (BMAA) and 124 µg/day (DAB), according to the recommended dose. Along with several studies showing adverse health effects associated with spirulina use, this study demonstrates the need for strict quality control of microalgae food supplements as well as more clinical trials to evaluate their health effects before they can safely be brought to the market.
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PA9

Study on the chemical constituents of the fruit of Areca catechu L.

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As an important Chinese herbal medicine, ‘Binglang’ (Areca nut) is known as the first of the four Southern medicine. It is recently reported that chewing of ‘Binglang’ is closely associated with the occurrence of oral cancer. The paper selected ‘Binglang’ as the object to study the chemical compositions and its compositions for the role of carcinogenic basis. One new indole alkaloids (17), sixteen low molecular compounds (1-16), along with twenty six known compounds (18-43) were isolated from the dried fruit of Areca catechu L. The structures were elucidated on basis of the 1D, 2D NMR data and MS. Compounds 7-11, 13-15, 18-28, 41-43 both the first time isolated from the plant and this Arecaceae. In addition, the volatile components of the petroleum ether extract of Areca catechu L fruits were analyzed by GC-MS. The results showed that the main fourteen compounds (44-57) were aliphatic hydrocarbons, fatty acids and their derivatives, and some volatile alkaloids.

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PA10

Qualitative and Quantitative Analysis of Different Varieties of Cannabis sativa L. using GC-FID

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Cannabis (family Cannabaceae) is one of the oldest plants cultivated for fiber, food, oil, medicinal and ritual use or as a recreational drug for millennia. The plant has been reported to contain more than 500 different compounds belonging to a diverse group of chemical classes, the most important of which is the cannabinoids. There are 120 phytocannabinoids reported so far. Among cannabinoids, D9-tetrahydrocannabinol (D9-THC), commonly known as THC, is the major biologically and most important psychologically active compound, which accumulates mainly in the glandular trichomes.

of the plant. Beside THC, cannabidiol (CBD) is another important compound which is non-psychoactive and highlighted for its activity against childhood epilepsy syndromes and other disorders.

As a plant, cannabis is normally dioecious and occasionally monoecious, wind pollinated species which is highly allogamous (cross fertilization) in nature. Whether the genus Cannabis contains one species or more has been a long matter of debate. Cannabis is treated as one species by some groups, while others describe it as two to four species. Presently, cannabis is considered a single, highly variable species Cannabis sativa L.

On the other hand, cannabis can also be classified based on their cannabinoids profile. In general, cannabis is categorized in three different popular chemotypes i.e. high THC varieties, high CBD varieties and mix or intermediate varieties. In the present study, different varieties of cannabis were grown to maturity and periodically analyzed (from vegetative stage to flowering) for different cannabinoids content using gas chromatography-flame ionization detector (GC-FID). Based on the cannabinoids profile and content, plants were screened and categorized in high THC, high CBD and intermediate varieties/chemotypes. Vegetative cuttings of these varieties are conserved for the future use.

This work was supported in part by the National Institute on Drug Abuse (NIDA), National Institutes of Health (NIH), Department of Health and Human Services, USA, under the contract no. N01DA-15-7793.

PA11
Method development for the certification of ginsenosides in Panax Ginseng rhizome, leaf, extract, and pills via liquid chromatography/tandem mass spectrometry

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Ginsenosides are the active ingredients found in the Panax plant family often used as medicinal herbs in Chinese medicine. While the root is the primary source of active medicinal ingredients in Panax ginseng (Chinese ginseng), the use of leaves has been reported in commercial products, thus, it is imperative to isolate and determine ginsenoside compositions found in other portions of the plant to identify potential product adulteration. Candidate Standard Reference Material (SRM) 3384 Panax ginseng Rhizome is ground up portions of the plant rhizome which sprouts adventurous roots. Since roots connect directly and ultimately pass water and nutrients to the rhizomes, understanding the ginsenoside makeup and concentrations in the rhizome is necessary.

Traditional analytical techniques for the determination of ginsenosides include liquid chromatography (LC) coupled with single quadrupole mass spectrometry (MS) detectors. The isolation of ginsenosides in the plant generally produces complex samples which requires higher resolution detection methods such as MS/MS. The work presented here shows the optimization of a LC-MS/MS method for the separation and determination of ginsenosides for SRM 3384 certification measurements. Candidate SRM 3385 Panax Ginseng Root Powder Extract and candidate SRM 3388 Ginseng-Containing Solid Oral Dosage Form were also evaluated for ginsenoside concentrations. Reversed-phase liquid chromatography was used to separate ginsenosides Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1 in the complex sample matrices after sonication extraction under basic conditions. Quantitation of these seven compounds was accomplished with 4-methylestradiol and SRM 3389 Ginsenoside Calibration Solution serving as an internal standard and calibrants, respectively. Understanding the ginsenoside compositions and concentrations in various parts of medicinal herbs are important for phytochemical determination of specific Panax species for dietary supplementation.

The authors acknowledge financial support from the Office of Dietary Supplements at the National Institute of Health.
A Versatile, Cost-effective Method for Analysis of Water-Soluble Vitamins in Dietary Supplements by UHPLC with UV or MS Detection Systems

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Many traditional high pressure liquid chromatography (HPLC)-UV methods for quantification of water-soluble vitamins use chemicals which are not compatible with mass spectrometers (MS). Recently, methods developed utilizing the increased speed and reduced solvent consumption in ultra high pressure liquid chromatography (UHPLC) also use expensive MS detectors that can be cost-prohibitive to laboratories which need multiple systems to meet the turnaround time demanded of them. To address the need, the objective of this study was to develop an extraction procedure with a MS compatible mobile phase system that could analyze commercial dietary supplement products using a lower cost UV detector on a UHPLC instrument. Optimization of extraction solvent systems using acidified water, acetonitrile, and ethanol in different ratios were utilized for sample preparation. Various finished product analytes such as thiamine, riboflavin, niacin, niacinamide, pantothenic acid, pyridoxine, high potency methylcobalamin, and ascorbic acid were quantified in finished products using UV detection. This validated method can also be used for the quantitative analysis of micro-ingredient vitamins such as biotin and methylcobalamin or investigation of unknown peaks on MS systems without the risk of damaging the quadrupole with incompatible chemicals. Furthermore, the method will be capable of quantifying or authenticating natural products such as natural vanilla flavor and other dietary supplements such as melatonin.

Quantitative Determination of Primiquine-5,6-ortho-quinone and Carboxyprimaquine-5,6-ortho-quinone in Human Erythrocytes by UHPLC-MS/MS

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Primauqine (PQ) has long been used for radical cure of Plasmodium vivax and P. ovale malaria and prevention of all forms malaria. PQ and carboxyprimaquine (CPQ), the major metabolite in human plasma, were found to undergo oxidation to primauqine-5,6-orthoquinone (POQ) and carboxyprimaquine-5,6-orthoquinone (CPOQ), respectively, in erythrocytes. POQ and CPOQ are presumed as toxic metabolites. The aim of the study was to develop and validate quantitative analytical methods for CPOQ and POQ in erythrocytes.

Chromatographic analysis of these metabolites in human subjects was achieved by ultra-high performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-QToF-MS) using gradient elution. Quantitative estimation of POQ and CPOQ was executed by monitoring their key fragments at m/z 175.05. The developed analytical method was linear, sensitive, precise and robust. Several liquid-liquid extraction methods using different organic solvents have been investigated. A mixture of water and methanol (1:1, v/v) was found to be the optimal extracting solvent. Preliminary analyses indicated that POQ and CPOQ were higher in subjects with higher methemoglobin. This validated method can be used for the exploration of quantitative estimation of major metabolites of PQ in human subjects.

This study was supported by the US Army Medical Research & Materiel Command Award (No. W81XWH-15-1-0704 and W81XWH-18-2-0029) to the University of Mississippi (LAW, NPDN and BLT).
Target isolation of Di-lignans from Magnolia obovata using Pre-Processed Mass Spectral Data

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The dried bark of Magnolia obovata Thunberg (Magnoliaceae) has been used for the treatment of gastrointestinal disorders, anxiety and allergic diseases in Northeast Asia. It has been reported that M. obovata contains various compounds including neolignans, sesquiterpenes, sesquiterpene-neolignans, trineolignans and alkaloids.

Molecular networking is one of the recent bioinformatics approaches. This technique estimates structural similarity by comparing MS/MS data. Molecular networking enables the detection of related analogues as well as the dereplication of known molecules from complex mixtures. Using this powerful tool superior to traditional dereplication strategies, we can identify potential analogues and prioritize secondary metabolites for isolation according to the aims of researchers.

This presentation will discuss target isolation of di-lignans from M. obovata using the LC-MS/MS-based molecular networking. Further, massive molecular networks that include bioactivity were used to highlight potential bioactive nodes within the chemical diversity of the hexane-soluble extraction. This workflow enables one to target predicted active metabolites. This approach for natural product drug discovery can speed up and rationalize the isolation of natural products with chemically novelty and bioactivity.

Chemical analysis of fusaricidins from the antifungal microbial strain Paenibacillus species

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Paenibacillus sp. MS2379 is a highly efficient microbial strain producing fusaricidins, a class of lipopeptides that have demonstrated strong antifungal activities against a broad array of fungal pathogens. An integrated approach combining chromatographic fractionation, UHPLC-QTOF-MS analysis, and NMR spectroscopic interpretation was employed to characterize antifungal metabolites produced by this microbial strain, resulting in the identification of 48 fusaricidins including 30 cyclic and 18 open-chain species. In this regard, UHPLC-QTOF-MS played a vital role in determining structures of 28 new fusaricidins through peptide fragment analysis. The structural determination of the new fusaricidins by the high-resolution mass spectrometry was validated by follow-up isolation and NMR spectroscopic analysis of representative compounds. It is worth noting that novel fusaricidins with amino acid residues of serine and g-aminobutyric acid were identified, which is of great biosynthetic significance for this biologically important class of compounds. The present study illustrates the power of UHPLC-QTOF-MS for structural identification of lipopeptides, and the structural diversity of the identified fusaricidins makes this microbial strain unique as a potential biocontrol agent.

This work was supported by Agricen Sciences and the USDA Agricultural Research Service Specific Cooperative Agreement No. 58-6060-6-015.
Flavonoids Characterization in USP Botanical Reference Standards by High Resolution Mass Spectrometry

Guiraldelli A., Maranhão R., Monagas M., Cuiying M., Gude K. and Giancaspro G.

Botanical ingredients used as foods, dietary supplements (DS) and herbal medicines (HM), are usually complex matrices constituted by several classes of phytochemicals, including flavonoids, exhibiting different physicochemical properties. This scenario represents a challenge for their suitable standardization and quality control. Candidate botanical reference materials containing flavonoids (i.e Citrus reticulata pericarp, Bitter Orange extract, Maritime pine extract, Guarana seed, European elderberry extract, Ashwagandha aerial parts and Scutellaria baicalensis root) were analyzed by liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC-ESI-QToF). Collision-induced dissociation (CID) experiments provided valuable structural information highlighting diagnostic fragment ions for the characterization of the flavonoid substitution pattern. Several subclasses of flavonoids including flavanones, flavones, flavonols (monomeric catechins and procyanidins), anthocyanidins, chalcones and isoflavones, were evaluated. Retro-Diels Alder (RDA) reaction plays a key role in the identification of flavonoids. RDA reaction in the C-ring may occur in all subclasses of flavonoids, and may undergo different pathways according to the structural subclass. The double bond present in the C-ring of flavones, flavonols, chalcones and isoflavones leads to an additional RDA mechanism compared with dihydroflavonols and dihydroflavanols resulting in structural evidences for the B-ring substitution pattern.

Carbohydrate units may also undergo fragmentation by RDA reaction after water loss by hydrogen rearrangement followed by a subsequent RDA fragmentation in the C-ring (losing the B-ring as neutral molecule), generating in ESI+ mode an important diagnostic ion for the glycosylation position. In the case of flavone-O-diglycosides found in Bitter Orange extract, such as naringin, the MS/MS spectrum (ESI+) shows the formation of the fragment ion at m/z 459.11 due to the elimination of the B-ring after RDA fragmentation indicating that the dissacharide is located in the A-ring and not at 3-O position. On ESI- mode the typical diagnostic fragment ion for the A-ring of flavanones/flavones derived from naringenin (m/z 151.008) due to RDA fragmentation was observed. The B-ring was assigned based on the neutral loss of 136.05 Da for eriocitrin/neoceriitcin, 120.05 Da for isonaringin/naringin/narirutin, 150.06 Da for hesperidin/neohesperidin and 118.4 Da for rhoifolin. Scutellaria baicalensis root accumulates large amount of flavones lacking the 4'-OH group in the B-ring which may be assigned by the 102.04 Da neutral loss due to RDA reaction and presence of the fragment ion at m/z 123.00 (trihydroxylated A-ring). The flavones baicalein and wogonin (Scutellaria baicalensis root) present a vicinal hydroxyl group in the A-ring, which may favor water loss. The H2 loss may also be observed due to homolytic cleavage of the hydroxyl bond forming a four-membered ring. This is also observed in methoxylated flavonoids with a vicinal hydroxyl/methoxyl group in which the loss of CH4 leads to the formation of a five-membered ring (-O-CH2-O). The fragmentation profile of polymethoxylated flavonoids shows that radical fragmentation competes with RDA reactions and are favored, yielding fragment ions with four-membered and five-membered ring fused with the A/B-ring due to CH3-CH2 and CH2 loss, respectively, as observed for nobiletin, tangeretin and 3,5,6,7,8,3',4'-heptamethoxyflavone in Citrus reticulata pericarp. The MS/MS spectrum acquired from the isoflavone USP Daidzein RS showed the formation of the ion [M-H-'H]' forming a resonance stabilized ion at m/z 252.04 followed by a second elimination of 'H (m/z 251.03) and sequential losses of two molecules of CO (m/z 195.04). These sequential neutral losses are typical signals to assign isoflavonoids skeleton in high-energy MS/MS experiments. Biochanin A, methoxylated isoflavonoid, also undergoes elimination of 'CH3 followed by 'H and two CO losses. This research demonstrates the important application of High Resolution Mass Spectrometry in the characterization of flavonoids in botanical materials used for the development of USP Reference Standards.

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Identification of Bioactive Compounds in Leaves Extract of Gundelia tournefortii from Chalus – Iran by Phytochemical Method

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Phytochemicals are bioactive, non-nutrient plant compounds in fruits, vegetables, grains and other plant foods that have been linked with reducing the risk of major degenerative diseases. Thus there is a need to evaluate the potential of local vegetables in relation to the provision of basic nutrients and phytochemicals, which will help in providing vital data for food processors, nutritionist, dieticians, as well as the consumers for the selection of proper green leafy vegetables. Medicinal plants are used locally in the treatment of infections caused by fungi, bacteria, viruses and parasites [1,2]. In this study, the leaves were collected and extract prepared from ethanol by microwave assisted extraction (MAE) method. The present study revealed that the phytochemicals analysis of nine different chemical compounds terpenoids (Salkowski Test), flavonoids (Alkaline Reagent Test), phenols (Ferric Chloride Test), coumarins (sodium hydroxide Test), tannins (Ferric Chloride Test), phlobatannins (HCl Test), cardiac glycosides (Keller-Killani test), quinones (H2SO4 Test), and saponins (Foam Test) were tested in ethanolic extracts. The results of the phytochemical screening of ethanolic extract of leaves of Gundelia tournefortii were flavonoids, coumarins, saponins and cardiac glycosides presented. Furthermore, in the present study the phytochemical screening of Gundelia tournefortii were found to be a powerful antioxidant, antibacterial agent and this study can be continued for their structural elucidation and pharmacological activity.

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In vitro Qualitative Phytochemical Analysis of Ethanolic Extract of Leaves of Datura stramonium from Karaj - Iran

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Medicinal plants are becoming more mainstream as improvement in analysis and quality control along with advances in clinical researches have shown the value of folk medicine in the treatment and prevention of diseases. The use of herbs to treat disease is almost universal among non-industrialized societies and is often more affordable than purchasing expensive modern pharmaceuticals, this enables herbal medicine to be as effective as conventional medicines but also gives them the same potential to cause harmful side effects [1,2,3]. The aim of this study was to evaluate the secondary metabolites of ethanolic extract of leaves of Datura stramonium belonging to the family Solanaceae. The leaves of...
Datura stramonium were collected from Karaj, Iran and ethanolic extract prepared by soxhlet method. The present study reveals that the preliminary phytochemical analysis of six different chemical compounds Terpenoids (Salkowski Test), Flavonoids (Alkaline Reagent Test), Phenols (Ferric Chloride Test), Tannins (Ferric Chloride Test), Cardiac glycosides (Keller-Killani test) and Saponins (Foam Test) were tested in ethanolic extract. The results of the phytochemical screening of ethanolic extract of leaves of Datura stramonium were flavonoids, terpenoids, saponins, tannins, cardiac glycosides, and phenols presented. Therefore, presence of above phytochemicals in Datura stramonium can be correlated with its medicinal potential. Similar reports on phytochemical composition of various medicinal plants were made earlier by many workers. However, it is very essential to isolate the bioactive fractions from these major groups so that it can be used further in designing specific drugs.

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PA19
Investigation of Secondary Metabolites from Methanolic Extract of Leaves Salvia officinalis from Tonekabon – Iran by Phytochemical Analysis

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The Salvia genus belongs to the subfamily Nepetoideae in Lamiaceae family. Many Salvia species are used as herbal tea and for food flavoring, as well as in cosmetics, perfumery and the pharmaceutical industries throughout world. [1,2,3]. This research set to assess phytochemicals in the methanolic extracts of Salvia officinalis leaves by qualitative screening procedures. The Salvia officinalis leaves were collected, and the extract was provided from methanolic by microwave assisted extraction (MAE). The phytochemical assessment was done applying standard methods. The phytochemical evaluation by using standard methods. Our preliminary phytochemical analysis of leaves extract using methanolic as solvent confirmed the presence of flavonoids, terpenoids, cardiac glycosides, phenols, tannins and saponins. The findings of the present study demonstrated the potential of phytochemicals from Salvia officinalis leaves, a natural source, in the pathway of developing a novel antibacterial agent able of treating bacterial infections.

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PA20
Screening of Secondary Metabolites from Aqueous Extract of Leaves of Callistemon citrinus from Tonekabon – Iran by Phytochemical Analysis

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Medicinal plants are the oldest known source for treatment of disease. Using pharmaceutical plants and plant extracts has been at great attention. Different extracts from traditional medicinal plants have been tested to identify the source of the therapeutic effects. As a result some natural products have been approved as new antibacterial drugs, but there is still an urgent need to identify novel substances that are active towards pathogens with high resistance [1,2]. The aim of this study were to investigate the preliminary phytochemical screening, aqueous extract of leaves of Callistemon citrinus belonging to family Myrtaceae. The dried leaves of Callistemon citrinus were collected and subjected to successive extraction by percolation method. The present study for phytochemical screening method of phytoconstitute by Trease and evans, Sofowara and Harbone were followed. The results of the phytochemical screening of aqueous extract of leaves of Callistemon citrinus were coumarins, flavonoids, phenols, terpenoids, quinones, tannins, saponins and cardiac glycosides presented. Conclusively, Callistemon citrinus is valuable source for new compounds and should receive special attention in research strategies to develop new antibacterial urgently required in the near future.

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PA21
A Dietary Supplement Ingredient Database (DSID) Botanical Initiative: Disintegration Testing of Turmeric Dietary Supplements.

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U.S. sales of botanical supplements have steadily increased for the last 13 years (to 7.7% of all DS sales in 2016). Botanical dietary supplements (DS) are sold as capsules (hard shell and soft gel), tablets, powders, and tinctures. As a part of the botanical initiative for the Dietary Supplement Ingredient Database (DSID), turmeric DS were identified using the Dietary Supplement Label Database (DSLD), industry sales data, the Internet and local store research. Selected products were analyzed for their phytochemical content and tablets, hard shell capsules and soft gels were tested for disintegration and rupture. Both simple (single botanical ingredient) and complex (includes other botanicals and/or other non-botanical ingredients) turmeric DS were tested. In-house quality control materials and duplicate products from a single lot showed consistent results when tested over time. For the 52 products tested for disintegration, the overall pass rate was 86.5% (n=45). All soft gels passed (n=5). Hard shell capsules passed at a rate of 89.4% (34 of 38). For tablets and caplets, the pass percentage was lower, at 66.7% (6 of 9). The testing of lot 2 disintegration for these supplements is underway. The capsule material may also play a role in the disintegration of DS. Products in capsules were categorized as having either cellulose-based (non-gelatin) or gelatin-based shells by reviewing label information. The pass rate of gelatin capsules was higher than non-gelatin capsules: 100% (n=15) vs. 81.48% (22 of 27). Our disintegration data are in line with the results of a recent study by Glube et al., 2013 (Results Pharma Sci., 3: 1–6), that indicated better performance of capsules made from gelatin vs. non-gelatin shells.

Most turmeric DS samples tested passed disintegration testing, however, the results varied by type of dosage form. When comparing these results to those from two green tea DS studies, the turmeric DS tested were much more likely to pass disintegration (overall pass rate for simple and complex green tea supplements was 55% and 66.6% respectively). All three studies follow the same patterns for the dosage form and capsule material: soft gel capsules were most likely to pass disintegration, hard shell capsules less likely, and tablets/caplets were least likely to pass disintegration.
PA22
Maged H.M. Sharaf
The International Association for the Advancement of High Performance Thin Layer Chromatography, CH-4310 Rheinfelden, Switzerland


To our collaborators worldwide and those who believe in the values of herbal drugs!!

PA23
The impact of food types on the formation of trans fatty acids during repeated deep frying
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Trans fatty acids (TFA) formed in processed foods have received great attention worldwide due to their association with cardiovascular diseases. Deep frying has regarded as one source to form TFA especially using repeatedly reutilized oil which is named thermally abused frying oil (TAFO). In addition to the excessive heat exposure, the composition of foods for frying may also impact the formation of TFA; this has not been well studied. Therefore, the goal of this study was to investigate the influence of food source on the formation of TFA during repeated deep frying. Frying experiments were performed with repeated frying cycles of three most consumed fried foods in the US: chicken, fish, and potato in soybean oil intermittently, up to 100 cycles at 180 ± 5°C. Oil and foods were analyzed from different cycles strategically. Four groups of TFA, i.e., trans 16:1, trans 18:1, trans 18:2, and trans 18:3 were identified and quantified using GC-FID-MS. Repeated exposure to high temperature frying led to significant deterioration of oil (darkened color and altered chemical properties) and a dramatic increase of 18-carbon TFA in all TAFOs. The amount of individual 18-carbon TFA was in the order of trans 18:3>trans 18:2>trans 18:1, confirming that the potential for isomerization increases with an increase in number of double bonds. The accumulation rate of TFA in TAFOs was highly dependent upon the food being fried: total TFA was in the order of chicken> potato> fish. The distribution profile of TFA in different TAFOs varied as well. Fish frying was found to accumulate monounsaturated TFA most rapidly whereas chicken frying resulted in the most dramatic accumulation of polyunsaturated TFA. These results indicated that food component may migrate into the oil medium and participate in or promote the formation of TFA. Differences in TFA formation in the fried foods will also be highlighted to investigate the related interaction between oil and food. This study clarifies the food-induced TFA formation during deep frying; this will enable industry to control the quality of fried foods and develop strategies to reduce the health risks associated with these highly desired foods.

PA24
Time on Target: Efficient preparative LC gradients for purification of natural products from calibration of analytical systems
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Preparative LC (liquid chromatography) is widely used to purify natural products. One bottleneck in the purification process is method development. Significant time can be required to produce an efficient preparative purification method that resolves the bioactive compound from impurities and minimizes both time and solvent usage. This work describes a simple method of calibrating analytical HPLC systems to match the preparative LC system using the existing scouting gradients typically employed by a research group. After the calibration is complete, the determined delay volume is applied to the scouting gradient. This delay volume encompasses any dwell volumes, column volumes, mixing volumes, solvent misproportioning, and other corrections required to match the analytical system to the preparative system. After completing the above calibration step, the user simply enters the retention time of the desired compound from the
analytical HPLC scouting run into their preparative HPLC to generate a focused preparative method. The retention time of targeted compounds may be set during the calibration step.

PA25

Estimated Intakes of Hydroxyanthracene Derivatives (HADs) from Consumption of Drinkable Aloe vera Products on the EU Market and Processes to Reduce HADs in Drinkable Aloe vera Ingredients

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HADs are a group of compounds with an anthranoid structure which naturally occur in many botanicals, including Aloe vera leaves where the extract/juice is used in foods and food supplements. The primary HADs found in Aloe vera leaves are aloins A and B. The European Food Safety Authority (EFSA) recently raised safety concerns of genotoxicity and carcinogenicity of HADs found in food supplements sold for laxative use in the EU but was unable to establish a safe intake level. A study was conducted to estimate aloins intake from drinkable Aloe foods and food supplements in Europe that are not intended for laxation; and the intake of known HADs from the normal diet; to determine whether HAD intake from these products are of concern, as well as exploring potential process improvements to reduce aloins in Aloe products. The levels of aloins were analyzed in 15 drinkable Aloe products from 6 European countries. Potential intake level from each product was calculated based on the daily recommended serving on product labels. HAD intake from the normal diet was assessed using literature reported concentrations of HADs in foods in combination with food consumption data from the 2015 EFSA Comprehensive Database. The estimated intake of aloins from the analyzed Aloe products ranged from <1 to 864 µg/person/day. HAD intake from the normal diet were estimated to range from <1 to 457 µg/person/day (mean) and 23 to 3,599 µg/person/day (high-level). The intake level of aloins from the Aloe products were below high-level background HAD intake and only one sample exceeded the mean estimated intake, demonstrating that dietary intake of aloins from drinkable Aloe products, that are not intended for laxation, are within the range of intake of HADs consumed as part of the background diet. Because Aloe vera leaf extracts can contain trace amount of aloins, a process improvement step was evaluated to see if the aloin levels could be further reduced in Aloe vera leaf extracts. Preliminary pilot trials utilizing a scalable process improvement step indicate a potential for a significant decrease in aloin levels. The combination of the intake assessment and the putative process enhancements provide consumers of Aloe beverages reassurance that these products do not lead to significant exposure of HADs and that substantially lower levels of aloins are possible.

PA26

Study on the Chemical Constituents of Lonicera macranthoides Hand.-Mazz

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To investigate the chemical constituents in the flower buds of Lonicera macranthoides Hand.-Mazz.. Methods: Various column chromatography were used to isolate the constituents from the EtOAc extracts of the dried flower buds of L. macranthoides. The chemical constituents of the plant were elucidated by spectroscopic methods, respectively. Results: 17 known compounds were isolated and identified which including 13 flavonoids named 4,2',4'-trihydroxychalcone (1), formononetin (2), liquiritigenin (3), taxifol (4), tricin (5), luteolin (6), quercetin (7), sinesetin (8), nobiletin (9), 5,6,7,8,4'-pentamethoxyflavone (10), 3,5,6,7,8,3',4'-heptamethoxyflavone (11), amentoflavon (12), 3''-O-methylamentoflavon (13) and 4 other compounds named angelin (14), psoralen (15), dehydrocostus lactone (16), pinoresinol (17). Conclusion: Compounds 1-5 and 8-17 were obtained from this plant for the first time.

PA27
Annonaceae Family: a Venture into the Unknowns

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Annonaceae family or custard apple family has over a hundred genera and thousands of species. This family is distributed worldwide and some species have been used traditionally for medicinal effects. The purpose of this investigation is to screen for anti-cell proliferative effects of a collection of Annonaceae plant extracts in colon cancer cells (HCT-116 and HT-29), to isolate and identify the active constituents of the extracts, and to potentially explore the mechanism of action of the active constituents. A collection of 85 Annonaceae plant extracts (16 genera, 32 species) from the repository of the NCNPR was screened for anti-cell proliferative activity. The cell proliferation was determined by a colorimetric method based on tetrazolium salts WST-8 in which viable cells are able to reduce WST-8 salts to water-soluble dye. The results indicated that of the 85 extracts, 25 extracts inhibited cell proliferation of colon cancer cells at the concentration of 50 μg/ml or less. Phytochemical investigations of the two most active extracts were performed. The structures of the compounds were elucidated with 1D and 2D NMR and results are in progress.

PA28
Supercritical Fluid Extraction and Physiochemical Properties of Avocado Oil

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Avocado is the fruit of avocado tree, known as Persiana americana. The fruit is prized for its high nutrient value because it contains substantial amounts of vitamins, phytosterols, and triacylglycerols with high contents of unsaturated fatty acids. In recent years, the production of oil from avocado fruit is highly promoted due to the issue of easy oxidation and short time of maturation of avocado fruit for producers. In addition, the oil has demonstrated huge potential for applications in cosmetics, food and pharmaceuticals industries.

Generally, avocado oil is extracted from avocado pulp by techniques of centrifugation, pressing and solvent extraction. These extraction methods are time consuming, economically unfavorable and not suitable for thermally unstable components. Furthermore, avocado oil that extracted using these methods always has a distinct green coloration due to the presence of chlorophyll. Chlorophyll often serves as a photosensitizer in oxidative processes, therefore, the high chlorophyll content may cause stability problems for avocado oil.
This investigation was conducted to study the supercritical fluid extraction (SFE) for oils from avocado pulp, skin and seed. The extraction time, temperature, pressure, supercritical carbon dioxide flow rate and various modifiers were evaluated. It has been shown that SFE can successfully extract high quality avocado oil from avocado pulp and skin. The seed only contains less than 2% of oil. The optimum extraction parameters were determined to be at 250 bar, 50 minutes, 10 mL/min pure CO\textsubscript{2} and 40 °C yielding an extraction that was 95% complete within 50 minutes. The shorter extraction time and lower temperature were used when compared to the conventional organic solvent extractions. In addition, the physiochemical properties, such as acid value, iodine value, saponification value and peroxide value were determined. This project demonstrated that SFE is ideal for the extraction of avocado oil and eliminates the undesirable compounds such as chlorophyll.

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PA29
Characterization and Authentication of Avocado Oil from Triacylglycerol and Fatty Acid Compositional Data

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Avocado (Persea americana Mill.) belongs to the family of Lauraceae and has the highest fat content among all known fruit and vegetable varieties. It has gained great popularity due to the various nutrients and bioactive phytochemicals present in avocado fruit. Avocado oil, traditionally extracted from the mature fruit flesh, has found a multitude of applications such as edible/culinary oil, an ingredient in healthcare products and cosmetics or in pharmaceuticals industry. Recently, avocado seed oil has appeared in the US market, and has been considered as a source of fatty acids, carbs in the form of starch, dietary fibre and a broad range of phytochemicals by some consumers.

About 98% avocado pulp lipid mass is represented by triacylglycerols (TGs), which are hydrophobic molecular species formed by esterification of three fatty acids (FAs) with a glycerol backbone. HPLC/MS has been the method of choice for TGs analysis. The characteristic fragment ions formed by IS-CID are usually diacylglycerols by loss of one acyl moiety. However, due to complexity of TGs differing in acyl chain lengths, numbers and positions of double bonds, and regioisomerism, as well as the lack of commercial standards, precise determination of individual TG with specific fatty acid chain position is full of challenge.

In the present study, an UHPLC/APCI-MS method was developed for the quantification of TGs for oils extracted from avocado pulp, skin and seeds. Three BEH C\textsubscript{18} column (2.1 x 100mm, 1.7µm) columns were connected together to provide the highest possible chromatographic resolution and to reduce the number of possible coelutions. The response factors (RFs) were determined using single-acid TGs such as tripalmitin (PPP, C16:0), triolein (OOO, C18:1), etc. The model for the calculation of RFs for mixed-acid TGs is proposed and applied for the determination of TG compositions in the authenticated avocado pulp, skin and seed oils, as well as 19 commercial oils claimed to extract from avocado pulp or seed. The quantification results of TGs in these oil samples were compared with fatty acid methyl esters (FAMEs) determined by GC/MS analysis. Possible adulteration of commercial oils with soybean and sesame oil has been identified and further confirmed with principal component analysis (PCA). It is concluded that the combination of TGs and FAMEs analysis using UHPLC/APCI-MS and GC/MS, and multivariate statistical analysis may provide comprehensive information for characterization, standardization and authentication of avocado oils.

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PA30
Characterization of Organic Acid Derivatives, Flavonoids, and Triterpene Glycosides from Eleutherococcus senticosus and Ci-wu-jia Tea by UHPLC-UV-QToF MS\textsuperscript{6060} Yan-Hong Wang\textsuperscript{1}, Yonghai Meng\textsuperscript{2}, Chunmei Zhai\textsuperscript{2}, Mei Wang\textsuperscript{1}, Bharathi Avula\textsuperscript{1}, Jimmy Yuk\textsuperscript{2}, Kerri M. Smith\textsuperscript{2}, Giorgis Isaac\textsuperscript{2}, Ikhlas A. Khan\textsuperscript{1,3}

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Eleutherococcus senticosus Maxim. (syn. Acanthopanax senticosus Harms) belongs to the Araliaceae family and usually grows in the forest or thickets where it is elevated from hundreds to above 2000 meters in altitude in China. E. senticosus is also known as ci-wu-jia in China. E. senticosus is in the same family as Panax ginseng and the leaves have been reported for glycosidase inhibition as well as having antibacterial properties. Therefore, E. senticosus leaves have been developed as a functional beverage called ci-wu-jia tea in China and Siberian ginseng tea in the United States and Europe. Phytochemical studies revealed that caffeoylquinic acid derivatives, triterpene glycosides, and flavonoids were the major secondary metabolites in leaves of E. senticosus.

Several analytical methods have been reported for the quantitative or qualitative analysis of caffeoylquinic acid derivatives, triterpene glycosides, and flavonoids in E. senticosus leaves, but no analytical method has done for the chemical analysis of caffeoylquinic acid derivatives, flavonoids, and triterpene glycosides in a single analytical method. Additionally, triterpene glycosides are difficult to identify by UV detection and contents of these compounds are low in E. senticosus leaves. In this study, a sensitive UHPLC method combining UV and MS/MS has been developed to characterize the triterpene glycosides along with organic acid derivatives and flavonoids from E. senticosus leaves. Fragmentation patterns of three sub-groups of triterpene glycosides in E. senticosus leaves were investigated. A compound screening library including 241 compounds reported in literature was created and used to confirm the compounds in the samples. In this work, 13 plant samples of E. senticosus and 11 ci-wu-jia tea products were analyzed. The developed UHPLC-UV-MS/MS analytical method combined with the UNIFI processing method can simultaneously characterize organic acid derivatives, flavonoids, and triterpene glycosides from E. senticosus. It provides a simple and sensitive way to perform quality control of E. senticosus and related ci-wu-jia tea products.

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PA31
A Sensitive UHPLC-MS/MS Method to Identify Harmful Amines in Bodybuilding Products

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Harmful amines such as 1,3-dimethylamylamine (DMAA), 1,3-dimethylbutylamine (DMBA), betamethylphenethylamine (BMPEA), and methylsynephrine can elevate blood pressure and lead to severe heart problems. The Food and Drug Administration (FDA) has sent out warning letters to remove dietary supplements with these amines from market. However, research has shown that these amines are still used in bodybuilding, athletic performance enhancer, and weight-loss supplements. Even worse, some products add analogues of these amines to replace the banned compounds. Therefore, it is crucial to develop a method to identify amines in various products.

A sensitive ultra-high performance liquid chromatography coupled with high resolution tandem mass spectrometry (UHPLC-HRMS/MS) method has been developed to identify harmful amines in bodybuilding products. Methanol aqueous extracts of bodybuilding products were reacted with 4-chlorobenzoyl chloride. The derivatives of amines and 4-chlorobenzoyl chloride can be determined easily by screening stable isotopic ions of chlorine on mass spectrometer. Amines were identified in 6 out 7 (86%) products. The limits of detection are below 40 ppm (40 µg/g sample weight) from a placebo sample spiked with 1,3-dimethylbutylamine (DMBA), 2-amino-6-methylheptane (DMHA), 5-methyl-2heptanamine (DMHA), 2-amino-4-methylhexane (DMAA), and phenethylamine (PEA). The developed UHPLC-MS/MS method is useful to determine harmful amines from dietary supplements.

This research is supported in part by “Science Based Authentication of Botanical Ingredients” funded by the Food and Drug Administration grant number 2U01FD004246-06.
PA32  
Botanical Dietary Supplement Standard Reference Materials and Quality Assurance Programs at the National Institute of Standards and Technology

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The National Institute of Standards and Technology (NIST) has been developing new Standard Reference Materials (SRMs) and analytical methods for botanical Dietary Supplements since 2001 through a multi-year interagency agreement with the National Institutes of Health’s Office of Dietary Supplements (NIH/ODS). Botanical SRMs typically include a wide range of assigned values for concentrations of organic and/or inorganic constituents. SRMs play a key role in ensuring quality control to promote Good Manufacturing Practices (GMPs) and assist in the verification of product label claims for commercially available botanical supplements sold at retail stores and online. Most consumers believe routinely taking botanical supplements will improve their health without normal medications side effects. Currently, two-thirds of adults in the U.S. believe they are active supplement users with an annual revenue of more than $25 billion. NIST has developed more than 20 botanical SRMs for the dietary supplement community including Ginkgo biloba (SRMs 3246 - 3249), Serenoa repens (SRMs 3250 and 3251), Green Tea (SRMs 3254 – 3257), etc. Additionally, the NIST Dietary Supplement Laboratory Quality Assurance Program (DSQAP) was established in 2007 through the agreement with NIH/ODS to enable members to comply with various regulations in GMPs. DSQAP provides supplement laboratories the opportunity to assess their in-house analytical measurements capabilities for active and/or marker compounds, nutritional elements, contaminants, and fat- and water-soluble vitamins. Reports and certificates of participation are provided and can be used to demonstrate compliance with regulated GMPs.

The authors acknowledge financial support from the Office of Dietary Supplements at the National Institute of Health.

PA33  
Reverse Phase Liquid Chromatography and Photodiode Array Detection for Determination of Phenolic Acids in Black Cohosh Standard Reference Materials

Wilson WB1 & Rimmer CA1

Chemical Sciences Division, Material Measurement Laboratory, National Institute of Standards and Technology

Black cohosh (Cimicifuga racemose (L.) Nutt.) roots and rhizomes have been used in the preparation of dietary supplements for the treatment of women’s disorders worldwide and has been classified as one of the top 10 best-selling herbs in the US. Several studies in the US have reported on the adulteration of black cohosh supplements and a described hepatotoxicity associated with the supplements prepared with other plant parts then the roots, rhizomes, or different Cimicifuga species. Phenolic acids are a primary group of active compounds present in black cohosh and work as antioxidants to prevent cellular damage due to free-radical oxidation reactions and help promote anti-inflammatory conditions in the human body. In this study, a new reversed-phase liquid chromatography (RPLC) method coupled to a photodiode array detector (PDA) was developed for the determination of phenolic acids in three candidate black cohosh Standard Reference Materials. The PDA simultaneously monitors different wavelengths throughout a chromatographic run. A typical two-dimensional chromatogram based on retention time (x-axis) and absorption (y-axis) is extended to a three-dimensional format with wavelengths (z-axis). The RPLC-PDA method used an ACE 3 C18 column to analyze black cohosh samples.

The authors acknowledge financial support from the Office of Dietary Supplements at the National Institute of Health.
Composition Analysis of Ashwagandha Dietary Supplement by NMR-guided Fractionation

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Dietary supplements, particularly botanical dietary supplements, are usually complex mixtures, they post challenges for composition analysis and quality control. The violations such as mislabeling and adulteration of the products are often hard to be found if the components in the products are not clearly identified. Nuclear magnetic resonance (NMR) is a powerful tool for structure determination and can be used for quantification as well. In the present study, a dietary supplement “Ashwagandha” collected from the market was studied by using NMR as a tool for identifying the unusual minor components in the product. 1D and 2D NMR techniques were applied for guiding chromatographic fractionations and structural identifications. Some nitrogen containing components were identified from the chromatographic fractions.

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NMR Fingerprinting and Chemometric Approach for Differentiation of Whey Protein Products

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A wide array of whey protein dietary supplements are marketed for bodybuilding and muscle strength boosting. There are three primary types of whey protein used as dietary supplements. In addition to whey protein, other ingredients, such as sugars, vitamins, amino acids, etc. may be added into the supplements. Many methods have been developed for protein fraction analysis so far. However, there is a necessity to develop a simple and efficient method for assessment of the quality of whey protein supplements. In this study, nuclear magnetic resonance (NMR) technique together with chemometric analysis were applied to investigate 20 whey protein commercial products. It was found that seven samples (including three outliers) showed significant difference in NMR fingerprint patterns compared to the other thirteen samples.

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PB1

Global Climate Change and Medicinal and Aromatic Plants in Turkey

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Global warming, drought, medicinal and aromatic plants

Turkey climate in the complex structure, particularly due to global warming, is one of the countries that will be most affected by climate change. Researchers in the average global temperature in 2050 is estimated to be a temperature rise of between 1.5 and 4.5 degrees. Hot and dry conditions will occur in the future, it should be studied to determine appropriate plant varieties for dry and hot conditions. Some farmers will have to change their crops according to the new climate conditions because of the drought. Some medicinal plants (Thymus L., Rosmarinus officinalis L., Carthamus tinctorius L., Calendula officinalis L.), which are resistant to temperature and drought, may be alternative crops for farmers in Turkey in future. These plants are used in such as health, food, spices, tea, and cosmetics. Some of them are exported. In the future, the presence of these plants in drought fields will benefit both farmers and sustainable agriculture.

PB2

Concept of Body Fat and its accumulation in Graeco-Arabian medicine (Unani Medicine)

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Graeco-Arabian Medicine or Medieval Islamic medicine or Unani medicine as it is called in Indian subcontinent derives its term from the original Greek medicine which was developed during the Arab civilization. In the Indian subcontinent, it is called Unani medicine out of adherence to its true historical derivation whereas European historians call it Arab medicine. Unani is derived from ‘Unan’, which is an Urdu language translation of Greece

Unani medicine may have disappeared from the country of its origin, but it has flourished in the Indian subcontinent. Arab traders who entered through the Western Ghats long before Mughals introduced Unani here.

Unani scholars have described fat in the context of organs which are homogeneous in structure throughout and known as “Mutashahibul-Ajza” or “A’za’ basitah” which means simple organs and smallest part of which exactly resembles the whole.


Temperament is a holistic sorting of behavior patterns among individuals in unani medicine. There are four such temperaments which will be discussed in the speech

Avicenna in Canon of Medicine has described composition of fat as “Fat is formed from wateriness and greasiness of the blood and cold coagulates it. This is why heat dissolves it.”

PB3

Examining the Association Between Green Tea Supplement Consumption, Liver Biomarkers, and Adverse Event Reports in the U.S.

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The safety of green tea supplements has been questioned due to isolated case reports of liver toxicity in individuals consuming supplements containing green tea extracts. The relationship between green tea supplement consumption and...
abnormal liver biomarkers in adults was evaluated using the 2009-2014 U.S. National Health and Nutrition Examination Survey (NHANES). An individual was considered to have an abnormal liver biomarker if any one of the liver biomarkers (bilirubin, gamma glutamyl transferase, alanine aminotransferase, aspartate aminotransferase, or alkaline phosphatase) exceeded the upper end of the age-and gender-specific normal reference ranges from the Centers for Disease Control and Prevention (CDC). Green tea supplements were identified as dietary supplements containing either green tea powder or extracts of green tea as indicated on the labels. Use of green tea supplements was quantified as consumption versus no consumption. The associations between green tea supplement use and the odds of having abnormal liver biomarkers were assessed using multiple logistic regression models (gender, age, race, medication use, alcohol use, waist circumference, and liver disease). This relationship was further examined using adverse event reports in the Center for Food Safety and Applied Nutrition (CFSAN) Adverse Event Reporting System (CAERS) database during the same time span. Adverse event reports received for suspect products classified under the Vit/Min/Prot/Unconv Diet(Human/Animal) industry category were extracted. Proportional reporting ratio (PRR) was used to detect signals of disproportionate reporting between green tea products and liver toxicity. Results showed that 23% of the individuals in the NHANES analysis had at least one abnormal liver biomarker, and 1.3% were users of green tea supplements. Consumption of green tea supplements versus no consumption was not associated with abnormal liver biomarkers after adjusting for confounding variables (OR=0.92; 95% CI: 0.51, 1.66; p=0.78). In the CAERS analysis, one potential signal was identified between a Green Tea Fat Burner product and jaundice (PRR=12.81; 95% CI: 4.29, 38.25). The alleged link between green tea supplement use and liver toxicity appears to be idiosyncratic in nature and dependent upon a variety of factors.

PB4
Differential Pathway Sensitivity Associated with various Natural products across Cancer signaling

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Complex molecular mechanisms contribute to the transformation of normal cells into cancerous cells and they result in the abnormal functioning of many signaling pathways that control cancer metabolism. The efforts to identify new therapy for cancer must be based on the activities of multiple signaling and considering the differential pathway dependencies, we developed a method to identify the agents that target these various pathways. We have collected a battery of 15 inducible luciferase reporter gene vectors where expression is driven by enhancer elements that bind to specific transcription factors. For this assay, cancer cells were transiently transfected with appropriate plasmid DNA using X-tremeGENE HP transfection reagent. After 24h of transfection, the cells were treated with test agents and 30mins later, they were induced with specific inducers of that pathway. After 4 or 6h of treatment, the cells were lysed with one-Glo luciferase reagent and measured. Over 100 pure compounds from natural sources belonging to several chemical classes were run through this high throughput screen at different concentrations. IC₅₀ values were determined and their structure-activity relationships were analyzed. Electrophilic compounds (e.g. naphthoquinones, chalcones, sesquiterpene lactones, and isothiocyanates) preferentially targeted NF-κB. This assay also provided additional information about the anti-inflammatory potential of compounds as they inhibit inflammatory pathways viz., STAT3, SMAD, NFκB, Notch and Wnt. When more than 700 crude plant extracts, (authenticated samples from our repository) were tested in combination, these signaling pathways are helpful to determine their synergistic or antagonistic potential during interactions. This is a useful screening tool for monitoring activity profile changes of a lead compound during chemical modification. (E.g. Structure-activity relationships of flavonoids).

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The extrapolation of a safe oral dose from lab animals to humans differs by agency

Brickel J.A.

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Guidelines for determining a safe, oral dose for a compound in humans using laboratory animal test data differ based on the source of exposure (e.g., as a drug or therapeutic, drinking water contaminant, pesticide residue, food ingredient, or dietary supplement ingredient). A lack of harmonization between methodologies introduces confusion and variations in the application of regulatory guidance and determinations of safe, oral doses in humans. For orally administered drugs, FDA recommends converting no observable adverse effect levels (NOAELs) established in lab animals to NOAEL human equivalent doses (HEDs) using body surface area conversion factors (BSA-CFs) to normalize for differences in body surface area (BSA) between the test animal and humans. An uncertainty factor (UF) (e.g., default of 10) is then applied to the NOAEL\textsubscript{HED} to derive Maximum Recommended Starting Doses (MRSDs) in clinical trials. EPA guidance recommends calculating oral reference doses (RfDs) by applying the appropriate composite UF to the point of departure (POD) (e.g., NOAEL, Lowest observable adverse effect level (LOAEL), or Benchmark Dose (BMD)), after the POD in lab animals is converted to a HED by physiologically based-toxicokinetic (PBTK) modeling or chemical-specific information. Absent such data, dosimetric adjustment factors (DAFs), based on body weight scaling, are applied to the POD in animals to calculate the HED. In contrast, BMD modeling and HEDs are not included in FDA regulations or guidance for food substances or dietary ingredients. When assessing the safe use of a food substance under the conditions of intended use (i.e., Generally Recognized as Safe (GRAS) dossier or Food or Color Additive petition), NOAELs are identified in lab animals, then the NOAEL from the most sensitive laboratory species is commonly selected, and an UF (e.g., default of 100) is applied to the NOAEL to determine the Acceptable Daily Intake (ADI) in humans. It is common for the NOAEL in a lab animal species to be directly extrapolated to humans based on body weight alone (1:1), to determine the margin of safety (MOS) between the (extrapolated) NOAEL in humans and estimated daily intake (EDI). For dietary supplement ingredients, an ADI may also be determined through the identification of a NOAEL in lab animals and application of a composite UF, without consideration for BMD modeling or BSA or body weight scaling. These differences afford the opportunity for inconsistencies and variations in translating safe, oral doses, highlighting the need for additional education within the scientific community, and consensus and harmonized guidelines between US regulatory bodies.

Investigations on the essential oil composition and biological activities of three Baccharis species from Brazil

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The genus Baccharis L. (Asteraceae) comprises about 435 species, distributed from Argentina to the USA. In Brazil, it is represented by 179 species. Several species of Baccharis are commonly used in traditional medicine as analgesic, antidiabetic, anti-inflammatory, digestive, diuretic and spasmolytic agents. Chemical compositions as well as antitrypanosomal and insecticidal activities of the essential oils of three Brazilian species, namely B. microdonta, B. reticularioides and B. sphenophylla, were studied. GC/MS analyses of these essential oils showed prominent differences in their chemical compositions. Some of the compounds were specific to these species hence could be used as chemical markers for species identification and authentication. The major compounds observed in these species included α-pinene (24.50%) in B. reticularioides; spathulenol (24.74%) and kongol (22.22%) in B. microdonta; and β-pinene (15.24%), limonene (14.33%) and spathulenol (13.15%) in B. sphenophylla. In vitro analyses for antitrypanosomal and insecticidal activities were conducted for all the three species. Essential oils of B. microdonta and B. reticularioides exhibited antitrypanosomal activities while B. sphenophylla showed strong toxicity to bed bugs in fumigation bioassay.

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research was supported by USDA-Discovery & Development of Natural Products based insect management for medical, veterinary & Urban (58-6066-6-043).

PB7
Ethnopharmacological investigations of Cuphea calophylla Cham. & Schtdl. subsp. mesostemon (Koehne) Lourteig.

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Several species of Cuphea (Lythraceae) are used medicinally and are reported to have anti-inflammatory, diaphoretic, diuretic, antihypertensive and cardioprotective properties. Cuphea calophylla subsp. mesostemon (Koehne) Lourteig is an herbaceous plant growing naturally in the fields, forest edges and along roadsides in Brazil. It shares similar morphological features with several other species of Cuphea creating confusions in the species identification. Due to the morphological similarity, different species known as seven-sangrias in Brazil are used interchangeably for the same therapeutic purposes. The main objectives of the present work were to characterize the morpho-anatomy of leaves of C. calophylla and to perform a whole-ethnopharmacological investigation of the cardiorenal properties of the ethanol soluble fraction from C. calophylla (ESCC) in Wistar rats. For the anatomy analysis, the plant materials were analyzed by light and scanning electron microscopy. The leaf is amphistomatic with uniseriate epidermis and dorsiventral mesophyll containing oil droplets. Anomocytic and diacytic stomata are present. The trichomes are of three types: a) glandular, multicellular, multiseriate; b) non-glandular, warty, adpressed; and c) non-glandular, multicellular, uniseriate. In cross-section, the midrib is slightly concave-convex in outline and possesses a central beam of bicollateral vascular system in open arch. For toxicological and pharmacological assays, the ESCC was obtained (by infusion) and its chemical profile was determined by LC-DAD-MS. In addition, an acute toxicity assay was conducted in female Wistar rats in order to observe any toxic effects after one single administration. Finally, the diuretic and hypotensive potential of ESCC (30, 100 and 300 mg/kg) were investigated in male rats followed by the evaluation of its possible effects on peripheral vascular resistance and tissue antioxidant system. Chemical compounds identified from ESCC were mainly glycosylated flavonoids and organic acids. No signs of toxicity as well as no changes in urine volume or electrolyte elimination were observed after ESCC acute treatment. On the other hand, prolonged treatment with ESCC (30 mg/kg) significantly increased urine volume and sodium excretion at 7th day of treatment, without affecting blood pressure, heart rate, or peripheral resistance in mesenteric vascular beds. Apparently, these effects are involved with an increase of tissue superoxide dismutase activity after prolonged treatment.

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PB8
Hypoglycemic activity of aqueous extract of Citullus colocynthis

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There are numerous plants worldwide, which have beneficial effects on the treatment of diabetes. Several studies have shown reassurance to the traditional medicine and natural therapies in the treatment of diabetes. In addition, WHO emphasizes on diabetes therapy with traditional treatments, particularly by natural hypoglycemic plants as a useful source of oral medications. Many traditional plants being used in the UAE as antidiabetic remedies, Citrullus colocynthis (Handal) is amongst one of them. The fruits is some times as large as an apple with a bitter taste that is why it is also called as bitter apple. Colocynth has been widely used in traditional medicine for centuries.
The *C. colocynthis* plant extract was suspended/dissolved in water before the administration to the animals (Treated group) orally by gavage feeding needle at the dose of 200mg/kg for 5 days and the water administered to the (control group) with the same volume and by the same route.

On the 5th day, using (Glucose analyzer GM9 machine) to read the glucose levels, the data collected, recorded, entered, arranged and analyzed using Student's T-test $P<0.05$. The *C. colocynthis* treated group exhibited hypoglycemic activity showing reduction of blood glucose level mean of 144 mg/dl as compared to the control group of 192 mg/dl, however, statistically significant. These results suggest that the aqueous extract of the plant possesses significant hypoglycemic effect in mice.

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**PB9**

**Anatomy of leaf, chemical composition, cytotoxicity and insecticidal activity of the essential oil from Ocotea porosa**


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*Ocotea porosa* (Nees & Mart.) Barroso, commonly known as “imbuia”, “canela-imbuia” or “imbuia-amarela” in Brazil, is a tree of the Southern Atlantic Forest. The present study investigates the anatomy of leaf, volatile oil chemistry, as well as cytotoxicity and insecticidal activity of the essential oil from *O. porosa*. Secretory cells with spherical to oblong shape and with light yellow lipophilic content reacted with Sudan III were observed in blade and petiole of *O. porosa*. Thirty five volatile compounds (92.54%) were identified in the essential oil from *O. porosa*. The major components were bicyclogermacrene (24.62%), α-pinene (19.71%), and β-pinene (13.86%). Cytotoxicity against human cancer cell (MCF-7), mouse cancer cell (B16F10) and mouse nontumoral cell (McCoy) was confirmed. The essential oil from *O. porosa* induced cell death with apoptotic characteristics as cell rounding, membrane blebbing, and chromatin condensation. However, this volatile oil showed a selectivity index (SI) of 1.05 and 0.05 for B16F10 and MCF-7 when compared to fibroblast normal cells (McCoy line), respectively. In addition, a weak insecticidal activity of the essential oil against susceptible ‘Ft. Dix’ bed bugs by topical application was demonstrated.

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PB10
Cytotoxic mechanism of French lavender essential oil against human non-small cell lung cancer cell line (Calu-3)

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The essential oil of Lavandula dentata L. possesses sedative, antioxidant, antibacterial, antifungal, carminative, antidepressant and cytotoxic properties. The aim of this study was to investigate the cytotoxic effects of L. dentata essential oil on Calu-3 lung cancer cell in both vapor and liquid phases and to provide further insights into its cytotoxic mechanisms. Usual MTT reduction cell metabolism test, sulphorhodamine B assay, and indirect analysis of cellular DNA content-propidium iodide fluorimetry were used for evaluating cytotoxicity. In addition, membrane integrity test by lactate dehydrogenase release, acridine orange with ethidium bromide, Hoescht 33342, propidium iodide, Rhodamine 123, and Annexin V analyses were performed in order to explore the cytotoxic mechanisms. Rhodamine 123 accumulation assay was used for evaluating P-glycoprotein inhibitory potential. Lavandula dentata essential oil provided a significant reduction of cell viability for all cytotoxic experiments by reaching 84% of cytotoxicity in vapor phase and showed a time-dependent profile. Both necrosis and apoptosis are involved in Calu-3 cell death by L. dentata essential oil. However, necrosis appears to be the dominant cell death pathway. Lavandula dentata essential oil did not inhibit P-glycoprotein.

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PB11
Hepatoprotective activity of Achillea millefolium against paracetamol induced liver damage in rats and its in vitro antioxidant effect

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Paracetamol (PCM) has an adequate safety profile when taken in normal doses. However, it could produce oxidative stress with liver injury when taken in an overdose. The aim of the present study was to evaluate the protective effects of Achillea millefolium extract (AME) against PCM-induced oxidative stress and liver injury in rats. In addition, to investigate its possible in vitro antioxidant effect. The in vitro antioxidant activity of AME was tested using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging. Five groups of rats were used for determination of hepatoprotective activity of the extract. The normal and hepatotoxic control groups received the vehicle while other groups were treated with silymarin (50 mg/kg) and AME (200 and 400 mg/kg), respectively for 7 days. Liver injury was induced on the 5th day by oral dosing of PCM (2 g/kg) to all rats except those in normal control group.

AME displayed in vitro antioxidant activity in a concentration-dependent way. The antioxidant status in liver such as activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and the levels of reduced glutathione (GSH) were significantly declined, while hepatic malondialdehyde (MDA) levels were significantly elevated in PCM alone treated rats. Hepatic enzyme markers as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and γ-glutamyl transferase (γ-GT) and level of total bilirubin (BRN) were significantly elevated, while total protein (TP) and albumin (ALB) were declined significantly in PCM-exposed animals. Administration of AME (400 mg/kg) prior to PCM, significantly reduced oxidative stress and prevented the increase in the serum levels of hepatic enzymes and BRN. The histopathological evaluation of the livers also revealed that AME
PB12

**Combating bacterial antibiotic resistance: Identification of a novel botanical-derived B-lactam antibiotic and B-lactam potentiation compound**

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Bacterial antibiotic resistance is a serious and growing phenomenon in contemporary medicine and has emerged as one of the eminent public health concerns of the 21st century. Botanical extracts with antibacterial activity are widely used in alternative medicine to treat bacterial infections. *Larrea tridentata* (commonly known as chaparral or creosote bush) is a prominent species in the deserts of southwestern North America. Our studies support the direct antibacterial activity of this botanical toward various bacterial genera, including members of *Staphylococcus* and *Streptococcus*. Through ELISA testing, we have demonstrated that extracts of *Larrea* contain a novel b-lactam type antibiotic which can be inhibited by b-lactamase and clavulanic acid. β-lactam antibiotics are a class of broad-spectrum antibiotics, consisting of antibiotic agents that contain a b-lactam ring in their molecular structures. Filamentous fungi are microorganisms of great biotechnological interest due to their ability to synthesize a variety of bioactive secondary metabolites including β-lactam antibiotics, such as penicillins and cephalosporins. Hydrophobic penicillins are only known to be produced by fungi, mainly *Penicillium chrysogenum* and *Aspergillus nidulans*, whereas hydrophilic cephalosporins are produced by both fungi and bacteria. Although we have not yet isolated the b-lactam active molecule from extracts of *Larrea*, the activity of the b-lactam is approximately 2000-8000 fold greater against *Staphylococcus* as compared to penicillin or ampicillin (based on b-lactam concentrations). This increased activity was found to be associated with a second constituent present in *Larrea* which can also lead to increased activity of several commercial b-lactam antibiotics dependent upon specific b-lactam structural features. These results support the identification of a potentially novel b-lactam antibiotic from a botanical source as well as a novel compound which can enhance the activity of current pharmaceutical b-lactam antibiotics.

PB13

**University of Mississippi Center for Clinical and Translational Science (CCTS): A Hub for Cooperative Research**


University of Mississippi: ¹School of Pharmacy, ²Center for Clinical and Translational Science (CCTS); University of Mississippi Medical Center: ³Department of Pathology, ⁴Department of Radiation Oncology, ⁵Department of Preventive Medicine, ⁶John D. Bower School of Population Health.

Established in the summer of 2018, The CCTS fosters cooperative clinical and translational sciences between the University of Mississippi School of Pharmacy (UMSOP) and the University of Mississippi Medical Center (UMMC). CCTS facilitates the translation of basic research discoveries into clinically validated therapies to improve the health of populations in Mississippi and beyond. To accomplish CCTS mission three overarching goals have been defined: I) Develop progressive and sustainable capacity for clinical and translational research; II) Promote interprofessional engagement in clinical and translational science; and III) Foster research collaboration among stakeholders.

To carry CCTS’s mission three research units have been established: 1) The Pre-clinical Research Unit: Develops processes to move basic science discoveries towards translation into research in humans. This unit provides guidance in the development of Investigational New Drug (IND) applications; and identifies and pursue opportunities to develop progressive capacities for *in vitro*, *ex vivo*, *in vivo*, and *in silico* approaches for evaluating new pharmaceutical and therapeutic agents. 2) The Clinical Research Unit: Transitions projects that have received IND approval into the first phase of clinical trials. It also transitions clinical trials from Phase I to Phase II and to Phase III; develops marketing plans, standard operating procedures (SOPs), personnel training plans, and policies to guide all clinical research; develops budgets and negotiate agreements with industry sponsors and governmental funding agencies; and assures compliance with all regulatory requirements. 3) Community/population Research Unit: Develops, coordinates, and facilitates research activities and translation between clinical and community/population research stages. To do so, this
unit works closely with the SOP community-based Research Program and the Population Health programs on the Oxford and Jackson campuses.

By promoting research collaboration between UMSOP and UMMC, CCTS is progressing in becoming a national leader in clinical and translational investigation.

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PB14
Critical Decision Points in Screening and Diagnosis of Prostate Cancer: The Role of Molecular Biomarkers as a Means to Personalize Cancer Screening and Treatment.


Prostate cancer (PCa) remains the most common form of cancer affecting men in the Western Hemisphere. Mortality rate is 130% higher among African-American men (AAM) than Caucasian-American men. As this trend is not new nor changing, there is an urgent need to identify markers with the ability to specifically distinguish aggressive PCa in the context of race. Gene expression patterns have been used as a tool to identify prognostic biomarkers for PCa to help reduce this disparity. Gene expression profiles reveal molecular mechanisms useful in understanding the biologic basis of tumorigenesis. Thus far, gene expression profiling analyses focused on race between AAM and Caucasian-American men (CAM) demonstrated distinct tumor microenvironments in the tumor-adjacent stroma and pathways associated with inflammation, lipid metabolism, and regulation of epithelial-to-mesenchymal transition. Additionally, we and others have established that hypoxia, another component of the tumor microenvironment, can been linked to malignant progression, metastasis, resistance to therapy, and poor clinical outcome in PCa. Gene expression panels, including distinct components related to the biology of PCa in AAM, may increase prognostic accuracy for this ethnic group. Furthermore, reference gene expression patterns, especially in the context of the emerging molecular taxonomy of PCa, would be buttressed by including more AAM in their development to consider the aspects of expression profiles differentially associated with race.

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PB15
Combination Treatment with Natural Stilbene and Epigenetic Agent Shows Better Effect in Prostate Cancer in vivo.


In our previous studies we found that MTA1 expression is significantly increased in prostate-specific Pten-null model, and that potent natural analog of resveratrol, pterostilbene (PTER), exerts its anti-tumorigenic effects by blocking MTA1-associated inactivation (deacetylation) of tumor suppressors. In the current study, we utilized the same mouse
model to evaluate the MTA1/HDAC mediated anti-cancer efficacy of combination treatment of PTER and clinically approved HDAC inhibitor, SAHA. We collected 30 prostate-specific luciferase expressing \textit{Pten} knockout (\textit{Pten}\textsuperscript{f/f}; Rosa26Luc\textsuperscript{+/-}; Pd-Cre4) male mice and randomized them into four groups: Vehicle control (10\% DMSO); PTER (10 mg/kg bw) alone, SAHA (50 mg/kg bw) alone, and PTER + SAHA. Compounds were injected daily, i.p., starting at 8 weeks of age. Mice were sacrificed at week 18. Histopathological (H&E, SMA), immunohistochemical (Ki-67, cleaved caspase-3, CD31) and molecular evaluation (MTA1, p21, p27, Ac-H3, HIF-1\alpha) of prostate tissues showed better beneficial effects of combination treatment compared to each agent alone.

This study was supported in part by the Department of Defense Prostate Cancer Research Program under Award W81XWH-13-1-0370 to A. S. Levenson. We are extremely grateful to K. Li for establishing PC3M\textsuperscript{-Luc} cell line, to A. Sinha for helping with ELISA assays, to A. Atfi for providing Luc mouse, and to A. Reddy for helping with TMAs (UMMC). We also thank R. L. Summers, S. Vijayakumar (UMMC) and J. M. Pezzuto (LIU) for their continued support.

**PB16**

\textbf{Memordica Charantia Induces CYP3A4 and 2C9 Through PXR activation mechanism}

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Herbal supplements are complex mixtures of phytochemicals that demonstrates medicinal and biological effects. These supplements are often consumed concomitantly with conventional drugs and as a result a pharmacokinetic and/or pharmacodynamic interaction may occur due to alteration in the activity of major drug metabolizing CYP450 enzymes. This interaction can lead to unfavorable outcomes such as drug toxicity or loss of efficacy. \textit{Memordica charantia}, a plant native to India that has been used as an alternative therapy for diabetes mellitus. There are some reports available on clinical use of MC in diabetes and cancer patients that have shown promising results. In this study, we investigated the effects of different concentrations of an ethanolic extract of \textit{M. charantia} on transcriptional activity of pregnane X receptor (PXR) and possible subsequent induction of mRNA expression of four selected CYP isoenzymes (CYP1A2, 3A4, 2B6 and 2C9) in a hepatic cell line (HepG2). Our results showed that \textit{M. charantia} extract was able to induce PXR activity by more than three folds at 60 \textmu{g/mL}. A qPCR analysis revealed significant increase in the mRNA expression of CYP3A4 and 2C9 (1.75 and 2.25 fold, respectively) in HepG2 cells treated with 30 \textmu{g/mL} of \textit{M. charantia} extract. Increased expression was reflected in increased enzymatic activity of CYP3A4 and 2C9 (3 and 7-fold increase respectively). The increase in CYP3A4 and 2C9 enzyme activity through activation of PXR by \textit{M. charantia} could pose a concern regarding the metabolism of co-administered drugs especially antidiabetic drugs which are substrates of CYP2C9. Further studies are encouraged to understand the clinical relevance of these observations.

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**PB17**

\textbf{Induction of CYP 3A4 and 2C9 Enzymatic Activity by Phyllanthus amarus Extract}

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\textit{Phyllanthus amarus}, a tropical plant, is being used in traditional medicine practices in several countries around the world. \textit{P. amarus} extracts have been reported to exhibit a variety of pharmacological effects, including antimicrobial, antiviral, anti-inflammatory and antitumor as well as chemopreventive and hepatoprotective effects. Herbal products are usually consumed in addition to conventional drugs. Pharmacological interaction could occur due to this co-administration, which could cause inhibition or induction of drug metabolizing enzymes, particularly cytochrome P450 that may lead to treatment failure or toxicity. In this study, we investigated the effects of an ethanolic extract of \textit{P. amarus}.
**PB18**

**Therapeutic Potential of Pien Tze Huang on Collagen-induced Arthritis Mice**

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Rheumatoid arthritis (RA) is an autoimmune disease characterized by synovitis. Pien tze huang (PZH) is a compound preparation refined from a variety of precious Chinese medicinal materials, with anti-inflammatory and immunomodulatory effects. This study aimed to investigate the effect of PZH on the collagen-induced arthritis (CIA) mice. DBA/1J mice were used to establish CIA animal model and then treated with PZH low dose (PZH-L, 0.078 g/kg/day), middle dose (PZH-M, 0.234 g/kg/day), high dose (PZH-H, 0.702 g/kg/day) for 4 weeks. Arthritis score was used to evaluate erythema and swelling in hind paws of CIA mice. HE staining was used to observe pathological condition of ankle joints. The expressions of IL-1β, IL-6 and IL-17 in serum and synovium were detected by ELISA and IHC, respectively. The results showed that PZH could reduce the arthritis score, improve the inflammation of ankle joints and decrease the levels of IL-1β, IL-6 and IL-17 in serum and synovium in CIA mice comparing with model group. This study demonstrated that PZH could improve the joint inflammation of CIA mice.

**PB19**

**Endophytic Bacterial Arginase: A Potent Therapeutic Agent for Cancer Therapy**

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Arginase is an enzymic protein, therapeutically used in treatment of arginine auxotrophic cancers but low antiproliferative potency, short serum half-life and limited proteolytic tolerance are some major shortcomings. Endophytes are the microorganisms, live inside plants and todays endophytes are explored to discover new compounds having extensive biotechnological and pharmaceutical applications. In this study, endophytic bacteria were isolated from various agronomic and medicinally important plants and screened them for extracellular arginase. Out of 116 bacterial strains, one strain which isolated from stem of Capsicum annuum exhibited highest arginase activity was identified as Pseudomonas aeruginosa IH2. Further, arginase was purified by sequential operation of ammonium sulphate precipitation, dialysis and ion-exchange and gel filtration chromatography. The complete purification procedure resulted in 95.67 fold purification with 20.66 percent yield and 102.37 IU mg⁻¹ specific activity. The relative molecular mass of purified arginase was found 75 kDa on native-PAGE, 37 kDa on SDS-PAGE and pl 6.2 calculated by IEF. Optimum activity of purified enzyme was achieved at pH 8 and temperature 35 °C. Purified enzyme showed prolonged serum half-life and strong proteolytic tolerance. Antiproliferative activity of purified arginase was evaluated against various cancer
cell lines and lowest IC₅₀ (0.8 IU ml⁻¹) was found against leukemia cell line HL-60. Phase contrast and fluorescent microscopy of HL-60 cells exhibited that arginase treatment induced apoptosis in dose dependent manner. Cell cycle analysis showed purified arginase arrest cell cycle in G0/G1 phase. Mitochondrial membrane potential analysis revealed that arginase treatment induced dysfunctioning of mitochondria. ROS and antioxidant analysis showed that arginase treatment increased ROS generation and decreased antioxidants. Purified arginase did not exert cytotoxic effects on human noncancer cells.

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PB20
Antibacterial Activities of Metabolites from Vitis rotundifolia (Muscadine) Roots against Fish Pathogenic Bacteria

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Abstract: Enteric septicemia of catfish, columnaris disease and streptococcosis, caused by Edwardsiella ictaluri, Flavobacterium columnare and Streptococcus iniae, respectively, are the most common bacterial diseases of economic significance to the pond-raised channel catfish Ictalurus punctatus industry. Certain management practices are used by catfish farmers to prevent large financial losses from these diseases such as the use of commercial antibiotics. In order to discover environmentally benign alternatives, using a rapid bioassay, we evaluated a crude extract from the roots of muscadine Vitis rotundifolia against these fish pathogenic bacteria and determined that the extract was most active against F. columnare. Subsequently, several isolated compounds from the root extract were isolated. Among these isolated compounds, (+)-hopeaphenol (2) and (+)-vitisin A (3) were found to be the most active (bacteriostatic activity only) against F. columnare, with 24-h 50% inhibition concentrations of 4.0 ± 0.7 and 7.7 ± 0.6 mg/L, respectively, and minimum inhibitory concentrations of 9.1 ± 0 mg/L for each compound which were approximately 25X less active than the drug control florfenicol. Efficacy testing of 2 and 3 is necessary to further evaluate the potential for these compounds to be used as antibacterial agents for managing columnaris disease.

Keywords: antibacterial; channel catfish; columnaris disease; Flavobacterium columnare; stilbenes; muscadine; pyranoanthocyanin

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Aphrodisiac activity of Vanari gutika formulated using milk obtained from Jersey cow (Bos taurus), indigenous cow (Bos indicus) and buffalo

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Erectile dysfunction (ED) or male impotence is a sexual dysfunction characterized by the inability to develop or maintain erection of the penis. Ayurveda recommends Vanari gutika prepared from Mucuna pruriens as one of the products suggested for the management of sexual dysfunction. We were interested to validate and compare aphrodisiac potential of Vanari gutika formulated as per the principles of Ayurveda while using milk obtained from Jersey cow (Bos taurus), indigenous cow (Bos indicus) and buffalo. In order to assess the aphrodisiac activity, Male Swiss Albino mice were used as experimental subjects (n = 6 animals per group) with oral administration of aforesaid three formulations of Vanari gutika with different doses i.e. 8.56 mg/ml, 17.14 mg/ml and compared with standard sildenafil citrate (5 mg/kg) and vehicle (milk + ghee) for 7 consecutive days. Animals were observed for mating behavior including mounting and libido followed by the treatment. Hormonal analysis, sperm count and histopathological studies were carried out to confirm physiological changes. The study exhibit marked changes in sexual behavior. Vanari gutika formulated using milk from indigenous cow at higher dose (17.14 mg/ml) showed significant increase in mounting frequency, hormone levels, sperm count and histopathology revealed the ruptured seminiferous tubules. These investigations confirmed significant intensification of sexual activities in experimental animals with the administration of Vanari gutika formulated using milk from indigenous cow as compared to other formulations.

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Application of Metabolomics for the determination of Hyperglycaemia-modulating metabolites

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Persistent hyperglycaemia characterizes prediabetes, usually progressing to diabetes. Its high prevalence and complications warrants further search for phytoconstituents with potential to prevent, reduce or delay hyperglycaemia and its progression to diabetes. Asteraceae species were evaluated for their in vitro ability to improve glucose-uptake (GU) in C2C12 myotubes, inhibit α-amylase activity and retard glucose diffusion. Metabolomics was applied to the observations in the assays targeting the active specialised metabolites. Metabolic fingerprints of the plant extracts obtained using UHPLC-UV(DAD)-MS(Orbitrap) were pre-processed in MzMine™, and the generated matrices analyzed in SIMCA using unsupervised (PCA) and supervised (PLS-DA) multivariate statistical analysis. By correlating the metabolic fingerprints with the plants’ bioactivities, significant discriminant metabolites (VIP score > 1) for each of the in vitro assays were determined to include a coumestan, flavonoids and sesquiterpene lactones, some of which are being isolated for further studies. Results of our study, which shall be presented, showed that metabolomics analysis, combined with bioactivity studies, offers a fast approach to determining phytoconstituents with potential to prevent, reduce or delay hyperglycaemia.

References


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PB23

Integrative Approach for the Safety Assessment of Dietary Supplements: Oxyelite Pro – New Formulatm as a Test Case

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Despite a general insufficiency of proof regarding their efficacy, multi-ingredient dietary supplements (DS) have gained wide-spread acceptance in the United States. Unlike conventional medications, DS are not required to undergo pre-market approval testing for safety or efficacy; thus, the toxicity potential of such products is not realized until after their ingestion by the consuming public. Of particular concern are dietary supplements marketed as weight-loss aids and exercise performance enhancers, a number of which have been linked to numerous cases of severe cardio- and hepatotoxicity and subsequently removed from the market. The most recent suspect in this controversy involved OxyELITE Pro (OEP), a DS whose original formulation was removed from the market due to serious adverse health effects. A new formulation (OEP-NF) was subsequently linked to a spate of severe liver injuries and also removed by the FDA soon after its appearance on the market. To investigate the hepato- and cardiotoxic potential of OEP-NF we utilized a multi-pronged approach. This tactic incorporated the analysis of various histopathological, physiological, biochemical, and molecular end-points in a multi-strain mouse model. Using this integrative approach, we investigated the general toxicity of OEP-NF and its underlying mechanisms using B6C3F1 mice; its potential for idiosyncratic hepatotoxicity using outbred CD-1 mice; and the role obesity/metabolic syndrome plays in augmenting OEP-NF toxicity using NZO/HILtJ mice. Results from this integrative approach attest to its utility for assessing DS safety.

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Cryopreservation of Clonally Propagated Cannabis sativa L. Plants

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Plant tissue culture and cryopreservation (storage at -196°C) are efficient biotechnological tools that can be used to conserve elite plant germplasm. Large scale and clonal propagation of improved cannabis varieties are possible through tissue culture and the long-term conservation of important varieties is feasible through cryopreservation. We have successfully adapted a simple and efficient protocol for the cryopreservation of elite C. sativa using axillary buds based on vitrification methods. Since our goal is to develop a secure and stable invitro clonal repository, the protocol developed would provide opportunities for propagating true-to-type cannabis varieties and their secure conservation.
This work was supported in part by the National Institute on Drug Abuse (NIDA), National Institutes of Health (NIH), Department of Health and Human Services, USA, under the contract no. N01DA-15-7793.

PB25

Sarsaparilla Identification via Orthogonal Approach

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Increasing popularity of standard methods as a means to botanical identification is prominent. Implementing an orthogonal approach provides the manufacturer with the confidence to properly identify botanical ingredients. Following training on botanical ingredient identification provided by the American Herbal Pharmacopoeia (AHP), Nature’s Way adopted an orthogonal approach to their botanical ingredient identification that includes an agreement from vendors to provide representative whole or semi-whole samples of botanical biomass that correlate with the purchased botanical powder or extract. Having biomass that is botanically intact facilitates proper identification. This has been an invaluable approach in quality control. One example of the importance and use of the orthogonal approach to identification entails differentiating bracken fern root from sarsaparilla root. A powdered sample of sarsaparilla (Smilax aristolochiifolia) was analyzed via High Performance Thin Layer Chromatography (HPTLC). It conformed to a verified botanical reference material (BRM) of Smilax aristolochiifolia. However, the sarsaparilla root biomass sample showed characteristics consistent with bracken fern root when examined via macroscopy and microscopy. A combination of HPTLC, DNA, macroscopy and microscopy, was necessary to differentiate between bracken fern root and sarsaparilla root.

We would like to thank Michayla Conrad for wild harvesting bracken fern in the Pacific Northwest. We would also like to thank Travis Borchardt, Brandon Podhola, and Sarah Hazart for their edits and reviews. Lastly, would like to thank Roy Upton and his team at AHP for their support and training on the orthogonal approach.

PB26

Astragalus Protects the Hippocampal CA2 Region against Ischemia/Reperfusion Injury via Regulation of Heme Iron

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The expression of Breast Cancer Resistance Protein (BCRP) was examined in rats following focal cerebral ischemia reperfusion with the traditional Chinese medicine Astragalus membranaceus. The rats were randomly divided into eight groups as follows: Normal group, Sham surgery group, model group, low-dose group of NTE (4.5g/kg), medium-dose group of NTE (9g/kg), high-dose group of NTE (18g/kg), Defriprone group (125mg/kg), Astragalus group (9g/kg). After 3 days of corresponding therapy by intragastric administration once a day, the regional cerebral ischemia reperfusion model was reproduced by the MCAO suture method with reperfusing after 2 hours. On the third day, the neurological behavior of the rats was analyzed by neurobehavioral assessment. BCRP in the hippocampal CA2 region was measured by western-blot, and the mRNA level of BCRP was detected by RT-PCR. The Astragalus group exhibited a lower neurological behavior score (P<0.05) and a higher level of expression of BCRP at the mRNA and protein level compared with model group (P<0.05). The study demonstrated that BCRP can protect the neuronal population in the hippocampal CA2 region by adjusting the expression of BCRP to balance heme iron levels following cerebral ischemia reperfusion.
PB27

Phosphatidic acid is critical for liver regeneration signaling after acetaminophen overdose

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Acetaminophen (APAP) overdose is the primary cause of acute liver failure (ALF) in the United States and several other western countries. Although N-acetylcysteine is a safe and effective treatment, it loses efficacy >24 h after overdose. After that, the only life-extending treatment is a liver transplant. Clearly, new treatments are needed. Liver regeneration is a major determinant of survival of ALF patients, but little is known about the basic mechanisms of regeneration after APAP overdose. We recently discovered that the endogenous lipid phosphatidic acid (PA) is elevated in both mice and humans after APAP overdose due to suppression of the enzyme lipin 1, and that inhibition of PA synthesis blunts liver regeneration. In the current study, we hypothesized that PA affects liver regeneration through Wnt/β-catenin signaling due to an effect on GSK3β. To test that, we treated mice with 300 mg/kg APAP at 0 h, followed by 20 mg/kg of the PA synthesis inhibitor FSG67 at 3, 24, and 48 h. Some mice also received 500 nmol of the GSK3 inhibitor L803-mts at the same time as FSG67. Blood and liver were collected at 6, 24, and 52 h. Consistent with our earlier results, FSG67 decreased markers of liver regeneration at 24 and 52 h. Phosphorylation of GSK3β was also dramatically decreased by 89±4% at 24 h, and active β-catenin was modestly reduced by 29±10%. Importantly, L803-mts completely restored normal liver regeneration. Finally, we confirmed the importance of PA in liver regeneration using mice with hepatocyte-specific lipin 1 overexpression, and by treatment with exogenous PA. Conclusions: PA is critical for liver regeneration after APAP overdose through its effects on GSK3β and Wnt/β-catenin signaling. Furthermore, PA, which is available over the counter as a dietary supplement, may promote liver regeneration in APAP-induced ALF.

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With the current trend of legalizing marijuana, as well as Epidiolex (>98% cannabidiol (CBD)) increasingly being prescribed to children, it is imperative to better understand the effects of developmental exposures to cannabinoids. Previously our lab found embryonic exposure to CBD to be more toxic compared to Δ⁹-tetrahydrocannabinol (THC) in zebrafish. The current mechanism of action for CBD and THC are not well understood and therefore, our goal was to investigate the roles of cannabinoid receptor 1 and 2 (cnr1 and cnr2) in CBD and THC exposures. In this study, cnr1 and cnr2 knockout zebrafish were nominally exposed to CBD (0.075, 0.15, 0.3, 0.6, 1.2 mg/L; 0.25, 0.5, 1, 2, 4 μM, respectively), THC (0.3, 0.6, 1.25, 2.5, 5.0 mg/L; 1, 2, 4, 8, 16 μM) or control (0.1% DMSO) from 6 to 96 hours post-fertilization (hpf). At 96 hpf, larvae were scored for developmental deformities and assessed using a ViewPoint Zebabox for their locomotive response to a light:dark:light cycle lasting a total of 30 minutes. Mortality, deformities, and behavior were compared between cnr2 and reference fish and differences were identified. Gene expression will be compared among the cnr1, cnr2, and reference fish following CBD and THC exposure to elucidate their roles in the toxicities observed. This study offers unique insight into the key roles of cnr1 and cnr2 receptors and their relation to the observed adverse effects following embryonic exposure to CBD or THC.

Supported by the National Institute on Drug Abuse R21DA044473-01

**Materials and Methods:** Mice were infected via the tail vein with live *C. neoformans*. Twenty-four hours post-infection, the mice were treated with different doses of IPD (0.0125 to 0.1 mg/kg) once a day (i.p.) or PPD (0.03125 to 1.0 mg/kg) once a day or twice a day (bid) orally, or with amphotericin B (Amp B) intraperitoneally (IP), or with fluconazole (Flu) orally for 5 days. The brains of all of the animals were aseptically removed and the numbers of live *C. neoformans* were recovered. In vitro toxicity of indolizidine alkaloids was determined in HepG2 cells.

**Results:** PPD showed to be potent in vivo activity against *C. neoformans* at a dose of 0.0625 mg/kg by eliminating ~76% of the organisms compared to ~83% with Amp B (1.5 mg/kg). In addition, PPD was found to be equally efficacious, but less toxic, at either 0.125 or 0.0625 mg/kg compared to Amp B (1.5 mg/kg) when it was administered bid (twice a day) by an i.p. route. When tested by an oral route, PPD (10 mg/kg) showed potent activity in this murine model of cryptococcosis with ~82% of organisms eliminated from the brain tissue, whereas Flu (15 mg/kg) reduced ~90% of the infection. Our results also showed that IPD exhibits potent in-vivo activity against *C. neoformans* by eliminating 48.5%, 71.6% and 76.3% organisms at doses 0.05, 0.025 and 0.0125 mg/kg in mice, respectively. *In vitro* results suggest that quaternary indolizidines were less toxic as compared to those of tertiary bases.

**Conclusion:** The results indicate that i.p. administration of PPD and IPD against *C. neoformans* infection showed better efficacy at lower doses. In addition, no signs of discomfort were observed in the mice treated with PPD or IPD, which were administered either once or twice a day. Higher doses of PPD were equally effective when they were given orally.
This work was supported by the Overhead Funds of NCNPR and in part by the USDA-ARS Specific Cooperative Agreement No. 58-6408-1-603.

PB30
Evaluation of Caffeine used in Combination with Selected Herbal Substances

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The prevalence and consumption of caffeine-containing dietary supplements are becoming increasingly common. These supplements often contain additional dietary ingredients, including botanicals that may modulate cytochrome P450 (CYP) activity. Caffeine is predominantly metabolized by the cytochrome P450 enzyme CYP1A2 to paraxanthine; therefore, botanicals that inhibit CYP1A2 may indirectly elevate systemic levels of caffeine. To identify specific ingredients of concern, a literature review was performed to evaluate the CYP1A2 inhibitory potential for five botanicals that are frequently combined with caffeine in dietary supplements: cayenne/capsaicin, Echinacea purpurea, ginger, Ginkgo biloba, and Panax ginseng. In vivo studies that reported indirect measurements of CYP1A2 inhibition (including changes in paraxanthine/caffeine serum ratio and changes in caffeine clearance) were preferred, although in vitro studies that identified IC₅₀ values were also considered. Based on the results of the literature search, capsaicin, Ginkgo biloba, and Panax ginseng were associated with minimal inhibition of CYP1A2 when co-administered with caffeine. Conversely, Echinacea purpurea was associated with moderate CYP1A2 inhibitory potential in vivo based on reduced oral caffeine clearance rates. Although in vitro data for ginger indicated limited CYP1A2 inhibitory potential, in vivo data are required to determine whether inhibitory potential exists at human-relevant dose levels. In conclusion, Echinacea purpurea may potentiate the effects of caffeine when consumed with a caffeine-containing dietary supplement, whereas further data are needed to evaluate the CYP1A2 inhibitory potential of ginger. Caution may be advised when ginger or Echinacea purpurea are used in combination with caffeine in dietary supplements.

PB31
Diligustilide Isolated from Ligusticum Porteri Releases H₂S and Stabilizes S-Nitrosothiols in Ethanol-Induced Lesions on Rat Gastric Mucosa

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(Z, Z’)-Diligustilide (DLG) is a dimeric phthalide isolated from Ligusticum porteri (Osha), an endemic Apiaceae species distributed in México and the southern United States. Several activities have been reported for compounds isolated from L. porteri including cardioprotective, anti-inflammatory, neuroprotective and anti-ulcer effects. In previous work, we studied the gastroprotective effects of DLG¹. However, its gastroprotective action mechanism has not been completely elucidated. We studied the contributions of hydrogen sulfide and S-nitrosothiols to the action of DLG. Animals were pretreated with freshly-formed in vitro nitrosothiol using Na₂S and sodium nitroprusside to elucidate participation in the action of DLG. We also evaluated the production of H₂S in vivo and in real time on the stomach via a specific electrode introduced into the stomachs of anesthetized animals pretreated with DLG. Treatment with 10 mg/kg DLG increases gastric H₂S production in vivo from 7.8 ± 0.81 ppm to 13.1 ± 3.01 ppm and prevents the decrease caused by absolute ethanol. In addition, it maintains endogenous concentrations of GSH and NO. Exogenous S-nitrosothiols protect the gastric mucosa from damage, suggesting that the action of DLG might be associated with S-nitrosothiol and H₂S formation³.

¹Velázquez-Moyado et al., Journal of Ethnopharmacology 174; 403-409 (2015)
³Velázquez-Moyado et al., Inflammopharmacology 26; 611-619 (2017)
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PB32
United States Pharmacopeia Safety Review of Chinese Skullcap Root

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Chinese Skullcap (CS) (Scutellaria baicalensis) root is an ingredient in some dietary supplements (DS) in the USA market. This review was conducted according to the Guideline for the Admission of DS Ingredients to the USP–NF Monograph Development Process for purposes of determining admission of ingredients for monograph development.

No clinical safety studies of CS root were found. One clinical study that administered extract intravenously for treatment of foot and mouth disease reported significantly higher incidences of elevated alanine aminotransferase and creatine kinase-MB levels, brainstem encephalitis, and neurogenic pulmonary edema in the treatment group, however the adverse events (AEs) were considered related to disease progression and not treatment. Two combination products containing CS root and Areca catechu were reportedly associated with liver damage, also reported in case reports and adverse event reports (AERs). In December 2017, FDA issued a consumer alert warning against a popular product containing CS root extract and requested the manufacturer to recall non-expired lots after determining the product was the likely cause of liver injury and hypersensitivity pneumonitis in 30 evaluable AERs out of 200 filed.

Animal toxicology studies reported a maximum tolerated dose for aqueous CS root extract as 2,000 mg/kg in mice. In rabbits and dogs the administration of 10.0 g/kg and 0.2–5.0 g/kg of CS root extract, respectively, caused no serious AEs. In rats CS root extract was associated with the formation of an extra rib and visceral abnormalities in premature pups, but this effect was later shown to be transient and absent in mature pups.

CS root extract is reported to inhibit platelet aggregation and thus concurrent use with antiplatelet and anticoagulant drugs could increase bleeding risk. Traditional texts and pharmacopeial monographs recommend intake amounts ranging from 3 to 9 g of dried roots used as an infusion or decoction. Labels in the NIH dietary supplements labels database recommend intakes ranging from 120 mg to 1 g of CS extract daily.

Based on this review, the USP Expert Committee admitted Skullcap for monograph development and recommended a cautionary statement to discontinue use and consult a healthcare practitioner if a consumer develops symptoms of liver trouble, such as abdominal pain, dark urine, or jaundice (yellowing of the eyes or skin).

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PB33
Investigation into the Mechanism of Costus speciosus in Inducing Apoptosis in Breast Cancer Cells

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Medicinal plants play important roles in the folkloric medicine and the modern one, as well. Costus speciosus rhizomes extract is reported to demonstrate in vitro inhibition of cell proliferation and induction of apoptosis in different cancer cell lines. The main mechanism by which it exerts this action is not yet known. One of the major pathways involved in breast cancer is mitogen activated protein kinase (MAPK) that consists of ERK, P36, and c-Jun N-terminal Kinase (JNK), which is involved in cell differentiation and apoptosis. This study aims at investigating the possible involvement
of MAPKs and other possible signaling cascades in the apoptosis induced by *C. speciosus* extract in MDA-MB-231 and MCF-7 breast cancer cells.

*C. speciosus* rhizomes were extracted in 80% methanol and the dried extract was fractionated, using vacuum liquid chromatography, into eleven fractions with different polarities. *C. speciosus* extract activity was evaluated on MDA-MB-231 and MCF7 breast cancer cells, using MTT assay, Western blotting and ELISA.

The extract significantly induced cytotoxicity and reduced the viability of MDA-MB-231 (22% ± 9.2) and MCF-7 (31.7% ± 6.4) breast cancer cells in a time- and dose-dependent manner when compared to controls. Additionally, the extract induced apoptosis and promoted proteolytic cleavage of PARP (31.3% ± 8 and 3.55 ± 1.54) and caspase-3 (17.6% ± 4 and 21.7% ± 4), which correlated with an increase in the cytoplasmic Bcl-xl (0.2% ± 0.02 and 0.13% ± 0.11) and Bax (0.7% ± 0.13 and 0.38% ± 0.14) protein degradation in both cell lines, respectively. More importantly, the extract specifically activated JNK in a time- and dose-dependent manner. These effects were significantly inhibited by the JNK selective inhibitor, SP60025. Moreover, the extract synergized the cytotoxic effect of doxorubicin.

This shows that *C. speciosus* can be a potential lead in cancer treatment. It showed synergism with doxorubicin that can culminate into dose reduction and, consequently, fewer side effects.

This project was supported by College of Graduate Studies, and Research Sector, Kuwait University (Grant Number YP04/16).

PB34

**Exposure of zebrafish (Danio rerio) during embryonic development to ∆^9-tetrahydrocannabinol or cannabidiol causes significant changes in gene expression.**

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Countries are relaxing laws regarding, and like Canada legalizing, the use of cannabinoids such as ∆^9-tetrahydrocannabinol (THC) and cannabidiol (CBD). Last year, the FDA approved Epidiolex (> 98% CBD) for treatment of epilepsy in children as young as 2 years old. As a consequence of their increased use, understanding potential adverse outcomes following exposure to cannabinoids during critical developmental periods is important. The objective of this experiment was to assess transcriptomic effects following a developmental exposure to THC or CBD. Zebrafish were waterborne exposed from 6 hours post fertilization (hpf) through the larval stage (96 hpf) to nominally 1.25 mg/L (4 µM) THC or 0.15 mg/L (0.5 µM) CBD. THC and CBD concentrations were selected to be below the lowest observed adverse effect concentrations for developmental toxicity. RNA sequencing (RNA-Seq) was conducted on zebrafish larvae and revealed differential expression of 1096 and 904 genes for CBD and THC, respectively. Among them, 351 genes were common to both treatment groups. Analysis of THC and CBD differentially expressed genes by Ingenuity Pathway Analysis (IPA) revealed that molecular pathways including hepatic effects, lipid/fatty acid metabolism, cell survival/viability and immune response were significantly increased, while pathways relating to inflammation were significantly decreased. For the 351 overlapping genes, the top two significantly modulated canonical pathways were FXR/RXR and PXR/RXR. The genes for *cyp3c1* and *cyp7a1a* were both be significantly upregulated following exposure to THC or CBD. IPA predicted significant toxicological effects related to the liver, kidneys and heart. Collectively, these results indicate developmental exposure to THC or CBD alters metabolism, hepatic, and immune function gene expression that may be linked to latent adverse outcomes. (Supported by the National Institute on Drug Abuse R21DA044473)
PB35
Immunomodulating Effects of Oplopanax Horridus in RAW264.7 Macrophage Cell Line

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Devil's Club, *Oplopanax horridus* (Sm.) Miq. (Araliaceae) (syn. *Fatsia horrida* (Sm) Benth. & Hook.; *Echinopanax horridus* (Sm.) Decne. & Planch; *Panax horridum* (Sm.)), is a deciduous shrub of the Araliaceae or Ginseng family native to the Northwestern United States. Native Americans of British Colombia and the Northwestern United States regard Devil's Club as an important medicinal plant. Traditional medicinal uses of *Oplopanax horridus* (OH) include its use as an antibacterial, antifungal, antimycobacterial, antiviral and as an immunomodulator for rheumatism and diabetes. Previously it has been shown that extracts of OH have antibacterial, antifungal, antiviral and anti-mycobacterial properties. The mechanisms of action of OH’s effects have not been established and there are few studies that have presented chromatographic profiles of OH extracts, although we are conducting a thorough phytochemical study. Previously we demonstrated a decrease in tumor necrosis factor alpha and nitric oxide (NO) production in a LPS-stimulated RAW264.7 macrophage cell line, which may partially explain the traditional use of OH as an immunomodulator for both rheumatism and diabetes. To further characterize the immunomodulatory effects of OH, LPS-stimulated RAW264.7 macrophage cells were treated with OH extracts and a cytokine array was performed that demonstrated a significant decrease in fifteen cytokine/chemokines, including tumor necrosis factor alpha, Interleukin 1 (IL-1), IL-6, IL-10. Prostaglandin E2 levels were also evaluated after OH treatment and demonstrated a significant decrease. This study demonstrates that OH extract has significant immunomodulatory activity in the RAW264.7 macrophage cell line. Further studies include antidiabetic and antiobesigeneic assays.

PB36
Comparative Morpho-anatomy and Qualitative Analysis of Tinospora Species

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A comparative morpho-anatomy of the leaves and stems of *Tinospora* species, known as heavenly elixir, was undertaken to elaborate on their distinguishing pharmacognostic features. Overlapping geographical occurrence, history of traditional use, confusion in species identification and morphological resemblances among various species are some considerations that necessitate the importance of qualitative analysis for efficient quality control. Detailed anatomical studies of the authentic materials of three species of *Tinospora*, namely *T. cordifolia*, *T. crispa* and *T. sinensis* were conducted by light microscopy. In addition, chromatographic profiles of the plant materials and commercial products derived from the *Tinospora* plants were obtained and compared by high-performance liquid chromatography with the aim to determine the quality of the products.

This study was supported by Science Based Authentication of Dietary Supplements and Botanical Dietary Supplement Research funded by the Food and Drug Administration grant #2U01FD004246-06. Fulbright Graduate Scholarship Program and United States Educational Foundation of Pakistan (USEFP) are acknowledged for financial support.
PB37
Proteomics and DNA Barcoding Analysis of Whey Protein Dietary Supplements

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A number of dietary supplements containing Whey proteins are being used especially for bodybuilding and muscle development. Whey is a by-product of the cheese-making process – the liquid left over once the milk has been curdled and strained. In its powder form, it’s one of the most popular sports nutrition products in the world because of its availability, cost and effectiveness. Commercial whey protein powder comes in four different forms: concentrate, isolate, hydrolysate and native. A variety of whey protein supplements are available in the market and most of them claim to contain the whey protein in any of its four forms.

At the molecular level whey protein contains three major globular proteins viz. α-lactalbumin, β-lactoglobulin and serum albumin. Twenty eight products were analyzed for the presence of three major peptides using different proteomics techniques. The total proteins were quantified using Bradfords assay. SDS-PAGE was used to separate the peptides on the gel and the peptides were identified using Mass spectrometry, LC-MS/MS. Of the 28 products tested, 27 products were found to contain proteins from bovine and one from plants (soy protein) that matches with the label claim.

Furthermore animal-specific and plant-specific DNA barcode primers were used to authenticate the source of proteins in the commercial samples. A primer set for a mini-barcode has been developed to detect animal DNA. It performed better than a previously published primer pair based on the COX1 genomic region. Animal DNA was detected in all of the twenty seven tested Whey products. PCR performed with primers detecting plant DNA did only result in products when a soy based protein product and known plant DNA (positive control) was used as template. Molecular characterization of 27 commercially available whey proteins was performed to provide methods of quality control of Whey protein supplements sold in the market regardless of the manufacturer. Periodic evaluation of products and labeling verification is recommended to ensure product quality for consumers.

PB38
Extracts of Nymphaea odorata and isolated methyl-gallate inhibit the growth of colon cancer cell lines HCT-116, by acting as HDAC inhibitors

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Nymphaea odorata Aiton (Nymphaeaceae; American white water-lily) is an aquatic perennial plant root used in First Nation, Ayurvedic and Chinese traditional medicine for the treatment of gastrointestinal, genital, and bronchial diseases. The leaves and roots have also been used externally, as infusions to treat lesions and inflammation associated with mucous membranes. In this work we have investigated N. odoratumethanol (NOM) extract and its active constituent methyl-gallate (MG) on the growth of several colon cancer cell lines including HCT-116, SW480, SW620 and CaCO2 in concentrations up to 20.0 mg/ml. Control cells were treated with vehicle solvent (DMSO 0.02%). Cytotoxicity and cell apoptosis were determined using the CellTiter-Glo® 2.0, Caspase 3/7 and 8 assay kits. The expression of Bax, Bcl2, HDACs 1-3, SIRT1 and 3 were determined by qPCR. NOM inhibited the proliferation of HCT-116, but not that of SW480 and SW620. In HCT-116 cells, the IC₅₀ concentration was 15.4 µg/mL. Bioassay guided fractionation of NOM led 26 fractions, of which seven were very active against HCT-116. These fractions were combined and methyl-gallate (MG) was isolated and identified as the active constituent. Gene expression data showed that both NOM and MG inhibited HDAC mRNA expression at non-cytotoxic concentrations suggesting that NOM and MG inhibit colon cancer growth by acting as HDAC inhibitors.
PB39
Toxicity of fourteen commercial essential oils against the common bed bug Cimex lectularius L.

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Cimex lectularius L., popularly known bed bug, is a hematophagous pest that feeds upon human blood. It is a difficult pest to control as it typically feeds at night, whereas, during the day, hides in the folds of furniture, inside cracks and crevices, wooden or non-wood structures. Essential oils ‘EO’ are known to possess insecticidal activity against different insect pests. The toxicity of essential oils (EO) viz, Ocimum basilicum, Callitris intratropica, Bosenbergia pandurate, Cinnamomum camphora, Fortunella japonica, Tanacetum vulgare, Melaleuca caujuputi, Eucalyptus-globulus, Kunzea ambigua. Cinnamosma fragrans, Citrus junos, Cananga odorata, Boswellia serrata was studied against two strains of bed bug (Bayonne ‘Insecticide resistant’ and Ft. Dix strains ‘Susceptible’) in three different delivery methods topical, residual and fumigation. In preliminary screening only E. globulus induced 30% (Bayonne,) and 50% (Ft. Dix.) mortality at 100 µg of EO/bug in 24 hr after treatment that increased to 46-66% on day 3, respectively. The O. basilicum and C. camphora were more toxic in their vapor form. O. basilicum induced 43% (Bayonne) and 76% (Ft. Dix) mortality while C. camphora killed 56% (Bayonne) and 93% (Ft. Dix) bed bug at 250µg/125mL of air in 24 hrs after the treatment fumigation bioassay. None of the oils produced significant mortality when exposed to their deposits on filter paper at 100µg/cm² in residual bioassay. The chemical composition of active EOs will be studied further.

This research is supported by USDA-Discovery & Development of Natural Products based insect management for medical, veterinary & Urban (58-6066-6-043). The bed bug strains (Bayonne and Ft. Dix) were provided by Dr. Changlu Wang, Department of Entomology, Rutgers University, New Brunswick, NJ.

PB40
Toxicity of traditional herb representatives and natural product-derived compounds against the common bed bug Cimex lectularius L.

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The common bed bug C. lectularius L. is known to have worldwide occurrence. Bed bug infestation has exponentially increased since the early 2000s. The National Pest Management Association (NPMA) of the USA reported a 500% rise in bed bug incidents. A large number of US pest control companies have reported a surge in the bed bug infestations. The recent resurgence of bed bug is largely attributed to the inappropriate utility of insecticides such as carbamates, pyrethroids, and organophosphates. Since there is no validated target in bed bug for insecticide action, in vivo screening has been a prevailing approach to identify lethal agents or repellents against bed bug. In this presentation, representatives of extracts of traditional herbs, pure phytochemicals of different classes, including phenolics, terpenoids, and alkaloids used in herbal medicine, derivatives of fatty acids, derivatives of benzoic acid, coumarin (a natural flavouring agent) and a number of its derivatives, were evaluated for their potential toxicity in topical and residual bioassays against the two strains of bed bug (Bayonne ‘Insecticide resistant’ and Ft. Dix strains ‘Susceptible’). Out of all, coumarin and clove oil were the more potent. Coumarin killed 40-50% of the bed bug (Bayonne and Ft. Dix strains) at 100 µg/bug in 24 hr that reached to 90% on days 3 after treatment. Mortality recorded at lower doses 25 and 50 µg/bug was (30-46%) and (50-70%) on day 3 after the treatment, respectively. None of the coumarin derivatives showed toxicity against the bed bug. Similarly, clove oil produced 40-60% mortality (Bayonne and Ft. Dix strain) in 24 hr that reached to 70% on day 3 after the treatment. The other tested substances were inactive.
This research is supported by USDA-Discovery & Development of Natural Products based insect management for medical, veterinary & Urban (58-6066-6-043). The bed bug strains (Bayonne and Ft. Dix) were provided by Dr. Changlu Wang, Department of Entomology, Rutgers University, New Brunswick, NJ

PB41
Access to the complementary medicine. Considerations about Cuba.

Remirez D, Cuba Regulatory Agency of Drugs and Medical Devices.

**Background:** Traditional and Complementary medicine (TCM) is an important and often underestimated part of health care found in almost every country in the world and the demand for its services is increasing. Complementary medicine (CM) of proven quality, safety, and efficacy, contributes to the goal of ensuring that all people have access to care. Access to medicine refers to the reasonable ability for people to get needed medicines required to achieve health. The objectives of this presentation are: to show the importance of the TCM access for people around the world, how to get the access, to explain WHO Strategy related with this subject, besides that, to illustrate the Cuba experiences about the integration of TCM in the health system in order to get the complete access of TCM.

**Focus of discussion.** In order to explain the objectives, the factors involved for getting access will be shown, such as: policy and regulations, the knowledge about appropriate use, cost, among others. The importance of the National Policy, the convergence process among countries, will be proved. Moreover will be illustrated as example, Cuba health system, which is for all the people, there are different levels of health care (primary, secondary and tertiary level), the regulations are based on risk approach, TCM is included in the list of essential medicines, etc. There is an Integrated System for TCM Dispensation thanks to the National Medicine Program.

**Implications.** In general, health systems around the world should take care about the use of TCM based on the quality, safety and efficacy.

PB42
Pharmacogenetics and herbal medicines

RemirezD, PhD Cuba Regulatory Agency of Drugs and Medical Devices.

**Background:** The science of pharmacogenomics has advanced significantly in the last five years, but it is still in infancy and is mostly used on research basis. The Pharmacogenomics helps identify interindividual variabilities in drug response (both toxicity and effectiveness). Due to the fast growing in the consumption of phytomedicines, it is necessary the investigation of mechanism of actions of these products with more rigor. The aim of this work is to present an updated report about this novel topic pharmacogenetic and its relation with herbal medicines’.

**Focus of Discussion.** The herbal medicines like synthetic drugs have been showed their bioactivation through cytochrome P-450, the main enzyme involved in the metabolism of xenobiotics. The main enzymes involved in the metabolism of phytomedicines, the advantages and disadvantages of bioactivation to metabolites less or more toxics are described as well as the pharmacodynamic interactions involving herbs. Moreover, the herbs which affect the P-glycoprotein activity in vitro will be showed. These studies strengthen and optimize the safety of herbal medicines.

**Implication** The hope for the future is that through personalized medicine, doctors and patients will be able to make better-informed choices about treatment. This treatment will avoid the adverse drug reaction to the medication and will improve the diagnosis diseases as well as the prevention and treatment of diseases.

PB43
Regulatory status of herbal medicines in Latin America. New focus for marketing authorization of herbal medicines as medical devices.

Remirez D, Cuba Regulatory Agency of Drugs and Medical Devices.

**Background:** In the last decade, there has been a global upsurge in the use of traditional medicine and complementary and alternative medicine in both developed and developing countries. This is one of the main reasons for reinforcing the
Focus of discussion: It is shown the differences between Cuba and Latin America regulations, taking into account the classification of the products, modalities approved, clinical trials quality specifications among others. The WHO strategy for the development of herbal medicinal product is also showed concerning to the strength of the quality, safety and efficacy policy through regulation of products, practices and professionals, the importance of clinical trials in order to guarantee the safety, quality and efficacy of Natural Health Product and the main mistakes in Clinical Trials of natural products are explained. Another important result is related with the register of herbal medicines (syrup, tablet etc) as medical devices, these products are characterized by metabolomics techniques and they have non pharmacological action for therapeutic indication is mainly through barrier and mechanical action.

Implications: Herbal medicines take special considerations in this moment, for its properties. Drug Regulatory Authorities should ensure the quality, safety and efficacy of traditional medicines.

PB44

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Millions of people consume botanical dietary supplements with continued growth in the industry expected. However, current regulatory guidance in the United States emphasizes a botanical safety framework that relies on history of safe use and traditional animal testing paradigms with virtually no utilization of in vitro and in silico approaches. There are thousands of botanical products that combine unique botanical ingredients. This makes it challenging to establish history of safe use for unique dietary supplements. Botanicals themselves are naturally complex and may be subject to contamination and adulteration. The 1994 Dietary Supplement Health and Education Act provides the statutory basis for regulating the safety of botanical dietary supplements and mandates that manufacturers ensure the safety of their products with the FDA bearing enforcement responsibility. Data supporting safe history of human use often provides the best indication of an ingredient’s safety but is not always available or sufficient. Animal studies are the current standard for assessing toxicity, but the number, complexity and variable nature of botanicals precludes their use as a pragmatic solution. Established in vitro and in silico approaches offer promise for bridging the gap, however there are numerous uncertainties in the appropriate application of these methodologies. The convergence of these factors calls for the development of a pragmatic strategy for evaluating the safety of botanicals. The Botanical Safety Consortium is a collaboration between scientists in industry, government and academia formed with the goal of providing a sound scientific basis for integrating existing data with the latest toxicology tools to evaluate botanical safety. Chemical characterization of complex botanical products and identification of fit-for-purpose assays for evaluating genotoxicity, hepatotoxicity, developmental and reproductive toxicity, cardiotoxicity, and repeat-dose, systemic toxicity are key areas to be explored by the Consortium. A botanical library containing ingredients with known in vivo toxicity, from animal studies or reports of adverse events, will be created and evaluated as part of the recommended battery of assays. Results from the Consortium will be shared through a publicly available database and recommendations published in the peer-reviewed literature. The Botanical Safety Consortium aims to enhance the botanical safety toolkit and bring clarity to botanical safety assessments for manufacturers and regulators of botanical ingredients. This session will outline the regulatory and safety challenges, fundamental developments and gaps from the botanical safety sciences, and key next steps of the consortium.

Presentations:

1. What is the Botanical Safety Consortium? Cynthia Rider (NTP/NIEHS)
2. Exploring Hepatotoxicity Using the Latest Toxicology Tools - Amy Roe (P&G)

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4. Next Steps – Sibyl Swift (US FDA)

Panel Discussion with BSC Steering Committee: Joe Dever, Stefan Gafner, Duffy MacKay, Dan Marsman, Cynthia Rider, and Sibyl Swift

PB45
Effects of ploidy level and genotype on chemotype in the Achillea millefolium complex

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The Achillea millefolium complex is a species aggregate composed of plants occurring across the globe and ranging in ploidy from diploids (2n=18) to octoploids. Common in the Northern hemisphere, this genus has a long history of medicinal use as a hemostat, vulnerary, febrifuge, anti-inflammatory, digestive aid, and anxiolytic. Current understanding dictates that the presence of the sesquiterpene chamazulene in the essential oil is in part dependent on the ploidy level, most frequently occurring in high amounts in tetraploids. However, crude hydro- or hydro-ethanolic extracts of the plant are the most common forms for medicinal use, and little is known about the effects of ploidy level or genotype on the presence and abundance of other compounds present in these preparations. We are cultivating 94 populations of A. spp. from the USDA and Kew MSB in a common garden, determining ploidy level through flow cytometry, analyzing SSR markers, and performing untargeted metabolomics analysis on leaf and flower tissue by reversed-phase HPLC-HRMS in positive and negative mode. The data are processed with XCMS and analyzed in Galaxy. SSR and metabolomics analyses are still underway. Flow cytometry data will be presented, as well as preliminary data on the correlation between leaf and floral chemotype in A. millefolium.

PB46
Cucurbitane type-triterpenes from bitter melon inhibit α-amylase and α-glucosidase and display in vitro anti-inflammatory activities

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Bitter melon (Momordica charantia L.) is a well-known plant species cultivated as a medicinal vegetable for the management of diabetes in tropical and subtropical areas such as East Africa, Asia, South America, and the Caribbean. To date, more than 230 bitter melon cucurbitane triterpenoids have been reported from different plant organs including leaves, stems, roots, fruits, and vines. Although there are numerous studies available on the anti-diabetic activities of whole bitter melon fruit or extracts, activities on pure compounds are limited. In the present study, two cucurbitane-type triterpene aglycones such as 3β,7β,25-trihydroxycucurbit-5,23(E)-di-en-19-al, charantal, and one sterol glucoside (25ξ-isopropenylchole-5, 6-ene-3-O-D-glucopyranoside) were isolated from bitter melon fruit for the first time. The structures of these compounds were elucidated by HRESIMS, 1D and 2D NMR. All purified compounds exhibited significant inhibition of α-amylase and α-glucosidase in vitro (56–79%) comparable to acarbose. They also exhibited significant anti-inflammatory activity, downregulating the expression of NF-kB, INOS, IL-6, IL-1β, TNF-α, and Cox-2 in lipopolysaccharide-activated macrophage RAW264.7 cells. Further molecular docking studies were carried out to understand the interactions between purified compounds and enzymes. Overall, the present results demonstrated that the purified compounds have the potential to use as antidiabetic and anti-inflammatory agents. Therefore, these compounds can be explored further for the development of a plant-based formulation for diabetic and inflammatory conditions.
PB47
Berberine protects C17.2 neural stem cells from oxidative damage and induces their neuronal differentiation

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Neurodegeneration is the progressive loss of structure or function of neurons, including death of neurons. Neurodegenerative diseases have been regarded as world-wide burden due to the dramatic increase of life span. The oxidative stress has been suggested as one of the common etiology in various neurodegenerative diseases. It is therefore necessary to find the effective medicines that can protect against oxidative damage and induce neurogenesis.

Berberine has been shown beneficial effects in various neurodegenerative and neuropsychiatric disorders. We hypothesized berberine could protect C17.2 neural stem cells from 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH)-induced oxidative damage and then promote neuronal differentiation.

Results showed that berberine was able to protect C17.2 neural stem cells from AAPH-induced oxidative damage. It lowered the cellular reactive oxygen species (ROS) level in C17.2 cells via NRF1/2-NQO1-HO1 pathway. It also down-regulated the apoptotic factor caspase 3 and Bax and the anti-apoptotic factor Bcl2. After AAPH damage, berberine-protected C17.2 cells were recovered for two days. Berberine could increase C17.2 cell viability via up-regulating ERK and pERK expression in this recovery period. Then cells were kept cultured for another week in differentiation medium with/without berberine. Berberine promoted C17.2 cell to differentiate into neurons and the differentiation mechanism involved the activation of WNT/b-catenin pathway as well as the upregulation of pro-neural factors like ASCL1, Neurog1, NeuroD2 and DCX.

Our work provides scientific evidence to support the use of berberine as anti-neurodegeneration medicine.

J.W. Shou was supported by the Hong Kong PhD Fellowship Scheme (PF15-16899).

PB48
4-O-Methylhonokiol Induces Developmental Abnormalities in Medaka Embryos

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The aim of this project was to characterize the teratogenic effect of 4-O-Methylhonokiol (MH), a major bioactive constituent of Magnolia grandiflora seed or Magnolia officinalis bark on Japanese medaka (Oryzias latipes) embryogenesis. Since the potential adverse effects of MH remain unexplored, the effects were assessed on medaka embryos. The embryos were treated with different concentrations of MH for 0-6 days post-fertilization (dpf) in embryo rearing medium (ERM) in a 48 well plate (1 embryo/ml/well) with 16L: 8D light cycle at 25±1°C. MH caused a reduction in heart beat, suppression in blood flow, and decrease in survivability in medaka embryos in a concentration-dependent manner. We analyzed the effect of MH on the major components of the blood coagulation pathway, because these factors can be activated by the lesions on the lining of the blood vessels. The blood vessel occlusion might be caused due to activation of FXI (plasma thromboplastin antecedent), FVII (Stable factor), and FX (Stuart-Prower factor) of the blood coagulation pathway. MH was also able to induce developmental abnormality, such as curved tail phenotype in a concentration-dependent way when the embryos were hatched into larvae. Our study suggested that MH influenced the normal development of medaka embryos.

This work was funded by the University of Mississippi OHA to ZSM and partially supported by the USDA ARS Specific Cooperative Agreement No. 58-6408-2-00.
Gastroprotective Effect and Chemical Constituents from the Ethyl acetate Fraction of the Leaf Extract of Flabellaria paniculata Cav. (Malpighiaceae)

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Leaves from Flabellaria paniculata Cav (Malpighiaceae) are used in traditional medicine for wound dressing, and to treat ulcers and inflammation in Nigeria. The present study evaluates the gastroprotective activity of the EtOAc fraction of the leaves and reports on the isolation and identification of compounds from the fraction. The crude extracts (methanol, ethanol and aqueous) and methanol derived solvent fractions (100 mg/kg, p.o.) were screened using an ethanol-induced ulcer model. The activity of the most active methanol derived EtOAc fraction (25, 50 and 100 mg/kg, p.o.) was further evaluated in indomethacin and pylorus ligation-induced ulcer models. The EtOAc fraction was chromatographed and chemical structures of the isolated compounds were elucidated by NMR spectroscopy. The MeOH extract of F. paniculata and the EtOAc fraction from this extract displayed significant gastroprotective effects. Column chromatography of the EtOAc fraction resulted in the isolation of two triterpenoids (friedelin (1) and friedelinol (2)), two steroids (sitosterol (3) and β-sitosterol-β-D-glucoside (4)) and a flavonoid glycoside (Kaempferol-3-O-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside (5)). The EtOAc fraction from the MeOH leaf extract of F. paniculata displayed gastroprotective effect and the identified compounds could be responsible in part for the observed effect.

Cannabidiol and Δ⁹-tetrahydrocannabinol reduce seizures in scn1a-mutant zebrafish

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Dravet syndrome is a pharmaco-resistant form of epilepsy wherein ~80% of the patients carry a mutation in the voltage-gated sodium channel Nav1.1 (scn1a). Cannabidiol (CBD) has become the focus of much research regarding treatment of childhood epilepsies such as Dravet syndrome. In scn1a-morphant zebrafish larvae, electroencephalograms indicate seizure-like activity, epileptiform brain activity, and hyperactivity; all neurological phenotypes consistent with human epilepsy. In this study, we utilized scn1a-mutant zebrafish to determine the effectiveness of cannabidiol and Δ⁹-tetrahydrocannabinol (THC) in reducing seizures as well as to determine which genes are differentially expressed compared to non-treated scn1a-mutant fish. At 120 hours post fertilization (hpf), scn1a-mutant larvae (24 larvae per treatment) were exposed to nominal concentrations of control (0.05% DMSO), CBD (0.25, 0.6, or 1 µM), THC (1 or 4 µM) or clemizole (positive control; 10 µM). Following 24 hours of exposure, larvae were screened for deformities and behavior was analyzed using the ViewPoint Zebabox for 45 minutes with 100% light. Zebrafish activity was significantly reduced by 0.96 µM CBD, 3.98 µM THC, and 10 µM clemizole compared to controls by 1.69-, 2.03-, and 2.11-folds, respectively.
2.33-fold, respectively. Genes involved in neurogenesis, metabolism, and liver toxicity are being analyzed to determine the mechanisms by which CBD and THC are reducing seizures. In conclusion cannabinoids show promise in treating Dravet syndrome but more research is needed to determine its mechanism of action.

Supported by the National Institute of General Medical Sciences (NIGMS) P20GM104932.

PB51

Uncovering the Molecular Mechanism of Shuang Huang Lian for Treatment of Upper Respiratory Tract Infections (URTIs) by Active Components and Network Pharmacology

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Introduction: Shuang Huang Lian (SHL) is a modern Chinese herbal medicine (CHM) formula derived from Lonicerae Japonicae Flos, Forsythiae Fructus and Scutellariae Radix. Despite of widely use of SHL in clinical practice for treatment of upper respiratory tract infections (URTIs), the molecular mechanisms of SHL remain unclear due to its multi-component and multi-target nature. Aim of the study: To understand the underlying action mechanisms of SHL using network pharmacology approach and active components. Materials and methods: The active components of SHL obtained by UPLC-QTOF-MS analysis, PubMed text mining, and TCMID and TCMSP data mining were used for target fishing with PharmMapper server and GeneCards database. The protein-protein interaction (PPI) network, component-target (C-T) network and target-biological function-pathway (T-B-P) network were constructed using String database and Cytoscape with ClueGo and CluePedia plug-ins, and molecular docking was performed on systemsDock server. Results: Twenty pharmacologically active components and 41 common genes related to the targets and the URTIs were uncovered. Out of 227 biological functions and 60 pathways found, 23 biological functions are considered to be relevant to immunostimulation, antibacterial and antiviral activities, and 11 pathways are associated with influenza A, Kaposi’s sarcoma-associated herpesvirus infection, and Staphylococcus aureus infection. Molecular docking simulation showed that two protein targets (CASP1 and AKT1) have docking scores greater than 8.0 for brusatol, forsythoside A, phillyrin, and rutin. These components may be lead compounds for new therapeutic agent development. Conclusions: Our studies suggest that SHL exerts its therapeutic effect against URTIs through multiple pathways, including B cell receptor signaling pathway, ERBB signaling pathway, Estrogen signaling pathway, and lipopolysaccharide-mediated signaling pathway, etc.

This work was supported by the funding provided by Cleveland State University, Office of Research Internal Funding Programs, Graduate Student Research Award. Dan Liu would like to acknowledge the support of a research fund (No. 201708420116) from China Scholarship Council.

 PB52

Hypothesis: the dectin-1b activating particulate beta glucans within mushrooms are derived from endophytic yeast

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Mushrooms contain beta glucan polysaccharides as a cell wall component. In the particulate/insoluble form, beta glucan binds to and activate the dectin-1b receptor resulting in stimulation of innate immune cells. The purpose of the current study was to evaluate the level of activity exhibited by particulate beta glucans within 13 different types of edible mushrooms. Activity due to particulate beta glucan was detected using HEK-Blue™ hDectin-1b cells, a cell line engineered to selectively detect activators of the dectin-1b signaling pathway. Dramatic variation was observed in the level of dectin-1b-dependent activation (active particulate beta glucan) between the different types of mushrooms as well as between different sources of the same mushroom. Furthermore, mushrooms that exhibited no detectable dectin-1b-
dependent activity had dramatically lower yeast CFU’s as compared to mushrooms containing high activity. This hypothesis-generating data suggests that the dectin-1b-dependent activity exhibited by these mushrooms is due (at least in part) to the particulate beta glucan derived from endophytic yeast.

This research was partly funded by the USDA, ARS Specific Cooperative Agreement No. 58-6060-6-015.

PB53

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The American Herbal Products Association (AHPA) conducts biannual tonnage surveys to quantify annual harvests of certain North American herbs in commerce. The first addressed only the plant goldenseal (Hydrastis canadensis) which was CITES listed in 1997 due to supposed overharvesting. Actual harvest data can enable informed decisions, and may serve to defend ongoing, sustainable use of these plants. This work has been recognized by the Fish and Wildlife Service of the U.S. Department of the Interior as “a vital index of native U.S. botanical consumption.” The data included here represent aggregate quantities of harvest data provided to AHPA by companies that serve as raw material suppliers to the trade. For each of the commodities that are the subject of the survey, respondents were asked to provide information on the amounts of both cultivated and wild-harvested material, and of both fresh and dried material.

Grateful acknowledgement is given to all the primary raw material suppliers who willingly volunteered harvest tonnage information.

Please contact mzimmermann@ahpa.org or visit the website for the American Herbal Products Association at www.ahpa.org for more information.

PC1
Use of Secondary Metabolites in Biological Fighting for Weed

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In recent years, the demand for agricultural products has increased with rapid population growth and climate change in the world, and it is aimed to obtain more yield from the unit area. While intense input with intensive agriculture yielded more efficiency than unit area, synthetic chemicals used in weed control had negative effects on ecological balance, biodiversity, human health and food quality. In recent years, with the increasing awareness of agriculture, these topics have been discussed more and alternative agricultural methods have been preferred to keep the ecological balance, do not harm the biodiversity and take the health and food quality criteria into consideration. Organic agriculture is preferred from these systems. In this agricultural system, alternative methods are preferred instead of chemical herbicides used in weed control. One of these alternative methods is the use of natural compounds which have allelopathic effect in the control of weeds. Etymologically, the combination of the words os Allelo “and Yunanca Pathos os with Greek origin meaning” mutual suffering Et; allelopathy with a complex genetic, physiology and mechanism; Secondary chemicals secreted by various organs in the plant are called to interfere or stop plant growth. Most of the secondary metabolites secreted by plants have allelochemical properties. Secondary methobilites with allelopathic potential are found in almost all plant tissues such as leaves, stalks, rhizomes, roots, flowers, fruits and seeds. For example, allelochemicals released from Centaurea diffusa leaves inhibit the germination of rye seeds by up to 80%. Allelopathy in the fight against weeds; natural mulch, covering plant, crop rotation plant, mixed planting, green manure, toxic extracts extracted from allelopathic plants, natural herbicides and allelopathic product types.

As a result of the use of secondary metabolites secreted by allelopathic plants in weed control instead of chemical herbicides, it will help to protect biodiversity and soil.
Phytochemical and biological study of fifty six traditional Egyptian medicinal plants with potential anticancer activity

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Twenty three percent of Egyptians rely on medicinal plants as remedies for their health problems. The number of Egyptian patients suffering from cancer is increasing and is estimated to be 70,000 per year with 40% of them coming from Upper Egypt, 35% from the Metropolitan areas, and 24% from Lower Egypt as reported by Egyptian National Cancer Institute in 2010. As part of our continuous efforts to find alternative treatments for cancer, we have studied fifty six plants collected from Upper Egypt. The alcoholic extracts of the fifty six plants were screened in vitro for their cytotoxicity using MTT assay against MRC-5 and leukemia K562 cell lines as well as brine shrimp lethality assay.

Selected extracts and their successive fractions were further screened against additional six cell lines; SK-MEL, KB, BT-549, SK-OV-3, LLC-PK1, and Vero cell line. Followed up phytochemical investigation toward the isolation of the bioactive markers is undergoing and the data will be presented.

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Chemical Constituents of Callistemon citrinus

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Callistemon citrinus (Myrtaceae) is commonly known as crimson bottlebrush, which is an indigenous plant to Australia and widely distributed in Asia and South America. The plant is used in traditional medicine for the treatment of gastrointestinal disorders, cough, bronchitis and infectious diseases. The phytochemical analysis of methanol extract of the leaves of Callistemon citrinus resulted in the isolation and identification of flavonoids, chromone glycosides, gallates and acylphloroglucinol glycosides. Their structures were elucidated on the basis of extensive analyses of spectroscopic data including 1D, 2D NMR and HRESIMS.

We are grateful to the Egyptian Government, and the NCNPR, School of Pharmacy, The University of Mississippi for the financial support.
Bioactive metabolites from the Red Sea soft coral Sarcophyton convolutum

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Sarcophyton genus soft corals are rich in secondary metabolites specially cembranoid diterpenes, which represent the main chemical defense of corals against their natural predators in addition to their myriad biological effects in humans. Cembranoids; such as sarcophine, 7β,8β-Dihydroxydeepoxy-sarcophine and other related compounds, one steroid; 3β,24R-ergost-5-en-ol and one sesquiterpenoid; prostantherol, have been isolated from the red sea soft coral Sarcophyton convolutum. The structures of the isolated compounds were elucidated with extensive spectroscopic data analysis including 1D-, 2D-NMR and MS data. In addition to known, several interesting metabolites were identified in these extracts for the first time. The anti-inflammatory and cytotoxic potential of these compounds are being evaluated. Along with biological properties, the details of structure elucidation, biogenesis, interrelationship between these unique secondary metabolites will be disclosed.

Keywords: soft coral; Sarcophyton convolutum; cembranoid; cytotoxic and anti-inflammatory activities; biogenesis.

Phytochemical and Biological Studies of Sphenocentrum Jollyanum

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Sphenocentrum jollyanum is a sour tasting plant which belongs to the family Menispermaceae. It is a perennial plant that grows naturally along the west coast sub region of Africa with expanse from Cameroon across Nigeria to Sierra Leone. It has found use as chewing sticks, relief for constipation, cough medicine, for sickle cell disease, rheumatism and other inflammatory conditions. Pharmacological activities include the use as antimalarial, antiviral, anti-angiogenic, analgesic, aphrodisiac and vermifuge. It has also been used in treatment of jaundice, breast engorgement, tumors, and dressing chronic wounds.
The ethyl acetate fractions of the crude methanol extract of *Sphenocentrum jollyanum* plant were found active at various concentrations against *Candida albicans* with a minimum inhibitory concentration (MIC) of 12.5 mg/mL compared to the reference standard Tioconazole cream 1%. The ethylacetate fraction also exhibited activities against the following species of Candida and plant fungi: *Candida valida*, *Candida pseudotropicalis*, *Candida tropicalis*, *Candida glabrata*, *Candida krusei*, *Trichophyton rubrum*, *Trichophyton interdigitalis*, *Trichophyton tonsurans*. Phytochemical investigation of *Sphenocentrum jollyanum* yielded a new and four known compounds which were identified by their NMR, IR and MS spectral analyses. The known compounds were elucidated as: polypodine B, ecdysterone, *polypodoaurein*, 20,26-dihydroxyecdysone.

The project was supported by American Association of University Women (AAUW), Faculty of Pharmacy, University of Ibadan, Nigeria, National Center for Natural Product Research, USA and in part by the USDA Agricultural Research Service Specific Cooperative Agreement No. 58-6060-6-015.

**PC6**

**Chemical Constituents of Sarcocephalus latifolius**

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*Sarcocephalus latifolius* (*Nauclea latifolia*) (Bruce. Smith) is an important genus of plants in the Rubiaceae family. It is an evergreen, multi-stemmed shrub or spreading tree native to Asia and Africa and widely distributed in the tropical rain forest of West African countries. Different parts of this plant are commonly used in Africa as a remedy for diabetes, dental cares, septic mouth, pain, diarrhea, leprosy, debility, malaria, hypertension, gastrointestinal disorders, prolonged menstrual flow and sleeping sickness. Phytochemical investigation of the root extract of *S. latifolius* resulted in the isolation and identification of six phytochemicals including, quinovic acid-3-0-β-D-fucopyranoside, quinovic acid-3-O-β-D-fucopyranosyl-28-O-β-D-glucopyranosyl ester, quinovic acid-3-O-α-L-rhamnopyranoside, quinovic acid-3-O-β-D-glucopyranoside, strictosidine and strictosamide. Their structures were elucidated on the basis of NMR spectroscopy and mass spectrometry. The absolute configuration of D-fucose unit in was qualitatively determined using an Ultra-performance Liquid Chromatography Mass Spectrometry technique (UPLC-UV/MS).

1. National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS
2. Tertiary Education Trust Fund (TetFund), Nigeria.

**PC7**

**Anti-Methicillin-Resistant Staphylococcus aureus (MRSA) and Biological Activities of Metabolites from Digitaria sanguinalis L.**

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Natural products comprise multiple useful entities ranging from pure bioactive compounds to more complex forms such as standardized extracts. Several natural products and bioactive compounds have been used throughout history to treat infections, which still give the promise to obtain valuable therapeutic agents for infectious diseases from plants. Natural products even provide immense opportunities for new drug leads because of the matchless availability of chemical
diversity. The research aims to evaluate antimicrobial, Anti MRSA, antiviral and cytotoxic activities of biologically active fractions of the alcoholic extract and seven isolated compounds of *Digitaria sanguinalis* aerial parts. Bioassay-guided fractionation of the ethanolic extract of aerial parts of *Digitaria sanguinalis* L. (Poaceae.) followed by using several chromatographic techniques resulted in the isolation of seven compounds; The structural elucidation of isolated compounds were established by spectroscopic analysis (UV, IR, ¹HNMR, ¹³CNMR, and HRESIMS). Antimicrobial, anti-MRSA, antiviral and cytotoxic activities were determined for n-hexane, ethyl acetate, n-butanol fractions of the ethanolic extract, in addition to the isolated compounds. Seven compounds were isolated: p-coumaric acid (1), tricin (2), p-hydroxybenzoic acid (3), stigmasterol (4), β-sitosterol-3-O-β-D-glucoside (5), tricin 7-O-β-D-glucopyranoside (6) and isoorientin (7). The results revealed that several isolated compounds from *Digitaria sanguinalis* exhibited marked antimicrobial, antiviral and cytotoxic activities, while compounds 1, 2, 5 and 6 showed significant activity against methicillin-resistant *Staphylococcus aureus* (MRSA). The study shows that several fractions and isolated compounds from *Digitaria sanguinalis* possess considerable promise as potential therapeutic agents, represented by their significant antimicrobial, anti-MRSA, antiviral and cytotoxic activities.

PC8

**Antimicrobial Effect, Antioxidant Potential and Cytotoxic Activity of the Crude and Green Synthesized Silver Nanoparticles’ Extracts of Silene conoidea L. Leaves Growing in Egypt**

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Nowadays, the field of nanotechnology is one of the most active researches in modern material science, among the utilized methods the eco-friendly methods “green mediated synthesis” of nanoparticles are the preferred because they are less toxic and economically effective.

In the current study, the crude methanol leaf extract of *Silene conoidea* L. (CS) was used for the synthesis of its silver nanoparticles (SCAgNPs). The formation of the nanoparticles was confirmed by UV–visible spectrophotometry. Their characterization was on the basis of Fourier Transform Infra-Red spectrometric analysis, which revealed the possible involvement of phytoconstituents in the formed silver nanoparticles of crude extract, moreover, field emission scanning electron microscope (SEM) was used for determination of their shape and size, the nanoparticles were spherical in shape having average particle size of 30 nm.

The green synthesized nanoparticles’ extract (SCAgNPs) showed more total phenolic compounds as well as flavonoid and proanthocyanidin contents compared to the crude extract, moreover it extracts exhibited enhanced antioxidant potential against stable DPPH radical (2, 2-diphenyl-1-picrylhydrazyl) using butyl hydroxyl toluene (BHT) as a reference standard, cytotoxic activity towards three cell lines namely HEP-G2, HCT-116 and MCV-7 using Cisplatin as a reference anticancer drug as well as significant antimicrobial effect.

The gained results support the advantages of using bio-green method for the production of nanoparticles having the potential of antimicrobial and cytotoxic activities.

PC9

**Cheminformatic Approach for Deconvolution of Active Compounds in a Complex Mixture - PhytoSERMs in Licorice**

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Studies on the efficacy of botanical natural products have been tackled using a myriad of scientific methods, all of which tried to solve a common challenge: how to deal with a complex mixture. This study is centered on the application of new strategies such as cheminformatics tools to identify the putative active constituents, their potential metabolites and implications on their biological functions including deleterious effects. Licorice is used throughout the world as a
common spice, a traditional medicine and a botanical dietary supplement to exert various biological activities. To demonstrate the utility of cheminformatics for the deconvolution of active compounds in a complex mixture, two species of commonly used licorice, Glycyrrhiza glabra and Glycyrrhiza uralensis, were selected. Using several computational tools along with crystal structures of two isoforms of human Estrogen Receptors (hERs), phytochemicals from Glycyrrhiza were probed for their isoform preference along with their putative functionality as agonists/antagonists of estrogen receptors. Potential estrogenic activity based on ensemble docking and a quantitative structure activity relationship prediction model (QSAR) drew attention to several compounds from each species. The model was able to identify many known phytoestrogens that are part of licorice in agreement with reported literature. The details of our study, critical interactions in hERs activation and the utility of in-silico predictive models will be presented.

PC10
Studies on Drug Interaction Potential of Cyanotis vaga and its Constituents

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Cyanotis vaga is a Chinese herbal medicine which belongs to Commelinaceae family. Its decoction was reported to be used as fever reducer as well as mood enhancer. Even though C. vaga has been used for several conditions there are no reports about its effect on drug metabolizing enzyme Cytochromes P450 to predict its interaction with concomitantly used drugs.

In this study, eight ecdysteroids were isolated from C. vaga including two major compounds (20-hydroxyecdysone and ajugasterone C). The plant extract and isolates were tested for their inhibitory effects on CYP3A4 enzyme which is a major CYP isoform involved in the metabolism of commonly used clinical drugs. The extract showed strong inhibition of CYP3A4 with an IC50 value of 8.5 μg/ml while 20-hydroxyecdysone and ajugasterone C did not inhibit CYP3A4.

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Twenty-four pure fragrance ingredients classified as skin allergens were subjected to forced degradation studies to understand whether chemical degradation may be linked to the formation of species of dermatotoxicological concern in regard to skin sensitization adverse effects. Stability studies were performed by irradiation through dark/light cycles in combination with oxidizing conditions (air flux). The chemical reactivity of the compounds upon degradation was investigated using the Direct Peptide Reactivity Assay (DPRA). The DPRA is a chemical method accepted by regulatory agencies for the characterization of molecular initiating event (MIE or Key event 1), as described by the Adverse Outcome Pathway for skin sensitization.

In the present work, the 24 chemicals were subjected to forced degradation for 150 days. At the end of the study, 67% of the compounds underwent chemical decomposition. Only four fragrance ingredients (coumarin, benzyl salicylate, benzyl cinnamate and hexyl cinnamal) remained non-reactive with both peptides. Oxidation of fragrance ingredients typically occurs through radical mechanisms which may lead to the generation of organic and inorganic peroxides, epoxides, unstable radicals or peroxyradicals. These pro-oxidants species formed upon degradation, may result in depletion of peptide without formation of apparent covalent adducts with the test chemical. In order to better understand the effect of pro-oxidant on peptide depletion, hydrogen peroxide was used as model pro-oxidant. The obtained results showed little effect of oxidative conditions on Lys-depletion, while Cys-depletion was significantly affected by concentrations above 1.1 mg/L of H2O2. Overall, chemical instability should be considered as a potential concern when assessing the skin sensitization potential of (un)known chemicals using non-animal methods.

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Applicability of in chemico methods for skin sensitization investigation of botanical ingredients

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Botanical ingredients are ubiquitously used in commercial preparations, such as aromatherapy, cosmetics, detergents, household and perfumes products. Skin sensitization is one of the most common adverse effects related to the topical application of botanical ingredients. Skin sensitization risk assessment of botanical ingredients is therefore necessary for consumers’ protection and occupational hazards identification. There are currently very few non-animal methods that can assist in the evaluation of complex mixtures. While animal methods are available for screening of botanical ingredients, the literature concerning the application of non-animal in vitro models is scarce, and in chemico approaches are practically non-existent. Chemical methods can be used to characterize the ability of potential sensitizers to trigger the first Key Event (haptenation). Estimation of chemical reactivity toward model nucleophiles (used as surrogate of skin proteins) can provide essential information in a timely manner and thus help to reduce the need for in vivo testing. The fluorescent in chemico high throughput method (HTS-DCYA) is suitable for the rapid evaluation of chemical reactivity toward a fluorescent nucleophile (dansyl cysteamine), which is used as a surrogate of skin proteins. In the current work, the applicability of the HTS-DCYA method for the systematic evaluation of chemical reactivity of complex
botanical mixtures was explored. Four extracts from unrelated plant species were obtained and tested with the high-throughput fluorescence method at three concentrations. To illustrate the minimal matrix effects of the tested extracts on the developed method, the least DCYA-reactive extract (R. canina) was spiked with known sensitizers at different concentrations. The data obtained from the four plant extracts, and the spiking experiments with known sensitizers, suggest that the HTS-DCYA method can be successfully applied for estimating the skin sensitization potential of complex botanical matrices.

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PC13
Secondary metabolites from high CBD Cannabis sativa L. and their Cannabinoids binding affinity

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Chemical study of high CBD Cannabis sativa grown at the University of Mississippi resulted in the isolation of 16 compounds including: 13 cannabinoids (1-13), one spiro-indane (14) and 2 flavones (15-16). Compound 1 was a new cannabinoid isolated for the first time from a natural source. The chemical structures of the isolated compounds were elucidated using 1D and 2D NMR, along with GC/MS experiments. The CB1 and CB2 receptors binding activities of the isolated compounds were evaluated. Compounds 4, 6, and 11 showed high binding affinity to both CB1 and CB2 (CB1/CB2 %) at 10 µM (95.3/95.2, 86.9/91.7 and 79.9/85.5, respectively), while compounds 1, 2, 5, 8-10, and 13 displayed good selective binding affinity to CB2 ranging from 71.9 % to 88.8%. Compounds 14-16 showed week binding affinity to both CB1 and CB2 receptors. The CB1 and CB2 functional assay for the active compounds will be evaluated and presented

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PC14

Drug Interaction Potential (CYP-PXR-P-gp) and Comprehensive Quantitative Analysis for Phytochemicals Derived from the Globalized South African Rooibos Tea (Aspalathus linearis) and Dietary Supplements Using UHPLC-PDA-MS

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Aspalathus linearis is a leguminous shrub endemic in South Africa, and exploited for production of the quaffable herbal rooibos tea.1-3 Although it is enjoyed in 37 countries, rooibos extracts were reported to inhibit CYP2C9, CYP2C8 and CYP3A4 isozymes.4-6 In our study, phytochemical investigation of unfermented (green) rooibos tea leaves and seeds resulted in the isolation and characterization of eleven unique flavonoid C- and O-glycosides (1-11) in addition to syringin (12). This prime study aims to investigate the toxicity profile for the unfermented rooibos tea and its isolated unique secondary metabolites focusing on drug metabolizing enzymes and efflux transporters (CYP, PXR, and P-gp). In addition, chemical fingerprint profile and comprehensive quantitative analysis of these phenolic compounds in the authenticated extract and 21 herbal supplements claimed to contain A. linearis were performed using UHPLC-PDA-MS.

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References:
New lignans from stem bark of Iranian Daphnae mucronata

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*Daphne mucronata* in traditional medicine is used for treating tumors and skin diseases. In previous study, six compounds including mucronin-A, mucronin-B, daphnecin, aquillochin, umbelliferone, and coumarin have been isolated from the whole plant of *D. mucronata* with antilukemic and antituberculosis properties. In the current presentation, chromatographic fractionation of the methanolic extract of this plant led to the isolation and structural characterization of two new and three known lignans: 3,3'-dihydroxy-4,4'-dimethoxy-7,9:7,9-diepoxy-3,4:3',4'-bis(methylenedioxy)-8,8'-lignan; and 4,4'-Dihydroxy-3,3',5,5'-tetramethoxy-7,9:7,9-diepoxy-lignan, two biflavonoid: genkawanol, and 4',4'',5,5'',7,7''-hexahydroxy-3,3''-biflavanone, one flavonoid: apigenine, three simple phenolic compounds: syringin, 4-hydroxy-3-methoxycinnamaldehyde, and 4-hydroxy-3-methoxybenzoic acid, two triterpenoids: oleaneolic acid, and betulinic acid, in addition to one new guaiane class sesquiterpene, and one new daphnane class macrocyclic diterpene.

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Stem bark of Iranian Daphnae mucronata: a rich source of cumarins, biocumarins, and lignocumarins

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*Daphne* species are shrubs, with upright or prostrate stems. Even though most parts of these plants are toxic, but they are used in as a traditional medicine in China, Iran and tropical part of Africa. Currently, more than 50 *Daphne* species have been discovered so far, from which *D. mucronata* L., *D. pontica* L., *D. mezereum* L., *D. oleoides* subsp. *kurdica*, *D. stapfii* L., *Daphne resshingeri* L., *D. laureola* L. have been reported in Iranian plateau. In recent studies, the antimicrobial, antiviral, antitumor and anti-inflammatory effects of coumarins were reported. *D. mucronata* in traditional medicine is used for treating tumors and skin diseases. In previous study, four coumarinolignans mucronin-A, mucronin-B, daphnecin, aquillochin, and two simple cumarin umbelliferone, and coumarin have been isolated from the whole plant of *D. mucronata*. In the current presentation, chromatographic fractionation of the methanolic extract of the stem bark of this plant led to the isolation and structural characterization of two new and one known coumarinolignans, daphnetocin, three known bicumarin including 7-methoxyedgeworthin, 7-O-glucopyranoeedgeworthin, and 6-O-glucopyranoeedgeworthin, in addition to four cumarin including 7-hydroxucumarin, 7-methoxycumarin, 7,8-dihydroxycumarin, 8-hydroxy-7-glucopyranocumarin.
PC17

Long-term monitoring of green tea supplements’ performance on USP disintegration tests and development of dissolution tests with epigallocatechin gallate (EGCG) as a marker compound.

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Background: In the US, meeting the United States Pharmacopeia (USP) standards is mandatory for drugs but not for dietary supplements (DS), a vast majority of which do not have USP monographs and detailed protocols for quality control. The USP publishes performance standards designed to detect problems with the release of active ingredients (AI) in a variety of dosage forms due to formulation design and manufacturing processes. Meeting these specifications also ensures that different batches of products release AI consistently.

Research Objectives: Using USP 39-40 general chapter standards, this work sought to determine whether commercially sold single-ingredient green tea (GT) DS consistently meet the standards for disintegration and whether they pass the dissolution test for immediate release forms of botanical DS.

Methodology: A disintegration test evaluates the ability of a tablet or capsule to break apart within a specified amount of time during agitation. Six units of each DS were individually immersed and agitated in USP-designated solutions. A formulation passed a disintegration test if, after ½ hour, only fragments of coating or capsule shell remained, but not a palpably firm core. A dissolution test measures the amount of marker compound (in this case, the primary green tea catechin, epigallocatechin gallate (EGCG)), released from a dosage unit into a stirred solution at pH 1.2. A product passes the test if, after 1 hour, > 75% of the amount of EGCG is released.

Results: For 29 products with 2-4 tested lots purchased over a 4 year time period, 9 (31%) always passed disintegration, 9 (31%) always failed disintegration and 11 (38%) were inconsistent in their results for multiple lots. Importantly, results for the same lot of the in-house control product were consistent over time when tested by the same laboratory.

In dissolution testing, none of the 22 DS passed the test in its initial design when filters with nylon membranes were used to filter dissolution medium samples. A series of 4-10 ml filter pre-flushes with EGCG solutions were not sufficient to saturate the nylon filter and to avoid EGCG loss. We concluded that use of nylon filters is unacceptable, and only polytetrafluoroethylene (PTFE) filters (that do not capture EGCG) should be used for EGCG measurements.

Eleven products from lot 3 testing did not require retesting as they did not disintegrate and released less than 6% of the measured EGCG in dissolution testing. For the 11 products which had initial dissolution rates of 20-69%, we tested a new, fourth, lot with PTFE filters. The overall success rate (based on the pooled lot 3 and 4 data) was 5 (~23%) out of 22 GT DS (21 capsules, 1 tablet). The pass rate was higher for products in gelatin capsules as compared to cellulose-based capsules: 4 out 8 (50%) vs. 1 out 13 (~8%). Thus dissolution testing with the correct filter indicated that select GT DS do meet USP standards.
A newly released USP 41 DS general chapter recommends testing failed gelatin capsules and gelatin-coated tables with enzymes added to the dissolution medium to reduce the impact of potential gelatin cross-linking. Five products with failed test results were retested with the gastric enzyme pepsin. The percentage of released EGCG, however, was only marginally increased compared to the no enzyme conditions (only 1 additional product passed). The new pass rate of only 27% is a basis for a strong concern that AI are not released properly from dosage forms in vivo. We are investigating if the lack of improvement in dissolution was caused by an EGCG inhibitory effect on pepsin activity rather than an unacceptable level of gelatin cross-linking.

**Conclusions:** Long-term monitoring of disintegration of single-ingredient GT DS using the USP standards indicated a vast disparity and unpredictability in their performance quality over different lots. Less than a third of products met the USP dissolution requirements. However, effects of botanicals on enzyme activity should be further investigated to ensure correct assessment of products. The design of testing protocols should take into account AI enzyme interaction and dosage form chemistry.

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PC18

**2-Benzoxazolinone-based 1,2,4-triazoles as proinflammatory cytokine inhibitors.**


A library of 20 novel benzoxazolinone-based 1,2,4-triazoles was synthesized and screened for their _in vivo_ anti-inflammatory and antinociceptive activities. The compound 18e exhibited potent anti-inflammatory activity with 68.75 and 55.20% inhibition in comparison to indomethacin which showed 65.62 and 60.41% inhibition after 3 and 5 h, respectively. The five active compounds, i.e. 2a and 17e–20e showing significant _in vivo_ anti-inflammatory activity were further screened for their _in vivo_ COX-2, TNF-a, IL-1β and NO inhibitory activities. The COX-2 selectivity index of 17e was found to be 42.30 and thereby showing a high selectivity towards COX-2 inhibition. In silico molecular docking studies have been done in order to get an insight into the binding modes of these molecules with TNF-a protein.

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PC19

Phytochemical Investigation of Mimosa pigra

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In Southern Brazil, more than 60 plants were screened for anti-dermatophyte activity, and dichloromethane fractions of methanolic extract of M. pigra showed the lowest MIC values (1.9 µg/mL) without DNA destruction at 10 and 50 µg/mL of cell viability of human leukocytes.¹ In relation, this research is aimed at isolating interesting secondary metabolites with promising antifungal activity and safe human toxicity profiles. Powdered leaves of M. pigra were extracted by percolation in methanol at room temperature and yielded crude extract after removal of solvent under reduced pressure. The crude extract was fractionated by VLC using reversed-phase C-18 silica with gradient elution of methanol-water (0:1 to 1:0), leading to 16 fractions. All of the fractions were tested against C. albicans and several of them showed promising anti-candida activity. The obtained fractions were subjected to repeated column chromatography over Sephadex LH-20, RP-18 silica, and normal phase silica to yield nine phenolic compounds. This presentation emphasizes on isolation and structure elucidation of these secondary metabolites.

PC20

Chemical constituents from the flower of Buddleja officinalis

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Buddleja officinalis Maxim is deciduous early-spring flowering shrub from family Loganiaceae¹¹. It is mainly found in P. R. China. Its flowers are used in Traditional Chinese Medicines (TCM) to treat eyes diseases. The major chemical constituents of this plant are flavonoids, phenylethanoid glycosides and terpenoids. The chemical constituents have been reported with antioxidant, anti-bacterial, and anti-inflammatory activities. In this study, we focused on the isolation and characterization of natural products from the flowers of this plant. So far, twenty two compounds have been isolated. These have been identified as syringaresinol-β-D-glucoside (1), dammadenyl acetate (2), δ-amyrenone (3), bessisterol (4), β-amyrin (5), acacetin (6), 4',7'-dihydroxy-5-methoxyflavone (7), apigenin-7-O-β-D-glu-4'-O-methyl ether (8), eleutheroide B (9), ethyl-β-D-glucopyranoside and glycerin (10), trehalose (11), fructose (12), (2R, 3R)-2,3,4-trihydroxyl butyrate (13), 2,2'-oxybis (14), bis(2-ethylhexyl) phthalate (15), phthalic acid isodibutyl ester (16), schisandrin (17), kadsuphilin B (18), 3-O-β-D-glucopyranosyl(3R)-1-octen-3-ol (19), crocusatin M (20), catechol (21), 5-hydroxyxypyrrolidine-2-one (22). Seventeen of these compounds were discovered from this plant for the first time. Structures of the isolated compounds were elucidated using 1D and 2D-NMR spectroscopy.

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PC21
Phytochemical and Biological Investigation of Silene Rubella Belonging to Caryophyllaceae

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The genus Silene is known to be a source of biological active compounds. The most important active compounds in Silene species are the phytocdysteroids, which have a similar chemical structure to molting hormones of insects. Some Silene species are used as ornamental plants and in folk medicine to treat inflammations, bronchitis, cold and infections or as a diuretic, antipyretic, analgesic and emetic. The genus also possesses anticancer, antihypertensive, antispasmodic, antimutagenic, antioxidant, anti-inflammatory and antimicrobial activities. Silene rubella L., is a flowering plant, belonging to genus Silene of caryophyllaceae family. The Ethanolic extract of S. rubella showed moderate antibacterial activity against Streptococcus aureus, Bacillis subtilis and Salmonella typhimurium and antiviral activity against HAV-10. It also showed moderate activity towards colon carcinoma cell line HCT-116 with IC50 23.8 µg/ml. Phytochemical investigation of the aerial parts of S. rubella yielded 21 known compounds which were characterized by NMR, Mass and IR spectral data analysis as four steroids: β-ecdysone (I), polypodine (II), spinasterol (III), spinasterol-3-O-glucoside (IV), four triterpenes: lupeol (V), ursolic acid (VI), oleanoic acid (VII), betulinic acid (VIII), eleven flavonoids: apigenin (IX), diosmetin (X), luteolin (XI), kaempferol (XII), quercetin (XIII), myricetin (XIV), isovitexin (XV), luteolin-7-O-glucoside (XVI), (S)-naringin (XVII), (R)-naringin (XVIII) rutin (XIX), one phenolic acid: chlorogenic acid (XX), and a sugar derivative: 3-O-methyl inositol (XXI). All of these compounds are reported for the first time from this species.

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PC22
Phytochemical Investigation on the Fruits of Tujia Ethnomedicine "heilaohu"

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*Kadsura coccinea* (Lem.) A. C. Smith belongs to the medicinally important genus *Kadsura* from the Schisandraceae family. It is mainly born in mountain forests, often wrapped around large trees and evergreen. Mainly distributed in some areas of southwestern China such as Hunan Province, Sichuan Province and Guangxi Province, etc. The roots and stems of *k. coccinea* are used by Chinese residents to treat stomach, duodenal ulcer, chronic gastritis, rheumatoid arthritis, bruises, dysmenorrhea and other diseases. In previous studies, people found monoterpenes, sesquiterpenes, triterpenes, lignans and other substances in *k. coccinea*. Among them, the amount of triterpenoids and lignans is the largest. Up to date, in the study of the chemical composition of the *k. coccinea*’s fruit, eight compounds were found and identified their structures, including four triterpenoids and three lignans.


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PC23
Discovery of novel bioactive constituents from actinomycetes toward various agricultural pathogens

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Finding natural product-based solutions to help manage agriculture pests is a major global challenge due to an increased incidence of chemical resistance by agrochemical pathogens. The aim of this project was to identify new natural product-based pesticides and biopesticides for use as plant protectants or phytochemicals in plant protection and production. We focused on unique actinomycete species not previously studied as a source of antifungal constituents. Four bioactive organisms were isolated that can effectively control banana fusarium wilt disease caused by the soil-borne *Fusarium*
oxysporum f. sp. cubense. These organisms were subsequently identified by sequence analyses of the ITS region of the rRNA genes as belonging to the species Streptomyces yunnanensis. The butanol extract of the culture filtrate from Streptomyces yunnanensis was analyzed by innovative technology and potent bioactive preparations were evaluated by various in-house rapid bioassays using an activity-directed fractionation approach to determine potential inhibitory effects towards phytopathogenic fungi included Colletotrichum fragariae, C. acutatum, and C. gloeosporioides. Further studies toward the identification of specific bioactive compounds and their detailed biochemical pathways and physiological studies are currently underway.

PC24
Ornamental plants as sources of medicine

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The over-reliance on the natural ecosystem has been a worry for conservationist and may lead to the extinction of the very useful plants including Taxus brevifolia and Podophyllum peltatum. Further, some ethnomedicinal practitioners are over-dependent on the natural forest for processing raw materials for export to other countries, thus promoting the degradation of the natural forest. In meeting the Sustainable Development Goals (SDGs) on promoting health, combating climate change and protecting forests, there will be the need to identify other viable sources of natural medicine in order to reduce this over-reliance on the natural forest. This brings into view the need to explore other natural sources including ornamental plants. Ornamental plants are usually cultivated for their aesthetic value. They are usually cultured, propagated and grown in lawns with best agronomic practices to ensure their growth. However, when grown, they are usually cut and burnt. Finding other useful application, including their use as medicine will go a long way in providing alternative uses to further justify their cultivation and maintenance. This presentation has the following objectives; isolation of active principles, identification of novel compounds and their biological evaluation.

PC25
Chemical constituents of Cayratia albifolia C. L. Li

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Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease with unknown etiology, which regarded by academia as an autoimmune disease, it seriously affects human health and quality of life. The pathogenesis of RA is related to a variety of complex factors such as heredity, infection, environment, body neuroendocrine, immune system, etc., but the pathogenesis is not completely clear, which brings certain difficulties to its treatment. For the treatment of RA, dong people have accumulated rich experience in medicine. Cayratia albifolia C. L. Li has been widely used in dong minority for a long time, and its effect on arthralgia syndrome is unique, it has been used clinically for nearly 30 years, with 2,000 cases. Its unique curative effect benefits many patients with RA, but its chemical composition and mechanism of action has not been studied at home and abroad. So we studied the chemical constituents of Cayratia albifolia C. L. Li, at present, eight compounds were isolated from the extract, including β-sitosterol, β-sitosterol glucoside, stigmast-3,6-dione, betulinic acid, glycerol monolinoate, eleutheroside A, resveratrol and paimitic acid.

This work was supported by Hunan Provincial Key Laboratory of Dong Medicine (2015TP1020-02), Hunan Key Laboratory of Druggability and Preparation Modification for Traditional Chinese Medicine(2017-04) and Students Research Innovative Program of Hunan Province(grant number 2018-206).
PC26
Exploring antimicrobial natural products as food preservatives

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Food preservatives are utilized to prolong the shelf life of food products due to their antimicrobial and/or antioxidant properties. They play an important role in ensuring the quality and convenience of life to a healthy living. Current food preservatives have been used for decades and have many drawbacks. To address the need for the development of safer food preservatives, we have established an in vitro antimicrobial assay panel to screen natural product extracts derived from plants, marine organisms, and microbes. We have also evaluated many small-molecule antimicrobial natural products that are available in the National Center for Natural Product Research Repository. Salient results and potential issues will be discussed in this presentation.

This work was supported by the USDA Agricultural Research Service Specific Cooperative Agreement No. 58-6060-6-015.

PC27
Chemical and Biological Evaluations of Asimina triloba

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Annonaceae is the largest plant family of the magnoliales, and it is found mainly in the tropics, subtropics, and some extend into temperate regions such as Asimina genus. Annonaceae plants are important in folk medicine, and they have been used to treat various tumors. They contain a broad spectrum of biologically active acetogenins that have displayed many biological activities including anticancer activity. Also, Annonaceae plants contain different sub-classes of alkaloids that derived from isoquinoline alkaloids, which have anticancer activity as well. Regardless the growth in popularity of Annonaceae plants and their potential effect on health support (especially in their anticancer activities), studies have shown the development of atypical parkinsonisms, progressive supranuclear palsy (PSP)-like disease (sporadic), in people who consume Annonaceous products continuously. The potential risks of neurodegeneration associated with chronic consumption of plants of the Annonaceae family emphasize the need of further studies to identify the neurotoxic compounds in the edible annonaceous plants and compare their risk to their benefits in defeating cancer. Our study is focused on one of the most important Annonaceae plant, which is Asimina triloba specifically on its alkaloids constituents due to the lack of studies on the isolation and identification of the alkaloids from this plant.
PC28

USP Cranberry Monograph: Progress Report

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Cranberry fruit is the largest individual botanical ingredient used in the dietary supplements in the United States. The widespread use of cranberry supplements by the female population to alleviate urinary tract infection symptoms demands creation of robust public standards to assure product quality.

On June 22nd 2017, USP hosted a Cranberry Standards Development Roundtable to foster discussion of key quality attributes essential for development of standards for cranberry ingredients and finished products. This report summarizes the progress made since then on: i) development of nomenclature for different types of cranberry dietary ingredients, ii) optimization of HPTLC identification methodology, and iii) development of a new HPLC-DAD ID test; both tests facilitating identification of cranberry marker constituents in marketed raw materials, as well as those of the potential adulterants (e.g. grape seed, peanut skin, and pine bark extracts).

Samples (n=50) representing different types of marketed ingredients were assembled to represent different ingredient types and processing schemes, falling into six categories. For identification by HPTLC, different sample preparation protocols, mobile phases and derivatization reagents were evaluated; a solid phase extraction method was selected and the method was optimized for detection of anthocyanins, flavonoids (i.e., flavonol glycosides and flavan-3-ols) and sugars in both dietary ingredients and finished dietary supplement products. For the HPLC ID test, a fast multi-wavelength separation was developed to detect anthocyanins (520 nm), flavonol glycosides (365 nm), organic acids (310 nm) and phenolic compounds in general (278 nm). HPLC quantification of anthocyanins was carried out and peak ratios between different anthocyanins allow establishment of acceptance criteria specific for the cranberry fruit. Mixtures of cranberry extracts with adulterants at different levels (5, 20 and 50%) were prepared and the resulting profiles at 278 nm were compared to that of cranberry. Both HPTLC and HPLC results indicated that while the low-molecular weight polyphenols profile of cranberry fruit is affected by the ingredient processing conditions, it may still be suitable for compendial identification of cranberry-derived ingredients.

PC29

Monoterpene glycosides from the aerial parts of Vangueria agrestis

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Vangueria agrestis (Schweinf. Ex Hiem) Lantz, formerly known as Fadogia agrestis, is an African plant used in the African traditional medicine as a remedy for certain health disorders. V. agrestis is prescribed in the folk medicine primarily for the treatment of erectile dysfunction and as an aphrodisiac. The stem bark of V. agrestis has been reported to show analgesic and anti-inflammatory properties. It also displayed a sedative effect comparable to that of acetyl salicylic acid. In the current presentation, fractionation of the methanolic extract of Vangoria agrestis using normal- and reverse-phase chromatography and combined with spectroscopic analysis led to the isolation and structural characterization of four monoterpene glycosides. Of these constituents, there are three hitherto unreported monoterpene glycosides, namely (2E,6E)-2,6-dimethyl-8-[O-α-L-rhamnopyranosyloxy](1→3)-(2-O-(2E,6E)-8-acetoxyl-2,6-dimethyloctadienoyl)-α-L-rhamnopyranosyl)oxy]-octadien-1-yl α-L-rhamnopyranoside, (2E,6Z)-2,6-dimethyl-8-[O-α-L-rhamnopyranosyloxy](1→3)-(2-O-(2E,6E)-8-hydroxy-2,6-dimethyloctadienoyl)-α-L-rhamnopyranosyl)oxy]-octadien-1-yl α-L-rhamnopyranoside, and (2E,6E)-2,6-dimethyl-8-[O-α-L-rhamnopyranosyloxy](1→3)-α-L-rhamnopyranosyl]-oxy]-octadien-1-yl α-L-rhamnopyranoside. The previously reported monoterpene glycoside was identified as: (2E,6Z)-2,6-dimethyl-8-[O-α-L-rhamnopyranosyloxy](1→3)-α-L-rhamnopyranosyl]-oxy]-octadien-1-yl α-L-rhamnopyranoside. None of these compounds exhibited cytotoxicity up to 25 µg/mL against the cell lines used in the testing, namely SK-MEL, KB, BT-549, and SK-OV-3.

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PC30
Potential Modulation by EGCG and its Metabolites of Human NAD[P]H-Quinone Oxidoreductase 1 (NQO1) – A Systematic Computational Study

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Green tea is one of the most consumed traditional beverages in the world and is prepared from the dried leaves of Camellia sinensis (L.) Kuntze. Among the polyphenols found in green tea, the catechin (−)-epigallocatechin-3-O-gallate (EGCG) is considered to be one of the major, active constituents and has attracted a great deal of attention due to its perceived health benefits, including weight-loss properties and even claims that it is a potential therapeutic agent for cancer, obesity, diabetes and neurodegeneration. By contrast, within the last decade or so, more than 50 reports in the medical literature of clinically apparent acute liver injury with jaundice have been attributed to green tea extracts. It has been reported that o-quinone metabolites of gallic acid or EGCG are causative agents for hepatotoxicity. A significant increase in cytotoxicity and ROS formation was noted for EGCG or gallic acid when they were incubated with dicumarol (a known potent inhibitor of NQO1) in isolated rat hepatocytes. However, no experimental information is available at the molecular level on the possible role of NQO1 in the detoxification of EGCG and its metabolites, including reactive intermediates. Herein, we investigated the possibilities of NQO1 inhibition by EGCG and its metabolites by studying their interaction profiles and binding mechanism at the active site of NQO1 using molecular docking, binding free-energy calculations and molecular dynamics (MD) simulations. The molecular docking studies and free-energy calculations showed that some metabolites exhibited strong binding affinity and found that the binding orientation of the EGCG metabolites overlapped with that of dicumarol as found in the NQO1 X-ray crystal structure. The results suggest that these metabolites may act as strong NQO1 inhibitors, highlighting the necessity of experimental validation with appropriate biological methods. In addition, the MD results revealed that selected EGCG metabolites formed a stable and strong complex with NQO1, with amino acids W105, Y126, Y128, H161, F178, H194, and F232 being critical for potential NQO1 binding. The current results together with experimental data as well as studies of the polymorphisms of NQO1 may explain the observed idiosyncratic hepatotoxicity caused by consumption of green tea and its constituents.

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PC31
Phytochemical Investigation, Safety and Quality Assessment of Two Tinospora Species

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Tinospora crispa Miers ex Hook.f. & Thomson and Tinospora sinensis (Lour.) Merr. (Family Menispermaceae) are indigenous to south Asia and south East Asia. They are used in traditional medicine and are reported to possess antidiabetic, antihypertensive, anti-inflammatory, anti-leishmanial, cytotoxic and immunomodulatory activity. Both species bear morphological resemblance which makes their distinction critically important as T. crispa has been reported to cause hepatotoxicity. Isolation and characterization of the marker compound may aid in exploring the chemical diversity of the two plants and hence help to resolve safety and quality issues. Phytochemical investigation of the stems of T. crispa and T. sinensis has been carried out, which led to the isolation and characterization of compounds such as borapetosides B, C, F and tinosineside A along with seven new source compounds from these species. The structures of these compounds were elucidated by spectroscopic methods, including 1D and 2D NMR experiments and confirmed by www.oxfordicsb.org | facebook.com/OxfordICSB | icsb@olemiss.edu
HRESIMS. Hepatotoxicity is a major concern with herbal botanical products in general; *T. crispa* is no exception. Quality and safety issues were investigated by evaluating the hepatotoxic potential of *T. crispa* compounds in murine model under health compromised conditions and developing a validated UHPLC-UV-MS method to aid in the identification and subsequent differentiation of the closely related *Tinospora* species, in plant samples and in botanical products.

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PC32
Degradation products from major steviol glycosides formed under acidic conditions.

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The manufacture of beverages using high-potency sweeteners has been driven in the past few years by the desire to avoid or decrease sucrose consumption and thus the calorie content of the drinks. *Stevia rebaudiana* (Bertoni) Bertoni is a plant species belonging to the Asteraceae family reputed for its sweet taste, hence, many relevant commercial applications have been found for its steviol glycosides. Several beverages have been commercialized with high-potency sweeteners from *S. rebaudiana*. However, it’s well-known that steviol glycosides undergo double bond isomerization with sugar cleavage and Wagner-Meerwein rearrangement under acidic conditions. Therefore, appropriate storage condition of acidic beverages is important for avoiding the loss of the palate and health benefits of the high-potency Stevia derived sweeteners. The aim of this work is isolation of the degradation products from rebaudioside A and stevioside formed under mild and vigorous acid hydrolysis and elucidate their structures. Exposure of rebaudioside A to vigorous acid conditions yielded three main aglycones which were purified by using normal phase high-performance chromatography and identified as isosteviol, steviol and the endocyclic isomer of steviol. Four new glucoside degradation products with an ent-kaur-15-en-19-oic acid skeleton together with three known compounds were obtained after mild acid hydrolysis from rebaudioside A and stevioside. Structures of the new *endo*-steviol glycosides were unambiguously elucidated based on HRESIMS, HRESIMS/MS, 1D and 2D NMR experiments as follows: *endo*-steviolmonoside, *endo*-rebaudioside G1, *endo*-steviolbioside and *endo*-rubusoside. Additionally, three previously reported compounds were also identified: *iso*-rebaudioside A (*endo*-rebaudioside A), *iso*-rebaudioside B (*endo*-rebaudioside B) and *iso*-stevioside (*endo*-stevioside). Crystal structure determination of the endocyclic isomer of steviol, *endo*-steviolbioside and *endo*-stevioside allowed confirmation of their structures by X-ray diffraction.
In silico studies of two new α-Pyrone derivatives isolated from the endophytic fungus Embellisia sp. as CRM-1 inhibitors

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Two new α-Pyrone derivatives have been isolated from Embellisia sp. Fungus and identified as 5-(3-Hydroxybutyl)-4-methoxy-6-methyl-2H-pyran-2-one (1) and 4-(4-methoxy-6-methyl-2-oxo-2H-pyran-5-yl)butanoic acid (2). In addition, it was reported before that α-Pyrone derivatives have antileukemic activities. Increased expression of Chromosome Region Maintenance (CRM-1)/exportin-1 (XPO-1) has been correlated with poor prognosis in several aggressive tumors as leukemia, making it an interesting therapeutic target for antileukemic agents. In this work in silico docking studies were carried out to estimate the binding affinity of compounds 1 and 2 against CRM1. This might provide insights to develop a new natural-based inhibitor to CRM1/XPO. The two isolated α- pyrone derivatives have structural similarities with Goniothalamin (natural CRM1 inhibitor). Therefore, Goniothalamin was used as a reference in docking studies. The results of docking studies revealed that the studied ligands have similar position and orientation inside the putative binding site of Goniothalamin. Comparing to the binding mode of Goniothalamin (ΔG = -14.49 kcal/ mol), it was found that compounds 1 & 2 have good binding modes with ΔG values of -13.32 and 12.05 kcal/ mol, respectively.

Furthermore, ADMET studies were determined for the two isolated compounds using Discovery studio 4.0 software. The compounds showed very low BBB penetration power, good intestinal absorption, good to moderate aqueous solubility and CYP2D6 non-inhibitory activity. Liver dysfunction is therefore unexpected upon administration of such members. Based on such results, these candidates may serve as useful lead compounds in the search for powerful and selective antileukemic agents.

A Novel Hyaluronic acid-Dihydroartemisinin Conjugate: Synthesis, Characterization and Anti-proliferative Effect on Lung Cancer Cells

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Dihydroartemisinin is a derivative of artemisinin, has been recently demonstrated anti-proliferative effects on various tumor cell lines such as breast, prostate, colon, pancreas, liver and lung. However, DHA is associated with some drawbacks, such as low bioavailability which is hampered by its poor aqueous solubility and its rapid metabolism in systemic circulation. Therefore, to overcome these limitations, in this study, we synthesized a novel hyaluronic acid-dihydroartemisinin conjugates in which the 10-hydroxy group of DHA was covalently linked to carboxylic group of HA. The conjugate was successfully characterized using ¹H NMR, Gel permeation chromatography and UV spectroscopy. The synthesized conjugate self-assembled into nanoparticles in aqueous solution. The developed nanoparticles were characterized for particle size, zeta potential, Transmission Electron Microscopy (TEM), X-ray Powder Diffraction (XRD) and loading efficiency. The nanoparticles exhibited better cytotoxicity than native DHA in lung cancer (A549) cell line which was determined using CCK-8 cell viability assay. The enhancement in cytotoxicity of self assembled nanoparticles was supported by Annexin-V-FITC-Propidium iodide apoptosis assay, generation of reactive oxygen species (ROS) and loss of mitochondrial membrane potential. The findings demonstrated the potential of HA-DHA conjugate to improve clinical outcome of DHA for cancer chemotherapy.

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Microbial Biotransformation and Biological Studies of Dianabol Metabolites

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Microbial biotransformation is a process that involves the enzyme system of microbes (bacteria, fungi, virus) to convert endobiotic or xenobiotic substances into more polar and water-soluble metabolites. These metabolites are the end product of classical phase I metabolism i.e. oxidation, reduction, condensation, hydrolysis and isomerization followed by Phase II metabolism using cytochrome P450 enzyme system of the microbes.

In the following study Dianabol, an anabolic steroid was subjected to biotransformation by using fungal enzyme system, Apspergillus niger. Metabolites were first extracted with ethyl acetate then isolated by column chromatography using silica gel. Primarily isolated metabolites were visualized using thin layer chromatography using ceric sulphate as an indicator. Three metabolites were isolated and subjected to modern spectroscopic techniques e.g. 1HNMR, 13CNMR, EI-MS and mass spectrometry for structural elucidation and chemical characterization. Spectroscopic analysis shows that all three isolated metabolites were hydroxylated derivatives of parent drug molecule. Furthermore, these metabolites were subjected to the different biological assays and one metabolite showed promising activities as urease, alpha glucosidase and xanthine oxidase inhibitor.

Chemical composition and Insecticidal activity of volatile oils of fresh and air-dried buds of three different varieties of cannabis

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The volatile oils of fresh and air-dried buds of three different varieties of cannabis namely CBD type, CBD/THC type and THC type were obtained by hydro-distillation. GC/MS analysis of the volatile oils resulted in identification of 65 compounds of which 30 were monoterpenes and 35 were sesquiterpenes. The oil obtained from the dried plants showed significant change in the chemical composition from that of the fresh material. Generally, the % of monoterpenes decreased upon drying. The volatile oil of the THC type showed an increase in the ratio of the sesquiterpenes to monoterpenes content. Terpinolene content was shown to decrease upon drying. The moderate increase in β-caryophyllene, and caryophyllene oxide was observed. There is no detectable change in the % of monoterpenes and sesquiterpenes content in the intermediate type upon drying. However, It was observed that the monoterpenes content of...
the volatile oil of CBD variety was higher in the oil of the dried material than in the fresh one. This unexpected observation will be verified/refuted in subsequent studies. The insecticidal activity of the volatile oils was evaluated. The oil obtained from the fresh and dried high CBD Cannabis showed good biting deterrent activity at 10 ug/cm² compared to DEET at 25 nmol/cm and good larvicidal activity.

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PC37

Study on the chemical constituents of Swertia punicea Hemsl.

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Ten compounds had been isolated from Swertia punicea Hemsl, named 1-hydroxy-3,7,8-trimethoxy xanthone (1), 1-hydroxy-2,3,5-trimethoxy xanthone (2), swerchin (3), swertianin (4), 1-hydroxy-3,5,8-trimethoxy xanthone (5), swerpunilactone B (6), deacetylcentapicrin (7), 1,3,5R,8S-tetrahydroxy-5,6,7,8-tetrahydroxanthone (8), 3S,5R,6R,7E,9S-megastiman-7-ene-3,5,6,9-tetrol (9), 1,3,5,8-tetrahydroxy xanthone (10). Compounds 3,5,7 and 9 were first isolated from this plant. Their structures had been identified by 1D and 2D-NMR spectroscopy.

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PC38
The Imprinting Equivalence Research of Lonicerae japonicae flos and Lonicerae flos

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"Imprinting equivalence" refers to the phenomenon that the two have different chemical structures but reflect the same (similar) "imprinting template". That was, different substances produced the same effect. The paper wanted to explore and confirm the imprinting equivalence of Lonicerae japonicae thunb and Lonicerae confusa which was based on the autonomous function of the supramolecular "imprinted template". Lonicerae japonicae flos dregs, Lonicerae flos dregs and Dudleyagreenei dregs were host molecules. The water extract of Lonicerae japonicae thunb, Lonicerae flos, Shuanghuanglian (Lonicerae japonicae flos), Shuanghuanglian (Lonicerae flos), Yinqiaosan (Lonicerae japonicae flos), Yinqiaosan (Lonicerae flos) were guest molecules. The host molecules selectively absorbed the six guest molecules. Water extract of guest molecules were tested by HPLC. And HPLC fingerprint were obtained. The MRT and their difference value were calculated by the total quantum statistical moment method. And the results were performed by T-test. The six guest molecules were selectively absorbed by Lonicerae japonicae flos, Lonicerae flos, Lonicerae japonicae flos, Dudleyagreenei dregs, Lonicerae flos and Dudleyagreenei dregs. The MRT difference value was conducted by T-test. The results were \( P_1=0.94>0.05, P_2=0.02<0.05 \) and \( P_3=0.04<0.05 \). All guest molecules were absorbed by Lonicerae japonicae flos and Lonicerae flos dregs. There was no significant difference in the MRT difference value. But when six guest molecules were absorbed by Dudleyagreenei dregs, There was significant difference between Dudleyagreenei and Lonicerae japonicae flos(Lonicerae flos dreg) in MRT difference value. The Statistical data indicated that similarity was existed in both "imprinted template" of Lonicerae japonicae flos and Lonicerae flos. But there was difference between Dudleyagreenei and Lonicerae japonicae flos(Lonicerae flos dreg). There was imprinting equivalence between Lonicerae japonicae flos and Lonicerae flos.

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Evaluation of HPLC fingerprints of Lonicerae japonicae flos and Lonicerae flos

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The commonly fingerprint analysis software and the total quantum statistical moment(TQSM) were used as the analytical means. The Lonicerae japonicae flos and Lonicerae flos were used as the model drugs. Two different fingerprint similarity analysis methods were used to analyze the fingerprint of Lonicerae japonicae flos and Lonicerae flos. The evaluation of HPLC fingerprints of Lonicerae japonicae flos and Lonicerae flos was carried out to compare two different analytical methods. The commonly fingerprint analysis software obtained similarity results: the similarity between the fingerprints of 10 batches Lonicerae japonicae flos samples and the generated control map R1 were all above 0.91. The similarity between the fingerprint of 10 batches Lonicerae flos samples and the generated control map R2 was above 0.98. The similarity results of the TQSM were as follows: the similarity between the fingerprints of the 10 batches Lonicerae japonicae flos were above 0.92, and the similarity between the fingerprints of the 10 batches Lonicerae flos were all above 0.93. From these results obtained by the commonly fingerprint analysis
software and the TQSM, the HPLC fingerprints similarity of *Lonicerae japonicae flos* samples and *Lonicerae flos* samples were similar. But TQSM can be used to characterize the curve of chromatographic fingerprints: area under curve (AUC$_T$) can be used for quantitative analysis and area under curve per weight (AUCPW$_T$), mean chromatographic retention time of total quantum (MCRT$_T$), variance of mean chromatographic retention time of total quantum (VCRT$_T$) can be used for qualitative analysis. So TQSM reflects the comprehensive features of the fingerprint.

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**Triterpenoid Saponins from Massularia Acuminata**

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*Massularia acuminata* (G.Don) Bullock ex Holy (Rubiaceae) is a tropical plant native to western Africa and locally known as pako ijebu or orin ijebu in Southwest Nigeria. The stem of the plant is traditionally used as chewing stick for oral hygiene and the decoction/infusion for aphrodisiac in Nigeria. Phytochemical screening showed the presence of alkaloids, saponins, anthraquinones, phenolics, flavonoids, and tannins in the extract. Phytochemical investigation of the stem of *Massularia acuminata* yielded six triterpenoid saponins including three new compounds. Structure elucidation was achieved by means of NMR spectroscopic and mass spectrometric techniques.
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