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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

Poster Abstracts

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PA-1 (ID#2)

A UHPLC-MS/MS method for quality assessment of star anise and detection of toxic contaminant

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Star anise (also known as Chinese star anise) is an anise-scented star-shaped fruit of the evergreen *Illicium verum* tree. Star anise is rich in a variety of flavonoids and polyphenolic compounds and has a distinct licorice-like flavor. It has been used as a spice in Asian cuisines and as folk medicine to treat childhood colic, abdominal pain, colitis, diarrhea, bloating, etc. Pure Chinese star anise is typically safe for most people; however, it may be contaminated with Japanese star anise (a fruit of *Illicium anisatum* tree with similar morphological characteristics with Chinese star anise) that is known to contain highly toxic anisatin and can lead to serious physical symptoms from diarrhea, vomiting, stomach pain to seizures, loss of consciousness, respiratory paralysis, and even death. We have developed a rapid, sensitive, and reliable UHPLC-MS/MS method for simultaneous determination of the quality markers of Chinses star anise (*i.e.,* quercetin, kaempferol, and luteolin) and the toxicity marker of Japanese star anise (*i.e.,* anisatin). In this work, quercetin-d3 was used as the single exogenous internal standard to overcome both matrix effect and the necessity of analyte reference standards. Chromatographic separation of the analytes was achieved within 3 minutes with high strength silica (HSS) T3 column by gradient elution using 0.1% acetic acid aqueous solution and 0.1% acetic acid in methanol as the mobile phase. The method has been validated per the guidelines of the U.S. Pharmacopeia Chapter <1225> "Validation of Compendial Methods" for accuracy, precision, specificity, LOD, LOQ, linearity, range, and robustness; and used for the quality assessment of star anise preparations obtained in the U.S. merchandise stores.

PA-2 (ID#9)

Herbal product authentication via GC- high-resolution MS/MS (GC-HRMS/MS) technology and multivariate statistics

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Botanical authentication is quite difficult due to the innately complex chemical composition of plant products. This is especially true for herbal products, whose chemical variations are largely due to their highly volatile, and thus unstable, essential oils. Gas chromatography-mass spectrometry (GC-MS) has been the standard approach for essential oil analysis, but may fail to capture all compounds and can be restricted in compound identification. To explore the potential of an innovative GC- high-resolution MS/MS (GC-HRMS/MS) technology, which we hypothesize would improve metabolomic analyses for botanical authentication, we used seven cultivars from three *Ocimum* species as a model system. Metabolome profiles were coupled with supervised and unsupervised statistical models to 1) determine metabolite patterns amongst the various species 2) examine how essential oils can be used to differentiate between basil species 3) use constructed models to predict the identity of an unknown sample and 4) identify compounds responsible for species distinction. The high-resolution accurate mass and detailed fragmentation trees obtained from GC-HRMS/MS enabled greater metabolome coverage and provided added insight into previously overlooked compounds for species differentiation, which could be a great asset to studies looking to resolve ambiguity in botanical samples and classify products based upon their metabolome make-up.

Acknowledgments
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PA-3 (ID#12)

New resources and insights from the NIH ODS analytical methods and Reference Materials Program

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The NIH Office of Dietary Supplements (ODS) Analytical Methods and Reference Materials (AMRM) Program supports the development of tools that permit the verification of dietary ingredient identity and the measurement of constituents and contaminants in botanical raw materials and finished dietary supplement products. AMRM goals are accomplished through funding and collaborative activities with dietary supplement stakeholders across academic research institutions, manufacturers, industry trade groups, third-party testing and standard-setting groups, and Federal agency communities. In recent years, AMRM has supported method development for the characterization of botanical dietary supplement ingredients, the development of certified reference materials, and administration of quality assurance programs which help laboratories improve through the assessment of method performance and identification of sources of measurement bias. These efforts have highlighted chemical composition variations that can help inform investigations of authenticity and may influence safety and efficacy assessments.

PA-4 (ID#24)

Comparison of pollen and novel microalgae diets in honey bees through untargeted LC- and GC-MS metabolomics

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Honey bees collect floral pollen as their source of macro- and micronutrients. Managed honey bees are fed artificial diets to compensate for a lack of pollen forage in the environment and to prevent nutritional deficiencies. In this study, we formulated microalgae feeds using *Chlorella vulgaris* and *Arthrospira platensis* (spirulina) biomass and provided them to young adult honey bee workers. Diet-induced changes in bee abdominal metabolite profiles were studied relative to a natural pollen diet using LC-MS- and GC-MS-based metabolomics approaches. Bees fed microalgae diets exhibited significant metabolite overlap with pollen-fed bees. The metabolomics results are useful to understand the mechanisms of body mass increases, elevated nutritional status, and potential for increased stress resistance in bees fed the microalgae diets. Overall, this study demonstrates that metabolomics approaches can provide high-resolution information on the effects of feed and could eventually help tailor diet interventions to achieve precision nutrition in honey bees.

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PA-5 (ID#31)

Rapid, accurate quantification of capsaicinoids in various chili pepper extracts with Absorbance-Transmittance fluorescence Excitation-Emission (A-TEEM) spectroscopy.

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Chili peppers, largely believed to be derived from *Capsicum annuum* in the United States, are valuable for many types of food preparations due to their heat compounds known as capsaicinoids. Conventional analysis of capsaicinoids usually involves liquid chromatography (LC) with UV, photodiode-array, fluorescence and/or mass spectrometry detection. Hence, analyses in the field and lab can be prohibitive with respect to cost and levels of expertise needed to operate and maintain the equipment. Here we investigated the quantification of the three major capsaicinoids, capsaicin (Cp), dihydro-capsaicin (DHCp), and nordihydro-capsaicin (NDHCp). Methanolic extracts were prepared from dried, pulverized chilis from eight different commercial extracts each analyzed in triplicate. The total capsaicinoid content (Cp+DHCp+NDHCp) of the final A-TEEM sample dilutions determined by LC varied 255 fold, ranging from 0.028 to 7.158 mg/L. Importantly, the A-TEEM method™ automatic inner-filter-effect (IFE) correction facilitates linear fluorescence instrument responses over a wide concentration range. The A-TEEM scans included 240-700 nm for excitation and 250-800 nm for emission both with 2 nm increments. While all capsaicinoids fluorescence was contained to the UV region (650 nm; hence IFE correction was important to correct for interferences from these compounds. Two sensitivity ranges were applied for the A-TEEM scans differing only in the total integration time where samples with 0.110 mg/L used only 25 s per A-TEEM acquisition; A-TEEM data were intensity normalized using water Raman Scattering units to account for the integration time. Simple linear regression of the unfolded A-TEEM fluorescence data yielded accurate single ex/em coordinate variable predictions for all three capsaicinoids and their total with the R^2 >0.994 and relative error of prediction (REP) significantly less than 1%. Â We conclude that the A-TEEM with IFE correction can serve as a simple, rapid tool for measuring capsaicinoid content over a wide range in a variety of commercial chili products.

PA-6 (ID#32)

Easy preparative supercritical fluid chromatography method development for natural products

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Supercritical fluid chromatography (SFC) is a “green” purification method that is useful for natural products purification. One barrier to preparative SFC is method development. This work describes a simple method of calibrating analytical SFC systems to match the preparative SFC 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, and other corrections required to match the analytical system to the preparative system. The calculation can then be applied to scouting gradients run on the preparative SFC system as well, for rapid method development when matching analytical and preparative columns are not available.

PA-7 (ID#35)

Comparative analysis of chemical profiles of radix Astragali between cell wall broken material and traditional slices

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Radix Astragali, one of the most popular herbs in traditional Chinese medicine (TCM), is used to strengthen the immune system, protect liver function, fight bacteria and viruses, and treat diabetes, heart failure, and seasonal allergies. In recent years, a new form of Radix Astragali material processed by the cell wall disrupting technology, namely ultrafine granular powder (UGP), has been introduced into the market. In order to determine the chemical consistency and the homogeneity of the UGP material prepared from sliced traditional materials (TM) of Radix Astragali, multiple batches of the UGP and TM samples derived from *Astragalus membranaceus* var. *mongholicus* were analyzed by UHPLC/DAD-MS using isoflavones and triterpenoid glycosides as marker compounds. The results demonstrated that the chemical profiles of UGP were identical or similar to that of TM, but UGP was highly homogeneous in terms of marker compound contents as assessed, e.g., by the relative standard deviation values of the nine marker compounds in the range of 8.55%−43.80% for TM2 compared against 1.70%−8.38% for UGP2. Macromolecular component preparation and ^{1}H NMR analyses indicated that TM4 and its corresponding UGP4 produced similar polysaccharides, but the latter had an approximately two-fold dissolution rate of the polysaccharides when compared to the former (yield 7.22 ± 0.35% v.s. 3.39 ± 0.20%). This study confirms that the UGP of Radix Astragali is chemically consistent and homogenous, supporting its use as an improved material in TCM prescriptions.

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PA-8 (ID#38)

Development of black cohosh Standard Reference Materials (SRMs) and an analytical method for determination of marker compounds

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Black cohosh supplements prepared from the roots and rhizomes of *Actea racemosa* L. (syn. *Cimicifuga racemosa* [L.] Nutt.) are among the most popular herbal products sold on the market. The flowering plant is native to North America. Besides its traditional use, black cohosh is consumed primarily to help women manage symptoms associated with menopause. However, there is an increasing concern about quality issues that could be associated with adulteration, especially with other species of *Actea*. The National Institute of Standards and Technology (NIST), in collaboration with the National Institutes of Health-Office of Dietary Supplements (NIH-ODS), is producing four black cohosh reference materials to support the determination of eight triterpenic glycosides including actein and 23-epi-26-deoxyactein. As these compounds have low UV absorbance, a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method has been developed using Selected Reaction Monitoring in a positive mode.

PA-9 (ID#46)

Development of a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for characterizing linalool and linalyl acetate pharmacokinetics in humans

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Lavender (*Lavandula angustifolia*), an ethnopharmacological plant, is commonly known as English lavender. Linalool and linalyl acetate are major phytochemical components in lavender essential oil (LEO) derived from the flower heads. LEO has been used in aroma or massage therapy to reduce sleep disturbance and mitigate anxiety. Recently, an oral LEO formulation was administered in human clinical trials designed to ascertain its anxiolytic effect. However, human pharmacokinetics and an LC-MS/MS method for simultaneous measurement of linalool and linalyl acetate are lacking. To address this deficiency, we conducted a pharmacokinetic study in which subjects (8-10/cohort) received an oral dose of either linalool (50 mg) or its naturally occurring pro-drug linalyl acetate (45 mg). Samples prepared by protein precipitation and liquid-liquid extraction were analyzed using a C18 reversed-phase column and gradient elution (acetonitrile/water, 0.1% formic acid). A Waters Xevo TQ-S tandem mass spectrometer (positive mode) was used to quantitatively determine linalool, linalyl acetate, and farnesol (IS) according to transitions of m/z 137.09→95.10 (tR 0.79 min), 137.09→95.10 (tR 1.38 min), and 205.22→149.11 (tR 1.56 min), respectively. The validated method was then used to characterize the oral pharmacokinetics of linalool. Following linalool administration, linalool absorption was rapid with a corresponding half-life of 3.9 hours. However, neither linalool nor linalyl acetate was detected in the plasma following administration of linalyl acetate. Based on our data, we hypothesize that linalyl acetate contributes little to plasma linalool exposure. However, future pharmacokinetic studies designed to evaluate the impact of linalyl acetate on linalyl exposure following oral administration of LEO are required to test this hypothesis.

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PA-10 (ID#47)

Development of a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for characterizing caffeine, methylliberine, and theacrine pharmacokinetics in humans

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Coffea liberica possesses stimulant properties without accumulating the methylxanthine caffeine. The basis for this peculiar observation is that methylurates (e.g., theacrine and methylliberine) have replaced caffeine. The stimulant properties of methylurates, alone and in combination with caffeine, have recently been investigated. However, human pharmacokinetics and LC-MS/MS methods for simultaneous measurement of methylxanthines and methylurates are lacking. To address this deficiency, we conducted a pharmacokinetic study in which subjects (n = 12) were orally administered caffeine (150 mg), methylliberine (Dynamine™, 100 mg), and theacrine (TeaCrine®, 50 mg) followed by blood sampling over 24 h. Liquid-liquid extraction of plasma samples containing purine alkaloids and internal standard (^{13}C-Caffeine) were analyzed using a C18 reversed-phase column and gradient elution (acetonitrile and water, both containing 0.1% formic acid). A Waters Xevo TQ-S tandem mass spectrometer (positive mode) was used to detect caffeine, methylliberine, theacrine, and IS transitions of m/z 195.11 → 138.01, 225.12 → 168.02, 225.12 → 167.95, and 198.1 → 140.07, respectively. The method was validated for precision, accuracy, selectivity, and linearity and was successfully applied to characterize the oral pharmacokinetics of caffeine,

methylliberine, and theacrine in human plasma. Successful development and application of LC-MS/MS-based methods such as ours for the simultaneous measurement of methylxanthines and methylurates are essential for the characterization of potential pharmacokinetic and pharmacodynamic interactions.

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PA-11 (ID#49)

Caffeine and methylliberine: A human pharmacokinetic interaction study

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Methylliberine and theacrine are methylurates found in the leaves of various *Coffea* species and *Camellia assamica* var. *kucha*, respectively. We previously demonstrated that the methylxanthine, caffeine, increased theacrine's oral bioavailability in humans. Consequently, we conducted a double-blind, placebo-controlled study pharmacokinetic study in humans administered methylliberine, theacrine, and caffeine to determine methylliberine's pharmacokinetic interaction potential with either caffeine or theacrine. Subjects (n = 12) received an oral dose of either methylliberine (25 or 100 mg), caffeine (150 mg), methylliberine (100 mg) plus caffeine (150 mg), or methylliberine (100 mg) plus theacrine (50 mg) using a randomized, double-blind, crossover design. Blood samples were collected over 24 hours and analyzed for methylliberine, theacrine, and caffeine using UPLC-MS/MS. Methylliberine exhibited linear pharmacokinetics that were unaffected by co-administration of either caffeine or theacrine. However, methylliberine co-administration resulted in decreased oral clearance (41.9 ± 19.5 vs. 17.1 ± 7.80 L/hr) and increased half-life (7.2 ± 5.6 versus 15 ± 5.8 hrs) of caffeine. Methylliberine had no impact on caffeine's maximum concentration (440 ± 140 vs. 458 ± 93.5 ng/mL) or oral volume of distribution (351 ± 148 vs. 316 ± 76.4 L). We previously demonstrated theacrine bioavailability was enhanced by caffeine; however, caffeine pharmacokinetics were unaffected by theacrine. Herein, we found that methylliberine altered caffeine pharmacokinetics without a reciprocal interaction, which suggests caffeine may interact uniquely with different methylurates. Understanding the mechanism(s) of interaction between methylxanthines and methylurates is of critical importance in light of the recent advent of dietary supplements containing both purine alkaloid classes.

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PA-12 (ID#50)

Calibrating quantification models of acid and neutral cannabinoids in flower extracts measured by HPLC with Absorbance-Transmittance fluorescence Excitation-Emission Matrix (A-TEEM) spectroscopy

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Recent interest in cannabinoids has shifted from the major D9-THC and CBD compounds to other minor compounds with purported beneficial medicinal properties. Thus, the need for rapid, accurate analytical methods to evaluate the complete cannabinoid composition has increased. One well-established method is HPLC which can quantify many compounds of interest

but is expensive, time consuming (10-20 min) and requires high-level operator skills. Here we evaluate the ability to validate the rapid (*Cannabis sativa*, including high-THC, high-CBD and intermediate-THC-CBD chemotypes. HPLC analysis quantified 14 acid and neutral cannabinoid compounds including in order of decreasing maximum concentration (dry weight %): THCA, CBDA, CBD, D9-THC, D8-THC, CBCA, THCVA, CBGA, CBN, CBG, CBC, THCV, CBDVA, and CBDV. THCA and CBDA represented the major components at 6.55 and 5.45% followed by CBD and D9- and D8-THC at 0.86, 0.8 and 0.45%, respectively. CBCA, THCVA and CBGA were around 0.34%, CBN was 0.235% and all other compounds were ^{2} values >0.987 and LOD values ^{2} (LOD) values of 0.973 (0.003%); compounds less than 0.1% exhibited average r^2 (LOD) values of 0.965 (0.0004%). Here the LOD values decreasing with % dry wt. is caused by the error weighting of the fluorescence signal for lower concentration compounds decreasing the standard error of the linear intercept. We conclude that the A-TEEM can be effectively calibrated to yield quantitative predictions comparable to HPLC for a wide range of major and minor cannabinoids in flower materials of commercial and medical interest.

PA-13 (ID#56)

Direct Mass Spectrometry: A Fast and Effective Tool for Authenticity and Substitution Detection

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Economically motivated adulteration is a growing issue in the food supply as consumer demand for products increases and supply chains are consequently strained. A common form of economically motivated adulteration is substitution fraud, where lesser valued ingredients are blended with higher value items and misrepresented as pure or authentic. Existing mass spectrometry-based methods have proven effective in identifying substitution fraud but can require existing knowledge of ingredient and adulterant biomarkers, advanced training in mass spectrometry, and experience with multivariate analysis tools. One solution to these challenges is the use of the RADIAN ASAP system with LiveID, an easy to use and fit-for-purpose mass detection system which enables rapid and direct sampling of materials for untargeted analysis and classification. Further, the RADIAN ASAP hardware and software are simple to learn and use, thus lowering the barrier to entry for those who want to implement fraud screening workflows into their laboratories and facilities. This presentation will consist of highlights from studies where this technology has been successfully applied to oregano, oolong tea, and Chinese *Baijiu*.

PA-14 (ID#57)

Trends in furanocoumarin profiles among 57 essential oils across 12 plant families

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Furanocoumarins (FCs), a large family of compounds commonly found in some essential oils (EOs), have the potential to elicit variable degrees of phototoxic skin reactions. Although the phototoxicity of some of these FCs is well known, the FC profile and phototoxic safety profile of all EOs are not well established. To better understand the risks within EOs, we quantified 14 FCs in 57 EOs across 12 different plant families using LCMS with LOQ of 0.0001-0.0050 ppm. This study, to our knowledge, represents the most comprehensive investigation of FC profiles across EO plant families to date. Among EOs that had detectable levels of FCs, 71% contained bergapten and herniarin. Potential correlations were observed between relative concentrations (calculated as % of the total FC content within each EO) of compound pairs bergapten and xanthotoxin, byakangelicol and 5-geranyloxy-7-methoxycoumarin, and bergamottin and herniarin within the Apiaceae family, as well as 5-geranyloxy-7-methoxycoumarin and bergamottin within the Rutaceae family. Among all 57 EOs tested, trioxsalen was the only FC not detected. Differences were observed in FC concentrations among multiple samples of *Citrus sinensis* (orange) oil

sourced from varying locations. Further research is needed to determine specific mechanisms that influence FC concentrations in different plant families.

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PA-15 (ID#58)

Metabolism of primaquine and alteration of endogenous metabolites in G6PD normal and deficient human erythrocytes after oral primaquine administration

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Primaquine (PQ), is one of the active drugs against *Plasmodium vivax* and *P. falciparum*, can cause red cell hemolysis in subjects with glucose-6-phosphate dehydrogenase (G6PD) deficiency. Understanding of the endogenous cellular metabolic responses associated with hemolysis is very limited. This study aims to give insights on the erythrocyte's metabolic changes upon oral administration of PQ to healthy human volunteers and its metabolism in erythrocytes. Mass spectrometry-based metabolomic analyses were performed on RBCs from G6PD normal (G6PDn), and deficient (G6PDd) human volunteers received primaquine orally. An increase in glycolysis was observed in G6PDd compared to G6PDn RBC. Upon PQ treatment, both categories of RBC showed enhancement of glycolysis. A compound responsible for redox cycling, GSH, was found elevated in G6PDn, and it was further increased upon PQ treatment. In contrast, in G6PDd RBC, GSH was reduced and was further decreased upon PQ treatment. Oxidative stress markers, e.g., allantoate, hypoxanthine, and spermidine, were reduced in G6PDn RBC and elevated in G6PDd RBC upon PQ treatment. Differences in saturated and unsaturated fatty acids were observed between G6PDd and G6PDn RBC, and also alterations in those fatty acids were observed upon PQ treatment. Other notable differences were found in fatty acyl composition, purine metabolites, and lipid oxidation products. Principle component analysis indicated significant differences in G6PDd RBC before and after PQ treatment, while a notable difference was also observed in G6PDn RBC. Metabolomic analysis identified several markers related to G6PD deficiency and the effects of PQ treatment in endogenous metabolites of RBCs. In spite of the limitation of the small sample size, this study points toward several candidate biological pathways altered upon PQ treatment in G6PDn and G6PDd RBC.

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PA-16 (ID#60)

Headspace sampling and analysis of essential oil volatile organic compounds using nanofiber sensors

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Essential oils are plant extracts that contain volatile organic compounds, many of which are aromatic. The aroma of an essential oil is linked to quality and benefits, so it is important to have markers by which aromatic compounds can be quantified. Gas Chromatography/Mass Spectrometry (GC/MS) is commonly used to analyze essential oils, but it does not characterize

aroma. Subjective organoleptic analysis defines aroma, but has drawbacks: It may be inconsistent due to transient states; it is subject to drifting baselines; extensive training is required; and it is non-quantifiable. This study evaluates the VaporSens Pilot, a novel chemical detector that features an array of sixteen unique sensors based on organic nanofibers with enhanced selectivity and sensitivity, in aromatic headspace sampling. The Pilot may provide an inexpensive, high-throughput, objective characterization of aroma to establish unique profiles even among closely-related samples of essential oil. In this study, we show that the aroma of different lavender oils which are difficult to distinguish organoleptically have measurably different aromatic profiles. This type of characterization may be applicable in research, quality, and botanical sourcing applications.

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PA-17 (ID#63)

Quantitative determination of CBD, THC and related cannabinoids from cosmetics containing *Cannabis sativa* by UHPLC-UV-MS

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Hemp-derived ingredients including CBD (cannabidiol) are increasingly being used in a variety of products including food, dietary supplements, cosmetics, and animal health products. The main reason for this trend is most likely due to the passage of the 2018 Farm Bill which excluded hemp from the DEA's Schedule I controlled substances list. Under the 2018 Farm Bill, Hemp is defined as *Cannabis sativa L*, an annual herbaceous plant belonging to the family Cannabaceae, with no more than 0.3% delta-9 tetrahydrocannabinol (THC) on a dry weight basis. The aim of this study was to develop a quantitative method capable of adequately measuring the levels of CBD, THC, and related cannabinoids in cosmetics claiming to contain Hemp or Hemp-derived ingredients. An ultra-high-performance liquid chromatography coupled with photodiode array and mass spectrometry detectors (UHPLC-UV-MS) method was developed. The method was validated in terms of extraction solvents, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ), and linearity range. Out of 69 cosmetic products tested, CBD was detected in 24 products (34.8%) at levels ranging from DUL (detected under limits of quantification) to 4.1% (w/w). THC was found in six products (8.7%), but no product contained THC content above the 0.3% (w/w) limit. Additionally, cannabinoids including cannabidiolic acid (CBDA), cannabigerol (CBG), and cannabinol (CBN) were identified in eight cosmetic products.

Acknowledgments
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PA-18 (ID#64)

Eleutherococcus senticosus reference material development at the National Institute of Standards and Technology

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Eleutherosides are the main identifying constituents of Eleuthero (*Eleutherococcus senticosus)* and have been reported to be a major source of the popular plant's adaptogenic properties. The root of Eleuthero is the primary source of eleutherosides in traditional processing for medicinal practices; however, adulteration is common due to the use of aerial parts, vernacular names, region confusion, and a lack of quality control. It is imperative to develop authentic Eleuthero reference materials to aid in the standardization of raw material testing for Eleuthero-containing dietary supplements. The work presented here, in collaboration between NIST and NIH ODS, shows the method development for eleutheroside value assignment in candidate Reference Material 8662 (*Eleutherococcus senticosus* Root) and 8663 (*Eleutherococcus senticosus* Root Extract). A liquid chromatography/tandem mass spectrometry method was developed for the separation and identification of eleutherosides after sonication extraction. Identification of eleutherosides in various complex sample matrices is critical for the phytochemical determination of eleuthero for use in dietary supplement products and research.

PA-19 (ID#68)

Development of a novel method for identification of American ginseng adulteration with Atmospheric Solid Analysis Probe-Mass Spectrometry (ASAP-MS)

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The National Institute of Standards and Technology (NIST), in collaboration with the National Institutes of Health, Office of Dietary Supplements (NIH-ODS), has worked to develop tools for the analytical characterization of dietary supplements. To date, these tools have been focused on quantitative measurements of specific analytes in specific matrices, however, non-targeted analysis (NTA) has emerged as a method for investigation of material identity and adulteration. To this end, work to study the spectral properties of authentic dietary supplement materials has commenced. Through mass spectrometric experiments and chemometric analysis of the chemical profiles, NIST is seeking to determine best practices for the development of suites of materials for determining the identity and quality of dietary supplement ingredients. *Panax quinquefolium* (American ginseng) was selected as the plant material for the pilot project. NIST and NIH-ODS have acquired seventeen powdered American ginseng root materials collected from farms in Wisconsin, USA and Ontario, Canada. These authentic verified samples were used to create and validate a statistical model. Four other materials (*Eleutherococcus senticosus* root and extract; *Panax ginseng* root and extract) were used as a test group. The samples from both groups were extracted with methanol and tested using the Waters Atmospheric Solids Analysis Probe (ASAP) with a RADIAN quadrupole mass spectrometer. The preliminary data allow for the differentiation of the American ginseng group and test group. The study will be expanded through the addition of closely related samples and spiking samples with known adulterates to test the chemometric model used for the NTA. Following this step, a suite of materials for confirmation of identity or adulteration will be curated and tested with the intention of distributing the suite to laboratories for use as a reference material.

PA-20 (ID#70)

Assessment of *Nigella sativa* seed and its adulterant through targeted, untargeted metabolomics and qualitative microscopy

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Due to the versatile pharmacological properties of *Nigella sativa* seed, researchers are keen to explore its bioactive constituents. But, *N. sativa* seed is frequently adulterated with similar-looking seeds to cope with its high demand. This work aimed to develop an approach combining quantification of bioactive constituents, metabolomics, histology, and histochemical evaluation to

differentiate *N. sativa* seed collected from different geographical locations and its adulterant. Quantitative estimation of *p*-cymene analogs (thymoquinone, thymohydroquinone, thymol, and *p*-cymene) found in *N. sativa* seed was performed by high-performance liquid chromatography (HPLC-PDA). Metabolomic differentiation was carried out through ultra-high-performance liquid chromatography combined with high-resolution mass spectrometry (UHPLC-MS). Micromorphology studies with general histology and histochemical localization were made to qualitatively analyze the genuine and adulterant seeds. The developed HPLC method is simple, sensitive, accurate, precise, and lacks matrix interference. A significant variation in the quantity of *p*-cymene analog was observed among *N. sativa* seeds collected from different geographical locations. UHPLC-MS profiling resulted in the identification of several specific constituents (tentatively identified) which can be used for markers to characterize for high yield of *p*-cymene analogs and to differentiate *N. sativa* from their adulterant. Major adulterants such as *Allium* and *Senna* spp. contains epicatechin, dihydrocapsaicin, 2,5-dimethoxyflavone, licarin c, and merazin, which are not present in *N. sativa* seed. Seed microanatomical structure shows the distinguishable characters between the genuine *N. sativa* and its adulterant *Allium* and *Senna* seeds. Also, qualitative localization of lipids using fluorescence staining is a remarkable feature to identify the genuine seeds from their adulterant. Findings from the current study will be utilized to differentiate *N. sativa* seeds based on micromorphology and chemical markers to identify its major adulterants. These results will be rational and reproducible for quality control in botanical authentication.

PA-21 (ID#71)

Quantitative determination of aromatic amines and dye intermediates in natural and synthetic hair colors by UHPLC-UV-MS method

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Hair dying has become a fairly common practice in recent years, with many people frequently using cosmetic dye to change or enhance the color of their hair. The beauty industry produces a wide range of hair colors by combining oxidative hair dyes, which mostly comprise aromatic amines, phenols, and other derivatives. In this study, a rapid, stable, and straightforward method has been developed for the quantitative analysis of thirteen commonly found oxidative intermediates in hair dye products. The developed method comprises simple solid-liquid extraction and analysis by reverse-phase UHPLC with gradient elution and detections with PDA and ESI-MS. The developed method displayed good linearity for all thirteen compounds within a range of 0.5-80 µg/mL, with the lower limits of detection and quantitation level being 30.2-214.5 ng/mL and 109.1-651.3 ng/mL, respectively. The recoveries of all compounds ranged between 86.3 and 99.9%, and the repeatability (r) and reproducibility (R) values (r 2.70-6.30% and R 3.61-5.34%) indicated that all thirteen compounds showed good precision. This method has been applied to hair color products. The most commonly found compounds in these samples were p-phenylenediamine, aminophenol, and resorcinol. The developed method is suitable and applicable for the identification and quantification of dye intermediates.

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PA-22 (ID#74)

An integrated chemico-biological standardization approach to ensure the quality of botanicals - Fatty acid profile in *Arthrospira/Limnospira* correlates with *in vitro* immune cell activation

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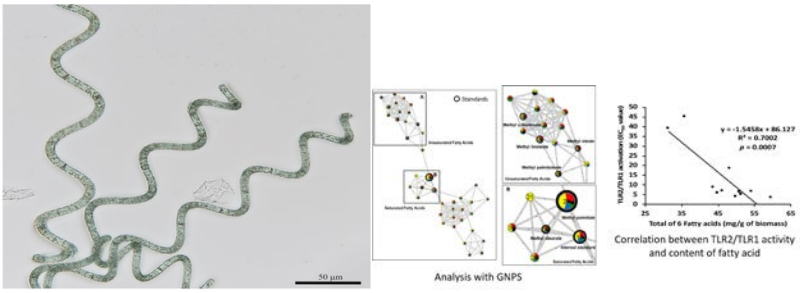
Limnospira (formerly, *Arthrospira*) is considered a worldwide food/dietary supplement due to its broad health benefits such as antioxidant[1], anti-inflammatory, and enhancing host immune resilience against viral infections. The main purpose of this study was to advance the standardization technology to ensure the overall quality of *Limnospira* which is often cultivated at various geographical locations in multiple batches, by developing an integrated chemico-biological standardization. Single-step transesterification of fatty acids into their corresponding methyl esters, aka FAMES, were utilized as reliable volatile organics to establish a quantitative GC-MS method. To probe the validity of such a method, 20 different batches of biomass from one commercial grower and 12 biomass samples from 10 different countries were analyzed. Further analysis with GNPS (global natural products social molecular networking) was instrumental in distinguishing the content and degree of unsaturation of fatty acids present in these globally-sourced samples. The sum (absolute quantity) of linoleic and γ-linolenic acids positively correlated with Braun-type lipoprotein immune-enhancing activity (activation of the TLR2/TLR1 signaling pathway). A large variation in the total quantity of linoleic and γ-linolenic acids as well as immune-enhancing activity was observed among the samples sourced from 10 different countries, highlighting the absolute necessity in developing integrated testing methods to assure the overall quality and consistency of *Limnospira* biomass produced by various manufacturers.



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PA-23 (ID#78)

Multi-class antibiotic screen in Royal Jelly, Propolis, and Bee Pollen by High-Resolution Accurate-Mass (HRAM) mass spectrometry

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A multi-class antibiotics screen in freeze dried royal jelly, propolis, and bee pollen powders was developed using HPLC-Orbitrap-MS and screened for the presence of sulfonamide, tetracycline, fluoroquinolone, nitrofuran, nitroimidazole, and macrolide classes of antibiotics. It is known that bee keepers will sometimes use antibiotics to control bacterial infections in bee hives, but improper dosage or use of unapproved antibiotics can lead to potentially concerning levels of antibiotic residues in honey products. This has led many countries to monitor antibiotic residues in honey. Honey is the most commonly and typically discussed commodity in analytical methods; however, royal jelly, propolis, and bee pollen also have the potential to be affected by antibiotic use. A multi-class screen is complicated by difficult sample preparation and the large chemical differences between classes affecting recovery and chromatography unequally. In particular, the tetracyclines proved to be difficult to recover from these matrices and had poor peak shape under typical conditions. In this method, an extraction using acetonitrile and acetic

acid without clean-up provided the best recoveries for all classes. Good separation and peak shape were achieved using a metal-free, highly end capped, hybrid silica C18 column. Finally, the selectivity provided by HRAM compensated for lack of sample clean-up. Together, this method is a quick, easy, and sensitive screen of antibiotics amenable for a high-throughput quality control setting.

PA-24 (ID#81)

AHP and USP Elderberry monographs: HPTLC analysis of different species, dietary supplement ingredients and products

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European Elderberry, *Sambucus nigra* L. has a long history of traditional use as a cold and flu remedy. However, increased demand due to COVID-19 and limited supply of traditional Elderberry ingredients has created a situation that increases adulteration risk. Major concerns related to adulteration are the substitution of Elderberry with black rice extract, the addition of synthetic dyes, and species mislabeling. This poster summarizes the progress made towards developing new American Herbal Pharmacopeia (AHP) and United States Pharmacopeia (USP) monographs for Elderberry, and the different categories of derived ingredients. A summary of HPTLC analysis of a total of 167 samples from different types of ingredients marketed as containing *S. nigra*, including whole fruits, juice concentrates, dry juices, fruit powders, liquid extracts, aqueous extracts, and Dietary Supplements (DS) products (capsules and syrups) is presented. Related species (*S. cerulea*, *S. canadensis*, *S. ebulus*, and *S. rubra*) and confounding anthocyanin sources were also analyzed, including black rice extract as the main adulterant of Elderberry ingredients. The HPTLC method published in USP *European Elder berry Dry Extract* monograph was optimized and applied following USP *<203> HPTLC for Article of Botanical Origin*. HPTLC images, peak profiles from images, and peak profiles from scanning densitometry (at 210, 254, 280, and 540 nm) were used to compare the fingerprinting of the different types of samples. According to the profile of flavonols and phenolic acids, samples corresponding to *S. nigra* berries could be classified into 4 different groups or chemotypes. In comparison to related species, the anthocyanins profile of *S. nigra* revealed an additional zone due to cyanidin-3-glucoside. Among the different types of ingredients, 55 ingredients met the characteristic zones for *S.nigra* for anthocyanins, flavonols, and phenolic acids. Adulteration by substitution with black rice extract was clearly detected in 5 samples. In some ingredients, particularly liquid extracts, dry juices, and fruit powders, the anthocyanin detection was very poor, but flavonols and phenolic acids were detectable. The same was observed for the syrups category and some capsule products. Other capsules products did not show characteristic zones in any detection mode, which might indicate either the absence of Elderberry ingredients or the large dilutions with excipients. In conclusion, the HPTLC method proposed in this work is selective enough to allow species differentiation, distinction from cofounding anthocyanin sources, and the detection of adulteration in both ingredients and DS products.

PA-25 (ID#89)

Ultrastructural and Energy-Dispersive X-ray Spectroscopy characterization of *Cetraria islandica* (L.) Ach.

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The lichen *Cetraria islandica* was used in traditional and modern medicines for its many biological properties such as immunomodulating, antioxidant, antimicrobial, anti-inflammatory activities. This species is gaining popularity in the market and interest among the many industries for selling as medicines, dietary supplements, and daily herbal drinks. Due to its high demand, adulteration is becoming a very common problem in cases of intentional and un-intentional add up. This study aimed to profile the morpho-anatomical features by light and scanning electron microscopy and analysis the presence of basic surface elements using Energy-Dispersive X-ray Spectroscopy (EDS). The microscopic studies show that the heteromerous foliose thallus is glabrous, flat, with brittle bands and the transverse section of the thallus, the outer epicortex of 0.5 -1.5 µm thick and covered with biofilm. Followed by the upper cortex, 30.05–50.6 µm wide, formed by the collocation of tightly packed cortical cells, loaded with fungal hyphae. The medullary region measures 70–100 µm wide and consists of loosely interwoven mycobiont hyphae that run horizontally and provide large airspaces for the phycobiont. The algal cell measures 3.25-12.5 µm in diameter and forms a bi-stratified structure. These phycobiont cells are located immediately below the upper cortex region of the medulla and absent or lack abundance in the lower medulla region. EDS analysis shows that the calcium is present in the upper surface and not in the lower surface. The trace amount of sodium is also observed in the upper surface along with the iron. This result will be a helping tool to authentic the samples sold in the market in the name of *C. islandica*.

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PA-26 (ID#94)

Chemical profiling of *Cetraria islandica* lichen using LC-DAD-QToF approach

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Phytochemical analysis of *Cetraria islandica* lichen was performed using high-resolution mass spectrometry combined with liquid chromatography system (LC-DAD-QToF). Total 37 compounds were identified and characterized based on comparing with literature data, retention times, and their mass fragmentation mechanism/s. The identified compounds were classified under five different classes i.e., depsidones, depsides, dibenzofurans, aliphatic acids, and others which contain simple organic acids in the majority. Four different solvent extracts were investigated and substantial variations were observed among chromatographic profiles of aqueous ethanolic, ethanolic, methanolic, and acetone extracts. Two major compounds (fumaroprotocetraric acid and cetraric acid) were identified in aqueous ethanolic and ethanolic extracts of *C. islandica* lichen. Apart from the identified and characterized compounds, 24 unknown compounds were observed and their molecular formulas were summarized based on exact masses. The developed LC-DAD-QToF approach for *C. isladica* lichen allowed us to characterize and classify the known secondary metabolites and preliminary confirmation of unknown compounds led to further isolation of compounds of interest and their pharmacological activities.

PA-27 (ID#95)

Quantification of anthraquinones from *Bulbine natalensis* and dietary supplements using Ultra-high-performance liquid chromatography-photodiode array-mass spectrometry (UHPLC-PDA-MS)

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A validated ultra-high-performance liquid chromatography-photodiode array-mass spectrometry method was developed for the quantification of seven anthraquinone-type compounds from *Bulbine natalensis*. The separation was achieved using a reversed phase (C-18) column, photodiode array detection, and a gradient of water/acetonitrile as the mobile phase. The seven compounds could be separated within 15 minutes using the UHPLC method with detection limits of 25 ng/mL with 2 µL injection volume. The analytical method was validated for linearity, repeatability, accuracy, limits of detection (LOD) and limits of quantification (LOQ). The relative standard deviations (RSD) for intra- and inter-day experiments were less than 5% and the recovery efficiency was 98-101%. Nine supplements labeled as containing *B. natalensis* were examined. Five of nine products contained anthraquinone-type compounds with total content ranging from 11.3 to 90.4 mg per daily dose. Compounds of *B. natalensis* were not detected in the four of nine supplements. The analytical method is simple, economic, rapid and especially suitable for quality control analysis of *B. natalensis*. LC-mass spectrometry coupled with electrospray ionization (ESI) was used for the identification and confirmation of compounds in plant samples and dietary products.

Acknowledgments
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PA-28 (ID#96)

A Comprehensive workflow for the analysis of bio-macromolecular supplements: A case study of twenty whey protein products

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The presence of bio-macromolecules as major ingredients is a primary factor in the marketing of many biologically derived macromolecular supplements. Workflows for the analysis of these supplements for quality assurance, adulteration, and other supply chain difficulties must include the analysis of both small molecule content and the macromolecular components. Twenty whey protein supplements were analyzed using an integrated workflow to identify protein content, protein adulteration, inorganic elemental content, as well as the macromolecular and small molecule profiles with orthogonal analytical methods including NMR profiling, LC-DAD-QToF analysis of small molecule components, ICP-MS analysis of inorganic elemental content, determination of protein content by a Bradford assay, SDS-PAGE protein profiling and bottom-up shotgun proteomic analysis by LC-MS/MS. The use of orthogonal, an integrated workflow allowed the detection of crucial product characteristics that would have remained unidentified using traditional workflows involving either analysis of small molecule nutritional supplements or protein analysis.

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PA-29 (ID#97)

Quantitative determination of curcuminoids from *C. longa* samples using UHPLC-PDA-MS and non-targeted analysis using liquid chromatography-diode array detector-quadrupole time of flight mass spectrometry (LC-DAD-QToF MS)

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Curcuma, an important genus in the family Zingiberaceae consisting of about 110 species and distributed in tropical Asia and the Asia-Pacific regions. According to the Indian Pharmacopoeia (1996), dried turmeric rhizomes should contain not less than 1.5% of curcumin (*w/w*). The Pharmacopoeia of People's Republic of China (2005) requires no less than 1.0% of curcumin content (*w/w*) in dried turmeric rhizomes. Curcuminoids in turmeric are primarily accumulated in rhizomes. Curcumin, demethoxycurcumin, and bisdemethoxycurcumin have been used as marker compounds for the quality control of rhizomes, powders, and extract as well dietary supplements, Ar-turmerone, a-turmerone, and b-turmerone may be used to control the product quality of turmeric oil and oleoresin products. Quantification of curcuminoids, ar-turmerone, tetrahydrocurcumin and sudan-I from *C. longa*, and dietary supplements was carried out using UHPLC-PDA-MS. The total content of curcuminoids (curcumin, desmethoxycurcumin, bisdesmethoxycurcumin) was found to be in the range from 1-7.2% and 0.3-1183 mg/daily dose in *C. longa* and dietary supplements, respectively. No dyes were detected in analyzed dietary supplements. Non-Targeted analysis was performed for *C. longa* and dietary supplements using LC-QToF-MS and 23 compounds were identified from *C. longa* samples.

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PA-30 (ID#98)

Comparative analysis of five *Salvia* species using LC-DAD-QToF

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Several *Salvia* species, commonly known as sage plants, are an integral part of various culinary and folklore preparations for the perceived wide range of effects from organoleptic to psychological. As a result, many of these species are an integral part of botanical drug applications, highlighting the need for accurate identification and quality control for consumer's safety. Five

closely related *Salvia* species (*S. officinalis*, *S. miltiorrhiza, S. divinorum*, *S. mellifera*, and *S. apiana*) within a same botanical family were analyzed and differentiated using LC-QToF. Accurate mass measurement (< 5 ppm) of protonated and deprotonated molecules together with resulting fragments and product ions allowed unequivocal or tentative identification of more than 180 compounds either by comparison with reference standards or literature data. The aerial plant parts were identified based on various phenolic acids, flavonoids as well as di- and tri-terpenoids. Polyphenolics, *viz.,* salvianolic A/B and rosmarinic acids in *S. officinalis*, lipophilic diterpenoids, viz., tanshinones in *S. miltiorrhiza,* and abietatriene diterpenes and triterpenoids (ursane-/olean-type) in *S. mellifera,* and *S. apiana* were identified as characteristic, significant components. In comparison, salvinorins and divinorins representing a class of neoclerodane diterpenoids were detected only in *S. divinorum*. The presented methodology can successfully be applied to qualitatively assess sage-based ingredients in various finished products and formulations.

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PA-31 (ID#99)

Quantitative determination and characterization of polyphenols from *Cissus quadrangularis* L. and dietary supplements using UHPLC-PDA-MS, LC-QToF and HPTLC

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Stem and leaf of *Cissus quadrangularis* L. (Vitaceae), indigenous to Asia and Africa, were used for both medicinal and dietary purposes but limited information is available about the phytochemistry of the plant. Stem and leaf samples were assessed for the simultaneous determination of polyphenolic compounds (catechin, epicatechin, quercetin-3-*O*-*β*-glucopyranoside, kaempferol-3-*O*-*β*-glucoside, quercetin-3-*O*-*β*-rhamnoside, leachianol F, amurensin A, pallidol, resveratrol, and quadrangularin A), using UHPLC-PDA-MS. The validation data showed that the method is precise, specific, accurate and linear over the range of 0.5-100 µg/mL. Reversed phase ultra-high-performance liquid chromatography (UHPLC) fingerprints of the crude methanolic stem and leaf extracts of *C. quadrangularis* were obtained at different wavelengths based on their l_{max}. Polyphenolics were characterized using both UHPLC-PDA-MS and LC-QToF analysis. From liquid chromatography quadrupole time of flight-electrospray ionization mass spectrometry (LC-QToF) spectra, over 40 components were structurally correlated and confirmation was based on the fragmentation characteristics and also from the information available in the literature. An HPTLC method was also developed for the rapid chemical fingerprint analysis of *Cissus* samples.

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PA-32 (ID#100)

Chemical profiling and characterization of phenolic acids, flavonoids, terpene glycosides from *Vangueria agrestis* using Ultra-High-Performance Liquid Chromatography/Ion Mobility Quadrupole Time-of-Flight Mass Spectrometry and metabolomics approach

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Vangueria agrestis is a shrub indigenous to tropical Africa, belonging to family Rubiaceae and traditionally is used as a decoction for treatment of fever, pain and malaria. The current study was undertaken to investigate the chemical constituents based on precursor exact mass and fragment ion information. The chemical profiling and structural characteristics of chemical constituents from methanolic extracts of dried aerial and roots of *V. agrestis* and dietary supplements were analyzed using UPLC-QToF coupled with UNIFI platform and multivariate analysis in both negative and positive ion modes. A non-targeted UPLC/MS analysis was carried out to profile the chemical constituents of crude extracts of *V. agrestis* and seventy-three compounds including reference compounds were identified. The fragments of flavonoids, monoterpene and triterpene glycosides revealed the characteristic cleavage of glycosidic linkages, and the fragmentation pattern provided identity of the sugars. This analytical method provides a fast method for quality assessment of dietary supplements. Finally, a chemometrics approach with multivariate statistical tools was used to visualize the differences between root and aerial parts of plant samples and to find the potential chemical markers that differentiate among these parts of *V. agrestis* samples and dietary supplements.

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PA-33 (ID#101)

Chemical fingerprinting and differentiation of *Panax ginseng*, *Panax notoginseng* and *Panax quinquefolius* using liquid chromatography-diode array detector-quadrupole time of flight mass spectrometry (LC-DAD-QToF MS)

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The roots of *Panax ginseng* (PG), *Panax notoginseng (PN)* and *Panax quinquefolius (PQ) were used in traditional medicine. All these roots contain similar ginsenosides (triterpenoid saponins) and other chemical constituents. Ginsenosides are the characteristic and principal components having various pharmacological activities.* American ginseng is substituted and/or adulterated by other cheaper species appears to be due to a considerable price difference in North America and China. Furthermore, in the market, American ginseng cultivated in China is often labeled as cultivated in North America. Therefore, accurate identification the species of American ginseng and discrimination of cultivation region are essential for quality control.

In addition, the ratios of Rg1/Rb1 and Rb2/Rb1 have been widely used to differentiate these two ginsengs. Ratios of Rg1/Rb1 less than 0.3 and Rb2/Rb1 less than 0.4 are indicative of American ginseng [14]. In contrast, significantly higher values of both ratios are characteristic of Asian ginseng. *A qualitative analysis was performed for the compound identification to differentiate these three species using* LC-DAD-QToF fingerprinting method. A total of 103 Ginsenosides (74 PG, 62 PN, 71 PQ) were detected in the extracted ion chromatograms. Secondly the samples were clustered into groups by chemometric analysis using PLS-DA and OPLS-DA models and 15 diagnostic markers were identified. The developed method can be applied for the quality assessment of Ginseng samples and dietary supplements.

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PA-34 (ID#105)

Solid-phase Microextraction (SPME): Method Development, Validation, and Application to *In vitro* Metabolism Study

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Although many technological advances have been made in the field of bio-analytical chemistry, sample preparation remains both a challenging and time-consuming process. Many techniques often require that the analyte(s) be removed from any sample matrix in order to be analyzed by instrumentation. Due to the complexity of many biological matrices, thorough analyte extraction can be a major challenge. Headspace solid phase microextraction (HS-SPME) is a rapid, sensitive, solvent-free, and economical method of extracting analytes from a variety of matrices by partitioning them from a liquid or gaseous sample into an immobilized stationary phase. Thus, HS-SPME eliminates the need to separate the analyte(s) of interest from biological matrices. Our goal was to develop and validate a HP-SPME method coupled with GC/Q-ToF in order to quantitate the in vitro metabolism of β-caryophyllene by both human liver microsome (HLM) and S9 liver fractions.

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PA-35 (ID#106)

Quality evaluation of copaiba essential oil/oleoresin using GC/MS combined with chemometric analysis

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For thousands of years in tropical areas of the world, essential oil (EO)/oleoresin obtained from members of the genus Copaifera has been used as a traditional medicine. Although primarily used for its purported anti-inflammatory and anti-septic properties, the EO/oleoresin has also been utilized to treat a variety of urinary, skin, and respiratory conditions by

practitioners of traditional medicine. In addition to its use as a traditional medicine, the oleoresin has also been used in a wide range of cosmetic and pharmaceutical preparations such as soaps, perfumes, ointments, and oral products. The yellow to light brown oleoresin is obtained through a perforation in the trunk of the tree, often being mixed with additional oleoresin obtained from several trees, while the EO is a distillation product of resin. Since the chemical composition of EO/oleoresin from different species can vary, standardization of Copaifera resin is challenging. Due to the time-consuming extraction process and the limited availability of the resin, product adulteration often occurs in order to reduce product cost and to maximize profits. Two methods of oleoresin adulteration have been reported: 1) adding either mineral or vegetable oil to authentic resin; or 2) adding cheaper essential oils of other plants which are similar in odor and density. Currently, the lack of an established standard for the chemical composition of copaiba oleoresin being sold to consumers poses both a health and safety risk. With this in mind, it is our goal to develop methods for quality control and product standardization. With the establishment of quality control methods, both the health and safety of consumers can be enhanced.

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PA-36 (ID#107)

GC/Q-ToF and chemometric analysis of five *Salvia* species

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Members of the plant genus, *Salvia*, have a long and rich history of use as a medicinal herb. Members of the genus *Salvia* have been purported to possess a wide range of pharmacological properties ranging from anti-inflammatory and anti-nociceptive to psychoactive effects. These pharmacological properties are varied among the members of the genus *Salvia*. With nearly 900 species included in the genus *Salvia*, identifying plant material and products can be a daunting task. Although GC/MS identification of *Salvia* species has been established as a means of species identification, it is often a time-consuming task which does not lend itself to high throughput applications. With this information in mind, our goals were to extract and identify marker compounds present in five species of *Salvia* (*apiana, divinorum, mellifera, miltiorrhiza*, and *officinalis*) utilizing gas chromatography/quadrupole time-of-flight mass spectrometry (GC/Q-ToF), to perform a principal component analysis (PCA) in order to differentiate *Salvia* species, as well as to establish a sample class prediction model (SCP) for quality evaluation of *Salvia*-based commercial products.

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PA-37 (ID#109)

Determination of five residual solvents in cannabis products by GC-MS

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The interest in medicinal cannabis has increased exponentially during the last few years. During the preparation of cannabis-derived products, many organic solvents are used with hexane and ethanol being the main solvents used in the preparation of cannabis extract. The marc following hexane extraction is washed with ethanol to produce placebo plant material. Hexane, ethyl acetate, and ethanol are used during the preparation of cannabidiol (CBD). Five solvents were involved in the preparation of THC including ethanol, methylene chloride, hexane, ethyl acetate, and methanol. So, there is a need to analyze the quality of cannabis-derived products for residual solvents to ensure the safety of these products for human consumption. The main target of most solvent-dependent procedures is to minimize the final residual solvents in the products as regulated by the guidelines of the U.S. Pharmacopeial Convention and the Food and Drug Administration.

In this study, a fast, accurate, and sensitive gas chromatography-mass spectrometric detection (GC-MS) method was developed and validated for the detection and quantitation of five residual solvents, namely, methanol, ethanol, dichloromethane, *n*-hexane, and ethyl acetate in cannabis-derived products. Here, a simple protocol is developed for the analysis of multi-solvent standard mixtures in less than nine minutes using DB-5ms (30 m x 250 µm x 0.25 µm). The method was linear over the range of 100-5000 ppm with $R^2 \geq 0.99$. The LOD and LOQ were 50 and 100 ppm, respectively. The method accuracy (% recovery) for all solvents ranged from 85%-116% and the precision (% RSD) was less than 15%. The method was also applied to determine the solvent residues in cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (Δ^9-THC) (both as active pharmaceutical ingredients), cannabis extracts of different varieties, and for placebo plant material. The method is accurate, reproducible, sensitive, and suitable for the determination of residual solvents in cannabis-derived products.

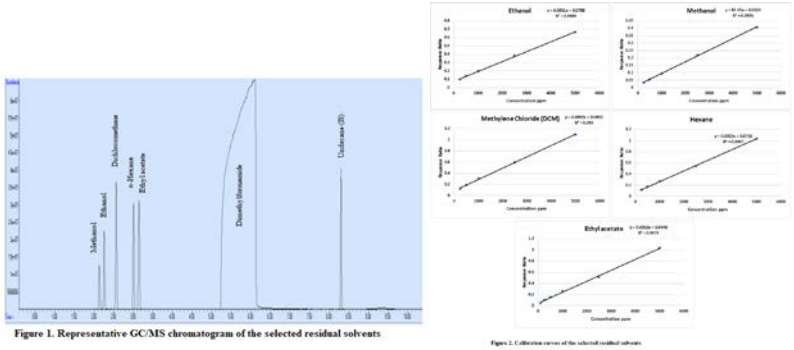

Figure 1. Representative GC/MS chromatogram of the selected residual solvents

Figure 2. Calibration curves of the selected residual solvents

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PA-38 (ID#110)

HPTLC fingerprint analysis for authentication and quality evaluation of *Prunus africana* and its dietary supplements

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Prunus africana is an economically important and endangered tree species in Africa listed in CITES Appendix list II. The bark of *P. africana* is popularly used as dietary supplement for benign prostatic hypertrophy. However, these supplements are not strictly regulated for efficacy or safety prior to sale. To develop HPTLC fingerprint method for *P. africana* bark, and apply the developed method to evaluate the authenticity of commercial *P. africana* products. HPTLC method was optimized to resolve the major compounds. Ethyl acetate: Chloroform: Methanol: Water: Formic acid (6:8:1.2:0.8:2) was used as the developing solvent and the plates were derivatized using 0.5% Vanillin in sulfuric acid and ethanol. The plates were examined under white light, Rf values and images were recorded using Camag TLC visualizer and VisionCATS. Three pattern of HPTLC fingerprint profiles

was observed among the 93 samples, and the samples displayed varying concentrations of secondary metabolites which in turn resulted in differing fingerprint patterns. Analysis of commercial products revealed that eight products labelled to contain *P. africana* bark did not show the presence of *P. africana* bark rather shown only β-sitosterol as a major compound in six products, and two products were similar to saw palmetto. On the other hand, all the analyzed *P. africana* commercial bark powders and seven capsules resulted in similar HPTLC fingerprint of the authentic *P. africana*. However, one out of ten bark powder and nine out of twelve capsule and all the three tablet products showed several fold higher concentrations of β-sitosterol which was not observed in authentic *P. africana* bark extracts. The developed HPTLC fingerprint for *P. africana* will be suitable for fast authentication and visual comparison of the differences among various *P. africana* samples collected in different region, and aids in authentication of *P. africana* commercial products.

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PA-39 (ID#121)

GC-FID method for 20 different acidic and neutral cannabinoids

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For decades, *Cannabis sativa* had been illegal to sell or consume around the world, including the United States. However, in light of the recent 2018 Farm Bill and the legalization of hemp across the US, various cannabis preparations have flooded the market, making it absolutely vital to be able to quantitate the levels of the different acidic and neutral cannabinoids in *C. sativa* to have a complete cannabinoid profile of the plant. A GC-FID method was developed and validated for the analysis of 20 acidic and neutral cannabinoids, namely cannabidivarinic acid (CBDVA), cannabidiolic acid (CBDA), cannabinolic acid (CBNA), cannabielsoinic acid (CBEA), cannabicyclolic acid (CBLA), cannabichromenic acid (CBCA), trans-Δ^9-tetrahydrocannabivarianic acid (Δ^9-THCVA), trans-Δ^9-tetrahydrocannabinolic acid A (Δ^9-THCA), cannabigerolic acid (CBGA), cannabidivarian (CBDV), trans-Δ^9-tetrahydrocannabivarian (THCV), cannabidiol (CBD), cannabicyclol (CBL), cannabichromene (CBC), trans-Δ^8-tetrahydrocannabinol (Δ^8-THC), trans-Δ^9-tetrahydrocannabinol (Δ^9-THC), cannabigerol (CBG), cannabinol (CBN), cannabitriol (CBT), and cannabielsoin (CBE). The method was applied for the analysis of different cannabis varieties grown at the University of Mississippi, National Center for Natural Products Research (NCNPR). The developed method is simple, sensitive, and reproducible for the quantitation of all 20 acidic and neutral cannabinoids with a limit of detection (LOD) as low as 0.1 ppm while limit of quantitation ranged from 0.25 ppm-0.5 ppm.

PA-40 (ID#122)

Rapid LC-MS/MS method for analysis of THC-COOH and THC-COOH glucuronide in human urine samples

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While cannabis (*Cannabis sativa*) remains federally illegal, many states have legalized medical marijuana. As a total of 37 states and four territories have regulated cannabis for medical use only, there is a need to analyze Δ^9-Tetrahydrocannabinolic acid (THC-COOH) and tetrahydrocannabinolic acid glucuronide (THC-COOH-GLU) as metabolites of Tetrahydrocannabinol (THC). We developed and validated a rapid, and sensitive LC-MS/MS method for the determination of tetrahydrocannabinolic acid (THC-COOH) and tetrahydrocannabinolic acid glucuronide (THC-COOH-GLU) in human urine samples. Urine samples were prepared by dilute and shoot technique, to eliminate tedious sample preparation. Furthermore, the target analytes were separated in just less than 4 minutes using a gradient elution of 0.1 % formic acid in water (mobile phase A) and 0.1 % formic acid in acetonitrile (40%- 90% B). The separation was achieved using Phenomenex C18 Synergi Hydro –RP (100A°, 50x3.00 mm) column. D6-THC-COOH was used as internal standard for the quantitation of THC-COOH and THC-COOH-GLU. The method was validated according to ICH guidelines, with respect to linearity (R^2 was ≥ 0.999) in the range of 1.0-100 ng/mL for both THC-COOH and THC-COOH-GLU. The limits of detection (LOD) and limits of quantitation (LOQ) were found to be 0.1 and 0.5 ng/mL. The method intraday and the interday precision (%RSD) was less than 9% for both THC-COOH and THC-COOH-GLU, while the method accuracy (calculated as % recovery) ranged from 93%- 108% for THC-COOH and 94%-103% for THC-COOH-GLU. The method was found to be simple, accurate, and precise when used for the determination of THC-COOH and THC-COOH-GLU in human urine samples submitted to our laboratory for analysis.

PA-41 (ID#128)

NMR approach for quality assessment of Copaiba oil

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Copaiba oil (oleoresin and essential oil) has been traditionally used for the treatment of cystitis, urinary incontinence, gonorrhea, syphilis, leishmaniasis, respiratory ailments, infections in the skin and mucosa, ulcers and lesions of the uterus, leucorrhea, anemia, headaches, and snake bites. It has also been used in the cosmetic industry as a fixative for perfumes and perfuming soaps. As an emollient, bactericidal, and anti-inflammatory agent, copaiba oil is used in the production of soaps, lotions, creams and moisturizers, bath foams, shampoos, and hair conditioners. Copaiba oil is obtained from *Copaifera* trees. There are more than 70 *Copaifera* species of copaiba trees distributed throughout the world, mostly in South and Central America. *C. langsdorffii, C. officinalis, and C. reticulata* are the most important commercial sources of copaiba oil, and the most prized copaiba oils are rich in *β*-caryophyllene. More than 230 constituents have been reported from *Copaifera* species. The chemical composition of copaiba oils obtained from different *Copaifera* species varies significantly. Adulteration of Copaiba oil by the addition of cooking oils or other cheap oils was reported. So far, there are no official standards for Copaiba essential oil or oleoresin existed, leading to difficulties in the quality control and the safety assurance of the products. The aims of this study are: i) to evaluate the variation and distribution of chemical composition in copaiba oil samples; ii) to detect the outlier samples and explore possible adulteration based on the NMR profile information. As the results, significant variation of the chemical composition was observed for some of the investigated samples by comparing their NMR spectral fingerprints, and the samples were classified into four groups (A – D) by the PCA analysis. 20 out of the 36 (~55.6%) commercial samples obtained from the market were found to be adulterated. Among those 20 commercial samples, 17 were adulterated with triglycerides, and three were identified to be adulterated with triethyl citrate or plasticizers (diethylhexyl phthalate and diethyl phthalate) based on 2D NMR analysis.

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PA-42 (ID#129)

Metabolite variation of licorice species and discrimination by HPTLC and NMR

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The roots and rhizomes of several *Glycyrrhiza* species are widely used as sweetening and flavoring agents in food, as well as important ingredients in formulations of traditional medicines [1]. Five *Glycyrrhiza* species, *G. uralensis*, *G. glabra*, *G. inflata*, *G. echinata*, and *G. lepidota* often share the name "licorice roots" in the botanicals' marketplace. Unfortunately, misidentification/mis-labelling is very common due to their similarities in morpho-anatomical features. Significant metabolite alterations among the different *Glycyrrhiza* species and their hybrids have been reported [2], suggesting that the biological activities could vary with the use of the licorice roots or products derived from different species. Developing simple and effective methods for species identification and differentiation is of key importance. In this study, 78 licorice samples were investigated by using HPTLC and NMR as analytical tools. Significant metabolite variations were observed between the five species. The species-specific fingerprint patterns for the five *Glycyrrhiza* species were determined with HPTLC and NMR; then applied to the sample identification and discrimination. The results obtained from these two orthogonal analytical methods were in consonance with each other. Furthermore, the NMR signals and the species-specific constituents that made significant contributions to the differentiation of the five *Glycyrrhiza* species were confirmed on the basis of the multivariate analysis of the NMR spectral data. Using the established OPLS-DA models, the classification of hybrids was evaluated and confirmed. The developed methods, particularly the HPTLC method with its simplicity and low cost, could be used as a rapid and reliable approach for the authentication of licorice species and quality control of licorice raw material and products.

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PA-43 (ID#133)

Quantitative determination and validation of 15 cannabinoids in cannabis plant materials and marketed products using HPLC/PDA

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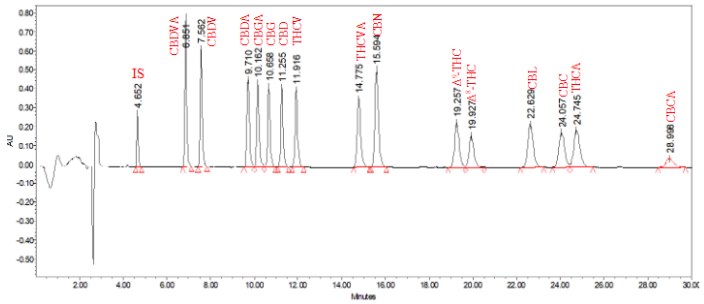
The increase in the production of cannabis for medical and recreational purposes in recent years has led to a corresponding increase in laboratories performing cannabinoid analysis of cannabis plant materials and marketed products. A validated analytical method using liquid chromatography coupled with PDA detector (HPLC-PDA) was established for the determination of 15 cannabinoids. The developed method is simple, reliable, specific, and accurate for the analysis of 15cannabinoids in cannabis plant materials and marketed products. The 15 cannabinoids are delta-9-tetrahydrocannabinol (Δ9-THC), delta-9-tetrahydrocannabinolic acid (Δ9-THCA), cannabidiol (CBD), cannabidiolic acid (CBDA), cannabidivarin (CBDV), cannabidivarinic acid (CBDVA), cannabigerol (CBG), cannabigerolic acid (CBGA), cannabichromene (CBC),

cannabichromenic Acid (CBCA), delta-9-tetrahydrocannabivarin (THCV), delta-9-tetrahydrocannabivarinic acid (THCVA), cannabicyclol (CBL), delta-8-tetrahydrocannabinol (Δ8-THC) and cannabinol (CBN). The analytical method was validated using a Waters Alliance HPLC System with a 2695 separation module and a 2996photodiode array (PDA) detector. A reverse-phase Luna®-C18(2)100 Å column (250x4.6mm ID, 3.0 µm), maintained at 40°C was used for the separation. A mobile phase of 75% acetonitrile and 0.01% formic acid in water was used isocratically for elution. Linearity of calibration curves in methanol was demonstrated with regressionvaluesR2≥ 0.999in the concentration range of 1.0–125µg/mL for all cannabinoids except CBCA (5-125µg/mL). LOQ of 1 µg/mL was achieved for all cannabinoids except CBCA which was 5µg/ml. No peaks or carryover were observed in methanol blanks before or after LOQ injections and S/N results were ≥ 10 for all cannabinoids at the LOQ level. Precision results (%RSD) were ≤ 10.1% and accuracy results (%recovery) ranged from 82%-111%, for the 15 cannabinoids. Forty-nine (49) cannabis plant materials of different varieties as well as eight E-cigarettes were analyzed using this method and the result will be represented in this poster. In conclusion, the developed HPLC method is suitable for a wide range of applications including routine analysis for neutral and acid cannabinoids in plant materials and for the analysis of marketed products for their cannabinoids content and compliance with label values.



PB-1 (ID#3)

Network-based pharmacology study reveals protein targets for medical benefits and harms of cannabinoids in humans

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This network-based pharmacology study intends to uncover the underlying mechanisms of cannabis that lead to a therapeutic benefit and the pathogenesis for a wide range of diseases claimed benefited from or caused by the use of the cannabis plant. Cannabis contains more than 600 chemical components. Among these components, cannabinoids are well-known to have multifarious pharmacological activities. In this work, twelve cannabinoids selected as active compounds through text mining and in silico prediction of drug-like properties were used for initial protein-target retrieval from the pharmacophore database. The disease-associated biological functions and pathways were enriched through GO and KEGG databases. Various biological networks [i.e., protein-protein interaction, target-pathway-disease, and target-(pathway)-target interaction] were constructed, and the functional modules and essential protein targets were elucidated through the topological analyses of the networks. Our study revealed that eighteen proteins (CAT, COMT, CYP17A1, GSTA2, GSTM3, GSTP1, HMOX1, AKT1, CASP9, PLCG1, PRKCA, PRKCB, CYCS, TNF, CNR1, CNR2, CREB1, GRIN2B) are essential targets of eight cannabinoids (CBD, CBDA, Δ^9-THC, CBN, CBC, CBGA, CBG, Δ^8-THC), which involve in a variety of pathways resulting in beneficial and adverse effects on the human body. The molecular docking simulation confirmed that these eight cannabinoids bind to their corresponding protein targets with high binding affinities. This study generates a verifiable hypothesis of medical benefits and harms of key cannabinoids with a model which consists of multiple components, multiple targets, and multiple pathways, which may be used to deploy preclinical and clinical studies of cannabis.

PB-2 (ID#4)

Unraveling the molecular mechanisms of fructus anisi stellati as remedy for infantile colic by network pharmacology

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Fructus anisi stellati (FAS) is an anise-scented star-shaped fruit from *Illicium verum* tree. It is commonly consumed in many cultures as food and medicine, particularly as a remedy for infantile colic (IC). The elucidation of molecular mechanisms of action would contribute to the understanding of the traditional therapy of FAS and help to guide the preclinical and clinical study of this herb. This work aims to investigate the key therapeutic compounds of FAS and to explore the underlying molecular mechanisms of FAS therapy. The chemical components of FAS were obtained through data mining on TCMSP and ADME screening, and the common protein targets of the FAS compounds and the IC-correlated diseases were obtained from PharmMapper, GeneCards, and OMIM databases. GO and KEGG databases were used for the enrichment of molecular functions and pathways. Cytoscape was used for network construction and analysis. SystemsDock was used for molecular docking. Three key compounds *(i.e.,* quercetin, luteolin, and kaempferol), 19 targets, 7 molecular pathways, and 12 IC-correlated diseases were identified to be involved in the molecular mechanisms of FAS for the treatment of IC. Our study showed that three therapeutic modules were primarily engaged in the molecular mechanisms of FAS for IC therapy, including inhibition of inflammatory reactions, stimulating immunoglobulin A production in the gastrointestinal tract, and enhancing the secretion of digestive enzymes.

PB-3 (ID#6)

Assessment of herb-drug interaction potential of five common species of licorice and their phytochemical constituents

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Dried roots and rhizomes of *Glycyrrhiza* species (*G. glabra*, *G. uralensis*, and *G. inflata*), commonly known as licorice, have long been used in traditional medicine. In addition, two more species, *G. echinata* and *G. lepidota* are also considered “licorice” in select markets. Currently, licorice is used as a sweetener, flavoring, or masking agent in many finished products, and it is an integral part of several botanical drugs and dietary supplements. To probe the botanicals’ safety and their herb-drug interaction potentials, the hydroethanolic extracts of five *Glycyrrhiza* species and some of their constituents were investigated in terms of their effects on pregnane X receptor (PXR), aryl hydrocarbon receptor (AhR), two major CYP450 isoforms (3A4 and 1A2), and the metabolic clearance of two antiviral drugs in hepatocytes. All extracts induced transcriptional activity of PXR and AhR (>2-fold) and increased the activity of CYP3A4 and CYP1A2. The highest increase in CYP3A4 was seen with *G. echinata* (4-fold), and the highest increase in CYP1A2 was seen with *G. uralensis* (18-fold) and *G. inflata* (16-fold). Among the constituents, glabridin, licoisoflavone A, glyasperin C, and glycycoumarin activated PXR and AhR, with glabridin being the most effective (6- and 27-fold, respectively). Licoisoflavone A, glyasperin C, and glycycoumarin increased 3A4 activity while glabridin, glyasperin C, glycycoumarin, and formononetin increased 1A2 activity (>2-fold). The metabolism of two antiretroviral drugs, rilpivirine, and dolutegravir, was increased by *G. uralensis* (2-2.5-fold) and its marker compound glycycoumarin (2.3-1.6-fold). The metabolism of dolutegravir was increased by *G. glabra* (2.7-fold) but not by its marker compound, glabridin. These results suggest that

licorice and some of its phytochemicals could affect the metabolism and clearance of certain drugs that are substrates of CYP3A4 and 1A2 isozymes.

Acknowledgments
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PB-4 (ID#7)

Evaluation of the herb-drug interaction potential of commonly used botanicals on the US market with regard to PXR- and AhR-mediated influences on CYP3A4 and CYP1A2

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In this study, hydroethanolic extracts of 30 top-selling botanicals (plants/herbs) commonly used as herbal dietary supplements in the US were screened for activation of the human pregnane X receptor (hPXR) and human aryl hydrocarbon receptor (hAhR) as well as the activities of hPXR- and hAhR-regulated drug metabolizing cytochrome P450 enzymes (i.e., CYP3A4 and CYP1A2, respectively). Of the 30 botanicals tested, 21 induced PXR and 29 induced AhR transcriptional activities. Out of the 21 botanicals that induced hPXR transcriptional activity 14 yielded >50% induction in CYP3A4 activity at concentrations ranging from 6-60 µg/mL and 16 botanicals out of the 29 botanicals that activated hAhR yielded >50% induction in CYP1A2 activity at concentrations ranging from 3-30 µg/mL. Moreover, eight botanicals (*G. gummi-gutta* [garcinia], Hemp [low and high CBD content], *H. perforatum* [St. John's wort], *M. vulgare* [horehound], *M. oleifera* [moringa], *O. vulgare* [oregano], *P. johimbe* [yohimbe] *and W. somnifera* [ashwagandha]) yielded >50% induction in both CYP3A4 and CYP1A2 activity. Herbal products are mixtures of phytoconstituents, any of which could modulate drug metabolism. Our data reveals that several top-selling botanicals pose a risk for herb-drug interactions (HDI) via CYP450 induction. While *in vitro* experiments can provide useful guidance in assessing a botanical's HDI potential, its clinical relevance needs to be investigated *in vivo*. Botanicals whose effect on hPXR/CYP3A4, and hAhR/CYP1A2 activity were most pronounced will be slated for further clinical investigation.

Acknowledgments
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PB-5 (ID#8)

Role of *Agrilus* sp. (Coleoptera, Buprestidae) in the production of Brazilian red propolis

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The species *Dalbergia ecastaphyllum* (Fabaceae) is well established as the primary botanical source of the Brazilian red propolis, along with *Symphonia globulifera* (Clusiaceae), as bees collect a reddish resin from the stems of these species to produce the red propolis. This species occurs in the coastal dune and mangrove ecosystems, where local beekeepers install their beehives for propolis production. However, some evidence suggested that the reddish resin available in *D. ecastaphyllum* stems is not produced spontaneously but induced by the presence of a parasitic insect that feeds on the stems of this species. Therefore, field research in apiaries of COPAER beekeepers' association was carried out in March and November 2019, which led to the capture of a new species of an insect belonging to the genus *Agrilus* (Coleoptera, Buprestidae). The expedition results confirmed that the production of the reddish resin of *D. ecastaphyllum*, rich in isoflavonoids, occurs only when adult insects of *Agrilus* sp. emerge from the plant stem by making a hole in which the plant exudates the resin. The chromatographic profiles of resin and plant sources will also be discussed.

Acknowledgments
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PB-6 (ID#18)

Antioxidant and in-vitro anti-diabetic activities of ethanolic leaf extract of *Nephrolepis exaltata* (l.) Schott (nephrolepidaceae)

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Diabetes mellitus is amongst the main diseases prevailing worldwide. α–amylase and α- glucosidase are responsible for postprandial glucose levels, thus interesting and novel therapeutic targets for diabetes mellitus treatment. This study aimed at investigating the antioxidant and *in vitro* antidiabetic potentials of the ethanolic leaf extract of *Nephrolepis exaltata.* The antioxidant assay was carried out using the DPPH free radical scavenging activity and lipid peroxidation assay while the antidiabetic assay was carried out using α-amylase and α-glucosidase inhibitory activity. The percentage inhibitions were calculated. Statistical analyses were carried out using two-way ANOVA (mixed model) at an alpha value of α = 0.05 (95%). Phytochemical screening showed the presence of phenols, tannins, flavonoids, alkaloids, resins, saponins, and carbohydrates. Radical scavenging assay showed no statistically significant difference at high concentration compared to the standard with IC50 of 5.83 µg/ml (DPPH assay (standard, 3.9 µg/ml)) and 0.04 µg/ml (lipid peroxidation assay (standard 0.02 µg/ml)). The α-amylase inhibition assay showed there is no statistically significant difference across all the concentrations with IC50 values of 0.02 µg/ml compared to 0.03µg/ml of the standard. For the α-glucosidase assay, the extract had inhibition comparable to the standard with no statistically significant difference at high concentrations (IC50 values of 0.05 µg/ml compared to 0.05 µg/ml of the standard). The ethanolic leaf extracts of *N. exaltata* possessed antioxidant activities useful in fighting free radicals which can cause oxidative cell damage and inhibited α–amylase and α-glucosidase which are responsible for postprandial glucose levels; therefore, the plant's extracts might decrease the postprandial blood glucose levels, thus being an interesting and novel therapeutic target for diabetes mellitus management.

PB-7 (ID#19)

Red Brazilian propolis: Antimicrobial potential against bacteria responsible for dental caries

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Brazilian red propolis produced by *Apis mellifera* has great economic importance due to its biological properties, including the activity against microorganisms causing human diseases. Thus, the hydroalcoholic extract of Brazilian red propolis (EBRP) was tested *in vitro* against a panel of bacteria responsible for dental caries. The results revealed that, EBRP showed promising antibacterial activity against all evaluated oral pathogens (OP). Then, nine metabolites were isolated from EBRP and also evaluated against OP, and oblongifolin B (1) was the most effective one (minimal bactericidal concentration values lower than 10.0 µg.mL-1). In addition, 1 in concentrations equal to or lower than 25.0 µg. mL-1, inhibit the biofilm formation of *Streptococcus sobrinus* (ATCC 33478), *Streptococcus mitis* (ATCC 49456), *Streptococcus mutans* (ATCC 25175), *Streptococcus salivarius* (ATCC 25975), *Streptococcus sanguinis* (ATCC 10556), *Lactobacillus casei* (ATCC 11578), and *Enterococcus faecalis* (ATCC 4082) in 50% or more. The results described here pointed out 1 as a natural prototype for further medicinal chemical studies against bacteria responsible for dental caries.

Acknowledgments

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PB-8 (ID#20)

Macropatterns and chemical compositions of crystals in the genus *Baccharis* (Asteraceae)

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Calcium oxalate crystals occur rather frequently in the plant kingdom. The crystals have various functions depending on their form and occurrence in plants, including defense against herbivory, cellular ion balance, detoxification of oxalic acid or heavy metals, calcium regulation, and light gathering and reflection. The size and location of the crystals may be influenced by physical, chemical, and biological circumstances such as ion concentration, temperature, pressure, pH, and herbivory. However, crystal formation in plants is under genetic control, hence the presence or absence of crystals in tissues or organs and their morphotypes are considered taxonomic features. Morphologies and distributional patterns of the crystals in the leaves and stems of 43 species of *Baccharis* were studied using optical and scanning electron microscopy. The chemical compositions of the crystals were determined by energy-dispersive X-ray spectroscopy and Raman spectroscopy. Different crystal shapes, such as prisms, druses, styloids, and sand crystals were present. Various forms of prisms, including tabular, cuneiform, arrow-shaped, trigonal, pyramidal, and bipyramidal shapes, were observed. The presence of two forms of hydration, namely whewellite (CaC2O4.H2O) and weddellite (CaC2O4.2H2O), were observed in the crystals by Raman Spectroscopy. All the crystals found in the studied species of *Baccharis* were formed by calcium oxalate. The crystals were more abundant in the stems than the leaves. Combinations of the morphotypes and occurrences of these crystals can be used to identify and differentiate between the species of *Baccharis*.

Acknowledgments
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PB-9 (ID#21)

Microscopic identification of *Cecropia pachystachya* and its adulterant *Tetrapanax papyrifer*

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Cecropia pachystachya Trécul (Urticaceae), popularly known as “ambay”, is a medicinal plant used in Central and South America as a diuretic, an anti-asthmatic, an anti-diabetic, an anti-inflammatory, and an anti-hypertensive. However, the same vernacular name is also applied to several other plants, such as *Tetrapanax papyrifer* (Hook.) K. Koch (Araliaceae), which exhibit morphological similarities. Due to this confusion, the species is often misidentified or adulterated. The present work provides a comparative leaf anatomy of the two species by optical and scanning electron microscopy. The main anatomical markers of *C. pachystachya* are the papillate adaxial epidermis, non-glandular and uniseriate trichomes on both sides, filariform trichomes and anomocytic stomata on the abaxial side, prismatic crystals on the leaf epidermis, and the petiole with perimedullar fibers. In *T. papyrifer*, 3-branched stellate trichomes on the adaxial leaf surface, 10-branched stellate trichomes and paracytic

stomata on the abaxial face, druses in the palisade parenchyma, and perimedullar and cortical fibers in the petiole are present. These anatomical characteristics can help to differentiate *C. pachystachya* from its adulterant plant *T. papyrifer*.

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PB-10 (ID#22)

Bioprospects of two unconventional edible grains for diabetes management

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Two unconventional edible whole grains used by the Gond and Korku tribes of the Vidarbha region, *Paspalum scrobiculaum* (Kodon) and *Panicum sumatrense* (Kutki), help with type 2 diabetes prevention and management by substituting refined, simple sugars in the diet, as these millets are sources of complex carbohydrates which leads to better blood sugar management compared to refined grains. Also, these take comparatively longer to digest, which results in a steady release of glucose into the bloodstream. Kodon and kutki are good sources of fiber, which helps slow the absorption of glucose.

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PB-11 (ID#28)

Evidence for involvement of TRPV1 receptors and potassium channels in the seizures induced by the alkamide alpha-sanshool

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Several plants of the genus *Zanthoxylum* (Rutaceae) are used as medicines in several native cultures from Africa, America, and Asia. Alkamides have a diversity of biological activities, including insecticidal, antibacterial, antifungal, anti-parasitic, analgesic, and local anesthetic. Alpha-sanshool is an alkamide isolated from the stem bark of *Zanthoxylum liebmannianum* (Engelm.) P. Wilson (Rutaceae), an anti-parasitic Mexican medicinal plant known as Colopahtle (scorpion's plant, in Nahuatl language). Previously, we reported that intraperitoneal administration of alpha-sanshool induced tonic-clonic seizures in mice. The present study was designed to elucidate the convulsive effect of alpha-sanshool and its mechanism of action using well-known convulsive and anticonvulsive drugs in an *in-vivo* approach. Alpha-sanshool showed a potent [ED_{50} ($CL_{95\%}$) = 3.06 (2.92-3.22) mg/kg] and immediate (2 ± 2 seconds) seizure effect after the intraperitoneal administration in mice. Alpha-sanshool was only less potent as a convulsive than strychnine [ED_{50} = 1.53 mg/kg], but more potent than bicuculline (ED_{50} = 4.78 mg/kg), 4-aminopyridine (ED_{50} = 12.10mg/kg), affinin (ED_{50} = 51.74 mg/kg), and pentylenetetrazol (ED_{50} = 73.61 mg/kg). The seizures induced by alpha-sanshool were reduced only by capsazepine and diazoxide, suggesting the involvement of TRPV1 and potassium channels in the convulsant mechanism of this compound. The administration of alpha-sanshool by oral route did not induce seizures. These results suggest that rather than an effect on the CNS, the drug may have a peripheral effect on nervous tissue.

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PB-12 (ID#29)

Valeiridoside, an iridoid xyloside from *Valeriana procera* with anxiogenic effect in mice

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Mexican valerian (*Valeriana procera* Kunth, syn. *V. edulis* ssp. *procera* (Kunth) F.G. Meyer, Caprifoliaceae) has been widely used in Mexican traditional medicine and the pharmaceutical industry to prepare phytomedicines expended as mild sedatives, tranquilizers, and sleep-aid agents. Pharmacological studies of this plant have demonstrated sedative, anxiolytic, and vasorelaxant effects. The chemical investigation of the ethanol extract prepared with the roots of this plant has led to the isolation of an iridoid xyloside which was named valeiridoside, a β-D-xyloside of the iridolactone patriscabrol. This compound, paradoxically, showed an anxiogenic effect in mice at 10 mg/kg (*i.p.*), which was inhibited by ketanserin (2.5 mg/kg, *i.p.*) and WAY 100635 (5 mg/kg, *i.p.*), but not by diazepam (2.5 mg/kg, *i.p.*) or NECA (0.00547 mg/kg, *i.p.*). These results suggest the participation of 5-HT2 and 5-HT1A serotonin receptors, but not the involvement of $GABA_A$ or adenosine receptors, in the anxiogenic effect of valeiridoside. Often, it is claimed that crude extracts are more effective than purified compounds, assuming a beneficial synergic effect. Antagonism is a less-studied phenomenon that also occurs in medicinal plants, in which, the opposite effects of active constituents are masked by other compounds in complex plant matrices. Valeiridoside showed an opposite effect to the depressant activity of the whole extract of *V. procera*, an effect that encouraged us to investigate its pharmacological action mechanism.

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PB-13 (ID#34)

Anti-coronavirus activity of *Artemisia annua* and the isolated compound, artemisinin

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The recent pandemic that was caused by the coronavirus, SARS-CoV-2, set forth an unprecedented increase in research regarding this virus. Our lab has been testing various botanical extracts to potentially target replication of this virus. From this, several botanical extracts were discovered which effectively inhibit the replication of SARS-CoV-2 with minimal cell toxicity. These top botanicals have also been tested against Mouse Hepatitis virus (MHV), another member of the coronaviridae family. Several botanicals demonstrated similar viral inhibitory activity against both coronaviruses, while others had more narrow viral inhibition against just SARS-CoV-2. This may suggest a different mechanism of action for these botanicals between these two coronaviruses. Of the botanicals tested, *Artemisia annua* was found to be highly effective against both MHV and SARS-CoV-2. The previously isolated compound from *Artemisia*, artemisinin, was found to be highly effective against SARS-CoV-2 but ineffective against MHV. This difference in activity of *Artemisia* and artemisinin against SARS-CoV-2 and MHV suggests *Artemisia* extracts may contain multiple anti-coronavirus compounds. Further research in this area has been targeted towards the isolation and identification of the active constituent(s) present in *Artemisia annua* extracts.

PB-14 (ID#37)

Determination of apigenin inhibitory activity on DNA repair enzymes

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The effects of certain natural compounds on DNA damage and repair aroused researchers' curiosity to understand their role in cancer therapy. Apigenin is a flavone widely found in many herbs, fruits, and vegetables and has been shown to possess various biological activities with the potential for health benefits and chemopreventive properties. Common cancer treatments induce cell death by direct or indirect DNA damage. However, tumor cells can initiate DNA repair pathways to resist chemotherapy or radiation therapy. Combining nuclear or mitochondrial DNA repair pathway inhibitors with anticancer agents may increase tumor cell sensitivity to these agents. Therefore, targeting DNA repair pathways may be a potential therapeutic approach for cancer treatment. Base excision repair (BER) is one of the pathways that repair oxidatively-modified DNA bases. In mammalian cells, a series of DNA glycosylases such as NEIL1, OGG1, and NTHL1 remove modified DNA bases by hydrolyzing the glycosidic bond, leaving behind an apurinic/apyrimidinic (AP) site. This is followed by the action of other enzymes to complete the DNA repair. Recently, NEIL1, OGG1, and NTHL1 were identified as potential targets in combination chemo- or radiation-therapeutic strategies. To understand the effect of apigenin on DNA glycosylases, γ-irradiated calf thymus DNA with multiple DNA base lesions was treated with NEIL1 (edited or unedited), OGG1, or NTHL1 in the absence or presence of apigenin. The released DNA base lesions, which are known substrates of these enzymes, were analyzed by gas chromatography-tandem mass spectrometry with isotope dilution. Apigenin exhibited a dose-dependent inhibition on NEIL1-unedited and NEIL1-edited at concentration levels from 0.5 mM to 20 mM. Conversely, apigenin had no significant effect on the activity of OGG1 or NTHL1. The data suggest a need to investigate further the potential therapeutic benefits of apigenin in cancer therapy as an inhibitor of NEIL1 enzymes.

PB-15 (ID#39)

Investigations into *Allium cepa* constituents as anti-inflammatory leads

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Since the pre-historic times, natural products, particularly phytochemicals, have been used in the treatment and prevention of many ailments. These phytochemicals show unique chemical and biological diversities, therefore, presenting an opportunity for novel drug discovery. Onion extract along with twelve fractions of the extract were investigated for their potential anti-inflammatory effects on house dust mite-induced bronchial inflammation and DSS-produced colitis murine models. Our data show that the extract and some fractions inhibited the inflammation in both models. In addition, the study revealed that combining the extract with steroids resulted in an enhanced anti-inflammatory effect via a synergistic action. GC-MS analyses of the active fractions identified several fatty esters and sulfur-containing compounds as the major potentially active components. These include methyl linoleate, methyl palmitate, allyl sulfide and 2,4-dimethyl thiophene.

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PB-16 (ID#41)

Rhinovirus inhibitory effects of *Echinacea purpurea* root ethanol extracts and alkamides

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The roots of *Echinacea* species have been used medicinally for over three hundred years. Consumer demand for *Echinacea* as an immunomodulatory agent to treat the common cold and flu has increased significantly over the past several decades. *Echinacea purpurea* is currently the most heavily cultivated and utilized species. Despite hundreds of *in vitro* and *in vivo* studies exploring *Echinacea* plant chemistry and bioactivity, there are still discrepancies about its therapeutic efficacy and confusion surrounding which plant parts, extraction methods, and compounds are implicated in the purported immunomodulatory, anti-inflammatory, and antiviral effects of the plant. Of the major medicinal compounds present in *E. purpurea* roots, the alkamides have been of particular interest for their purported immunomodulatory effects related to the endocannabinoid system. Our research has focused on the characterization of the immunomodulatory and anti-rhinovirus activity of *E. purpurea* root extracts and the role of the isolated alkamides. Notably, the alkamides were found to have strong direct antiviral activity against rhinovirus targeting viral binding and uptake into the cell. This research may provide insight into the development of effective *E. purpurea*/alkamide therapeutics for the common cold.

PB-17 (ID#42)

Contributions of ploidy and genotype for chemotaxonomy of *Achillea*

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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, febrifuge, anti-inflammatory, and digestive aid. Proazulene-rich individuals produce the preferred blue essential oil while others, high in allergenic sesquiterpene lactones, have a clear to pale yellow essential oil; previously this difference was attributed to ploidy, but base genomes play a bigger role. However, crude hydro- or hydro-ethanolic extracts of the plant are the most common forms for medicinal use, and less is known about the effects of ploidy level or genotype on the presence and abundance of other compounds present in these preparations. We cultivated 115 populations of *Achillea* spp. from the USDA Agricultural Research Service Germplasm Resources Information Network and the Kew Millennium Seed Bank in a common

garden, determined ploidy level through flow cytometry and chromosome counts, analyzed genetic structure with SNPs, and performed untargeted metabolomics analysis on leaf and flower tissue by reversed-phase HPLC-HRMS. As a whole, we found that genotype correlated well with geographic origin and species distinctions, and that chemical profiling described similar clusters to those of genotype. Ploidy had minimal effect, except where it was also associated with speciation. We will describe our chemotaxonomic findings as correlation plots compared to the genotype, ploidy level, and current morphological taxonomic descriptions.

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PB-18 (ID#53)

Drug interaction potential of *Zingiber officinale* and its constituents

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Ginger (*Zingiber officinale*) belongs to the family Zingiberaceae and is widely used as a spice flavoring agent. Ginger has also been used in traditional medicine for the treatment of several human ailments such as the common cold, inflammation, rheumatic disorders, and gastrointestinal discomfort. With the increasing popularity of botanical dietary supplements, consumption of ginger-containing products is on the rise. Chronic intake of such supplements with conventional drugs may pose a risk for herb-drug interactions. This study was carried out to evaluate ginger's pharmacokinetic drug interaction potential in an intestinal cell line (LS174T) through pregnane X receptor (PXR)-mediated modulation of drug-metabolizing enzymes and transporters. An ethanolic extract of ginger caused a 2.3-fold increase in transcriptional activity of PXR at 20 µg/mL in LS174T cells and significantly increased the expression of CYP1A2, CYP2B6, and ABCB1 (P-gp) mRNA. Among pure constituents, 6-paradol, 6-shogaol, and dehydro-6-gingerdione were more effective in activating PXR (>2 fold) and increasing the expression of *CYP* and *ABCB1* genes. 6-gingerol and 6-gingerdiol were less effective (<2 fold) in activating PXR. The results indicate that ginger extracts may affect the pharmacokinetics of conventional drugs by modulating the activity of drug-metabolizing enzymes and transporters. Chronic intake or overconsumption of ginger-containing supplements along with concomitant drug administration may pose a risk for herb-drug interactions. However, more in-depth studies are warranted in order to determine the clinical relevance of these findings.

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PB-19 (ID#54)

Clinical assessment of the drug interaction potential of the psychotropic natural product Kratom

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Oral supplements from the leaves of the kratom (*Mitragyna speciosa*) plant are increasingly used for their opioid-like effects to self-manage opioid withdrawal and pain. Calls to US poison centers involving kratom exposures increased 52-fold from 2011-2017, one-third of which reported use of kratom with drugs of abuse. Many of these drugs are eliminated from the body through extensive metabolism by the cytochrome P450 (CYP) enzymes, particularly CYP2D6 and CYP3A, raising concerns for potentially dangerous kratom-drug interactions. The objective of this work was to conduct a powered clinical kratom-drug interaction study using a well-characterized kratom product and the probe drug substrates dextromethorphan (CYP2D6) and midazolam (CYP3A). Twelve healthy adult volunteers participated in an open label, two-arm crossover, fixed sequence study. Midazolam (2.5 mg) and dextromethorphan (30 mg) were administered orally with water to obtain baseline pharmacokinetics. At least one-week later, participants were administered the probe drugs 15 min after consuming a low dose (2 g) of the kratom product as a tea. Plasma samples were collected (0-24 h) and analyzed for the probe drugs using LC-MS/MS. Pharmacokinetics were characterized *via* noncompartmental analysis. The geometric mean ratio (90% confidence interval) of the area under plasma concentration-time curve (AUC) and maximum plasma concentration (C_{max}) in the presence to absence of kratom were determined. Kratom showed no effect on dextromethorphan AUC and C_{max} ratios [0.97 (0.81-1.16) and 0.96 (0.78-1.19), respectively] but a modest increase in corresponding midazolam ratios [1.38 (1.23-1.57) and 1.50 (1.32-1.70), respectively]. Lack of change in midazolam half-life [1.07 (0.98-1.17)] and the higher increase in C_{max} compared to AUC indicated that kratom primarily inhibited intestinal CYP3A. This work is the first to provide direct evidence of the pharmacokinetic drug interaction potential of kratom. Co-consuming kratom with other drugs extensively metabolized by CYP3A may precipitate serious interactions. These data fill critical knowledge gaps about the safe use of this increasingly popular natural product, thereby addressing ongoing public health concerns.

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PB-20 (ID#61)

Botanical aphrodisiacs for women's health

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Female Sexual Dysfunction (FSD) affects nearly 40% of women in the United States. While males have five FDA-approved drugs for erectile dysfunction, there is only one FDA-approved as-needed treatment for premenopausal women with acquired, generalized Hypoactive Sexual Desire Disorder (HSDD), the most prevalent FSD. The lack of approved drugs is largely due to the biopsychosocial complexity of HSDD; however, there are neurobiological underpinnings evident. Due to the lack of safe, efficacious treatment options, we hypothesize that botanical species traditionally used as aphrodisiacs may be promising leads, and exert their effects via activation of melanocortins, an excitatory circuit implicated in sexual function, specifically at MC4R. We conducted a review of the aphrodisiac products in the U.S. dietary supplement market to determine their levels of ethnobotanical and clinical evidence and narrow species selection. Utilizing market data, we found that 53 species were used for female-specific sexual complaints; concluding that there is little to no clinical evidence from the literature to substantiate their use. We selected five plants with reasonable evidence for further evaluation, *Corynanthe yohimbe, Labisia pumila, Asparagus racemosus, Tribulus terrestris,* and *Trigonella foenum-graecum*. Species were sequentially extracted, concentrated, dried, and tested to determine a NOAEL before subjection to an MC4R assay. Results showed four extracts demonstrated significant activation over control ($p < .05$). Further fractionation of one of the hits, *L. pumila* (H_2O), resulted in a loss of activity. Large-scale isolation of *L. pumila* and preliminary analysis of fractions utilizing database services has resulted in multiple compounds of interest, and structure elucidation is ongoing. Additional experiments are being conducted to determine whether isolated

specialized metabolites of those active extracts also activate the MC4R, and those which show activity will subsequently be tested for activity at the MC3R as there is a 75% similarity between these two receptor subtypes. To analyze any potential downstream effects of this binding, we will utilize the GT1-7 cell line which secretes Gonadotrophin Releasing Hormone in response to depolarization.

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PB-21 (ID#62)

In-vivo models for evaluating Immulina™ for increasing resilience to influenza type A (H1N1) viral infection

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Influenza, or flu, is a viral infection that mostly targets the respiratory system. Influenza A (H1N1) viral infection has resulted in numerous global fatalities with an average of 30,000 annual deaths in the United States alone. This viral infection usually becomes fatal for immune-compromised individuals or children and elderly people with a weak immune system. Tremendous research is being carried out to develop different drugs and strategies to combat influenza infection. One of the strategies is to boost the host immune system by oral administration of natural products that modulate mucosal immunity to boost resilience against influenza A (H1N1) viral infection. At the National Center for Natural Products Research, we have established three non-lethal *in vivo* murine models (prophylactic, prodromal, and recovery) for evaluating resilience against influenza A virus infection. All these models differ in the treatment regimen. Furthermore, we have evaluated the effect of oral administration of ImmulinaTM (an extract of the botanical dietary supplement *Arthrospira platensis*) in these infection models. The most beneficial effects of ImmulinaTM for increasing resilience against Influenza A viral infection were observed in the prophylactic and prodromal treated mice. Both male and female mice exhibited similar responses. The results from the present study indicate that oral administration of ImmulinaTM to mice resulted in decreased weight loss, decreased clinical signs of infection, and reduced lung-body weight ratio as compared to the infected control group.

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PB-22 (ID#69)

Macro/microscopic identity challenges: The case of *Euphrasia officinalis* and its adulterants from the genus Odontites

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Eyebright (*Euphrasia officinalis*) is a well-known medicinal, used in Europe as a remedy for eye-related health issues. Determining morphologic and genetic delineations among Euphrasia species has been difficult because many morphological features can overlap between species and sometimes other genera within the family Orobanchaceae. Euphrasia commonly hybridizes within its species complex and has two pollination systems (insect- and self-pollinated); it has a diverse range of morphological characters, is small in stature, and is equipped with complex tissues and anatomy. Recent HPTLC studies of powders and tea-bag cut materials labeled as Euphrasia determined that the genus is highly variable in its chemical composition.

In addition, several samples were found to be from the genus Odontites, a close relative, presumably substituted for Euphrasia. Substitution of the target ingredient signals probable economically motivated adulteration since Odontites species grow much taller and hence harvesters can collect larger volumes in a shorter time. Results from both genetic and chemical methods indicate that they can typically distinguish between the two genera, but what role, if any, can classic botanical identity methods play in resolving identity issues? While *Odontites* species are clearly distinguishable from Euphrasia species in the field or as whole material, it has not yet been determined whether differentiation between the two taxa is possible at the decidedly smaller particle sizes used in supply chain and finished products. Euphrasia is considered unresolved taxonomically, and determining species based on macro- and microscopic features is problematic. Here, two botanical samples labeled as "eyebright" but assigned to Odontites based on genetic and HPTLC testing were examined using macro- and microscopic techniques and compared to authenticated Euphrasia materials, to determine the initial feasibility of correctly identifying each genus at low- and high-magnification. A set of potentially useful features to distinguish the two genera are presented here. The study also examined powdered materials determined by HPTLC as possessing "no detectable material" but billed as Euphrasia, to see if any further microscopic identifications of the contents can be made.

PB-23 (ID#73)

Elucidating mechanisms underlying the Goldenseal-Midazolam interaction using a modeling and simulation approach

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Goldenseal is a clinical inhibitor of cytochrome P450 (CYP) 3A activity, as evidenced by an ~50% increase in plasma AUC of the probe drug midazolam after coadministration with goldenseal. The collective *in vitro* and pharmacokinetic (PK) studies prompted development of a physiologically based PK (PBPK) model to 1) predict plasma exposure to the goldenseal alkaloids berberine and hydrastine, 2) distinguish contributions of reversible and time-dependent CYP3A inhibition by each alkaloid, and 3) determine the primary anatomical site of the interaction. A PBPK inhibitor model was developed for each alkaloid using Simcyp™. Physicochemical properties, CYP3A inhibition kinetics, and PK data obtained from the literature were incorporated into the models. Models were refined by addition of the dissolution rate profile and apparent permeability for berberine and hydrastine, which were determined using fasted state simulated intestinal fluid and Caco-2 cells, respectively. The inhibitor models were combined with the midazolam substrate model to simulate alkaloid exposure and predict the magnitude of the goldenseal-midazolam interaction. Simulation outcomes were verified with data from an independent PK study. Total berberine and hydrastine content dissolved by >80% within 20 min. Hydrastine was ~60x more permeable than berberine ($9.5x10^{-6}$ *vs* $1.7x10^{-7}$ cm/s). Predicted plasma AUC of each alkaloid was within 2-fold of observed AUC. PBPK models incorporating experimentally determined dissolution profiles and permeability adequately predicted alkaloid plasma exposure. Simulations implicated hydrastine as a major precipitant of the goldenseal-midazolam interaction, primarily inhibiting gut CYP3A *via* time-dependent inhibition. Modeling and simulation can be applied to other natural product-drug interactions to further understand underlying mechanisms.

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PB-24 (ID#75)

Identification of the putative binding site of a novel benzimidazole (Etazene) and its metabolites with μ-opioid receptor: A systematic computational study

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The abuse of novel psychoactive substances (NPS) has risen dramatically worldwide, especially among teenagers and young adults. The absence of pharmacological and toxicological data of new, illicitly-used, and abused opioids, has resulted in serious adverse effects, including death. The synthetic benzimidazole opioid Etazene gained popularity as a recreational drug on the illegal/darknet market; however, no experimental information is available at the molecular level on the binding mechanism and putative binding site of Etazene and its metabolites at the μ-opioid receptor (MOR). In the present study, we investigated the possibilities of MOR activation by Etazene and its metabolites by studying their binding mechanism and interaction profiles at the active-state MOR using homology modeling, molecular docking, binding free-energy calculations, and all-atom molecular dynamics (MD) simulations. The putative metabolites of Etazene were predicted using ADMET Predictor 10.1. The molecular docking studies and free-energy calculations showed that Etazene and its *O*-dealkylated metabolite exhibited strongly predicted binding affinity at MOR and showed overlapped binding orientation with MOR bound agonist BU72 co-crystalized in the MOR X-ray crystal structure (PDB ID: 5C1M). These results suggest that Etazene and its metabolites may act as a strong MOR agonist, highlighting the necessity of experimental validation. Identification of key interactions between Etazene and its metabolites and the MOR is a step towards a better comprehension of the target protein and the design of new analogs.

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PB-25 (ID#79)

Antimicrobial natural products as food and cosmetic preservative

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Microbial spoilage of foods due to contamination by bacteria and fungi is one of the major concerns. These microorganisms initiate undesirable reactions in the food that include unpleasant taste, smell, and toxicity. In cosmetics and personal care products, antimicrobial contamination-like growth of molds, yeasts, and bacteria are major concerns, that can cause skin irritation, allergy, or infections. Preservatives are natural or synthetic compounds that retard or prevent microbial growth in various types of products, including pharmaceutical drugs, foods, and personal care products. In recent years, several reports have indicated that synthetic preservatives have an effect on several cellular targets and might exert toxic effects on the consumer. With the extensive use of synthetic preservatives, the emergence of microbial resistance is also a major threat. Therefore, the identification of alternative natural preservatives is immensely needed with better antimicrobial efficacy and safe for human health. To address the need for the development of safer food/cosmetic preservatives, we have established an in-vitro antimicrobial assay panel including mold, fungi, and Gram +ve/-ve bacteria to screen natural product extracts derived from the plants. Several natural products including small molecules and extracts that are available in the National Center for Natural Product Research repository have been screened for preservative efficacy.

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PB-26 (ID#87)

PPARα and PPARγ agonistic effects and increase in glucose uptake by *Aquilaria sinensis* flower extract

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Aquilaria sinensis (Lour.) Spreng. is known for its resinous secretion (agarwood), often secreted in defense against injuries. We investigated the effects of *A. sinensis* flower extract (AF) on peroxisome proliferator-activated receptors alpha and gamma (PPARα and PPARγ), liver X receptor (LXR), glucose uptake, and lipid accumulation (adipogenesis). Activation of PPARα, PPARγ, and LXR was determined in hepatic (HepG2) cells by reporter gene assays. Glucose uptake was determined in differentiated muscle (C2C12) cells using 2-NBDG (2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose). Adipogenesis was determined in adipocytes (3T3-L1 cells) by Oil red O staining. At a concentration of 50 µg/mL, AF caused 12.2-fold activation of PPARα and 5.7-fold activation of PPARγ, while the activation of LXR was only 1.7-fold. AF inhibited (28%) the adipogenic effect induced by rosiglitazone in adipocytes and increased glucose uptake (32.8%) in muscle cells at 50 µg/mL. It was concluded that AF acted as a PPARα/γ dual agonist without the undesired effect of adipogenesis and exhibited the property of enhancing glucose uptake. This is the first report to reveal the PPARα/γ dual agonistic action and glucose uptake enhancing property of AF along with its antiadipogenic effect, indicating its potential in preventing the symptoms of metabolic disorder.

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PB-27 (ID#88)

Daniellia oliveri (Rolfe) Hutch and Dalziel: antimicrobial activities, cytotoxicity, and phytochemical identification by GC-MS.

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Daniellia oliveri (*D. oliveri*) has been reported to treat intertrigo and candidiasis in an ethnobotanical survey conducted in Togo. The present study has been performed to investigate firstly, the antibacterial, antifungal, as well as the cytotoxic activities of raw extracts and their subsequent fractions from the leaves, trunk barks, and roots of *D. oliveri*; secondly, the chemical composition of the most active fractions of *D. oliveri*. The antibacterial activity was evaluated by determining Minimum Inhibitory Concentration (MIC) using the broth dilution method. The most active fractions were subsequently evaluated for cytotoxicity exploiting normal human cells (MRC-5 cells) via the MTT assay. The most active and not toxic fractions were finally evaluated for phytochemical investigation by GC-MS to provide a preliminary analysis of the chemical components present in *D. oliveri*. All the raw extracts and fractions were active against the bacteria tested, with MICs ranging from 16 µg/mL to 256 µg/mL. Interestingly, the raw extracts and fractions of *D. oliveri* were more active against Staphylococcus species, including Methicillin-Resistant Staphylococcus aureus. No toxicity was observed against MRC-5 cells with the most active fractions, except for the butanol and water fractions of the trunk barks. Unfortunately, no activity was observed against *Candida albicans* even at 256 µg/mL, the highest tested concentration. Then, the most interesting fractions were screened for phytochemical analysis using GC-MS. To the best of our knowledge, most of the compounds identified during the phytochemical investigation by GC-MS (pentacyclic triterpenes, phytosterols, phenolic compounds, fatty acids, and other terpenes) are reported for the first time in *D. oliveri*. This study is the first to propose a deep investigation on all the parts of *D. oliveri*, including raw extracts and fractions. In the end, our results demonstrate that *D. oliveri* possesses valuable antibacterial activities, which agrees with the data obtained after interviews with the traditional healers in Togo.

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PB-28 (ID#119)

Computational tools to expedite the identification of potential PXR modulators in complex mixtures – A case study with five closely related licorice species

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The genus *Glycyrrhiza,* comprising over 28 spp., possesses complex structural diversity and is also documented to possess a broad spectrum of biological activities. Detection and comprehension of the mechanisms of efficacy or safety for a plant-based therapy are very challenging, yet it is crucial and necessary to understand the polypharmacology of traditional medicines. Licorice extract was shown to modulate the xenobiotic receptors, which might manifest as a potential route for natural-product-induced drug interactions (NPDI); however, different mechanisms could be involved in this behavior. Since the induced herb-drug interaction of licorice supplements *via* Pregnane X receptor (PXR) is poorly studied, we ventured out to analyze the potential modulators of PXR in complex mixtures such as whole extracts by applying computational mining tools. A total of 518 structures from five species of *Glycyrrhiza*: 183 (*G. glabra*), 180 (*G. uralensis*), 100 (*G. inflata*), 33 (*G. echinata*), and 22 (*G. lepidota*) were collected and post-processed to yield 387 unique compounds. For further study, a visual inspection of top candidates based on favorable ligand-PXR interactions and the highest docking scores were selected. The *in vitro* testing revealed that glabridin (GG-14) is the most potent PXR activator amongst the tested compounds, followed by licoisoflavone A, licoisoflavanone, and glycycoumarin. A 200 ns molecular dynamics study with glabridin confirmed the stability of the glabridin-PXR complex, highlighting the importance of computational methods for rapid dereplication of potential xenobiotic modulators in a complex mixture instead of undertaking time-consuming classical biological testing of all compounds in a given botanical.

Acknowledgments
This research was supported by the "Holistic Approach for Potential Drug Interactions with Botanical Drugs – Importance of Chemical Fingerprinting and Biosimilarity" funded by the US Food and Drug Administration grant # HHSF223201810175. Excellent technical support in the bioassays by Ms. Olivia Dale and Ms. Katherine Martin is also acknowledged.

PB-29 (ID#120)

A novel approach for rapid dereplication of herb-drug interaction causative agents in botanical extracts – A molecular networking strategy to identify potential PXR modulators in Yohimbe

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It is a common and costly bottleneck in natural products isolation efforts; despite promising extracts' bioassay results, the active compound(s) may not be isolated during subsequent bioassay-guided purification or classical pharmacognostic investigations. Indeed, most bioassay-guided fractionation efforts result in either repeated isolation of already described molecules or the failure to identify the molecule(s) responsible for the observed bioactivity. The isolation procedure must be rationally applied to these substances to better detect bioactive molecules before undertaking extensive pharmacognosy steps. For example, the bark of *Pausinystalia johimbe* (K. Schum.) Pierre ex Beille (known as Yohimbe) has been traditionally used in herbal medicine as a

general tonic, an aphrodisiac, and a performance enhancer. More recently, *P. johimbe* bark has been an integral part of various dietary supplements. In our continued quest on the safety of botanical ingredients, the hydroethanolic extract of *P. johimbe* has been identified to cause at least 3-4-fold increase in transcriptional activity of Pregnane X receptor (PXR), which is the central nuclear receptor responsible for regulating the expression of key drug-metabolizing enzymes and transporters. For rapid identification of causative agents responsible for PXR activation in the botanical mixtures, an MS/MS-based molecular networking analysis was integrated with the PXR's modulation data as a proof-of-concept. This novel approach resulted in the dereplication of several corynanthine-type alkaloids as significant inducers. Further analysis of the nearest nodes within the molecular network of biologically active fractions identified three oxindole alkaloids, eleven indole alkaloids, and two *N*-oxide indole alkaloids as the main contributing agents for PXR modulation. Importantly, the identity of these compounds was unambiguously established by comparison with reference standards' chromatographic and mass spectral data. Furthermore, tentative structure-activity relationships revealed the formation of a methoxy-acrylate moiety (an open form of E-ring) as a common structural feature among the observed PXR inducers. The exact details on how molecular networking strategy was effectively utilized to identify PXR modulators in a Yohimbe extract will be presented.

Acknowledgments
This research is supported in part by "In vitro screening of a unique botanical extract collection for herb-drug interaction (HDI) potential" funded by the National Center for Complementary & Integrative Health, NIH grant number 5R21AT011107, and "Science-Based Authentication of Botanical Ingredients" funded by the Center for Food Safety and Applied Nutrition, US FDA grant number 5U01FD004246.

PB-30 (ID#125)

Botanical and chemical characterization of seeds called "Ting Li Zhi"

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The botanicals called "*Ting li zhi*", in Traditional Chinese Medicine (TCM) is the seeds of *Lepidium apetalum* and *Descurainia sophia*, of Brassicaceae. Morphologically, both look very similar in color as well as size and shape. in TCM Traditionally, the name called "*Nantinglizi*" describes the *Descurainia* seeds, the latter is called "*Beitinglizi*" *Lepidium* seeds. Based on the Pharmacopoeia of the People's Republic of China, it shows that the *Descurainia* Seeds as southern lepidium and *Lepidium* seeds as northern Lepidium, these two species used for the same therapeutical usage. Thus, this study focused to see the morphology and chemical similarity between these two species to understand the usage of two different seeds as one. The microscopic observation looks more similar between the two species and these characteristics were highlighted in this study. The chemical composition differences of these seed samples were investigated by the chemical fingerprinting approach.

Acknowledgments
This research is supported by the "Science-Based Authentication of Botanical Ingredients" funded by the Center for Food Safety and Applied Nutrition, US Food and Drug Administration grant number 5U01FD004246.

PB-31 (ID#126)

Characterization of botanicals named "Mu Tong and Wei Ling Xian" in traditional Chinese medicine

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In Traditional Chinese Medicine (TCM), the usage of the name " *Mu Tong*", "*Wei Ling Xian*" "*Fang Ji*" or even "*Ma Dou Ling*" is highly interchangeable with one another herbal used. This leads to confusion in the use of the genuine root of *Aristolochia* spp. and certainly several species of genus *Clematis*, *Akebia*, *Stephania,* or *Cocculus*. It is well-known fact that *Aristolochia* species contain aristolochic acid, which is a carcinogen. Thus, many industries, botanical products use the other species of *Aristolochia* or substitute with the above-mentioned genus. For Example, in Belgium the ingredient *Stephania tetrandra* was substituted for *Aristolochia fangchi.* Thus, this study focused on the identification and authentication of the botanical sold in the name of "Mu Tong or Wei Ling Xian" in Traditional Chinese Medicine. Samples were subjected to macro, microscopical evaluation, and chemical profiling to differentiate the chemical variation of the species studied. The observation of the results shows that the samples sold are mostly the root and stem parts of *Clematis* sp. and stem of *Akebia* sp., this may be due to usage of alternative sources of *Aristolochia* in the herbal products.

Acknowledgments
This research is supported by the "Science-Based Authentication of Botanical Ingredients" funded by the Center for Food Safety and Applied Nutrition, US Food and Drug Administration grant number 5U01FD004246.

PB-32 (ID#130)

Natural products offer eco-friendly alternatives to snail problems on Mississippi catfish farms

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The snails *Biomphalaria havanensis* and *Planorbella trivolvis* (Family: Lymnaeidae) are an essential link in the transmission of several important trematode parasites, most notably *Bolbophorus damnificus*. The diseases transmitted by these parasites cause significant qualitative and quantitative losses to catfish farms in the southeastern US. Currently, copper sulfate ($CuSO_4$) has been practiced as an effective management tool, that do not come without risk and must be applied judiciously. The National Center for Natural Products Research (NCNPR), The University of Mississippi; The Mississippi State University College of Veterinary Medicine, the Mississippi Agriculture and Forestry Experiment Station and the USDA Catfish Genetics Research Unit teamed up to investigate eco-friendly natural molluscicide alternatives. The plant originated natural products are known to be toxic to insect pests and snails, but unlike other chemicals, are relatively innocuous to plants and do not leave harmful environmental residues. To study molluscicidal toxicity of plant derived natural products, the NCNPR established a high-tech rearing facility for both snail species. Subsequently, dose and time – lethality bioassays were performed against adult snails to determine lethal parameters. Several essential oils and saponin extracts from plants listed by the FDA as GRAS (Generally Regarded As Safe) were evaluated and compared to a $CuSO_4$ positive control. $CuSO_4$ induced toxicity in *B. havanensis* at 6.96 ppm (LC50) and 12.96 ppm (LC90) in 24-hr exposures. Comparably, *P. trivolvis* was more sensitive to these $CuSO_4$ treatments, with an LC50 of 1.65 ppm and LC90 of 6.12 ppm after 3-day exposures. While, the median lethal dose for *B. havanensis* for the saponin extract was 10.5 ppm, with an LC90 of 15.6 ppm in 24 hr. The 3-day LC50 and LC90 for *P. trivolvis* was 10.1 and 28.8 ppm, respectively. The active fraction of the saponin extract induced 100% mortality against *P. trivolvis* at 2.5 ppm after a 3-day exposure. Similarly, bioassays revealed the essential oil candidate EO-1 induced 100% mortality at 25 ppm in *B. havanensis*

after just 24 hr. These promising natural molluscicides will be further investigated for synergism and active compounds will be identified by bioassay-guided fractionation.

Acknowledgments
The study at NCNPR was supported by the U.S. Department of Agriculture grant 10001598. The project was supported by the Mississippi State University College of Veterinary Medicine, the Mississippi Agricultural and Forestry Experiment Station (MAFES), and Mississippi State University under MAFES Project Number MIS-371592 and the U.S. Department of Agriculture–Agricultural Research Service SCA586066-6-021.

PB-33 (ID#134)

Predictive machine learning *vs.* traditional QSAR modeling: A case study of known PXR activators

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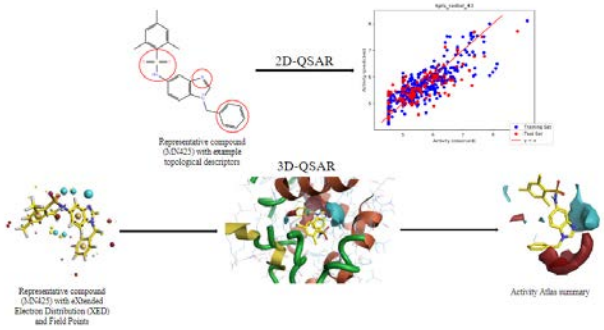
Recent studies have added multiple active constituents of herbal medicines to the list of xenobiotics as potent activators of the pregnane X receptor (PXR), which is responsible for recognizing and detoxifying a diverse array of xenobiotics encountered by the human body. Considering the wealth of compounds from the literature that have been experimentally-validated as agonists of PXR, quantitative structure-activity relationship (QSAR) modeling is a compelling choice for assessing the activity associated with PXR agonists due to its competency with high throughput screening. In this study, a range of QSAR techniques were probed using 500 known, structurally-diverse PXR agonists to establish the requisite features of PXR agonists. Traditional two-dimensional (2D) QSAR, machine-learning-based 2D-QSAR, field-based three-dimensional (3D) QSAR, and machine-learning-based 3D-QSAR models were built and validated. Across all generated QSAR models, an external set of botanical triterpenes experimentally-determined to be PXR agonists were used for model validation and cross-confirmation. The best 2D-QSAR model produced an external validation correlation coefficient (R2) of 0.24, whereas the best 3D-QSAR model yielded an R2of 0.675. QSAR data analysis revealed that machine learning-based 2D-and 3D-QSAR models were more accurate in predicting PXR agonism versus traditional QSAR methods. Additionally, a visual summary of the shape, hydrophobics, and electrostatics representing the ligand-binding domain of PXR was presented from the field-based 3D-QSAR model. From this study, a robust groundwork for assessing PXR agonism from a variety of chemical backbones has been established, and access to such models is anticipated to expedite the identification of the potential causative agents in a complex mixture, viz, botanical extracts and other finished products.



Acknowledgments
This research is supported in part by "Science-Based Authentication of Botanical Ingredients" funded by the Food and Drug Administration grant number 2U01FD004246, the United States Department of Agriculture, Discovery & Development of Natural Products for Pharmaceutical and Agricultural Applications No. 58-6060-6-015. Also, thanks to the Computational Chemistry and Bioinformatics Research Core of the US National Institute of General Medical Sciences of the National Institutes of Health (NIH) under Award Number P20GM130460 for use of Cresset software.

PC-1 (ID#65)

Late-stage diversification of lipoic and arundic acids - Synthesis of novel hybrid chemical entity with potential synergistic antioxidant and neuroprotective properties.

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From accessible materials, natural products can be modulated from being single-targeted ligands to multi-targeted ligands, providing superior pharmacological potency and a better understanding of the pathogenesis underlying complex diseases, particularly Alzheimer's disease (AD). AD is one of the most progressive neurodegenerative disorders that causes irreversible memory impairment and continuous decline in carrying out daily tasks independently, which results in severe complications and death. Throughout the years, studies and reviews were primarily dedicated to two hallmarks, namely: amyloid-ß (Aß) protein and neurofibrillary tangles (NFTs) of hyperphosphorylated tau protein. Despite the colossal investment in disease-modifying drugs aiming to target either Aß or Tau proteins, they continue to fail with more setbacks than successful treatment. Other factors including neuroinflammation and oxidative stress are currently considered as hallmarks of the disorder, but little is known about their contributions to the pathology and whether each factor is regarded as a cause or a bystander. Up-to-date, there is no cure for AD, and most of the approved drugs are merely used for symptomatic purposes to improve cognitive performance and quality of life. Our research emphasizes the multifactorial nature of AD, thus, the importance of the paradigm shift in drug development for AD from single-targeted ligands to multi-target directed ligands. Hereby, by combining the structural features of the natural antioxidant —lipoic acid, and the synthetic neuroprotective agent — arundic acid, hybrid small molecules of multifunctional ligands were designed. This approach will improve the overall antioxidative and neuroprotective traits of novel lipoic acid analogs.

PC-2 (ID#66)

Late-stage diversification of a monoterpene as an insect growth regulator and its impact against (*Cimex lectularius* L.)

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Besides therapeutics, natural products applications can be used for protecting human health and reducing environmental pollution and hazards. Bed bugs *(Cimex lectularius L.)* are reported the most in urban areas and have raised many concerns regarding public and environmental health. The bugs survive solely on the blood of humans and animals causing skin rashes, bleeding, inflammation, as well as psychological impacts including sleep difficulties, anxiety, and stress as a result of the continuous and overwhelming infestations. Thus far, there are two commercially available juvenile hormone analogs (JHAs), namely methoprene and hydroprene. JHAs are insect growth regulators (IGRs) that act by mimicking insect juvenile hormone, a pivotal developmental hormone that plays a critical role in regulating insect's life-cycle, keeping insects in immature stages, and preventing their reproduction. Although JHAs are considered optimal for their proven effectiveness and relatively low toxicity to non-arthopods, their efficacy for bed bug management remains, nevertheless, inconsistent. Because some strains of bed bugs developed resistance to common insecticides, JHAs have shown insecticidal activity only when used at higher application rates than the labeled rate. Based on that, observations such as aquatic toxicity and human overexposure to

insecticides through inhalation or dermal contact have been recorded. Hence, this study focuses on developing a novel natural product-derived analog as an IGR for managing bed bugs with a preferable safety profile compared to other available IGRs.

PC-3 (ID#72)

Generation of reference compounds of kratom alkaloids from *Mitragyna speciosa*.

Rangel-Grimaldo M, Oberlies NH

Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, North Carolina, USA.

Kratom [*Mitragyna speciosa* (Korth.) Havil. Rubiaceae] is growing in popularity in the United States for self-treatment of pain and opioid withdrawal symptoms. Recently, there has been an explosion in the biomedical and pharmacological literature regarding the potential of this plant and its indole/oxindole alkaloids. The most abundant and studied kratom alkaloid is mitragynine, which has been reported as a μ-opioid receptor (MOR) agonist. In vitro and in vivo studies report that 7-hydroxymitragynine (7-HMG), a mitragynine derivative, is a product of CYP3A4 in the human body and is a more potent and efficacious opioid than its parent, mitragynine. A recent in vitro study demonstrated that 7-HMG is converted in human plasma to mitragynine pseudoindoxyl, an opioid that is even more potent than either mitragynine or 7-HMG, and this activity may contribute to the pharmacology of kratom. However, MOR activation is also associated with serious side effects, such as tolerance, physical dependence, and risk of abuse. Therefore, the discovery of a new class of MOR agonists that retain potent analgesic actions but with reduced side effects and abuse potential is a long-term goal. However, the availability of 7-HMG and mitragynine pseudoindoxyl is a serious challenge, due to difficulties in the isolation of these metabolites from a biologic system and the amounts necessary to perform bioassays. To increase our understanding of the behavior of kratom alkaloids and their metabolites, we strove to generate well-characterized standards for mitragynine and its derivatives, 7-HMG, and mitragynine pseudoindoxyl, as well as other indole and oxindole alkaloids present in the plant. We started by isolating reference standards of mitragynine and its diastereomers. Additionally, we generated mg quantities of 7-HMG and mitragynine pseudoindoxyl using a semi-synthetic approach by using mitragynine as a starting material. Future studies are expanding this approach, so as to generate 7-hydroxy and/or pseudoindoxyl derivatives of other kratom alkaloids, thereby providing the tools needed to further our pharmacological knowledge of *M. speciosa*.

PC-4 (ID#90)

Undescribed benzoylcyclopropane derivatives from *Hypoxis hemerocallidea* corms

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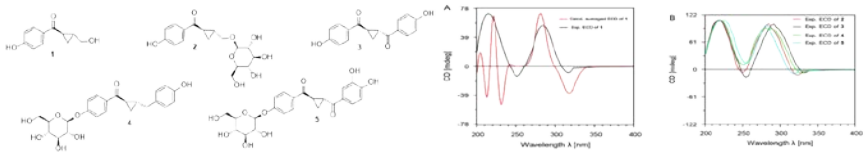
Hypoxis hemerocallidea Fisch., C.A.Mey. & Avé-Lall. (synonym *H. rooperi T. Moore*) (Hypoxidaceae), is commonly known as African Potato. The corms of the plant are utilized by the indigenous populace of South Africa in a variety of ways and for numerous restorative purposes as well as for sustenance. The medicinal uses of *H. hemerocallidea* include the treatment of hypertension, urinary infections, prostate hypertrophy, cancer, benign prostatic hyperplasia, and central nervous system disorders. In recent years, this plant has become a prevalent ingredient of numerous commercial products and dietary supplements with various remedial claims. Two monobenzoylcyclopropane [hypoxhemerol A (1) and hypoxhemeroloside G (2)] and three dibenzoylcyclopropane [hypoxhemerol B (3), hypoxhemeroloside H (4), and hypoxhemeroloside I (5)] derivatives were isolated from the hydro-alcoholic extract of *Hypoxis hemerocallidea* corms. This is the first instance where benzoylcyclopropane analogs were isolated from any natural source. Structure elucidation was mainly based on 1D- and 2D-NMR and HRESIMS data. The absolute configuration (2*R*, 4*R*) of 1 was determined via NOESY NMR and experimental and calculated ECD data

analyses. The isolated benzoylcyclopropane derivative could be used as markers for authentication and standardization of *H. hemerocallidea* commercial preparations.



Acknowledgments
This research is supported by the "Science-Based Authentication of Botanical Ingredients" funded by the Center for Food Safety and Applied Nutrition, US Food and Drug Administration grant number 5U01FD004246.

PC-5 (ID#91)

Phasvulic acid: an undescribed cyclohexane carboxylic acid derivative from Black Turtle Bean (*Phaseolus vulgaris* L.)

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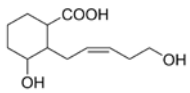
Black turtle bean is a variety of the common bean belonging to the *Phaseolus vulgaris* L. species of the Fabaceae family and is one of the most important varieties of edible cultivated food legumes like kidney, pea, white, yellow beans, etc. In this study, a targeted phytochemical investigation of the ethanolic extract of its seeds, focusing on the constituents other than the lipophilic metabolites, resulted in the isolation and characterization of five compounds of diverse classes. Phasvulic acid, a previously undescribed cyclohexane carboxylic acid derivative, was characterized as (*Z*)-3-hydroxy-2-(5-hydroxypent-2-en-1-yl)cyclohexane-1-carboxylic acid based on various spectral data including one and two dimensional NMR (^{1}H, ^{13}C, COSY, HSQC, and HMBC) and high-resolution electrospray ionization mass spectrometry (HR-ESI-MS). Other compounds were found to be formerly described as dihydrophaseic acid, uridine, stigmasterol-3-*O*-*β*-D-glucopyranoside, and *β*-sitosterol-3-*O*-*β*-D-glucopyranoside.



Phasvulic acid

Acknowledgments
This work was supported by Tertiary Education Trust Fund (TETfund) Nigeria through Federal University Dutsin-Ma, Katsina State, Nigeria under the Academic Staff Training and Development 2018-2019 (merged intervention).TETF/DASTD/UNIV/KATSINA STATE/TSAS/2019/VOL.1 and the Natural Centre for Natural product Research School of Pharmacy University of Mississippi USA.

PC-6 (ID#92)

Neo-clerodanes from *Teucrium divaricatum* and their potential antiinflammatory, antimicrobial, and antimalarial activities

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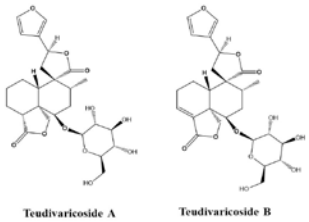
Teucrium L., one of the 245 genera of the Lamiaceae family, is represented with approximately 370 taxa in the world. Its members are mainly distributed in the Mediterranean region with a considerable number of species occurring in Spain, Algeria, Morocco, Italy, Greece, and Turkey. Çeçen et all described the last taxa as *T. turcicum* and by last revision of the genus, 50 *Teucrium* taxa (38 species) are found in the Flora of Turkey and 19 of them are endemics in Turkey. *T. divaricatum* and *T. chamaedrys* have traditionally been used as a tonic, carminative, spasmolytic, diuretic, antiseptic, antirheumatic, antipyretic, and anthelmintic. We reported the isolation and structural identification of 15 *neo*-clerodane diterpenoids including two undescribed *neo*-clerodane glycosides, an iridoid glycoside, and a phenylpropanoid glycoside from the whole plant of *T. divaricatum* subsp. *divaricatum. Neo*-clerodane diterpenoids were evaluated for their potential anti-inflammatory, antimicrobial, and antimalarial activities.



Teudivaricoside A **Teudivaricoside B**

New *neo*-clerodane glycosides from *Teucrium divaricatum* subsp. *divaricatum*

Acknowledgments
This project was supported by Ege University Scientific Research Project-18-ECZ-013.

PC-7 (ID#93)

Phytochemicals of *Teucrium pruinosum* and their antibacterial, anti-inflammatory, and antimalarial activities

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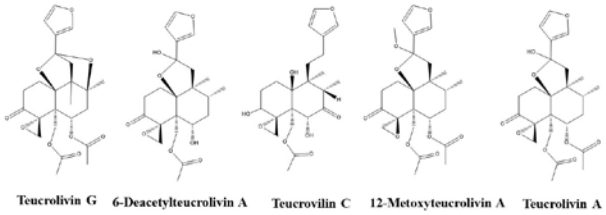
Teucrium L. (Lamiaceae) is a large and highly polymorphic genus that includes more than 300 species distributed in Europe, America, Asia, and more prevalent in the Mediterranean region like Turkey, Spain, Italy, and Morocco. *T. polium* and *T. chamaedrys* species are generally named as “germander”, and especially are worldwide used in folk medicine to treat many diseases related to obesity, diabetes, hemorrhoid, gastrointestinal, respiratory, and nervous systems. In Turkey, 50 *Teucrium* taxa are described and several of them are widely used in Anatolian traditional medicine as antiseptic, antibacterial, carminative, spasmolytic, diuretic, antiseptic, antirheumatic, antipyretic, and anthelmintic. *Neo*-clerodane diterpenes have been reported as the major constituents in *Teucrium* L. As part of our ongoing investigation on the secondary metabolites of the *Teucrium* genus, we attempted to reveal comprehensive secondary metabolite profiles of the roots and aerial parts of *T. pruinosum* Boiss., which resulted in the isolation of five *neo*-clerodanes and two abietene diterpenoids, together with eight flavonoids, one sesquiterpene, one phenylethanoid, and three iridoid glycosides. Diterpenoids and flavonoids were tested for anti-inflammatory, antimicrobial, and antimalarial activities.



Neo-clerodanes from *Teucrium pruinosum*

PC-8 (ID#108)

Isolation and characterization of lipophilic metabolites of *Garcinia cambogia*

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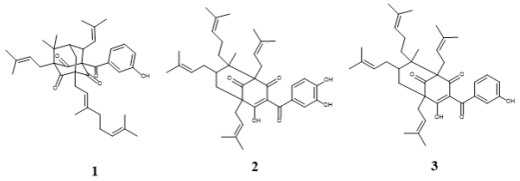
Garcinia cambogia is a medicinal herb used in well-known traditional medical philosophies such as Ayurveda, traditional Chinese medicine, and complementary and alternative medicine. The popularity of *G. cambogia* has been dramatically increased as weight loss remedies, resulting in its wide range of formulations marketed with various claims. Hydroxycitric acid (HCA) is a major component of *G. cambogia* and the free acid is unstable and is converted to its more stable lactones. Recent studies showed that HCA has a curbing effect on appetite. Besides this, a variety of oxygenated and prenylated xanthones and polyisoprenylated benzophenones were identified that possess a wide range of pharmacological activities like antioxidant antiparasitic, antiviral, antifungal, antibacterial, and cytotoxic activity, etc. Previously, various polar metabolites have been isolated *from Garcinia,* but very limited information is available on its non-polar metabolites. This study deals with the isolation and characterization of various polyprenylated benzoylphloroglucinol derivatives from the DCM soluble part of the ethanolic extract of *G. cambogia* fruit that are listed below as compound 1, 2 and 3.



Acknowledgments
This research in part is supported by the Food and Drug Administration "Science Based Authentication of Botanical Ingredients" award number 2U01FD004246-06.

PC-9 (ID#117)

A molecular networking-based discovery of dimeric flavonoids from *Fridericia cinnamomea* (Bignoniaceae)

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Bignoniaceae is a pantropical family with a regional localization to Brazil, with 33 of the 82 genera being found within the country. Several species from this family are highly characterized in folk medicine and are recognized for producing bioactive compounds. For example, bioactive phytochemicals, such as brachydins (dimeric flavonoids with unusual structures), were identified from *Fridericia platyphylla*, a plant popularly used in Brazil to treat kidney stones and painful joints. Other species from Fridericia were also reported as sources of new compounds, such as xanthones and anthocyanidins. Due to the importance of this genus as a source of novel compounds, samples of the leaves and branches of F*ridericia cinnamomea* (DC.) L.G. Lohmann was collected from Adolfo Ducke Forest Reserve (Amazon, Brazil) for phytochemical analysis. The air-dried and ground plant material was exhaustively extracted with hexane and subsequently with methanol. All extracts were analyzed through HPLC-MS/MS; the resulting mass spectral data was instrumental in generating molecular networks with the aid of the GNPS platform. Molecular networking analysis allowed the identification of clusters believed to be several brachydin-like metabolites; therefore, targeted isolation procedures were implemented to obtain such compounds. The MeOH extract was resuspended in MeOH: H_2O (8:2) and partitioned to afford CH_2Cl_2 (DCM), EtOAc, and hydroalcoholic (HA) extracts. The presence of dimeric flavonoid derivatives was identified in DCM extracts. Further fractionation, sequential purifications, and characterization with analytical tools allowed us to identify two new brachydins, and the exact details will be presented.

PC-10 (ID#118)

Design and synthesis of tetrahydroisoquinoline derivatives as kappa-opioid receptor ligands for the treatment of addiction

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Kappa-opioid receptor (KOR), a G-protein-coupled class of receptors (GPCRs), are expressed at particularly high levels within brain areas implicated in the modulation of emotion, and cognitive function, when activated by endogenous opioid dynorphin. Chronic activation of KORs in animal models has been found to aggravate depression and propensity to engage in drug-seeking behavior. Thus, KOR antagonists are being considered to treat a variety of neuropsychiatric conditions, including depression, anxiety, and substance abuse disorders. JDTic, a 4-phenyl piperidine derivative (see figure), is a selective KOR antagonist that displayed robust activity in rodent models of depression, anxiety, stress-induced cocaine relapse, and nicotine withdrawal. However, it has a very long duration of action caused by altered activity of c-Jun *N*-terminal kinases (JNK) and poor brain-to-plasma concentration ratio. Adverse cardiac events in patients enrolled in phase I clinical trial resulted in discontinuation of further drug development to treat cocaine abuse. Therefore, new analogs with favorable drug profiles (e.g., short-acting, improved brain penetration, etc.,) need to be developed. We have designed and synthesized several simple tetrahydroisoquinoline derivatives that are structural mimics of JDTic. The majority of these compounds exhibited greater than sixty percent inhibition against KOR at 10 μM concentration and showed good isoform selectivity (κ vs μ) amongst opioid receptors. The primary binding assay of these compounds showed low micromolar activity (binding affinity, Ki ranging from 1-5 μM) against KOR receptors.

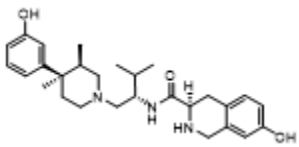

Figure. Chemical structure of JDTic

Acknowledgments
This research is supported by the US - Egypt Science and Technology Joint Fund, Grant #1086, Cycle 18, from the National academy of Sciences (USA) and Science, Technology, and Innovation Funding Authority (Egypt).

PC-11 (ID#131)

Schottiin, a new prenylated isoflavone from *Psorothamnus schottii* and antibacterial synergism studies between Methicillin and Fremontone against MRSA

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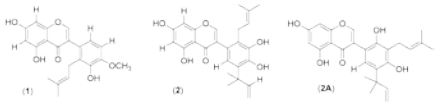
Bioactivity-guided isolation of an ethanol extract of the root of *Psoromanthus schottii* (Family Fabaceae) afforded a new prenylated isoflavone, named schottiin (1), together with four other isoflavones, including fremontone (2), 5,7,4´,5´-tetrahydroxy-2´-(3,3-dimethylallyl)-isoflavone (3), glycyrrhisoflavone (4) and fremontin (5), of which 3 and 4 identified as isomeric mixture. Structures of 1-5 were determined by full spectroscopic analyses. A comprehensive 2D NMR spectral data has allowed revising the structure of fremontone as 2 from previously reported 2A. Compound 2 showed weak *in-vitro* antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). A combination study using a checkerboard assay between fremontone (2) and methicillin exhibited synergistic activity with an 8-fold decrease in MIC of methicillin, as well as an additive effect with vancomycin against MRSA ATCC 1708. Compounds 1 and 2 also showed moderate antiplasmodial activity against chloroquine-sensitive (D6) and -resistant (W2) strains of *Plasmodium falciparum* with no cytotoxicity to mammalian Vero cells.



Acknowledgments
This work is supported by USDA-ARS SCA # 58-6060-6-015.

PC-12 (ID#132)

Development of CBD buccal film using Hot Melt Extrusion Technology

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Hot-Melt Extrusion (HME) is widely used to enhance the solubility of poorly water-soluble Active Pharmaceutical Ingredients (APIs) by melt-mixing process of the API and polymeric carriers. Moreover, there are other applications for HME including the development of modified-release drug delivery systems and to mask drug bitter taste. Cannabidiol (CBD) is a white crystalline cannabinoid isolated from *Cannabis sativa*. CBD is reported to have anti-seizure, anxiolytic, antipsychotic, and anti-inflammatory activities with very low binding affinity to CB1receptors, which explains its lack of psycho activity. However, CBD has low bioavailability (estimated at 6%) due to its low water solubility, and the excessive first-pass metabolism. To date, there is only one commercially available CBD pharmaceutical product (Epidiolex®). This is because of the CBD formulation challenges. The main objective of the current project was to develop CBD buccal film using HME technology to enhance the CBD solubility and mitigate its first-pass metabolism. Differential Scanning Calorimetry (DSC)(TA DSC 25) was performed to evaluate CBD miscibility with polymeric carriers and other excipients. A 10% w/w CBD was mixed with different ratios of poly ethylene oxide N80 (PEO N80), hydroxypropylcelleluse (Klucel™ EF), Sodium lauryl sulfate (SLS), d-α-tocopheryl polyethylene glycol 1000 succinate (Vitamin E TPGS), and Carbopol® 934 and 980.Then, the physical mixtures were extruded using Thermo Scientific HAAKE MiniLab II (Thermo Fisher Scientific, Waltham, MA, USA).The resulted films were evaluated for CBD physical state, drug content and drug release profiles. The DSC thermogram showed that the CBD has an endothermic peak at 65℃ which

disappeared in the HME films. This indicates the transformation of CBD`s physical state from crystal form to amorphous form. HPLC analysis of the developed films showed CBD content to be in the range of 95 to 105% of the nominal expected value. The drug release profile of the extruded films was significantly improved by 2to 5-foldcompared to the control (CBD powder) using the same condition for the dissolution test. In conclusion, HME was successfully used to develop an enhanced CBD buccal film.

Acknowledgments
We thank Coy Waller lab at the University of Mississippi for providing CBD for this study

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