Scientific Poster Abstracts

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PA-1: Analytical Chemistry Aspects of Botanicals

New Natural Product Certified Reference Material Resources Supported by the NIH Office of Dietary Supplements

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The NIH Office of Dietary Supplements (ODS) Analytical Methods and Reference Materials (AMRM) Program supports the development of tools that promote and facilitate rigorous and reliable characterization of dietary supplement (DS) identity, composition, and purity, as well as assessments of authenticity and contamination of botanical and other natural product raw materials and finished products. AMRM goals are accomplished through development and validation of quantitative and qualitative methods, production of certified reference materials (CRMs), and support of dietary supplement focused laboratory quality assurance programs. Recent advances in DS analytical resources supported by AMRM include an expanded availability of CRM calibration solutions for key bioactive and marker phytochemical constituents of several popular botanical DS ingredients, and the development of two new cyanobacteria biomass CRMs to for the determination of cyanotoxins. The current portfolio of AMRM-supported CRM calibration solutions in partnership with MilliporeSigma includes mixture solutions of ginger gingerols and shogaols, ashwagandha withanolides and withanosides, kavalactones, echinacea phenolic compounds and isobutyl amides, and Silybum silybins, silychristin, and silydianin. AMRM partnered with NRC Canada to support the production of a cyanobacteria CRM with comprehensive non-targeted analyses and value assignments for the major classes of cyanotoxins (i.e., microcystins, nodularins, anatoxins, cylindrospermopsins, and saxitoxins) and a non-toxic Aphanizomenon sp. Control CRM, which are anticipated to be available in 2024. These newly available resources should benefit natural product researchers and industry scientists, expanding the analytical resource toolkit to better support safety assessments or to improve our understanding of how the chemical composition of DS links to their effects their effects on health.

PA-2: Analytical Chemistry Aspects of Botanicals

Benefits and Challenges in Botanical Analysis Using High-Performance Thin-Layer Chromatography (HPTLC)

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In recent years, the total number of herbal supplements (HS) and botanical products, such as plant extracts, have increased with new botanical supplements expected to be introduced to the market in coming years. Dietary supplement manufacturers have the responsibility to use appropriate, scientifically valid methods to identify their ingredients, which can be challenging if their ingredient is a complex botanical extract or plant material. To prove identity and perform specification testing, High-Performance Thin Layer Chromatography (HPTLC) is one of the techniques used by the industry to establish identity of the raw material or final herbal product. HPTLC offers a rapid and reliable technique for quality assurance and offers benefits such as low solvent use, minimum sample clean-up, and low cost compared to gas and liquid chromatography. In addition, identification can be done using visual comparison of HPTLC fingerprints. However, its use as the sole method for identity purposes is challenging, especially when the raw material is mixed with other plant species or when there is the need to differentiate closely related species. Further, the chemical composition of a plant material may vary due to the age of the plant, geographical origin, and harvesting technique with various components potentially hindering accurate identification. Additionally, standard reference materials might not be available for identification using HPTLC, especially for novel herbal ingredients. In this poster we will provide an overview of the benefits of the HPTLC technique and its limitations for the identification of botanical materials.

PA-3: Analytical Chemistry Aspects of Botanicals

Dietary Supplement Laboratory Quality Assurance Program (DSQAP): Natural Product Interlaboratory Comparison Studies at NIST

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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 developed multiple measurement tools, including natural product reference materials, to support dietary supplement (DS) analytical communities. In addition to reference material development, a Dietary Supplement Laboratory Quality Assurance Program (DSQAP) was initiated in 2007 to provide measurement tools to laboratories for the improvement of measurements of chemical constituents in DS ingredients and products. The DSQAP provides an opportunity for DS testing laboratories to participate in interlaboratory comparison studies aimed at improving comparability and accuracy of their measurements. DSQAP exercises also provide NIST and NIH ODS a means to identify community needs for reference materials, workshops, and other measurement services. This poster will highlight previous and current natural product focused DSQAP studies which includes study design, observations, and overall technical recommendations provided to the dietary supplement measurement community.

PA-4: Quality Aspects of Botanicals

Value Assignment of NIST Reference Material 8210 Hemp Plant

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The National Institute of Standards and Technology (NIST) has prepared a hemp plant reference material (RM 8210) to aid *Cannabis* and forensic laboratories in the validation of their methods, support with the development of new analytical methods, and as a quality control material for routine analysis. RMs play an important role in promoting compliance with current and future legislation, labeling accuracy, and good manufacturing processes. However, RMs are a critical measurement service that is presently lacking in the cannabis industry. RM 8210 has non-certified mass fractions for cannabinoids, total Δ9-THC, total CBD, and toxic elements on a dry-mass basis. The need to accurately measure cannabinoids in hemp plants became significantly important after passage of the 2018 Farm Bill. New legislation legalized hemp in the US by removing hemp from the DEA Scheduled 1 controlled substance list and defined it as *Cannabis sativa* with a total Δ9-THC mass fraction of less than or equal to 0.3 % on a dry-weight basis. Mass fractions (%) were assigned for eight cannabinoids by NIST using LC-UV. Cannabinoids were identified using retention times, absorbance spectra, and peak purity evaluation using a PDA detector to compare absorbance spectra across the entire peak. Contaminants such as toxic elements are the next largest analytical measurements required by *Cannabis* laboratories for hemp plant samples to ensure materials are safe. Across states, regulatory guidelines have been set for toxic elements in hemp plant products to currently include at a minimum As, Cd, Pb, and Hg, while additional toxic elements are on a state-to-state basis. Several of the other RM 8210 toxic elements were selected since identified by the US FDA Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke: Established List. Mass fractions (µg/kg, dry-mass) for toxic elements were assigned at NIST using ICP-MS, ICP-MS/MS, ICP-OES, and direct combustion AAS.

PA-5: Analytical Chemistry Aspects of Botanicals

Characterization and Identification of Sesquiterpene Lactones from *Centaurea benedicta* using Liquid Chromatography/Electrospray Ionization Quadrupole Time-of-Flight Mass Spectrometry

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Centaurea benedicta (L.) L. (Syn. *Cnicus benedictus* L.) of family Asteraceae is an annual herbaceous plant commonly known as blessed thistle or St Benedict's thistle. It is native to the Mediterranean regions of Europe, Africa, and Asia. Liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry (LC/ESI-QToF) was used to identify and characterize eleven sesquiterpene lactones (cnicin), and one lignan (arctiin), in mixed parts of *C. benedicta*. A generalized fragmentation pathway was proposed by comparing the spectra acquired for all classes of compounds. The sesquiterpene lactones in *C. benedictus* have been classified into three groups: germacranolide, guaianolides and eudesmanolides. Using the ESI-QToF method, the major core peak ions generated by germacranolides, eudesmanolides, and guaianolides were investigated. From the QToF-MS/MS spectra, fragmentation reactions of the [M+NH4]+ and [M+Na]+ ions were recorded to provide structural information about the aglycone moieties. The data illustrates the ability of positive mode ESI for the identification of sesquiterpene lactones.

PA-6: Analytical Chemistry Aspects of Botanicals

Use of Statistical Models in Untargeted Metabolomics Analysis of Açaí (*Euterpe oleracea* Mart.) Fruit, Food Powder, and Botanical Dietary Supplement Extracts for Quality and Safety

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Euterpe oleracea Mart., commonly known as açaí, is a palm fruit native to the Amazon region which has gained popularity due to its health benefits including antioxidant and anti-inflammatory activities. The objective of this work is to develop an efficient method for the untargeted characterization of various açaí extracts that can be potentially used for the chemical characterization to evaluate efficacy and safety of açaí botanical dietary supplement (BDS) products.

Açaí fruits, food products, and two BDS brands were extracted with a variety of solvents with varying polarities. Dried extracts were then reconstituted and analyzed with high-resolution LC-MS for in-depth untargeted chemical fingerprinting. The full scan mass spectra were used for multivariate statistical analysis including principal component analysis and hierarchical cluster analysis. Tandem mass spectrometry data obtained using the same liquid chromatography separation were then used to obtain positive and tentative identifications of compounds.

We demonstrate the utility of this method for the characterization of extracts of açaí from multiple origins. This workflow allowed the identification or tentative identification of 173 compounds of which 138 were described in açaí for the first time. Principle component analysis revealed that features obtained using positive mode ESI provided a better model than features obtained in negative mode. This analysis also illustrated that açaí fruits from Hawaii and food products from Brazilian-grown fruits were very similar to each other in chemical composition when extracted with water or acidic methanol but not when extracted with more lipophilic solvents such as ethanol or methanol. For one BDS brand, the two separate lots were very similar in chemical composition for both. However, the second BDS brand showed the greatest difference between lots.

PA-7: Analytical Chemistry Aspects of Botanicals

Development of a Comprehensive Analytical Method for Simultaneous Quantification of Furanocoumarins and Nootkatone in Grapefruit Essential Oils Using UHPLC-MS/MS

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Furanocoumarins and nootkatone are abundant in grapefruit essential oil. These compounds are associated with phototoxicity, raising safety concerns due to their frequent use in cosmetics and perfume. Furanocoumarins have been identified for their interference with several medicines. Their impact on intestinal cytochrome P450 enzymes, especially CYP3A4, can result in increased drug bioavailability, posing a risk of potential overdosing.

The study employed ultra-high-performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS) for various grapefruit essential oils. A rapid, robust, and sensitive analytical method was developed to accurately quantify 15 furanocoumarins, named psoralen, 5-methoxypsoralen, 8-methoxypsoralen, 8-geranyloxypsoralen, bergamottin, epoxy bergamottin, byacangelicin, byacangelicol, oxypeucedanin, oxypeucedanin hydrate, imperatorin, isoimperatorin, phellopterin, heraclenin, and isopimpinellin, and one sesquiterpene nootkatone in different types of grapefruit essential oils. The developed method was validated for precision, robustness, accuracy, the limit of detection (LOD), and the limit of quantification (LOQ). The preliminary results demonstrated that the white grapefruit essential oils contained more furanocoumarins than other essential oils. The developed method is economical and can be applied for routine analysis of furanocoumarins and nootkatone in plant samples and various products.

PA-8: Analytical Chemistry Aspects of Botanicals

Quantitative Determination of Cinnamaldehyde and Related Metabolite Cinnamic Acid from Biofluids of Human Liver Microsomes Assay by UHPLC-MS/MS

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Cinnamon bark oil is a unique kind of essential oil because of the pharmacological activities and other health benefits of cinnamaldehyde, which is primarily obtained from the dried bark of *Cinnamomum verum* (syn. *C. zeylanicum*) that belongs to the family Lauraceae. Cinnamon bark contains 1 to 4% cinnamon oil, which comprises the highest quantities of trans-cinnamaldehyde (65–80%), eugenol (5–10%), and (5–10%) trans-cinnamic acid. For centuries, cinnamon oil has been used in traditional medicine, such as Ayurveda, Unani, and Chinese medicine, to manage oxidative stress and stomach diseases, especially gastritis, blood circulation, and liver diseases [1,2].

This study aimed to develop a sensitive method to measure the levels of cinnamaldehyde and its metabolite cinnamic acid in human liver microsomes (HLMs), human liver S9 fraction (HLS9), and primary human hepatocytes (PHHs). We developed an Ultra-High-Performance Liquid Chromatography coupled with a tandem mass spectrometric detector (UHPLC-MS/MS) method for analyzing the cinnamaldehyde and cinnamon oil after a variety of human liver microsomes bioassays. Validation parameters included sample preparation, precision, accuracy, the limit of detection (LOD), the limit of quantitation (LOQ), and the linearity range. The recovery rates ranged between 100% and 112%. Relative standard variations (RSD) of intra- and inter-day studies were below 1.85 % and 2.43 %, respectively. The LOD and LOQ were 0.2 and 0.5 ng/mL for cinnamaldehyde and cinnamic acid, respectively. This method has been successfully applied to the quantitative determination of cinnamaldehyde and cinnamic acid from human liver microsomes assay biofluids.

PA-9: Quality Aspects of Botanicals

Streamlined Sample Preparation for LC-MS and GC-MS Multi-Pesticide Residue Analysis in Botanicals and Oils Using Novel Pass-through Cleanup.

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One of the biggest challenges in routine pesticide residue analysis in botanicals is addressing the complex and varying nature of those matrices. Historically, separate preparations for LC-MS and GC-MS were necessary to achieve acceptable and consistent recoveries in botanical matrices. The LC-MS method utilized an AOAC QuEChERS extraction followed by a MgSO4, primary secondary amine (PSA), and C18E d-SPE cleanup step. The GC-MS method utilized an original QuEChERS extraction followed by an SPE cleanup containing PSA and graphitized carbon black (GCB). The GCB necessitated an elution solvent containing toluene to free the planar analytes from the GCB. These separate methods come with complications of allocating additional time to sample preparation, high cost per sample due to different sorbents used, and introducing points of contamination by additional interactions with the sample. These points make it difficult to implement on a larger or routine scale. There was a need for sample preparation that can cleanup a diverse number of matrices while not trapping any pesticides. A method was developed with Agilent's Captiva EMR that answered many of the above problems. This was accomplished largely in thanks to the unique composition of these cartridges that can trap pigments without the drawback of also trapping planar analytes. The new method utilizing the Captiva EMR cartridges allowed simultaneous preparation for analysis by both LC-MS and GC-MS, saving on analyst time and cost per sample. This method has shown an increased number of analytes able to be recovered on difficult matrices such as Ginger Root Powder, Peppermint Essential Oil, Turmeric Extract Powder, and various other botanical extracts. All monitored analytes met the SANTE guidelines for analyte recovery and linearity. This streamlined procedure has allowed an uninterrupted workflow that is flexible enough to handle a wide range of botanicals and robust enough to produce consistent data.

PA-10: Quality Aspects of Botanicals

HPTLC identification and differentiation of Juniper species

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An HPTLC method has been developed to identify juniper (*Juniperus communis*) berries for use in dietary supplements. Presently there is no official method for identification of the fruit of *Juniper communis*; the current methods focus on the identification of essential oil. This method can differentiate between three common *Juniper* species: *J. communis*, *J. virginiana*, and *J. horizontalis*. Samples were a combination of commercially available botanical reference material as well as commercial samples and field collected specimens. In addition to HPTLC, high resolution accurate-mass LC-Orbitrap mass spectrometry with statistical analysis was employed to help identify marker compounds which can be further used to aid in identification of true juniper and discriminate from other species. Several potential markers were identified to aid in differentiation of the species.

PA-11: Quality Aspects of Botanicals

Ramps Metabolomics

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Ramps are wild broadleaf *Alliums* consisting of two primary species, *Allium tricoccum* and *A. burdickii*. These are widely harvested and consumed across Appalachia and the midwestern U.S. They have been used for hundreds or thousands of years as a "spring tonic" herb to promote general wellness and for many other ethnomedicinal uses. They are also a prized ephemeral culinary herb and are popular with chefs. Festivals are held each spring to celebrate these wild vegetables. To date, no phytochemical analyses have been done on the less common species, *A. burdickii*, and only limited research into *A. tricoccum,* with vitamin analysis in the 1970's and sulfur analyses conducted in the 1990's. My research used LCMS (Orbitrap) analysis paired with online metabolomics machine-learning platforms (Metaboanalyst and GNPS) to characterize several classes of important bioactive molecules in both species. Semi-quantitative comparisons (based on peak areas) were performed, and results described here. Many of these compounds are characterized for the first time in both species (anthocyanins, steroidal saponin compounds). The sulfur compounds previously characterized in *A. tricoccum* are described for the first time for *A. burdickii*. Water-soluble vitamins were also quantified via HPLC-UV/DAD and standard calibration curves. The ramps were harvested across Pennsylvania, northern West Virginia, and eastern Ohio in April- May 2023. Instrumental analyses were conducted at Penn State University from May through December 2023.

PA-12: Analytical Chemistry Aspects of Botanicals

Wastewater Whispers: CNS Pharmaceuticals Revealed

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The study of sewage-based epidemiology (SBE) and drug utilization trends has concentrated primarily on large social and public events. High-population events (e.g., intercollegiate sports and concerts) often draw tens of thousands of attendees per event in the southeastern region of the United States of America. Increased drug use has been observed at such events, and the use of illicit drugs and stimulants is a growing socioeconomic burden. These substances are recognized for heightening sensory perception and improving mood over extended periods, ultimately contributing to drug dependency, rising crime rates, and declining human health. Furthermore, the continuous occurrence of CNS drugs in wastewater may result in harmful consequences downstream for fish and other aquatic wildlife.

This study used an LC-MS-MS method to identify CNS stimulants and their metabolites in wastewater from several southern towns and cities and a university campus. Specifically, a novel analytical method involving solid phase extraction of various sludge samples collected in and around Oxford locations was quantified for several CNS drugs, such as fentanyl and alprazolam, in wastewater streams. The details of the sample collection, analytical methodology, results, and findings will be presented. Our efforts are expected to impact the forensic science community significantly and aid law enforcement agencies in implementing such analytical methodologies as a frontline defense to monitor local drug abuse trends.

PA-13: Analytical Chemistry Aspects of Botanicals

Rapid, Accurate Detection of Goldenseal (*Hydrastis canadensis*) Adulteration with Yellowdock (*Rumex crispus*) Using A-TEEM Spectroscopy

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Goldenseal (GS) resources are limited by overharvesting of wild supplies and slow adaptation of cultivation. Hence the availability and price of GS can be a motive to adulterate it with Yellow Dock (YD) roots. GS contains highly colored and or fluorescent isoquinoline alkaloids compounds including berberine, canadine and hydrastine. Standard analysis of these GS compounds involves HPLC-UV and >10 min per sample. Here we investigated GS adulteration using the patented A-TEEM method requiring < 1 min per scan. Matching w:v ethanolic extracts of three individual GS lots were adulterated with extracts from three YD lots from 1 to 100% in a nine-point calibration yielding an 81-sample mixture matrix. The data were evaluated using Gray Classical Least Squares (Gray-CLS), Partial Least Squares (PLS) and Locally Weighted Regression (LWR) (Eigenvector Inc. Solo v9.3). Key performance indicators included the R2, the Root Mean Square Error (RMSE) of Cross Validation and limits of detection/quantification (LOD/LOQ). Pure GS extract exhibited higher absorbance at 345 nm (>9x) and fluorescence EEM intensities (>30x) at ex/em 345/550 nm than pure YD. Unique GS EEM contours resolved using Parallel Factor Analysis were attributed to the aforementioned alkaloids. Using Gray-CLS with the optimized residual weighting value the cross-validated RMSE for all 81 samples was 2.44% with an R2=0.9948; this compared favorably to respective PLS and LWR model solutions of 2.13 and 1.75% with R2 values of 0.996 and 0.997. The best LOD/LOQ values were from LWR at LOD = 3.3 and (SE Intercept/Slope) = 1.45% giving LOQ = 3.3 × LOD = 4.7%. We conclude that GS adulteration by YD can be detected using A-TEEM at levels below commercially relevant adulteration scenarios. Further method improvements with respect to optimizing variable selection, signal to noise and extraction efficiency as well as direct quantification of the alkaloid composition will be discussed.

PA-14: Analytical Chemistry Aspects of Botanicals

Analysis of Absorbance-Transmittance Excitation Emission Matrix (A-TEEM) Data for Natural Product Extracts Facilitated by Linear Correlogram and Gray-CLS Methods

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A-TEEM spectroscopy rapidly (s-min) quantifies specific chemicals from complex natural product extracts. Conventional A-TEEM analyses mostly focus on inverse chemometric methods such as Partial Least Squares (PLS) and other nonlinear machine learning methods including Extreme Gradient Boosting (XGB) and Support Vector Machines (SVM). These methods are prone to the common pitfalls of over- or under-fitting. Diagnosis of these fitting errors is often difficult due to complex interactions among multiple pre-processing and hyper-tuning parameters. Here we evaluate linear, first principles based analytical methods for A-TEEM data with natural product examples. The methods include linear correlogram analysis, where concentration-dependent correlations of the calibration data set are plotted as a function of single or summed A-TEEM variables; the linear slope, intercept and R2 are simply computed and applied to test data. The second linear method is Gray-Classical Least Squares (Gray-CLS) using Eigenvector Solo v9.3. Gray-CLS optimizes a single clutter-removal filter variable based on the CLS model residuals with no additional pre-processing of the spectral or concentration data. Test cases evaluated include anthocyanins in wine, capsaicinoids in chili peppers and cannabinoids in flower extracts. Independent test data are compared for their Root Mean Square Error of Prediction (RMSEP), the Coefficient of Variation (R2) and the Linear Slope and Intercept. When target compound spectral signals are well-resolved and or strong relative to other components in the matrix, linear correlogram models can be fit-for-purpose. In other cases where the matrix spectral components are complex or interfere with the target compound spectra, Gray-CLS models can perform with close to or better statistics than PLS or XGB. In conclusion, linear, univariate solutions can eliminate fitting ambiguity and increase model robustness for A-TEEM applications.

PA-15: Chemistry, biology, and safety of volatile organics from aromatic and medicinal plants

Phytochemical Composition of *Vincetoxicum* Extracts Recovered by Pressurized Acetone, Ethanol and Water and their Antiviral Activity Against Zika Virus

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Vincetoxicum is a genus of flowering plants in the family Apocynaceae, which is native to Europe and Asia. Considering widely documented health benefits (antioxidant, anticancer, anti-inflammatory, antiviral and other bioactivities), there is an interest in a more systematic evaluation of *Vincetoxicum* spp. (poly)phenolic and other bioactive compounds as well as their properties. For instance, quercetin, luteolin and rutin were reported as the strongest antiviral flavonoids, which may have antiviral activity against numerous enveloped RNA viruses including Flavivirus. The present study aimed at preliminary screening of phytochemical composition of extracts isolated from *V. hirundinaria*, *V. nigrum* and *V. luteum* with pressurized acetone, ethanol and water by UPLC-QTOF-MS/MS. In addition, considering possible inhibitory activity of phenolic compounds against different microorganisms, extract activity was evaluated against the Zika virus *in vitro*. Firstly, an MTT assay was performed to determine the non-cytotoxic doses of the extract using human lung epithelial cells A549. Then, the effects of extracts on ZIKV-MR766 (ZIKVGFP) were evaluated by flow cytometry assay. A concentration-dependent toxicity studies revealed that the cytotoxic concentration required to reduce mitochondrial activity by 50% (CC_{50}) was from 200 to 500 µg/mL. Flow cytometry assay showed that extracts demonstrated a dose dependent anti-ZIKV activity. UPLC-QTOF-MS/MS analyses revealed the presence of many compounds, quercetin, isoquercetin, luteolin and rutin being the major flavonoids according to the ion intensity. Several other compounds have also been tentatively identified: chlorogenic acid, quercetin 3-O-pentoside, kaempferol, antofine. In summary, a well-designed extraction process of *Vincetoxicum* spp. leaves enabled to obtain the extracts containing different classes of biologically active compounds with potent antiviral properties.

PA-16: Agrochemical Aspects of Botanicals

Adopting Agro-ecological Zoning Model to Analyse Replacement of Paddy Cultivation with Medicinal Plants in the Indian State of Punjab

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Punjab, an Indian state, heavily relies on agriculture, contributing 29% of the nation's rice and 20% of its wheat. This monoculture is leading to depletion of soil nutrients, necessitating increased chemical inputs. Moreover, paddy cultivation, requiring substantial water, is depleting Punjab's groundwater by 0.5 meters annually. To enhance diversity, scientists propose replacing paddy fields with alternate crops. The Ministry of AYUSH, Government of India is exploring medicinal plant cultivation as a profitable venture. Presently, less than 2% of Punjab's land is dedicated to this. Aim: With no clear policy to suggest the right medicinal plants in the right location, this study is focused on introducing medicinal plants like Andrographis paniculata and Chlorophytum borivilianum to replace paddy. Methods: Agro-ecological zoning, using meteorological data of past 20 years from IMD (Indian Meteorological Department) and PAU (Punjab Agriculture University) stations, determined temperature and rainfall ranges. The soil characteristics were determined using benchmark soil network. GIS Arc.GIS 10.3 helped to create digital soil maps, overlaying native crop requirements to identify suitable areas. Results and discussion: Andrographis paniculata, a Kharif crop, suited Punjab's Roopnagar, Hoshiarpur, and Pathankot districts (Zone I) due to its high-water requirement. With increased irrigation, it can thrive in other zones. In Punjab, the average high and low temperatures during the kharif season varied from 34.4 to 36.7°C and from 22.4 to 26.7°C, respectively. With 500–1500 mm of rainfall during the Kharif season, zones I, II, and III are ideal for *Chlorophytum borivilianum*, while Zone V is less suitable due to lower rainfall and higher soil pH. Conclusion: As per projection by the PRECIS model and given the industrial demand and climatic suitability, these medicinal plants could replace paddy cultivation, contingent on assured marketing connections.

PA-17: Quality Aspects of Botanicals

Reference Standards as Tools for Dietary Supplement Quality Control

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Reference standards are highly characterized substances intended for use in conducting the quality control tests and analytical procedures associated with documentary standards (written monographs that describe specifications and test methods for identity, purity, strength, and limits on contaminants). These materials are critical components of quality systems to help ensure accuracy of labeling. They may also be used for non-quantitative identity testing and as system suitability standards to ensure adequate performance of the chromatographic system. Based on the intended use, reference materials may be pure compounds or matrix materials, such as botanical extracts or enriched fractions. Dietary supplement cGMPs expect specifications including the use of criteria for selecting standard reference materials used in performing tests and examinations [21CFR111.315(d)]. USP and NIST reference materials serve as a widely acknowledged quality benchmark in establishing quality of dietary supplement products and their ingredients.

This session will discuss perspectives from reference standards producers and users on the terminologies, stringency of development, qualification, value assignment, and use of reference standards to exchange views and share experiences on the different issues related to the subject. The information sharing by the panel and the discussion with the participants on reference standards and related topics is expected to provide a better understanding of the use of reference standards.

PA-18: Quality Aspects of Botanicals

USP Standards for Mate (*Ilex paraguariensis*) and Guayusa (*Ilex guayusa*) Leaves: Development and Validation of a UPLC-DAD Method for the Simultaneous Analysis of Methylxanthines and Caffeoylquinic Acids

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Mate (or Yerba Mate) (*Ilex paraguariensis*) and Guayusa (*Ilex guayusa*) leaves have been traditionally consumed as infusions in South America. The international market has shown a growing interest in this category of food. Both Mate and Guayusa are rich sources of methylxanthines and caffeoylquinic acids, which confer stimulant and antioxidant properties for applications in novel energy beverages and the formulation of dietary supplements. Mate and Guayusa leaves are very similar botanicals belonging to the Aquifoliaceae family, which creates taxonomic challenges. In addition, different commercial and quality grades exist for both leaves, usually based on the allowable amount (%) of stems and post-harvest processing, which creates additional challenges in the selection of the appropriate materials for DS applications. Ingredients derived from Mate and Guayusa leaves occur in the form of cut leaf, powdered leaf, and aqueous or ethanolic extracts containing different caffeine vs caffeoylquinic acids ratios. Due to the increasing demand for these ingredients, there is an imperative need to create pharmacopeial standards to ensure the quality and safety of these ingredients and to support the specifications for the different articles of commerce. This poster summarizes the work carried out by USP for the development and validation of a UPLC-DAD method for the simultaneous analysis of methylxanthines and caffeoylquinic acids to be proposed as identification and composition tests in the creation of new standards for Mate and Guayusa leaves. Quality by Design (QbD) approach was applied for the sample preparation optimization for the efficient extraction of these compounds in the different matrixes and UPLC method development. The method was validated according to the requirements of USP-NF General Chapter <1225> Validation of Compendial Procedures, regarding specificity, linearity, accuracy, precision, robustness, and stability of solutions.

PA-19: Quality Aspects of Botanicals

Applicability of LC-QToF and Microscopical Tools in Combating the Sophisticated, Economically Motivated Adulteration of Poppy Seeds

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Intentional adulteration of poppy seeds is common, often combined with immature, less expensive, exhausted, or substituted with morphologically similar seeds, viz., amaranth, quinoa, and sesame. For a safer food supply chain, preventive measures must be implemented to mitigate contamination or adulteration. Morphine and codeine are the two principal opiates found in the opium poppy (Papaver somniferum L.) and are therapeutically used for pain management. Poppy seeds with low opiates are primarily used for culinary purposes due to their nutritional and sensory attributes. Moreover, the simultaneous analysis of P. somniferum and its adulterants is largely unknown. Pre- and post-processing further complicate the alkaloid content and may pose a significant health hazard. Considering the challenges, two independent methods were investigated with eight botanically verified and fifteen commercial samples. Microscopical features were established for the authenticity of raw poppy seeds. Morphine, codeine, and thebaine quantities ranged from 0.8–223, 0.2–386, and 0.1–176 mg/kg, respectively, using LC-QToF. In the majority of situations, conventional opiates have a higher content than papaverine and noscapine. The proposed and developed method/s provided a chemical profile of 47 compounds that can be effectively applied to distinguish poppy seeds from their adulterants and may serve as an effective tool to combat ongoing adulteration.

PA-20: Quality Aspects of Botanicals

6-Oxofurostane and (iso)Spirostane Types of Saponins in *Smilax sieboldii*: UHPLC-QToF-MS/MS and GNPS-Molecular Networking Approach for the Rapid Dereplication and Biodistribution of Specialized Metabolites

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Identification of novel phytochemical secondary metabolites following classical pharmacognostic investigations is tedious and often involves repetitive chromatographic efforts. During the past decade, Ultra-High Performance Liquid Chromatography-Quadrupole Time of Flight-Tandem Mass Spectrometry (UHPLC-QtoF-MS/MS), in combination with molecular networking, has been successfully demonstrated for the rapid dereplication of novel natural products in complex mixtures. As a logical application of such innovative tools in botanical research, more than 40 unique 3-oxy-, 3, 6-dioxy-, and 3, 6, 27-trioxy-steroidal saponins were identified in aerial parts and rhizomes of botanically verified *Smilax sieboldii*. Characteristic mass fragmentation patterns of aglycones, diosgenin, sarsasapogenin/tigogenin, or laxogenin were critical to establishing the unique nodes belonging to six groups of nineteen unknown steroidal saponins identified in *S. sieboldii*. Mass fragmentation analysis resulted in the identification of 6-hydroxy sapogenins, considered to be key precursors in the biogenesis of characteristic smilaxins and sieboldins, along with other saponins identified within *S. sieboldii*. These analytes' relative biodistribution and characteristic molecular networking profiles were established by analyzing the leaf, stem, and root/rhizome of *S. sieboldii*. Deducing such profiles is anticipated to aid the product integrity of botanical dietary supplements while avoiding tedious pharmacognostic investigations and helping identify exogenous components within finished products.

PA-21: Quality Aspects of Botanicals

Identification of Botanical Ingredients with Athletic-Performance-Enhancing–Effects in Dietary Supplements

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Since the US Food and Drug Administration (FDA) banned ephedra from dietary supplements in 2004, supplement manufacturers have promoted a complex variety of alternative botanical compounds for athletic performance enhancement. The FDA does not preapprove these ingredients, or any supplement ingredient, for either efficacy or safety before their introduction, but FDA inspections have found that supplement manufacturers often fail to comply with basic manufacturing standards, such as establishing the identity, purity, or composition of the final product. Given the products' potentially complex physiologic effects and concerns regarding manufacturing quality, we determined the accuracy of dietary supplement labels declaring halostachine, octopamine, and turkesterone. Eighty-nine percent of dietary supplement labels did not accurately declare the ingredients found in the products, and 12% of products contained FDA-prohibited ingredients. In the current study, which to our knowledge is the first to quantify these five supplement ingredients, only 11% of products were accurately labeled and three different FDA-prohibited ingredients were found, including an unapproved drug available in Russia (i.e., omberacetam), three drugs formerly available in Europe (i.e., octodrine, oxilofrine, and deterenol), and one drug that has never been approved in any country (i.e., 1,4-dimethylamylamine).

PA-22: Quality Aspects of Botanicals

Determining Quality of Ashwagandha: Current Challenges and Possibilities

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Ashwagandha (*Withania somnifera* (L.) Dunal) has gained significant attention in recent years for its adaptogenic properties that are supported with preclinical and clinical studies. Steroidal lactones and their glycosides, commonly known as withanolides, are generally perceived as the active constituents. Accordingly, the extracts 'standardized' to contain known concentrations of withanolides are used in dietary supplements and complementary medicines across the globe. Pharmacopoeial monographs on Ashwagandha and its extracts are available in USP, EP, BP, IP and API which describe analytical methods based on withanolides, mainly by HPLC and TLC. Our work on Ashwagandha focused on the following points:

1. Can Ashwagandha be evaluated just based on content of withanolides for quality? What are the pitfalls of this approach of excessive focus on content of withanolides?
2. Pros and cons of currently available analytical methods for withanolides.
3. Are the current methods adequate to identify mislabeling and adulteration?
4. Impact of COVID and climate change on supply chain and quality of Ashwagandha roots

We wish to elaborate our findings and thoughts on the above points in this presentation. We expect our findings to ignite further deliberations on the topic towards developing a better understanding on quality of Ashwagandha.

PA-23: Quality Aspects of Botanicals

Development and Validation of Fast GC/FID Method for Cannabinoids Analysis in Cannabis Plant Material of Different Chemovars

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With the legalization of *Cannabis* for medical and recreational purposes in many states, the demand for reliable and accurate cannabinoid analysis has increased. To meet this demand, a simple and fast gas chromatography-flame ionization detection (GC-FID) method was developed and validated for the quantification of the eight main cannabinoids in *Cannabis*. These cannabinoids are cannabidivarin (CBDV), tetrahydrocannabivarin (THCV), cannabichromene (CBC), cannabidiol (CBD), and Δ8-tetrahydrocannabinol (Δ8-THC), Δ9-tetrahydrocannabinol (Δ9-THC), cannabigerol (CBG), and cannabinol (CBN). The method provided baseline separation of the usually coeluted pairs of cannabinoids; CBD /CBC and CBG/CBN. The calibration curve was found to be linear between 5 and 100 µg/mL for all of the target analytes, with a significantly high value of regression coefficient ($r2 > 0.99$). The limit of detection (LOD) and the limit of quantification were 1 µg/mL and 5 µg/mL, respectively. The inter-day and intra-day precisions were less than 15% [relative standard deviation (%RSD)] and the accuracy ranged from 85 to 115 as % recovery. The method was applied for routine analysis of the eight major cannabinoids in different *Cannabis* biomass and *Cannabis* plant extracts. This method provides a reliable and efficient way to analyze the potency of different *Cannabis*-based products and for routine quality control testing for regulatory compliance and can help ensure the safety and consistency of *Cannabis* materials.

PA-24: Quality Aspects of Botanicals

Characterization of Black Cohosh Standard Reference Materials (SRMs) with Mass Spectrometry Detection

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This study presents the characterization of four Black Cohosh Standard Reference Materials® (SRM 3295 – Rhizomes, SRM 3296 – Leaves, SRM 3297 – Rhizome Extract, and SRM 3298 – Solid-Oral Dosage Form) developed collaboratively between the National Institute for Standards and Technology (NIST) and the National Institutes of Health Office of Dietary Supplements (NIH ODS). A targeted liquid chromatography-mass spectrometry (LC-MS) method employing selected ion monitoring (SIM) in negative mode was used for value assignment of key bioactive or marker compounds, including cimiracemoside C, cimigenol-3-β-D-xyloside, 23-epi-26-deoxyactein, cimiracemoside D, 23-epi-26 deoxycimicifugoside, cimicifugoside H-1, and cimicifugoside H-2 within the homogeneous black cohosh matrices. These new SRMs and the validated analytical method employed in their value assignment contribute to the quality assurance and standardization of black cohosh products and preparations used in clinical research, ensuring their reliability and safety in dietary supplement formulations.

PA-25: Quality Aspects of Botanicals

Microscopy, HPTLC, and LC-DAD-Q-ToF Validation of Nut-based Weight-Loss Dietary Supplements, *Aleurites moluccanus* (Candlenut) and *Bertholletia excelsa* (Brazil nut)

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Aleurites moluccanus (candlenut) and *Bertholletia excelsa* (Brazil nut) are marketed as dietary supplements for weight loss. These dietary supplements have been found to sometimes be adulterated with toxic nuts/seeds from *Cascabela thevetia*, commonly known as yellow oleander or lucky nut. This study emphasizes the key identification parameters to differentiate the genuine and adulterated nuts. Samples were obtained from authenticated sources of the nuts and from commercial sources of dietary supplements. The presence of yellow oleander was confirmed in all commercial dietary supplement samples marketed as candlenut as well as in commercial samples of Brazil nut. This study provides simple key identification characters using micro-morphology and histochemical localization of cardio glycosides in the commercial nuts, HPTLC fingerprints, and LC-DAD-Q-ToF analytical parameters to detect and identify adulteration in commercial products.

PA-26: Quality Aspects of Botanicals

Advance Microscopy, GC/Q-ToF, and LC/Q-ToF Characterization of *Salvia mellifera* (Black Sage), *Saliva apiana* (White Sage) and their Varieties

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Salvia mellifera (black sage) and *Salvia apiana* (white sage) are known for complex introgressive hybridization. Morphological variations between these two *Salvia* species and their varieties present overlapping characters that may cause confusion in identifying the genuine species in nature. Since these plants may be candidates for investigation of botanical-based drugs, this study aimed to differentiate the two *Salvia* species from each other and to group the closely resembling varieties. With many *Salvia* species offering health benefits, the morphological analysis and chemical fingerprinting of these two species will benefit their potential consideration for further evaluation of their health benefits. Detailed histology, histochemistry, and chemical characterizations were used to identify distinguishing characteristics of genuine *S. mellifera* and *S. apiana* along with two varieties resembling *S. mellifera* or *S. apiana*. Macroscopic and microscopic characterization of leaf lamina and types of trichomes can differentiate the morphology of *S. mellifera* from *S. apiana*. The histochemical analysis of glandular trichomes revealed the presence of flavonoids, terpenes, and other common secondary metabolites. Micropatterns of calcium oxalate crystals were identified as being characteristic and aiding in differentiation. Even though the external morphological characters of the two varieties resemble an intermediate between *S. mellifera* and *S. apiana*, chemical qualitative analyses via LC/Q-ToF and GC/Q-ToF indicate a chemical fingerprint more similar to that of *S. apiana*. This analysis uses various approaches including morpho-anatomy, trichome micromorphology, and chemical similarities to identify and differentiate between *S. mellifera*, *S. apiana*, and their close varieties.

PA-27: Quality Aspects of Botanicals

Using Isotopic Characterization to Identify the Authenticity of *Ilex paraguariensis* Brands Marketed in Southern Brazil

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Yerba mate (*Ilex paraguariensis*) has economic and cultural importance in South America. However, adulteration can occur due to a lack of quality control during processing. The study aimed to measure adulteration of yerba mate brands marketed in southern Brazil with sucrose, through isotopic characterization. Fifteen brands from local grocery stores were analyzed using eight samples of yerba mate derived from plants of different environments. Five samples of other species of *Ilex* genus and *Ligustrum japonicum*, which commonly contaminate yerba mate, were also tested. The isotopic composition of C ($\delta^{13}C$) and % N pointed out that some brands may have been adulterated with sucrose and a combination of % N and $\delta^{15}N$ may be sufficient to identify contamination by leaves from other species. This study shows that isotopic characterization can potentially identify yerba mate that is adulterated with sucrose, and this method can also be used for quality control in the food industry and to warrant further investigation.

PA-28: Quality Aspects of Botanicals

Chemical Characterization and Quantitative Determination of Flavonoids and Phenolic Acids in Yerba Santa (Eriodictyon spp.) Using UHPLC/DAD/Q-ToF

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Eriodictyon species, commonly known as yerba santa, are plants native to the Southwestern US and northern Mexico. The plants are known for their medicinal properties and are used to treat various ailments, in particular, respiratory conditions. Despite a long history of traditional use, many of the species have never been fully chemically characterized, and the constituent range of the species has not been comprehensively reported. In an effort to establish a quality control and chemical characterization method, an extensive set of *Eriodictyon* species including *E. californicum, E. angustifolium*, *E. trichocalyx*, *E. crassifolium*, *E. tomentosum*, *E. traskiae*, and *E. capitatum* were investigated. Fourteen compounds were quantified utilizing a UHPLC/DAD method. The results from the method validation demonstrated excellent linearity (R2 > 0.99) and sensitivity as evidenced by LOD (0.01–0.1 µg/mL) and LOQ (0.05–0.2 µg/mL). Likewise, the method was found to be precise (RSD < 2.78%) with recoveries between 88.9 and 103.2%. To the best of our knowledge, this work encapsulates the most comprehensive data set currently available for the chemical characterization and quantification of the primary constituents in Eriodictyon species. Additionally, results of this study also demonstrated the applicability of the developed method for quality assessment of raw material and commercial herbal products containing different *Eriodictyon* species.

PA-29: Chemistry, biology, and safety of volatile organics from aromatic and medicinal plants

Effect of Light Quality and Natural Ventilation System in Adventitious Roots Induction and Podophyllotoxin Content from *Hyptis suaveolens* (L.) Poit. (Lamiaceae)

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Hyptis suaveolens has podophyllotoxin (PTOX) in its roots, which is a natural product precursor for anticancer chemotherapy, including the semi-synthetic derivatives etoposide, etoposide phosphate, and teniposide. Adventitious roots *in vitro* tissue culture has been increasingly used to produce rare and high-quality medicinal compounds. Aimed to investigate the induction of adventitious roots in *H. suaveolens* leaves under different light qualities and natural ventilation systems. Firstly, the induction of adventitious roots was evaluated under different light conditions and, in a second one light condition were combined with a natural ventilation system. Plantlets of *H. suaveolens* were the explant donors for both experiments, 48 days after seed inoculation. Leaves presenting excised edges at the size of 1×1 cm were used. The Murashige and Skoog (MS) with 0.25 mg/L IBA, 2 mg/L NAA, 30 g/L sugar, and 5.5 g/L agar was used as the basic culture medium. Four explants were inoculated per flask. The first experiment evaluated different light (LED) qualities: T1) red, T2) blue, T3) white, T4) fluorescent, T5) dark (no light). In the second experiment, 6 treatments were evaluated in total: 2 types of ventilation systems (without porous membrane and 4 porous membranes) and 2 qualities of light (monochromatic red and absence of light). The first experiment was evaluated at 42 days and the second at 48 days regarding root dry weight (RDW) and root PTOX content. Direct adventitious roots emerged from *H. suaveolens* leaf explants only in the absence of light and under red monochromatic light. The RDW were statistically equal, on average 25.82 mg per bottle. PTOX content were 10,72 µg/g and 9,93 µg/g, respectively. In the second experiment, the dark condition without membranes accumulated greater RDW (84.02 mg per bottle) and PTOX (46.68 µg/g). In conclusion, adventitious root formation and PTOX accumulation was observed in leaves grown in semisolid MS medium in dark conditions without natural ventilation.

PA-30: Trade Aspects of Botanicals

Chinese Medicinal Herb Production in Southwestern US: Factors Influencing the use of Complementary and Alternative Medicine.

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The use of complementary and alternative medicines, referred to as CAM, has increased significantly over the last several decades. Interest in this growing market from farmers in the Southwestern U.S. has also increased, as medicinal herbs are well suited to the climate and serve existing strong cultural traditions in alternative healing in New Mexico (Moore 2008). CAM therapies include meditation, yoga, herbal medicine and acupuncture, lesser-known products and practices such as Reiki (an energy-healing practice) and biofeedback. Previous works have suggested many factors impacting the rise in alternative medicine such as rising dissatisfaction with traditional medicine and diagnosis of chronic health conditions and new health-conscious individuals demanding organic consumption. To address objectives to support farmers contemplating crop changes to adapt to market and climate challenges, our team sought to contribute to an identified need for a better understanding of the strength and character of the market through identifying the socio-economic factors driving the increased usage of CAM. We used data from a 2019 national consumer survey to assess consumer preferences for CAM after basing the survey on a CDC publication on the increased use of CAM. We used a bivariate logistic regression or binary logic model to identify significant predictors of CAM use while the survey questions focused on participants' use and perceptions of CAM therapies, with an emphasis on herb use. Results show that inclusive socio-economic factors over time have included age bracket, educational level, health conditions and family size. This market characterization helps break the gap between alternative medicine, consumers and farmers who will be growing the herbs. Future research includes an in-depth survey of producers and buyers to better understand crop and production preferences.

PA-31: Regulatory Aspects of Botanicals

Identity Authentication of Botanical Materials for New Dietary Ingredient Notifications

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The Food and Drug Administration (FDA) regulates dietary supplements by the authority outlined in the Food, Drug, and Cosmetic Act (FD&C Act) and the Dietary Supplement Health and Education Act of 1994 (DSHEA). These acts define a dietary ingredient and provide the basis for the FDA to evaluate a new dietary ingredient (NDI) as the subject of an NDI notification. Herbs or other botanicals, as well as their concentrates, metabolites, constituents, and extracts can be dietary ingredients. In order to fulfill the FD&C Act dietary ingredient definition in §201(ff)(1)(C) or §201(ff)(1)(F), the botanical materials from which an NDI is produced must be identified. Challenges can arise in authenticating the identity of botanical materials used to produce an NDI, such as a lack of sufficient detailed information intended to uniquely characterize and verify the taxonomy, origin, supplier, form, and/or composition of botanical materials. This project aims to illuminate common challenges associated with identity verification of botanical materials and propose practical suggestions for ensuring adequate identification of botanical sources. By improving the quality of NDI notifications, and subsequently improving the scientific evidence to support an NDI, the project can contribute to enhancing regulatory compliance within the dietary supplement industry.

PA-32: Quality Aspects of Botanicals

Is There One Method to Rule Them All? Comparing DNA Barcoding, HPTLC, and Untargeted Metabolomics for Herbal Product Identification.

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Botanical identity is a key component of herbal supplement quality programs; however, the innate complexity of plant products complicates testing results and drawing relevant conclusions. There are a few standard approaches to herbal identification testing with a history of success for numerous herbs and formulations, including High-Performance Thin Layer Chromatography (HPTLC) and DNA barcoding. Literature suggests that modern high-resolution mass spectrometry instrumentation is the next step for improving identity evaluations. To date, few studies have directly compared the identification capabilities of these technologies. Thus, we compared the performance of ISSR DNA barcoding, HPTLC, and untargeted metabolomics for identifying *Ocimum* (basil) herbal products. Due to limited reference standards, we generated a reference library by growing 30 *Ocimum* varieties, belonging to three species, in a greenhouse. We first evaluated the ability of each method to separate the three *Ocimum* species using unsupervised statistical models. Next, we investigated which, if any, method can reliably predict the species of commercially available *Ocimum*. Notably, DNA barcoding was unable to reliably produce data for the consumer product, making the approach unsuitable for predictions. Ultimately, each method varied in its classification and prediction capabilities.

PA-33: Agronomical Aspects of Botanicals

The Effect of Nitrogen-fixing Bacteria on Grain and Oil Production of Mustard (*Brassica juncea*) in Rainfed Conditions

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Diazotrophs are the mini-nitrogen factories that convert available atmospheric N2 to ammonia through a process known as “biological nitrogen fixation” which is then taken up by the plants for its metabolic functioning. Free living nitrogen fixing bacteria has been considered as low cost biofertilizer in agricultural production. This study was carried out to investigate the effect of three isolates of nitrogen-fixing bacteria on brown mustard grain and oil production in rainfed conditions .The experiment includes 3 types of nitrogen-fixing bacterial isolates and 7 fertilizer levels; 1) control (no bacteria or chemical fertilizers), 2) bacterial isolate 3MDP-1, 3) bacterial isolate 3MDP-6, 4) bacterial isolate 2MDP-10, 5) chemical fertilizer 250 kg/ha of urea and 150 kg/ha of phosphorus based on soil testing + bacteria 3MDP-1, 6) chemical fertilizer + bacteria 3MDP-6, and 7) chemical fertilizer + bacteria 2MDP-10. The results show that grain yield and yield components, as well as the oil percentage and oil yield of brown mustard grains were not affected significantly by treatments. The results of this research indicate that in rainfed conditions, application of biofertilizers alone or in combination with chemical fertilizers has a similar effect on grain yield and oil production of brown mustard. Therefore, it is possible to reduce the consumption of chemical nitrogenous fertilizers by replacing them with biofertilizers. This could lead to reduced mustard grain and oil production costs and healthier products as well as avoiding environmental pollution.

PB-1: Biological Aspects of Botanicals

Antidepressant Effect of Andrographolide in Chronic Unpredictable Stress Zebrafish Model.

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Recent studies have shown that *Andrographis paniculata* (*A. paniculata*) has an anti-depressive effect on rodents. Zebrafish have also become a valuable complementary model for studying antidepressant drug discovery. This study aimed to investigate the anti-depressive effect of *A. paniculata* extract and andrographolide in a zebrafish model of chronic unpredictable stress (CUS). Four groups of zebrafish (n=10/group) were tested in open-field and social interaction tests 24 hours after treatment: control, CUS (stressed, untreated), CUS+ *A. paniculata* (100 mg/L), and CUS+fluoxetine (0.01 mg/L). After screening the extract, the behavioural and cortisol responses of andrographolide (5, 25, and 50 mg/kg, i.p.) and fluoxetine (10 mg/kg, i.p.) were evaluated. Before the behavioural study, acute toxicity and characterization of *A. paniculata* extract using UHPLC-ESI-MS/MS were performed. The results showed a significant reduction in freezing duration in the *A. paniculata*- (t-test, p=0.0234) and fluoxetine-treated groups (t-test, p<0.0001) compared to the CUS group. Only the fluoxetine-treated group showed a significant increase in total distance travelled and contact duration (t-test, p=0.0007) and (t-test, p=0.0207), respectively. Both treatment groups showed a significant increase in highly mobile duration. Acute andrographolide treatment (50 mg/kg, i.p.) showed a significant reduction in freezing duration (p=0.0042), duration in a dark area (p=0.0338), and cortisol level (p=0.0156), as well as an increase in total distance travelled (p=0.0144). LC-MS/MS detected twenty-six compounds in the *A. paniculata* extract, with andrographolide content at 0.042 µg/g. According to cortisol analysis, *A. paniculata*'s LC50 is 627.99 mg/L, while andrographolide's EC50 was determined as 26.915 mg/kg. Further examination of the cellular and molecular mechanisms of andrographolide's anti-depressive effects is strongly encouraged to assess its potential as an antidepressant.

PB-2: Biological Aspects of Botanicals

Evaluating the Metabolite Profile and Ethnopharmacological Relevance of *Melastoma malabathricum* L. Leaf and Flower Extracts with Reference to Antioxidant and Antithrombotic Capacity: A Comparative Study.

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Melastoma malabathricum L., is an herbaceous shrub, widely used in Indian folklore medicine. This study aims to scientifically validate the traditional claims and explore the medicinal importance of *M. malabathricum* L. by highlighting the metabolite profile and correlating it with the antioxidant capacity, anticoagulant efficacy and in vitro thrombolytic properties of the leaf (MMLE) and flower (MMFE) extracts of *M. malabathricum* L. GC-MS/MS based untargeted metabolite profiling established the presence of 108 and 107 metabolites in MMLE and MMFE, respectively, with only four metabolites in common between the two extracts suggesting their differential pharmacological properties. The major bioactive metabolites identified in MMLE were squalene (12.63%), catechol (6.04%), and beta-sitosterol (5.99%); whereas 5-hydroxymethylfurfural (17.97%), and diosgenin (10.09%) were the core metabolites identified in MMFE. Free radical scavenging activity assays affirmed the strong antioxidant properties of both MMLE and MMFE. It was also observed that MMLE and MMFE had better proteolytic specificity towards the blood coagulation factor fibrinogen than casein. MMLE exhibited αβ-fibrinogenase activity and could also degrade the γ-band of fibrinogen. MMFE also demonstrated αβ-fibrinogenase activity but without any effect on the γ-band of fibrinogen. MMLE exhibited dose dependent anticoagulant properties and inhibited the prothrombin activation property of Factor Xa. However, MMFE showed no significant effect on the blood coagulation process, in spite of its ability to inhibit Factor Xa. Both MMLE and MMFE also had significant *in vitro* clot (thrombus) lysis property. These findings suggest the potential candidature of MMLE and MMFE which can be explored further for the development of conventional antithrombotic and/or thrombolytic agents.

PB-3: Biological Aspects of Botanicals

Anti-inflammatory and Anti-oxidative Effects of STW 42 and Root Extract of *Althaea officinalis* L. on endothelial cells, fibroblasts and macrophages in vitro.

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The root extract of *Althaea officinalis* L. (REAo) has been used since ancient times to treat mild stomach/gut discomfort and dry cough. Application of STW 42 (Phytohustil®), which contains REAo, induces regeneration of the lesioned mucosa. In this context, mucosa wound healing depends on the recruitment of several cell types, e.g., endothelial cells, fibroblasts, and macrophages (MΦ). Reactive oxygen species (ROS)-induced damage is associated with mucosal infiltration of activated leukocytes, such as MΦ, which produce excessive ROS and pro-inflammatory cytokines that may overwhelm the antioxidant defenses and exacerbate mucosal inflammation. Additionally, fibroblasts exhibit a metabolic adaptation to control inflammation-related oxidative stress.

We aimed to investigate the anti-inflammatory/-oxidative properties of STW 42 and REAo on human dermal fibroblasts (NHDF), human umbilical vein endothelial cells (HUVEC), and human acute monocytic leukemia (THP-1) differentiated MΦ, critical cellular components of the gastrointestinal and oral mucosa. Pre-treatment (24 h) of HUVEC, NHDF or THP-1-MΦ with STW 42 or REAo (100–1000 µg/mL) significantly inhibited the H2O2-induced intracellular ROS production by 30.0% to 58.7%. Whereas 24 h (HUVEC, NHDF) or 48 h (THP-1-MΦ) pre-treatment with STW 42 or REAo (10–1000 µg/mL) inhibited the LPS-activated IL6 release by 25.0–67.0%. Additionally, 48 h pre-treatment of THP-1-MΦ with 50–500 µg/mL STW 42 or REAo significantly inhibited LPS-induced TNF-α release by 21.5% (50 µg/mL) to 52.0% (500 µg/mL), in comparison with LPS-treated MΦ. The observed effects of STW 42 or REAo were similar to 25–100 µM diclofenac, which was used as an anti-inflammatory control.

These anti-inflammatory and antioxidant properties may support the benefit of STW 42 in patients during the treatment of irritated laryngopharyngeal diseases; however, they may also have protective/reparative effects on the gastrointestinal mucosa.

PB-4: Biological Aspects of Botanicals

Inhibitors of Multidrug Efflux Pumps from Natural Sources: An *In-Silico* High-Throughput Virtual Screening and *In-Vitro* Validation & Formulation

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Antimicrobial resistance in bacterial pathogens is a challenge that is associated with high morbidity and mortality. Antibiotic resistance happens when germs like bacteria and fungi develop the ability to defeat the drugs designed to kill them. Overuse of antibiotics is the principal cause of resistance evolution. Incorrectly prescribed antibiotics also contribute to the promotion of resistant bacteria. Acquisition of genetic material that confers resistance is possible through all of the main routes by which bacteria acquire any genetic material: transformation, transposition, and conjugation. There are multiple components in the bacterial cell that may be targets of antimicrobial agents, and there are just as many targets that may be modified by the bacteria to enable resistance to those drugs. Efflux pumps allow microorganisms to regulate their internal environment by removing toxic substances, including antimicrobial agents, metabolites and quorum sensing signal molecules. We have screened an *in-sillico* library of natural compounds to evaluate their ability to inhibit MexAB-OprM [PDB ID: 1lnw and 5daj] efflux system of *Pseudomonas aeruginosa* and MepA [PDB ID: 3ECO] efflux system of Staphylococcus aureus by binding specific efflux protein. We employed HTVS (High Throughput Virtual Screening) docking using MOE docking software to identify hits from the Pubchem database subset. A total of 20 compounds were shortlisted against MepA and MexABOprm considering the site of interaction suitable efflux pump inhibitory compounds were selected based on predicted binding energy. Molecular docking calculations identified Hesperidin from *Citrus aurantium* peels and carnosic acid from *Rosmarinus officinalis* leaves as good candidates to inhibit the MexAB-Oprm efflux pump of *P.aeruginosa* and 3ECO efflux pump of *Staphylococcus aureus*. These results are further validated by *in vitro* microbiological assays that showed their activity.

PB-5: Biological Aspects of Botanicals

Antidiarrheal Coumarins from *Psydrax schimperianus* (A. Rich.) Bridson Roots

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Psydrax schimperianus (A. Rich.) Bridson. roots are used for the treatment of diarrhea in the West Arsi zone, Ethiopia. This study aimed to investigate the in vivo antidiarrheal activity of crude extract and coumarins isolated from the roots of *P. schimperianus* to provide a pharmacological basis for its traditional use as an antidiarrheal agent in Ethiopia. The crude root extract of *P. schimperianus* was tested *in vivo* for antidiarrheal efficacy in mice utilizing castor oil-induced diarrhea, gastrointestinal transit time, and enteropooling models at doses of 100, 200, and 400 mg/kg. Phytochemical investigation of the crude root extract led to the isolation of two coumarins, isoscopoletin, and scoparone. Isoscopoletin and scoparone were evaluated for antidiarrheal activity against castor oil-induced diarrhea model at 10 mg/kg and 20 mg/kg doses. The crude root extract of *P. schimperianus*, at doses of 100, 200, and 400 mg/kg, inhibited defecation by 37.5, 46.2, and 61.2%, respectively. At a dose of 20 mg/kg, scoparone and isoscopoletin reduced defecation by 61.2 and 66.6%, respectively. The study warrants further investigation of isoscopoletin and scoparone towards development as a novel treatment for diarrheal diseases.

PB-6: Biological Aspects of Botanicals

Development and Evaluation of Crocetin-containing Nanogel for the Treatment of Skin Cancer

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Skin cancer is a growing health concern with increasing prevalence and mortality rates worldwide. While treatment options exist, they remain limited against aggressive and metastatic cancers. Naturally occurring compounds like crocetin have demonstrated promising anticancer potential; however, their clinical translation remains suboptimal due to poor solubility, instability, and bioavailability. The current study aimed to isolate crocetin from the seeds ethanolic extract of *Nyctanthes arbortristis* Linn to develop crocetin-loaded nanogel to enhance its delivery topically to skin tissues for potential treatment benefits. Biocompatible chitosan nanogels were synthesized to encapsulate lipophilic crocetin using an ionic gelation method optimized by Box-Behnken design. The size of the vesicles for the formulations was between 202 ± 14.2 nm and 374 ± 127 nm, while the entrapment efficiency was between 61.22 ± 0.23% and 85.16 ± 0.24%, and the drug release percentage after 8 h was between 48 ± 0.82% and 76 ± 0.52%. *In vitro* studies demonstrated the potential of the nanogel in significantly inhibiting the proliferation of skin cancer cells, evidenced by its cytotoxic effects on A375 and B16F10 cell lines while exhibiting minimal impact on healthy skin cells. Additionally, *ex vivo* studies of the nanogel showcased efficient penetration into deeper skin layers, promising enhanced drug delivery to the tumor site.

Furthermore, animal studies conducted on DMBA-induced skin cancer animal models corroborated the therapeutic efficacy of the crocetin-loaded nanogel, revealing marked suppression of tumor growth compared to conventional treatment by marketed gel or untreated groups. These findings supported the potential of this crocetin nanogel formulation as a promising strategy for skin cancer therapy, offering targeted delivery, reduced systemic toxicity, and improved treatment outcomes. Preclinical development of this targeted delivery system can facilitate the clinical advancement of this natural bioactive.

PB-7: Biological Aspects of Botanicals

Red Ginseng Extract Inhibits Platelet-leukocyte Aggregates in LPS-induced Septic Mice.

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Platelet–leukocyte aggregates (PLAs) play important roles in cardiovascular disease and sepsis. Red ginseng extract (RGE) has been well-studied for its antiplatelet and anti-inflammatory activities. However, the potential inhibitory effects of RGE on PLA have not been investigated. Six-week-old ICR mice were given oral gavage of RGE for 7 days, followed by an intraperitoneal injection of 15 mg/kg of lipopolysaccharide. Mice were euthanized 24 hours later, and blood samples were collected for further analysis. Flow cytometry was utilized to sort populations of PLAs and platelet–neutrophil aggregates (PNAs). PNAs were validated using confocal microscopy. Morphological changes in platelets and leukocytes were visualized with scanning electron microscopy. Tissue factor (TF) and platelet factor 4 (PF4) expressions were investigated using enzyme-linked immunosorbent assay. Populations of activated platelets, PLAs, and PNAs were significantly increased. Treatment with 200 and 400 mg/kg of RGE decreased platelet activation.

Moreover, the populations of PLAs and PNAs were reduced. PNAs were visible in the septic mice's blood, which was attenuated by treatment with 400 mg/kg of RGE. Morphologically, sepsis-induced platelet activation and fibrin formation in the blood. This was reduced with RGE treatment. Sepsis-induced increase in the plasma levels of TF and PF4 was also reduced with RGE treatment. This study shows that RGE is a potential therapeutic that reduces the activation of platelets and targets PLA and PNA formation. Detailed inhibitory mechanisms of RGE should be studied.

PB-8: Biological Aspects of Botanicals

Histomorphometric Lung Density Evaluation of Immulina™ Treatment Using a Murine Influenza Pneumonia Model

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Histomorphometric lung density measurements were used to evaluate the effects of Immulina™ on mouse pneumonia. Mice were intra-nasally exposed to the H1N1 influenza virus at a dose of 50,000 PFU/50 µL/mouse. Lung density was measured using the NIH ImageJ software program. Density values were compared to semi- quantitative pneumonia severity scores. Lung photomicrographs were evaluated at 25-×, 40-×, and 400-× magnification. The study included viral inoculated con- trols (IC) and non-inoculated controls (NC) and mice either treated or not treated with Immulina. Three doses of Immulina were included (25, 50, or 100 mg/kg) and administered using 3 protocols: prophylactic treatment (P), prodromal treatment (PD), and therapeutic treatment (TH) (note that in most of the evaluations of the data for the three treatment protocols were combined). Groups of mice were evaluated on days 3, 5, 7, 10, and 15 following exposure. The “digital pneumonia” (DP) occurrence was defined as a density measurement above the 95% confidence limit of the corresponding NC values. A significant reduction in the occurrence of DP with Immulina treatment at the higher doses compared to IC was seen as early as day 3 and persisted until day 15. There were also statistically significant dose-variable reductions in lung density in response to Immulina. The study suggests early administration of Immulina (P or PD protocols) may enhance resistance against influenza-induced viral pneumonia. A moderate correlation between pneumonia severity scores and lung density was observed for the 25-× and 40-× images (R = 0.56 and 0.53, respectively), and a strong correlation (R = 0.68) for 400-× images.

PB-9: Biological Aspects of Botanicals

The Comparison Studies of Protopanaxadiol and Protopanaxatriol: Efficacy in Blood Circulation

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Ginsenosides can be grouped into protopanaxadiol (PPD)- and protopanaxatriol (PPT)-types of molecules. Previous reports have shown that many ginsenosides exhibit anti-platelet activity. However, the comparison of the anti-platelet activity of PPD and PPT was not investigated previously. We conducted *in vitro* and *ex vivo* platelet aggregation studies in Sprague-Dawley rats. For *in vitro* studies, rat blood was collected via cardiac puncture, and platelets were collected after treatment with an agonist and samples. For *in vivo* studies, PPD and PPT were treated orally in rats for 3 consecutive days, and platelet aggregation was induced by collagen or ADP. Granule secretion, cAMP, cGMP, and TXB2 were investigated via ELISA; fibronectin adhesion was also investigated using fibronectin-coated plates. Our findings revealed that PPT was more effective than PPD regarding platelet aggregation in vitro. However, ex vivo experiments revealed that PPD was more effective than PPT; PPD effectively prevented platelet aggregation induced by collagen and ADP, reduced ATP secretion, serotonin release, and fibronectin adhesion. This is an interesting finding, and we hypothesize that the different delivery routes of PPD and PPT have affected its efficacy. PPD consists of Rb1, Rc, Rb2, Rb3, and Rd, whereas PPT consists of Rg1, Re, Rf, and Rg3. Ginsenosides were widely studied for their anti-platelet activities, and most ginsenosides from PPT (Rg1 and Rg3) were reported to prevent platelet aggregation. We hypothesize that the metabolism of PPD ginsenosides has affected its anti-platelet activity. In conclusion, PPD has more effective anti-platelet activity than PPT when administered orally.

PB-10: Biological Aspects of Botanicals

The Protective Effect of *Hypericum ascyron* L. Extract on Airway Inflammation.

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Industrialization has caused an increase in particulate matter and fine dust in the air we breathe and at the same time increases the risk of asthma and breathing difficulties. We aim to investigate the analgesic properties of *H. ascyron L.* (HA) in airway inflammation and unravel its mechanism of action. HA reduced CFA-induced NO without exhibiting cytotoxicity in MH-S cells. HA also reduced the mRNA expression of pro-inflammatory cytokines and increased the expression of proteins in the NFkB and MAPK pathways. In a mice model of CFD-induced airway inflammation, HA effectively reduced neutrophil infiltration in BALF and increased the amount of T cells in the BALF, PBMC, and blood while reducing all other immune cell subtypes to reduce the airway inflammatory response. CXCL-1, IL-17, MIP-2, and TNF-α expression in the BALF was also reduced and HA effectively reduced MIP-2 and TNF-α mRNA expression in the lung tissue of the mice. In a nutshell, HA is effective in preventing airway inflammation induced by CFA in MH-S cells as well as inflammation induced in mice by a combination of particulate matter and diesel particulate matter.

PB-11: Biological Aspects of Botanicals

Anticancer Activity of *Annona squamosa* Seed Isolate in Ovarian Cancer

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Cancer is a broad category of illnesses that can originate in nearly any organ or tissue in the body when aberrant cells proliferate out of control, cross normal boundaries to infect other body parts, or spread to other organs. In India, the Indian Council of Medical Research (ICMR) has disclosed in its bi-annual study that more women than males are receiving cancer diagnoses. The disease is expected to affect 1.57 million people in 2025, up from 1.46 million this year. Ovary, cervix, and corpus uteri were the next most common cancers in women, with breast cancer having the greatest prevalence. The study's main aim is to determine the anticancer activity of isolate derived from hydroalcoholic extract of *Annona squamosa* (AS) seeds and identify the compound responsible against Ovarian cancer lines.

Hydroalcoholic extract of *Annona squamosa* seeds was purified using column chromatography to yield two isolates, I1 and I2, and tested using the sulforhodamine B assay method on ovary cancer cell lines where phytochemical analysis was performed using the LCMS method. The phytochemical characterization was done using the LCMS method, which showed 15 different molecular weight compounds. The extract showed an average *in vitro* anticancer activity at 100 µg/mL concentration against ovary cancer cell lines. The phytochemical analysis using the LCMS method showed a wide range of phenols and flavonoids, which show anticancer activity of AS seeds isolate I2.

PB-12: Biological Aspects of Botanicals

Regioselective Claisen–Schmidt Adduct of 2-Undecanone from *Houttuynia cordata* Thunb as Insecticide/Repellent against *Solenopsis invicta* and Repositioning Plant Fungicides against *Colletotrichum fragariae*

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The U.S. Department of Agriculture (USDA) has established research programs to fight the phytopathogen *Colletotrichum fragariae* and the invasive red imported fire ant, *Solenopsis invicta*. *C. fragariae* is known to cause anthracnose disease in fruits and vegetables, while *S. invicta* is known for its aggressive behavior and painful stings and for being the cause of significant damage to crops, as well as harm to humans and animals. Many plants have been studied for potential activity against *C. fragariae* and *S. invicta*. Among the studied plants, *Houttuynia cordata* Thunb has been shown to contain 2-undecanone, which is known for its antifungal activity against *Colletotrichum gloesporioides*. Based on the mean amount of sand removed, 2-undecanone showed significant repellency at 62.5 μg/g, similar to DEET (N,N-diethyl-meta-toluamide), against *S. invicta*. The 2-undecanone with an LC_{50} of 44.59 μg/g showed toxicity against *S. invicta* workers. However, neither *H. cordata* extract nor 2-undecanone had shown activity against *C. fragariae* despite their known activity against *C. gloesporioides*, which in turn motivates us to reposition 2-undecanone as a selected candidate for a Claisen–Schmidt condensation that enables access to several analogs (2a–f). Among the prepared analogs, (E)-1-(3-methylbenzo[b]thiophen-2-yl) dodec-1-en-3-one (2b) and (E)-1-(5-bromothiophen-2-yl) dodec-1-en-3-one (2f) showed promising activity against *C. fragariae*, revealing a distinctive structural activity relationship (SAR). The generated analogs revealed a clear regioselectivity pattern by forming the C=C alkene bond at position C-1. These data open the window for further lead optimization and product development in managing *C. fragariae* and *S. invicta*.

PB-13: Biological Aspects of Botanicals

Comparative Study on Sub-critical Extracts of *Elaeocarpus sylvestris* var. *ellipticus* Leaves as Potential Cosmetic Ingredients

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Elaeocarpus sylvestris var. *ellipticus* (Thunb.) H.Hara (Elaeocarpaceae) is an evergreen tree with shiny and oblanceolate leaves. Since it is susceptible to cold weather, the distribution area is limited to subtropical regions such as Jeju Island (Korea), Japan, Southern China, and Taiwan. *E. sylvestris* contains polyphenolic compounds such as tannins and flavonoids, which exhibit antioxidant, antiviral, and antibacterial activities. In this study, we prepared extracts from each part (fruits, leaves, stem) in *E. sylvestris* by using the sub-critical extraction method and fermentation method by *Latilactobacillus curvatus* extracted with 70% EtOH to determine the efficient extraction conditions for *E. sylvestris*. The HPLC profile showed that each extract had similar patterns, but the sub-critical water leaf extract had the highest peak intensity at 280 nm. Gallic acid, the active and major compound in the extract, was quantified by HPLC, showing the highest content in the sub-critical water leaf extract among six samples. The antioxidant, anti-acne, and whitening effects were proportional to the gallic acid content. The findings suggest that subcritical leaf extracts of *E. sylvestris* have the potential to be effective materials for cosmetic applications such as skin whitening and anti-acne applications.

PB-14: Biological Aspects of Botanicals (OSD)

Menthalactone, a Potential Natural-product–based Bioherbicide Selective Against Creeping Bentgrass

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The persistent challenge of managing invasive weed species continues to confront the agricultural industry, presenting ecological, economic, and agronomic hurdles causing over $100 billion in annual crop losses globally. One such concern is the management of *Agrostis stolonifera*, commonly known as creeping bentgrass, which is particularly concerned due to its ability to form hybrids. This scenario underscores the urgent need for innovative, effective, and environmentally sustainable herbicides, steering the focus toward natural substances as potential candidates. In collaboration with USDA-ARS, we report a promising natural lactone, known as menthalactone, derived from *Mentha cordifolia*. The phytotoxic activity was assessed against the monocot, bentgrass (*A. stolonifera* - Penncross variety), and dicot, lettuce (*Lactuca sativa*—Iceberg A Crisphead cultivar, Burpee Seeds). Menthalactone displayed outstanding activity against the monocot bentgrass and was further evaluated for in-depth phytotoxic characteristics. The germination of *A. stolonifera* seeds was significantly inhibited with an IC_{50} value of 4.9 ± 1.2 µM.

Contrary to bentagrass seeds, *Lemna pausicostata* plants did not respond greatly to menthalactone with an IC_{50} of 293.4 ± 70.6 µM. Both species are monocots, and the results suggest that menthalactone might have a destructive effect on seed germination but does not effectively impact the metabolism in green tissues. The susceptibility of menthalactone on three common, obnoxious weed species, i.e., ryegrass (*Lilium perenne*), barnyard grass (*Echinochloa crusgalli*), and crabgrass (*Digitaria sanguinalis*) was assessed. Menthalactone at 1000 µM completely hampered the germination of all three grass species, while 330 µM was ineffective at inhibiting germination in less than 50%. Post-emergence application of menthalactone at 1% did not produce a significant effect against ryegrass, barnyard grass, or crabgrass.

PB-15: Biological Aspects of Botanicals

Pyrrolobenzodiazepines: Natural Sources, Therapeutic Uses, and Future in Neurological Treatments

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Pyrrolobenzodiazepines (PBDs) are a potent class of compounds that have been analyzed since the 1960s and are found naturally in a wide variety of species. They may be obtained through extraction from natural sources or through synthetic routes. Various studies have indicated remarkable anti-tumor, anti-bacterial, analgesic, and anti-neurodegenerative activities of PBDs. This review will examine natural sources of PBDs, their biological activities, newer synthesis strategies, and the effect of structural modifications, specifically from a neurological standpoint. Emerging studies on pyrrolobenzodiazepines offer encouragement for further research on their neurological activities, potentially leading to therapeutic uses in addition to that as an oncological agent.

PB-16: Biological Aspects of Botanicals

Bioaccessibility and Metabolic Stability of Cinnamaldehyde: A Major Constituent of Cinnamon Oil and its Interaction with Xeno-receptors

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Cinnamon has been used for a long time as a condiment in various food delicacies as well as an ingredient in herbal formulations. Cinnamaldehyde is a primary constituent of cinnamon, which substantially contributes to the food additive and medicinal properties of cinnamon. To understand its ADME properties, the present study evaluated cinnamaldehyde's bioaccessibility, metabolic stability, and interaction with xeno-sensing receptors (PXR and AhR). The results of dissolution studies showed that over 100% cinnamaldehyde was bioaccessible in fasted and fed-state simulated gastric and intestinal fluids. Metabolic stability studies suggested that upon incubation with HLMs and HLS9 fraction, cinnamaldehyde (in its pure form or the form of cinnamon oil) rapidly oxidized into cinnamic acid. However, incubation with primary human hepatocytes revealed moderate stability. Cinnamon oil dose-dependently activated AhR in human AhR-reporter cells, but cinnamaldehyde or cinnamic acid (in pure form) did not affect AhR. In addition, cinnamaldehyde significantly activated PXR in human hepatic (HepG2) and intestinal (LS174T) cells. On incubation with translationally matured CYPs, tested compounds partially inhibited the catalytic activity of CYP3A4 and 1A2. Our findings indicated that highly bioaccessible cinnamaldehyde possesses moderate hepatic stability with a reasonable PXR activation. Hence, controlled ingestion of cinnamon-containing foods or dietary supplements can boost health, but overconsumption may induce modest HDIs, especially in chronic pathophysiology.

PB-17: Biological Aspects of Botanicals

Fatty Acids from Natural Sources as Potential Moisturizing and Anti-aging Agents.

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Oxidized saturated fatty acids, a relatively unexplored category of oxidized lipids in the realm of food science, are investigated for their potential impact on skin hydration and anti-aging properties. While the conventional belief suggests that oils can enhance skin hydration by reducing trans-epidermal water loss, this study delves into the specific effects of oxidized saturated fatty acids derived from natural sources on key moisturizing and anti-aging mechanisms.

The primary objective of this research is to elucidate whether the observed skin hydration attributed to oxidized saturated fatty acids is linked to the upregulation of Hyaluronic Acid Synthase (HAS) and Cluster of Differentiation 44 (CD44) expression in human monocytes and keratinocytes. The study involves testing seven fatty acids on CD44 expression using human keratinocytes and THP-1 cells. Results indicate that 9-oxo-2-decenoic acid and 8-oxodecanoic acid significantly elevate HAS2 and HAS3 mRNA expression in human keratinocytes by Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR). Other fatty acids examined were found to be inactive in this regard. Furthermore, 8-oxodecanoic acid demonstrated a noteworthy increase in CD44 expression, as evidenced by fluorescence microscopy and flow cytometry analyses in human keratinocytes and THP-1 cells.

In conclusion, our findings suggest that 8-oxodecanoic acid, an oxidized saturated fatty acid derived from natural sources, holds promise as a potential moisturizing and anti-aging agent. These results contribute valuable insights for developing functional foods and cosmetic applications to enhance skin hydration and combat aging effects.

PB-18: Biological Aspects of Botanicals

Anti-obesity and Anti-diabetic Effects of Damiana (*Turnera diffusa*) Leaf Extract through Inhibiting Adipocyte Differentiation and Enhancing Glucose Uptake in Myocytes.

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Turnera diffusa leaf has been used in traditional medicine as an aphrodisiac, tonic, and in the management of diabetes. Based on the traditional use and recent evidence of antidiabetic activity, we investigated the effects of hydroethanolic extract of *T. diffusa* leaf extract (TDE) on a series of ligand-activated transcription factors, namely PPARα, PPARγ, LXR, and NRF2, which are involved in the regulation of metabolic pathways associated with obesity, diabetes, and inflammation. Further, the effects of TDE on lipid accumulation in adipocytes (adipogenesis) and glucose uptake in myocytes were also evaluated.

TDE demonstrated strong agonistic effects on LXR, resulting in an increase of >2-fold in LXR activity, while the activation of PPARα, PPARγ, and NRF2 was in the range of 1.15 to 1.82 folds under similar experimental conditions. At a 100 µg/mL concentration, TDE decreased lipid accumulation in adipocytes (55.3%) and increased glucose uptake in muscle cells (91.3%). The adipogenic effect induced by a full PPARγ agonist (rosiglitazone) was antagonized by TDE, showing a decrease of 57.6% in lipid accumulation. This is the first report to reveal the agonistic action of TDE on multiple nuclear receptors along with its glucose uptake enhancing and antiadipogenic effects. The results indicate the potential utility of TDE in alleviating metabolic syndrome symptoms and preventing the undesired adipogenic effects of antidiabetic drugs of the glitazone class. Phytochemical analysis of TDE to identify potential bioactive constituents is underway. Further studies in type II diabetes animal models would further corroborate our initial findings.

PB-19: Biological Aspects of Botanicals

Comprehensive Investigation of Psilocybin Mushrooms.

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Neuropsychiatric disorders (ND) pose significant challenges in the US, affecting various aspects of society, including the economy. Individuals with untreated neuropsychiatric disorders are at higher risk of involvement with the criminal justice system and face increased suicide rates. Non-hallucinogenic psilocybin-related metabolites could be transformative for the treatment of ND devoid of abuse liability. ND therapeutics, such as opioids, carry high abuse liability and unsafe drug-drug interactions. Psychedelic psilocybe mushrooms, also known as “magic mushrooms”, contain the psychedelic compound psilocybin and consist of over 200 species and have been used for centuries for their psychoactive and therapeutic effects. Psilocybin is the main active component in psilocybe mushrooms that metabolizes to the hallucinogenic psilocin. They target the human serotonin 2A receptor (5-HT2AR) and have shown promise in treating ND; however, their associated hallucinogenic effects are hampering their use. FDA granted "Breakthrough Therapy" status to psilocybin-assisted therapy for the treatment of resistant depression. We studied three selected strains of *Psilocybe cubensis*, namely Golden Halo, Hillbilly, and B+, at 3, 5, and 9 weeks of growing stages. Chemical analysis revealed at least a 20% increase in the psilocybin content at week 5 compared to 3 in the B+ strain, while considerable variation has been noticed for B+ and Hillbilly strains between week 5 and 9. LC/MS analysis has revealed the tentative identification of 14 compounds from B+, 12 from Hillbilly, and 9 from Golden Halo. Many non-reported related minor metabolites have been detected that warrant further large-scale investigation.

PB-20: Biological Aspects of Botanicals

Morphine Dependence is Attenuated by a Combination Treatment of Red Ginseng and Polygalae Radix in Mice.

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As part of ongoing efforts to alleviate morphine dependence, we screened for the anti-narcotic effects of natural products through behavior experiments. In addition, microRNAs, small noncoding RNA molecules that regulate post-transcriptional gene expression, and various genes in the brain of morphine-addicted mice. Polygalae radix is the dried rhizome of *Polygala tenuifolia* Wild. We compared each treatment alone with ginseng, polygalae radix, and a combination of the two in various ratios for morphine-induced dependence on mice. Mice were pretreated with red ginseng, polygalae radix, and a combination in various ratios once a day, 30 minutes before the morphine treatment. Morphine was injected twice a day at 12-hour intervals for 9 days to induce morphine dependence. Physical dependence was assessed using a naloxone-induced morphine withdrawal jumping behavior, and psychological dependence was measured by conditioned place preference (CPP) score. The scores of naloxone-precipitated jumps and CPP were significantly suppressed by the combination treatment of a specific ratio rather than the single treatment of red ginseng or polygalae radix. In addition, PCR and western blot experiments confirmed that the combination of red ginseng and polygalae radix in a specific ratio decreased morphine dependence. Therefore, this study suggested that a specific combination of red ginseng and natural products has more potential for the treatment of morphine dependence than treatment with either product alone.

PB-21: Biological Aspects of Botanicals

The Effects of Gintonin on the Activation of Macrophage through the MAPK Pathway

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The objective of the study is to examine the effect of macrophage activation by gintonin isolated from *Panax ginseng*. Macrophages are known as immune cells responsible for innate immunity. *Panax ginseng* has been traditionally used for a long time in Asia, including Korea and China, to remedy various diseases. One of the functions of polysaccharides is thought to increase immunity in the body by activating immune cells. Recently, we have isolated the gintonin compound with about 17 kDa as a novel compound of *Panax ginseng*. Gintonin consists of constituents of lysophosphatidic acids and ribonuclease-like protein. Therefore, we verified whether gintonin could increase the activation of macrophages. As a result, gintonin treatment increased the expression of CD11b, CD80, and MHC-II, a common marker for macrophage activation. Also, gintonin increases mRNA expression of TNF-α, IL-6, IL-1β, NF-kB, IkBα, p65, NOS2, COX-2, JNK, p38, Ephb1, PERK, NLRP3, and caspase-1. Gintonin increased protein levels such as iNOS, COX-2, IL-1β, caspase-1, TNF-α, TGF-β, MAPK, and NF-kB signaling. Namely, the treatment of gintonin up-regulated the protein expression of iNOS and COX-2 almost 2-fold in the present study. IL-1β, caspase-1, TNF-α, TGF-β, and MAPK phosphorylation had significantly increased compared to the control. Our findings showed that gintonin can increase macrophage functions by influencing gene or protein expression. Specifically, we observed that the NF-kB/MAPK signaling pathway plays an essential role in the upstream of cytokines.

PB-22: Biological Aspects of Botanicals

Activation of Natural Killer Cell by Korean Red Ginseng and its Mechanism

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Recently, studies have shown that Korean red ginseng has anti-inflammatory, antioxidant, and anticancer effects. However, the effects of Korean red ginseng and its immunomodulatory function on porcine are poorly understood. The pig (*Sus domesticus*) has very high similarities to humans in immune system functions (more than 80% in contrast to the mouse with only about 10%), e.g., the presence of tonsils, which are absent in rodents. Therefore, in the present study, we aimed to examine the immune functions of Korean red ginseng by natural killer (NK) cell activation as well as signaling molecules and cytokines on porcine. Korean red ginseng was supplied by Korea Ginseng Corporation (KGC, Daejeon, Korea). For the study, about 20 kg pigs were divided into four groups in each group. In the KRG groups, animals were treated with 3 and 6 g KRG because if 3 or 6 g Korean red ginseng is administered to a 20 kg pig, it corresponds to 150 or 300 mg/kg concentration, corresponding to the doses commonly treated in rodents. The results showed that Korean red ginseng treatment increased the CD4+CD8+T cell population and CD3-CD172-CD8+NK cells. These results indicated that Korean red ginseng affected these immune cells in porcine. The cytotoxic property target cells were analyzed. A significant increase in cytotoxic function was observed. These results show Korean red ginseng could have more cytotoxic activities against target cells. IL-4, IL-6, and IL-10 levels were also increased after treatment with Korean red ginseng. Also, we have studied NK cells' surface marker expression, i.e., NKp46, NKp44, and NKp30. As a result, our data showed that NKp30 and NKp46 were significantly more abundant than the normal group. NKp44 significantly increased in the 6 g KRG group. Taken together, we have concluded that when Korean red ginseng is administered on porcine, immune responses of NK cells increase, which is thought to be due to the mechanism of cytokine secretion.

PB-23: Biological Aspects of Botanicals

Pharmacology and Micro-morphological Analysis of *Houttuynia cordata* Thunb.

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Houttuynia cordata Thunb is widely distributed as an edible plant in many Asian regions. It plays a key role in traditional health care management, where its stems and leaves have been used in China to treat pneumonia and lung abscesses. Micro-morphological analysis of the stem using an Olympus BX53 fluorescence microscope shows two different structural outlines. The stem internodes are circular and symmetrical, whereas in the nodal region, the stem shows a concave shape on one side and a circular to oval on the other. The transverse section of heart-shaped leaves comprises an outer epidermal layer covered with a thin cuticle layer. In our in vivo study, rats infected with acute pneumonia caused by intravenous administration of oleic acid into their tail veins were treated with a combination of *H. cordata* and Immunorm® for 12 days. Rats were sacrificed 3, 7, and 14 days after acute pneumonia was simulated. On day 7, uneven thickening of the interstitium appears in the lung tissue due to cellular infiltration. On day 14, uneven narrowing of the lumen due to spasm is noted in the lung vessels. Our data suggested that combinational therapy of *H. cordata* and Immunorm® reduced the inflammatory histopathology of the lungs, as evidenced by the decrease in interstitial inflammation and an increase in the interstitial area, resulting in the restoration of lung tissue. HR-MS analysis of *H. cordata* methanol extract has revealed the tentative identification of four compounds commonly named as imenine; 5-methoxy, houttuynoid A, houttuynoid C, and strigol, 5-ketone, where the detailed biological contribution of the identified metabolites to the overall activity is undergoing.

PB-24: Biological Aspects of Botanicals

MR Antagonism of Rg3(R) Modulates FLG Expression via SIRT1 to Mitigate Excessive Skin Barrier Disruption Induced by Glucocorticoids.

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Panax ginseng root, widely embraced in traditional herbal medicine throughout Korea, China, and Japan, is renowned for its immune-enhancing properties. Ginsenosides, its primary pharmacological components, exhibit structural resemblance to nuclear hormone receptors(NRs), allowing them to modulate activation or inhibition by binding to NRs. Notably, glucocorticoids(GCs) can bind to mineralocorticoid receptors (MR) with high affinity due to their structural similarity. MR, inappropriately activated by excessive GC levels, significantly contribute to developing GC-mediated skin side effects. As highlighted by previous studies, the NAD-dependent protein deacetylase SIRT1 plays a crucial role in cell survival, aging and stress response, particularly in GC-induced skin complications. Our prior research confirmed Rg3(R) as an MR antagonist, illustrating its efficacy in mitigating GC-induced skin barrier breakdown by regulating FLG expression. In this study, HaCaT cell experiments showed decreased SIRT1 expression and increased acetyl p53 and p21 expression following GC treatment. Strikingly, treatment with the MR antagonist Rg3(R) reversed these trends, underscoring its potential to modulate FLG expression through the SIRT1 pathway, thereby alleviating GC-induced skin barrier disruption. These findings offer valuable insights for effectively managing skin complications associated with excessive GC exposure using herbal remedies and molecular modulators at the molecular level.

PB-25: Biological Aspects of Botanicals

Antimicrobial Evaluation of Various Extracts of *Hypsizygus ulmarius* (Bull. ex Fr.) Redhead: A Cosmeceutical Important Mushroom

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A variety of medicinal products from mushrooms may be used in cosmeceutical products applied topically, such as creams, lotions, and ointments. Cosmeceuticals incorporating mushrooms include those for skin care, such as anti-aging, antioxidants, skin revitalizing, skin whitening, and hair products. In some cases, topical preparations can also be prepared from the topical extraction of mushrooms. A mushroom polysaccharide has increased interest as a skincare ingredient due to its high moisture content in the epidermis and stratum corneum and its ability to retain moisture. Additionally, these compounds hydrate the stratum corneum and improve the physical and chemical properties of the skin, providing a smooth-soft texture. The *Hypsizygus ulmarius* species possess several nutritional and medicinal properties such as antitumor, immunomodulatory, antioxidant, anti-inflammatory, anti-allergic, hypocholesterolemic, and antimicrobial activities. The powdered *H. ulmarius* mushroom was successively extracted with various solvents such as ethanol, water, and ethanol/water through an orbital shaker. The mushroom extracts were tested for antimicrobial activity against *E. coli*, *P. aeruginosa*, *S. aureus*, *S. pneumonia,* and *C. albicans* by 96 well method at various concentrations (0.20 to 1.56 mg/mL) of extracts. All the extracts were effectively tested against microorganisms and compared with standard antibiotics (Ciprofloxacin and Amphotericin B). The maximum antimicrobial activities were obtained in water (200 mg/L to 6.25mg/mL), followed by ethanol/water and ethanol extracts. All three extracts were active against all the microorganisms, and strong activity was recorded against *C. albicans*. The present results indicated the potentiality of this mushroom extract, which can be utilized as a cosmeceutical product to control several skin infectious diseases caused by microorganisms.

PB-26: Biological Aspects of Botanicals

Examination of Essential Oil Combinations Against Bacteria Underlying Nosocomial Infections

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Nosocomial infections are causing an increasing problem in healthcare. In the case of hospital-acquired infections (HAI), it is common for the planned nursing time to be extended, and the patient needs to spend more time in the hospital ward, thus multiplying the probability of infection and increasing the use of antibiotics. Essential oils are complementary therapeutic possibilities, so their popularity is constantly increasing. The antibacterial effect of essential oils is known, but we have less information about the combined effect of essential oil samples used in different combinations.

Our studies aimed to test essential oil combinations against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Our essential oil samples included cinnamon bark essential oil, thyme essential oil, and clove essential oil. To carry out our research, we performed an *in vitro* checkerboard titration. The fractional inhibitory concentration index (FICI) value was calculated from the obtained values.

Based on our results, it can be said that all three strains of bacteria reacted sensitively to the essential oil treatment. During our combination studies, the essential oil of thyme and cinnamon bark proved synergistic for all three bacteria. In the case of *P. aeruginosa*, the combination of clove and cinnamon bark essential oil showed an additive effect, similar to the *S. aureus* clove-thyme and thyme-Ceylon cinnamon bark essential oil combination. In the case of *E. coli*, the combined use of clove and thyme essential oil resulted in an additive effect. During our research, we proved that different essential oils and their combinations show a higher degree of effectiveness in the case of different strains of bacteria. Furthermore, it was confirmed that certain essential oils can enhance each other's activity when used together, and a synergistic effect can be detected among them.

PB-27: Biological Aspects of Botanicals

The Combination of Natural Compounds Crila® and Epigallocatechin Gallate Showed Enhanced Antiproliferative Effects on Human Uterine Fibroid Cells Compared with Single Treatments

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Crila® and epigallocatechin gallate (EGCG) combined effects were investigated on human uterine fibroid cells (HuLM). HuLM cells were treated with different concentrations of Crila, alone or in combination with EGCG. Cell proliferation, drug synergy, protein, and gene expression of a proliferation marker and an apoptosis marker using western blot and qPCR were performed. Results showed that tested Crila concentrations, combined with 25 and 50 µM EGCG, exerted synergistic growth inhibitory effects on HuLM viability. This inhibitory effect on HuLM cell viability was mainly due to decreased cell proliferation, as shown by a decrease in the proliferation marker, proliferating cell nuclear antigen, at mRNA and protein levels. Our study concludes that further investigation is warranted for the combination treatment as a potential therapy for uterine fibroids. The utility of natural compounds may provide a safe and cost-effective alternative to currently used short-term hormonal therapies against uterine fibroids.

PB-28: Biological Aspects of Botanicals

Natural Molluscicides to Control the Intermediate Host Snails for the Trematode *Bolbophorus damnificus* in Catfish Aquaculture

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Infection by the digenean *Bolbophorus damnificus* causes substantial economic loss to catfish aquaculture in Mississippi and Louisiana. The catfish acquires the infection from the intermediate hosts, ramhorn snails (*Planorbella trivolvis*), and the invasive snail, *Biomphalaria havanensis*, prevalent in catfish production ponds. The infection is controlled by reducing the snail population, mainly using copper sulfate. Even though copper sulfate effectively controls the snail population, its usefulness is limited due to its accumulation in the environment, toxicity towards phytoplankton, and a small margin of safety between molluscicidal and ichthyotoxic concentrations.

This study aims to identify inexpensive natural molluscicides with limited toxicity to fish and phytoplankton. Saponins have been shown to possess potent molluscicidal activity. We evaluated the molluscicidal activity of five commercially available saponin extracts from plants (tea, alfalfa, yucca, English ivy, soya) listed by the US Food and Drug Administration as generally recognized as safe (GRAS). One of these commercial extracts showed potent molluscicidal activity, and the major compounds isolated from this extract showed activity comparable to copper sulfate. The molluscicidal activity of these extracts and compounds and their structure-activity relationships will be presented.

PB-29: Biological Aspects of Botanicals

Pharmacological Effects and Mechanism of Action of Xuetongsu in Alleviating Bone Destruction: Targeting RANKL-Related Osteoclastogenesis

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Rheumatoid arthritis (RA) is a progressive autoimmune disease that causes joint inflammation and bone destruction. Long-term severe bone destruction can cause joint deformity and disability, which seriously damages the health. Osteoclasts play a crucial role in bone destruction, as their excessive activity leads to cartilage matrix degradation. Currently, conventional anti-RA drugs used in long-term treatment often cause various side effects including gastrointestinal discomfort, hepatic and renal toxicity, and cardiovascular complications. This study was to examine the anti-bone destruction effects of Tujia ethnomedicine Xuetongsu on RA and explore ability to inhibit RANKL-mediated osteoclast differentiation and promote osteoclast apoptosis. RAW264.7 cells were cultured with 50 ng/mL RANKL to differentiate into osteoclasts. 36 SD rats were injected subcutaneously with complete Freund's adjuvant emulsion containing heat inactivated Mtb at the base of the tail to establish an AIA rat model. Osteoclasts were treated with different concentrations of Xuetongsu (0, 4.5, 9, and 18 μM) or methotrexate (4.5 μM). The Xuetongsu group received varying doses of Xuetongsu (1.0, 2.0, and 4.0 mg/kg), while the normal and model groups were treated with the same volume of 0.3% CMC-Na. The rats in positive control group were given 1.0 mg/kg methotrexate solution. Xuetongsu effectively mitigates RA-induced bone destruction in AIA rats by inhibiting osteoclast differentiation through direct targeting of RANKL and promoting osteoclast apoptosis via the activation of the Caspase-3/Bcl-2 pathway. Importantly, Xuetongsu exhibits favorable biosafety profiles in vivo. In conclusion, Xuetongsu inhibits RA bone destruction by targeting RANKL-Related osteoclastogenesis.

PB-30: Biological Aspects of Botanicals

Acupuncture and Moxibustion Modulate Gut Microbiota and Metabolism to Alleviate Colonic Inflammation in Crohn's Disease Rats: Implications for Therapeutic Mechanisms

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The mugwort in Hunan Province possesses significant medicinal value. The practice of igniting moxa sticks, made from the processed leaves of mugwort, for therapeutic purposes is known as moxibustion. Moxibustion is extensively employed in Asian regions and demonstrates efficacy in treating gastrointestinal disorders. In this study, we explored the effects of acupuncture and moxibustion on colonic inflammation in Crohn's disease (CD) rats and investigated the underlying mechanisms involving gut microbiota and metabolism modulation. CD rats were induced using 2,4,6-trinitrobenzene sulfonic acid (TNBS) and treated with acupuncture and moxibustion at specific acupoints for 7 days. We observed significant improvements in disease symptoms and colonic inflammation, accompanied by changes in the colonic microbiota composition and metabolic profiles. Treatment increased Proteobacteria and reduced Firmicutes abundance, along with alterations in key metabolites involved in various metabolic pathways. Our findings suggest that acupuncture and moxibustion alleviate inflammation in CD rats by modulating gut microbiota and metabolism.

PB-31: Biological Aspects of Botanicals

GOQDs-loaded Sinomenine Hydrochloride Nanocomplexes Synergistically Target Macrophage Repolarization and Proliferation of Rheumatoid Arthritis Fibroblast-like Synoviocytes for Treatment of Adjuvant-induced Arthritis and Collagen II-induced Arthritis in Rats

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The characteristic features of the rheumatoid arthritis (RA) microenvironment are synovial inflammation and hyperplasia. Therefore, there is a growing interest in developing a suitable therapeutic strategy for RA that targets the synovial macrophage and fibroblast-like synoviocytes (FLSs). In this study, we used graphene oxide quantum dots (GOQDs) to create a nanomedicine system (GP@SIN NPs) by loading anti-arthritic sinomenine hydrochloride (SIN). Our molecular mechanism studies demonstrated that this nanomedicine system was effective against RA by promoting the transition of M1 to M2 macrophages and inhibiting the abnormal proliferation of FLSs in vitro. To assess its therapeutic potential, we employed two preclinical models of RA: adjuvant-induced arthritis and collagen-induced arthritis in rats. Importantly, we modified the surface of the hybrid membrane (RFM)-coated biomimetic nanomaterial with hyaluronic acid (HA) (HA@RFM@GP@SIN NPs) to target inflammatory articular lesions. This modification allowed for the synergistic regulation of macrophage polarization and synovial hyperplasia, ultimately preventing cartilage destruction and bone erosion in vivo. Metabolomics demonstrated that HA@RFM@GP@SIN NPs exerted their anti-arthritic effects through the regulation of steroid hormone biosynthesis, ovarian steroidogenesis, tryptophan metabolism, and tyrosine metabolism. More notably, transcriptomic analyses and protein validation revealed that HA@RFM@GP@SIN NPs inhibited the abnormal proliferation of RA-FLS by interfering with the PI3K/Akt/SGK/FoxO signaling pathway, leading to a decrease in cyclin B1 expression and cell cycle arrest in the G2 phase. Additionally, HA@RFM@GP@SIN NPs exhibited favorable biocompatibility and biosafety in both in vitro and in vivo experiments. Overall, these multifunctional nanoparticles offer a promising therapeutic approach for patients with RA.

PB-32: Biological Aspects of Botanicals

Microbial Metabolism of the Sesquiterpene Carotol: Cytotoxicity Evaluation and *In Silico* Studies of its Metabolites

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Microbial cultures serve as efficient biocatalysts, offering a diverse array of derivatives that are often challenging to obtain from mammals or through synthetic methods. This technique has facilitated the study of xenobiotic metabolism. Carrot (*Daucus carota* L.) is a root vegetable where its seed essential oil is a common aromatic component in cosmetics and perfumes. Beyond its fragrance applications, it boasts therapeutic uses, particularly in treating skin conditions. Notably, carotol, a sesquiterpene alcohol, stands as the main compound found in carrot seed oil. This study aimed at exploring the microbial capacity to metabolize carotol into various derivatives, producing quantities sufficient for assessing their cytotoxic effects. All initial screening and preparative-scale experiments followed the standardized two-stage protocol. Carotol, prepared as a 10% solution in DMF, was introduced into the 24-h-old stage II culture medium of the microorganisms at a concentration of 0.1 mg/mL of medium. Substrate and culture controls were also prepared. Following a 2-week incubation period, each test and control underwent harvesting and subsequent analysis. Metabolites were isolated and purified using Si gel columns and identified through NMR and X-ray diffraction spectroscopy. The pure metabolites' cytotoxic activities were assessed against HepG-2, HTC-116, MCF-7, A-549 carcinoma cells, and the normal cell line MRC-5, in comparison to those of carotol and the control cis-platin. Molecular docking of carotol and the metabolites was conducted using MOE. Out of the seventeen microbial cultures screened for their capability to biotransform carotol, Absidia coerulea ATCC 6647 produced three hydroxylated metabolites (CM1, CM-2, and CM-3). Among these metabolites, CM-2 exhibited the highest activity against all tested cell lines. However, all metabolites demonstrated lower activities with high affinity and free binding energy to NADPH oxidase compared to carotol.

PB-33: Biological Aspects of Botanicals

Betulin Exhibits Antimigration Activity Against MCF7 Cells

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This work examines the antimigration activity of betulin against MCF7 breast cancer cells using a scratch wound assay. Betulin was isolated from the bark of *Parartocarpus venenosus* (Zoll. & Mor.) Becc. It precipitated out as a white powder from the fraction that eluted out of the crude ethyl acetate extract in a normal phase vacuum liquid chromatography using 7:3 (v/v) hexane: ethyl acetate. It was purified with reverse phase HPLC and characterized via 1D- and 2D-NMR, UPLC-HRMS, FTIR, and melting point. This is the first report of the isolation of betulin from *P. venenosus*. Prior to the scratch assay, betulin toxicity was evaluated against liver (HepG2), kidney (HK-2), and heart (H9c2) cells with an LDH quantification assay. It exhibited low cytotoxicity against the three cell lines at 1, 5, and 20 uM but was already nephro- and cardiotoxic at 50 uM. Betulin inhibited the migration of MCF7 cells at 22.6 uM. Although betulin has been shown to exhibit anticancer properties, this is the first report of its antimigration activity against MCF7 cells, suggesting its potential against HR+ breast cancers.

PB-34: Biological Aspects of Botanicals

Studies of St. Johns's Wort (*Hypericum perforatum*) Dry Extracts: No Hints on Mutagenicity

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The genotoxic safety of herbal medicinal products (HMPs) from preparations of *Hypericum perforatum* L., herba have already been assessed and reviewed over the last years. These HMPs play an important role in the treatment of e.g. mild to moderate depressive episodes and of mental exhaustion. One traditional form is the comminuted or powdered herbal substance alone. The way to test their genotoxic potential is the so called "bracketing and matrixing concept", where three extracts with different polarity are tested. The purpose of this concept is to obtain a representative sample of all components of the drug in these three extracts as a whole. Additional data on the genotoxic potential are therefore desirable to assess the therapeutic safety of these HMPs, using the Ames test according to the genotoxicity guideline of the Herbal Medicinal Product Committee HMPC of the European regulatory agency EMA. Three dry extracts of *Hypericum perforatum*, representing the whole spectrum of polarities of the extraction solvents (water – 50 % ethanol (v/v) – n heptane) were tested in the Ames test, according to the OECD and HMPC guidances.The extracts showed no mutagenic effect, even not in the highest concentrations according to the OECD guidance. The results of the three tested dry extracts can be extrapolated by a "bracketing and matrixing concept" for other dry extracts of *Hypericum perforatum* in the tested polarity range of the extraction solvents. The data support the therapeutic safety of the extracts and the drug powder and add the assessment in the HMPC monograph of *Hypericum perforatum*.

PB-35: Biological Aspects of Botanicals

Antimicrobial, Antibiofilm and Cytotoxic Assessments of Pakistani Traditional Medicinal Plants

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Traditional medicinal plants are a primary source of natural products which are used for the prevention and treatment of various infections throughout the world. This study documents the ethnobotanical investigation and bioactivities of 17 traditional medicinal plants belonging to 12 families from the Swat region of Pakistan. The plants were collected after interviewing local ethnomedicinal knowledge holders and confirmation of their effective use by the local population. The extracts (85) were prepared in five different solvents (hexane, acetone, ethanol, methanol, and water), and were tested for bioactivity such as antibacterial (5 Gram-positive and 9 Gram-negative bacteria), antifungal (6 yeasts), and cytotoxicity (cancerous and non-cancerous cell lines). Results demonstrated that 25.06% extracts showed pronounced activity (IV>50%) against different planktonic microbes, 35.3% extracts showed pronounced activity against biofilm strains of bacteria and fungi. Cytotoxicity was often observed against a tumor cell, but rarely against non-tumoral cell lines. Moreover, ethanol was found to be the best extractant solvent compared with other solvents. Based on the bioactivities observed in the study, plants like *Juglans regia*, *Punica granatum*, *Artemesia maritima*, *Aesculus indica*, *Thymus linearis*, *Nasturtium officinale*, *Berberis lyceum*, *Dysphania ambrosioides*, and *Mentha spicata* are recommended for further studies to be used as a potential source of novel drug discovery. The comprehensive pharmacological studies including extraction, bioassay guided fractionation, isolation, characterization, in vitro and in vivo assessments, understanding mechanism of actions and synergistic effects of the bioactive compounds from the mentioned plants could pave the way for the development of various drugs for various health conditions.

PB-36: Biological Aspects of Botanicals

Natural Products and Herbal Medicine from Dong Ethnic Medicine

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Dong minority is one of 56 races in China, with a population of 3 million scattered in the mountain areas of southwestern China, about 90 percent of Dong people living in the boundary of Hunan, Guangxi and Guizhou provinces. Due to the inconvenient traffic situation and poor medical health system. Dong people developed and inherited their own herbal medical knowledge based on the abundant natural plant/animal resources and the widespread basic TCM ideas. There are more than 300 kinds of herbal medicines summarized in total, while only parts of the resources have been researched currently. Here several kinds of herbal medicine with good therapeutic effects were subject to pharmacological research with modern technology. Such as Madengai, the rhizome of *Potentilla freyniana* Bornm. (Rosaceae), been used as a folk medicine in clearing heat, treating canker and external bleeding. Investigations disclosed that the plant contained lots of ursolic and oleanolic type triterpenoids, especially A-ring contracted triterpenoids madengaisu A and B, the structures were elucidated on NMR, HRESI-MS and other spectrum methods. Bioassay on isolated compounds showed good anti-inflammatory and anti-tumor activity.

PB-37: Biological Aspects of Botanicals

Ecological Momentary-assessment Study of US Adults who use Kratom Accompanied by Kratom Product Assay

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Aim: 1) to characterize proximal motivators, effects, and patterns of kratom use and to assess whether use frequency is associated with motivations, effects, past-year substance use disorder for kratom; 2) to quantify alkaloid content of the kratom products participants used.

Between July-November 2022 US adults regularly using kratom were screened and enrolled into a national convenience sample. Following baseline survey completion, participants underwent ecological momentary assessment (EMA) for 15 days.

Of the 2,235 candidates screened, 395 were enrolled. A total of 13,401 distinct kratom use events were recorded. Whole leaf/loose powder was most commonly used. Peak effects were 40–80 minutes post-dosing. Participants reported overall motivators of use on the baseline survey that involved managing psychiatric and SUD problems, but proximal motivators evaluated with EMA were situation-specific (increasing energy, alertness, productivity; decreasing pain). Acute effects were considered congruent with daily obligations. Use patterns, despite having some distinguishing features, were generally similar in their motivators and effects; participants used kratom predominantly during the daytime. Higher use patterns were associated with symptoms of physical dependence (withdrawal, tolerance). Co-used substances included caffeine, nicotine, tea, vitamins, and cannabis. The 330 kratom products collected were analyzed for 10 major and minor kratom alkaloids using a validated ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method. Among the analyzed commercial kratom products, mitragynine, paynantheine, speciogynine, and speciociliatine were found to be major alkaloids, and very low concentrations (<0.02%) of 7-hydroxymitragynine, corynoxine, corynoxine-B, and mitraphylline were observed. Corynoxine and corynoxine-B were correlated, with all remaining alkaloids correlated with one another.

PB-38: Biological Aspects of Botanicals

Discovering Antiviral Agents from Plant Sources

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Infectious diseases caused by emerging or re-emerging pathogens pose serious health problems and viral diseases dominate the World Health Organization (WHO)'s list of ten threats to global health. The recent Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) and monkeypox/mpox outbreaks have unmasked the urgent need for antiviral drugs that can be rapidly deployed to combat viral infections before a vaccine is developed. Thus, there is an urgent need for new and readily affordable drugs against viral infections and drug-resistant pathogens. Medicinal plants have been used against viral infections for centuries, but several of these plants remain uninvestigated both pharmacologically and chemically. Our work investigating the anti-mpox, and anti-COVID-19 activities of medicinal plant extracts will be presented as well as some biologically active compounds we isolated and characterized.

PB-39: Biological Aspects of Botanicals

Moringa oleifera Anticancer and Radio Sensitizing Agent on Cervical and Aero-digestive Cancer Cell Lines

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In this study, we evaluated the anticancer effect of the methanolic extract of *Moringa oleifera* leaves. Tested on HeLa and FaDu cancer cell lines, the leaf extract showed remarkable cytotoxic, antiproliferative and radio-sensitizing properties. Cell survival was significantly low in both cell lines when treated with the extract followed by irradiation at 2 Gy. Furthermore, a striking reduction (approximately 70–90%) in cell proliferation was observed when they were treated with the extract plus irradiation than with the extract alone. In addition, the radiosensitivity test carried out on these cervical and aerodigestive cancer lines showed a remarkable reduction in the repair of double-strand breaks after irradiation at a dose of 4 Gy. In addition, the analysis of the cell cycle showed an enrichment in G2/M indicating that the extract effectively blocks cell multiplication at the G2/M phase. Finally, GC-MS analysis of the spectrum of the total extract revealed the presence of numerous compound peaks. This suggests that the anticancer properties of *Moringa oleifera* could be attributed to bioactive compounds present in the methanolic extract of the plant's leaves. This is a unique study because no article has yet been published on the radio-sensitizing effect of *Moringa oleifera* extracts on the HeLa and FaDu lines. Thus, our study is the first of its kind to evaluate the cytotoxic, antiproliferative and radio-sensitizing properties of the methanolic extract of *Moringa oleifera* leaves. These results suggest that the leaves of *Moringa oleifera* collected in Senegal have anticancer activity, therefore a candidate drug for developing new anticancer drugs.

PB-40: Biological Aspects of Botanicals

An *In Vitro* Evaluation of Botanical Extracts on CES1 Catalytic Activity

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Carboxylesterase 1 (CES1) is the most abundant hepatic drug metabolizing enzyme and is capable of metabolizing endogenous and exogenous compounds including an array of medications such as cardiovascular, anticancer, and antiviral agents. CES1, a serine hydrolase, catalyzes the cleavage of amides, esters, and thioesters resulting in the activation of prodrugs (i.e., oseltamivir), and deactivation of active drugs (i.e., methylphenidate). Concerns have been raised over botanical-drug interactions (BDIs) and their potential to impair enzymatic function. Indeed, BDIs have been investigated with the cytochrome P450 superfamily of enzymes; however, CES1 BDIs have remained largely uninvestigated. This study aimed to assess the in vitro inhibitory potential of popular botanical extracts using an established CES1 incubation assay and LC-MS/MS analysis. Botanical extracts were generously provided by Finzelberg & Co. KG (Andernach, Germany) and the National Center for Natural Product Repository (University of Mississippi). Of the investigated extracts, ashwagandha, saw palmetto, St. John's wort, turmeric, and yohimbe all demonstrated significant impairment of CES1 metabolism. These extracts reduced CES1's catalytic activity by 50% or more, with turmeric having an astonishing 95% inhibition of CES1 at 10 µg/mL. Echinacea, ginseng, green tea, pitcher plant, and valerian also produced notable CES1 impairment by 20% or more. Turmeric demonstrated a lack of irreversible inhibition of CES1; however, it had a potent inhibitory effect on CES1 with an IC50 of 1.01 µg/mL. All other extracts that demonstrated a 50% inhibition of CES1, like ashwagandha, will be assessed for irreversible inhibition. Further in vitro work will explore the inhibitory potential of the individual constituents of the botanical extracts. These in vitro investigations are a necessary step in elucidating BDIs that may be clinically relevant and informing further in vitro, modeling, and clinical work.

PB-41: Biological Aspects of Botanicals

GC-MS Based Metabolites Profiling, *In-silico* Analysis and *In-vitro* Antiproliferative Activity of Bioactive Phytocompounds from the Ethanolic Extract of *Evolvulus alsinoides* L. Aerial Parts on MCF-7 Human Breast Cancer Cell Lines

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The study was designed to identify main bioactive phytocompounds and to evaluate the in-vitro antiproliferative effect of the ethanolic extract of Evolvulus alsinoides aerial parts against MCF-7 breast cancer cell lines using the MTT colorimetric assay. Flash chromatographic separation technique was used to isolate the main bioactive components followed by Gas Chromatography– Mass Spectrometry (GC–MS) analysis. A commercial mass spectral library was used for the depiction of individual phytocomponents. Fatty acids, phytosterols, alkaloids and flavonoids were identified from E. alsinoides. Bioassay analysis against breast cancer cell lines and molecular docking studies revealed the potential medicinal activities of active compounds like narcissidine, 9- methoxycamptothecin, dasycarpidanone from the plant. In addition, the binding energies were found to be -4.42, -4.71, -7.49 respectively, indicating their affinities towards Estrogen (PDB ID: 6CBZ) receptor alpha. The results showed a significant ($p < 0.01$) antiproliferative activity against MCF-7 cells breast cancer cell lines using the Micro Culture Tetrazolium (MTT) assay. From the present study it was concluded that the herbal drugs can be potentially used to control the proliferation rate of cancer cells. The present investigation may be quite useful as these medicinal plants are highly valued in traditional systems of medicine.

PB-42: Biological Aspects of Botanicals

Study on Chemical Constituents and Bio-activity of *Andromeda polifolia*

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The chemical constituents and antitumor activity ofthe methanol extract of *Andromeda polifolia* L. were investigated in this paper. *A. polifolia* belongs to *Andromeda* genus in Ericaceae family, which is mainly distributed in northern North America, northern Europe and northern Asia. It was first discovered in China in 2012, and was mainly growing on wetland, swamp and towers. At present, there are few studies and only nine compounds have been reported in this genus. Therefore, we decided to conduct systematic research on the chemical compositions in *A. polifolia*. To obtain a series of compounds with novel structure and significant activity, we will provide a theoretical basis for the subsequent pharmacodynamic substance base. In this study, the methnol extract of *A. polifolia* were separated and purified by normal phase silica gel and ODS column chromatography and Sephadex LH-20 gel column chromatography. Resulted in the discovery of 17 compounds and their chemical structures were elucidated by the physical and chemical properties, spectral data (1H-NMR and 13C-NMR), as well as by comparison of the spectral data with the literature. They were identified as quercetin (1)、quercetin-3-O-α-L-arabinopyranoside (2)、querce-tin-3-O-β-D-galactoside (3)、avicularin (4)、quercetin-3-O-α-L-(5 ″ -O-acetyl)-arabinofuranoside (5) 、 quercetin-3-O-α-L-arabinopyranosyl-(1→2)-β-D-galactopyranoside (6)、myricetin (7)、myricetin 3-O-ga-lactoside (8)、betmidin (9)、myricitrin (10)、syringin (11)、dehydrosyringin (12)、vanillic acid 4-O-β-D-glucoside (13)、lyoniside (14)、nudiposide (15)、(7R, 8S)-3',4,7,9-tetrahydroxy-3-methoxy-8-O-4'-neolignan-9'-O-α-L-rhamnopyra- noside (16)、(7R, 8R) -3', 4, 7, 9 - tetrahydroxy-3-methoxy-8-O-4'-neolignan-9'-O-α-L-rhamnopyranoside (17). To sum up, the chemical contituents of *A. polifolia* were enriched, which provided a theoretical basis for the development and utilization of *A. polifolia*.

PB-43: Biological Aspects of Botanicals

Screening Approach to find Natural Products that can be Utilized to Treat or Prevent Metabolic Syndrome

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Metabolic syndrome (MS) is a rising global health concern. MS is comprised of obesity, typeII diabetes, and hypertension which is also associated with a high degree of chronic inflammation. Nuclear receptors (NRs) are ligand activated transcription factors that regulate various metabolic pathways as well as inflammatory signaling. This study was carried out to screen a collection of medicinal plants for potential agonistic effects on selected NRs (LXR, PPARα, PPARγ, and NRF2) that are involved in the progression of metabolic syndrome using a series of high throughput reporter gene assays performed in hepatic cells. PPARα, PPARγ, and LXR play key roles in lipids, glucose, and energy homeostasis. NRF2/KEAP pathway is known to protect cells from oxidative stress and inflammation. The hydroethanolic extracts of a number of plants showed strong agonistic effects toward select NRs. Potential candidates were selected and further screened for their effects against obesity and diabetes based on established in vitro models that utilize differentiated adipocytes and muscle cells.

Out of about one hundred plant extracts screened, twenty extracts exhibited significant agonistic effects on multiple NRs indicating a modulation of multiple pathways. *Hydrastis canadensis* (root) and *Piper nigrum* (fruit) showed agonistic effects towards all four NRs. *Serenoa repens* (fruit) activated PPARα only while *Nigella sativa* (seed) activated PPARγ only. *Pueraria lobata* (root) was specific for LXR and *Withania somnifera* (leaf) was specific for NRF2. In the follow up assays, *Withania somnifera* (leaf) showed capability to reduce fat accumulation in adipocytes, and *Tribulus terrestris* (fruit) was effective in enhancing the glucose uptake in differentiated muscle cells. The study suggests the potential utility of selected medicinal plants in treating symptoms of MS. Further investigations are warranted in animal models of type II diabetes and obesity.

PB-44: Biological Aspects of Botanicals

In Vitro Antibacterial Activity of Crude Extracts of Some Fresh Water Cyanobacteria

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Antibiotics are increasingly becoming ineffective due to the astronomic rise of antimicrobial resistance (AMR), therefore, there is a need for the discovery of new antibiotics and antioxidants from natural products. This study aimed at exploiting the potential metabolite reservoir of cyanobacteria towards the development of novel antimicrobial compounds. Freshwater cyanobacteria, *Cylindrospermum alatosporum* NR125682.1 and *Loriellopsis cavenicola* NR117881.1, were utilized in this study and isolated from Vulindlela area, KwaZulu-Natal, SA. They were propagated on BG-11 and identified through 16S rDNA sequencing. The cyanobacteria biomass was sequentially extracted with hexane, dichloromethane (DCM) and ethanol, then screened for their antioxidant capacity using artificial and biological radicals, *in silico* molecular interactions against β-lactamase and *in vitro* antibacterial potential against some select gram-positive and gram-negative clinical isolates. The effect of the extracts on the bacterial membranes was also evaluated using the lactate-dehydrogenase assay, and the efflux-pump inhibitory potential was investigated by measuring the percentage of cytoplasmic accumulation of rhodamine. *In-vitro* beta-lactamase inhibitory potential was investigated along with synergistic potential when combined with erythromycin, their effect on DNA damage was also evaluated.

PB-45: Chemistry, biology, and safety of volatile organics from aromatic and medicinal plants

Antifatigue, Memory Enhancing and Blood Circulation Effects of Korean Red Ginseng

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Korean red ginseng (KRG) had been reported in many *in vivo* and clinical studies to exhibit various beneficial biological effects such as immunologic, antifatigue, antineoplastic, neuroprotective, hepatoprotective, antidiabetic, antistress, lowering blood pressure, memory enhancing, anti-inflammatory, antihyperlipidemic, improve blood circulation and antioxidative properties, etc. In this study, we share the antifatigue, memory enhancing and blood circulation effects of KRG.

KRG has been used in Asian countries to enhance vital energy since ancient times. Many *in vivo* and clinical studies have demonstrated that Korean red ginseng exhibited anti-fatigue activities (Brekhman, 1960; Chang, 1989). Li Zhang et.al 2019 evaluated the safety and antifatigue effect of KRG through a randomized, double-blind, and placebo-controlled clinical trial and found that KRG has a potent antifatigue effect disprove the common conception of “fireness” related to KRG.

KRG had been reported to be helpful for brain related diseases such as Alzheimer’s disease (AD), memory deficits in both *in vivo* and clinical studies. Young rats with hippocampal lesions displayed significant deficits in place learning tasks (PLT). Treatment with KRG significantly ameliorated place-navigation deficits in young rats with hippocampal lesions in the PLT. The results suggest that KRG ameliorates learning and memory deficits through effects on the central nervous system, partly through effects on the hippocampal formation (Nishijo et. al. 2004).
KRG platelet aggregation by regulating the synthesis of prostacyclin (PGI2), which has an antagonistic mechanism toward platelet aggregation, as well as thromboxane A2 (TXA2) and serotonin, which promote platelet aggregation, thus suppressing the generation of thrombi and improving blood circulation (So et al., 2018). Administration of KRG to healthy subjects significantly inhibited ADP-induced and collagen-induced platelet aggregation. KRG has a potential

PB-46: Molecular Biology Aspects of Botanicals

Advances in Biotechnology of Cannabis Mass Propagation

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Cannabis is a source of unique group of compounds called cannabinoids. So far, more than 550 constituents have been isolated from *Cannabis*, of which 125 are cannabinoids. Among phytocannabinoids, D9-tetrahydrocannabinol (D9-THC) is reported to be the most psychoactive compound with a wide spectrum of therapeutic potential in cannabis. On the other hand, cannabidiol (CBD), a non-psychoactive compound, is reported to contain very promising pharmacological activities as an antiepileptic agent, particularly for the treatment of intractable pediatric epilepsy. *Cannabis* is a dioecious and wind-pollinated species. A significant plant-to-plant variation in its cannabinoids profile and content is observed within a single *Cannabis* variety. For the production of a biomass product that is consistence in phytocannabinoids, sinsemilla (seedless female) plants are preferred. To obtain sinsemilla, male plants are removed from the cultivation site as soon as they appear, female plants with desirable profiles are screened and selected as mother plants for the future. Selected mother plants are then multiplied by using vegetative propagation or by using biotechnological techniques including micropropagation. In this presentation, we focus on our efforts on conservation and mass propagation of elite *Cannabis* plants using the biotechnological tools.

PB-47: Molecular Biology Aspects of Botanicals

Cultivation of Magic Mushrooms for Psychoactives

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Psychedelic fungi have experienced a surge in interest in recent years due to the therapeutic potential based on the fungal secondary metabolite, psilocybin. The psilocybin mushrooms also referred to as "magic mushrooms", have the potential to treat addiction, depression, anxiety, and other mental health concerns. This has escalated the demand for the natural products derived from the mushrooms. In the United States, psilocybin and psilocin are listed as Schedule 1 Drugs. There are hundreds of species belonging to at least 7 genera of psilocybin producing fungi of which *Psilocybe cubensis* is the most well-known *Psilocybe* species. With the growing interest in the medicinal benefits of psilocybin for treating mental health problems, an attempt has been made in our laboratory to cultivate different strains of *P. cubensis* which include B+, Hillbilly, and Golden Halo. The different stages of growth involved a selection of suitable commercial spores, development of spawn, substrate preparation, optimizing different growth conditions for fruiting and harvesting. Biomass produced for the three strains will be used for further research activities at NCNPR. All the growing activities were performed under the DEA (License # 18185/8.2) compliance.

PB-48: Agronomical Aspects of Botanicals

Effect of Magnesium on Cannabis Biomass Yield and Cannabinoids Content: Evaluating Different Application Rates and Methods

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Magnesium deficiency (MGD) is a severe problem in plants. Magnesium (Mg) is one of the important nutrients involved in many enzymatic activities and rarely studied in cannabis plats. In this study, the effect of application of different concentrations (2 mL/L of 'Advanced Hemp Factor X' equivalent to 10 mg/L of Mg and 4mL/L of 'Advanced Hemp Factor X' equivalent to 20 mg/L of Mg) and methods of application (drench vs. foliage application) of Magnesium was studied on useable biomass yield and cannabinoids content on two chemotypes (high CBD and high CBG) of *Cannabis sativa* L. Plants grown through feminized seeds, from the beginning, were divided into two groups, control (no treatment) and treated with different concentrations of Mg (10 mg/L of Mg drench, 20 mg/L of Mg drench and 3.75 mg/L of Mg foliage spray). Plants of both groups were grown side by side in an identical environmental condition in a polytunnel and, were watered and fertilized normally. Application of different concentrations of Mg was started from the first week of flowering until maturity. At maturity, both groups of plants were harvested and processed for usable dry biomass. Plants of both groups were compared for biomass production per plant and cannabinoids content.

Our results show that among all the treatments (drench and foliage application), in 'CBD chemotype' plants, maximum increase in cannabis biomass/plant was achieved by the application of 10 mg L-1 Mg whereas, it was achieved highest by the application of 10 mg/L Mg in 'CBG chemotypes'. On other hand, among all the treatments, maximum increase in CBD content/plant (in 'CBD chemotype' plants) and CBG content/plant (in 'CBG chemotype' plants) was observed by the application of 10 mg/L Mg drench as compared to those as 'control' plants.

PB-49: Technological Aspects of Botanicals

Utilization of GlyCORE Imaging Core in Advance Plant and Animal Biological Research

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The Glycoscience Center of Research Excellence (GlyCORE) is a Phase I Center of Biomedical Research Excellence sponsored by the NIH. It was established in 2020 at the School of Pharmacy, University of Mississippi. GlyCORE comprises three core facilities: Analytical and Biophysical Chemistry Core, Computational Chemistry and Bioinformatics Core, and The Imaging Core. The GlyCORE Imaging Core offers a range of services such as live cell imaging, FRET, FLIP and FRAP photobleaching measurements, immuno- and lectin staining, SEM coupled with EDS, and other imaging and image processing services. GlyCORE houses state-of-the-art instruments such as Stereomicroscopy, Epi-fluorescent and Bright Field Microscopy, Imaging Flow Cytometer, and Field-Emission Scanning Electron Microscope (FESEM) with multiple detectors: Energy Dispersive X-Ray Spectrometer (EDS). Confocal Laser Scanning Microscopy (CLSM). The Core's Leica SP8 CLSM has powerful multichannel capabilities with a resonant scanner (faster scanning) and HyD PMTs (super sensitive). It has an excitation of 405 nm, 442 nm, and a white light laser (WLL). The white light laser has continuous excitation lines from 470 nm up to 670 nm, and is pulsatile with a frequency of 80 Hz, allowing excitation to be tunable in 1 nm increments up to 8 laser lines simultaneously, such that almost any visible dye or fluorescent protein can be excited. This scope is highly sensitive, multispectral, and fast, enabling many advanced confocal techniques, such as FRET and FLIM. The JSM 7200FLV FESEM is another cutting-edge instrument found in the GlyCORE Imaging Core. The FESEM is highly versatile; it offers a new level of expanded performance using an in-lens Schottky Field emission gun. Advanced nanostructure analysis and determining the sample's elemental composition through X-ray spectroscopy (EDX) is possible. The GlyCORE Imaging Core specializes in supporting glycoscience research and advanced botanical research.

PB-50: Biological Aspects of Botanicals

Korean Red Ginseng Alleviates Choroidal Neovascularization and Fibrosis by Regulating Crystallin Alpha B Function

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Age-related macular degeneration (AMD) is a disease that leads to vision impairment as a result of the deterioration of the macula's function, which is located in the central part of the retina. It is a major cause of vision loss in old age. The current treatment for AMD not only involves the inconvenience of intravitreal injection of anti-vascular endothelial growth factor (VEGF), but also has a 45% risk of fibrosis within three years. Korean Red Ginseng (KRG, *Panax ginseng* C.A. Meyer) is recognized for its protective effects against fibrosis in various diseases. In this study, we investigated the anti-fibrotic and anti-neovascularization effects of Korean Red Ginseng water extract (RGWE) and ginseng total saponin (GTS) in both *in vivo* and *in vitro* AMD models. Additionally, the study measures the binding affinity of ginsenosides and crystallin alpha B (Cryab), a small heat shock protein to understand the mechanism underlying KRG effects. In ARPE-19 cells, RGWE, GTS, and ginsenosides decreased vascular endothelial growth factor (VEGF) and α-smooth muscle actin (αSMA). Furthermore, we showed that RGWE and GTS reduced CNV lesion area and VEGF and αSMA expression in laser-exposed mouse eyes. Additionally, we showed that ginsenosides interact directly with Cryab, as demonstrated by the pull-down assay. In conclusion, these results suggest that KRG may play a role in preventing the deterioration of macular degeneration through the regulatory effects of ginsenosides on Cryab function.

PB-51: Toxicological Aspects of Botanicals

Predicting Human Liver Injury and Botanical-drug Interactions with 3D Liver Microtissues and High-throughput Transcriptomics

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Human liver injury from botanical products and food additives continues to be a leading concern around the world, which is complicated by the vast array of varied compositions of commercially available products and related claims for health benefits. In order to address these challenges, an international, cross-sector consortium was formed with the goal of identifying useful test systems to study these complex mixtures being intentionally exposed to humans with little-to-no information on their potential for adverse health effects. In recent years, advances with physiologically relevant culture models and assay platforms revealed important opportunities to extend these systems to transform methods for evaluating natural products to predict human toxicity and drug interactions. In this study, we describe our use of patient-derived 3D spheroid cultures of primary human hepatocytes and high throughput transcriptomics to evaluate botanical safety with 13 botanical mixtures alongside 9 reference drugs with established clinical outcomes. Our work has demonstrated the translational utility of this integrated omics platform, in combination with benchmark concentration modeling, to predict human liver injury, estimate the potencies botanical-drug interactions, identify mechanistic signatures of biological response similarity across botanical extracts, and accurately estimate the potencies for in vivo liver weight increases. Ultimately, these data will be integrated with other toxicity endpoints to identify efficient methods for rapidly assessing the safety of natural products.

PB-52: Toxicological Aspects of Botanicals

Inter-strain Variability in Response to a Single Administration of Cannabidiol-rich Cannabis Extract in Mice

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Cannabidiol (CBD) has gained widespread popularity; however, its pharmacological and toxicological profiles in the context of human genetic diversity remain largely unexplored. Here, we investigated the variability in metabolism and toxicity of CBD-rich cannabis extract (CRCE) in genetically diverse mouse models: B6C3F1, C57BL6J, and NZO/HlLtJ strains. Mice received a single dose of CRCE containing 58.9% CBD at dosages of 0, 246, 738, and 2,460 mg/kg of CBD. All strains showed minor weight loss at 24 h post dosing. No significant alterations were detected in the organ-to-body weight ratios, nor were there appreciable histomorphological changes in these organs. However, plasma bilirubin levels increased markedly in all strains at the highest CBD dose, with C57BL6/J mice showing a significant increase at the 738 mg/kg dose. Elevations in liver enzymes ALT and AST were particularly pronounced in NZO/HlLtJ mice at the 738 mg/kg dose. C57BL6/J mice displayed significant increases in ALT levels at the two higher concentrations. While B6C3F1 and NZO/HlLtJ mice had negligible plasma CBD levels at 738 mg/kg, C57BL6J mice exhibited levels exceeding 7,000 ng/mL. At 2,460 mg/kg, high CBD concentrations were found in B6C3F1 and C57BL6J mice, but markedly lower levels were seen in NZO/HlLtJ mice. These patterns were mirrored in the plasma concentrations of CBD metabolites. Gene expression profiling showed a significant increase in Cyp2b10 across all strains, but varying responses in Cyp1a1 expression, indicating strain specific CYP dysregulation. Genetically similar and diverse mice exhibited differential pharmacological and toxicological responses to CRCE as evidenced by significant variations in CBD and its metabolites' circulation levels, metabolism, and liver injury biomarkers. Our results suggest a high potential for inter-individual variability in the pharmacology and toxicology of CBD in humans, underscoring the importance of considering genetic diversity in future research.

PB-53: Toxicological Aspects of Botanicals

An Evaluation of the Interaction Risk between Herbal and Pharmaceutical Medicines Used Concurrently for Disease Management in Blantyre, Malawi

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Herbal medicines have been used globally for centuries for myriad health benefits. These ubiquitous and readily available natural products are often used concurrently with pharmaceutical drugs, which can lead to altered drug efficacy or increased toxicity. Therefore, understanding potential herb-drug interactions (HDIs) is imperative to improve patient safety. Previous research in this field examined interactions between certain herbal medicines and antiretrovirals. The evolving global disease scenario and growing reliance on herbal medicines for non-communicable diseases necessitated a broader investigation into HDIs, particularly within the context of the healthcare system in Malawi. The objective of this study was to investigate the concurrent use of herbal and pharmaceutical medicines as treatment for diabetes and hypertension in Blantyre, Malawi, where this practice was identified as widespread. Through patient-reported data and literature-based case studies, results highlight the potential for HDIs, calling attention to the urgent need for more research and data collection, particularly within Malawi and other low- and middle-income countries. Examples of potential HDIs include those involving the popular herbal medicine moringa and anti-diabetes drug metformin. Results from this study could help inform the documentation of herbal medicine use in healthcare records and increase education for healthcare providers and patients about potential safety and efficacy concerns regarding HDIs. This survey and analysis could be adapted and expanded to other African regions and low- and middle-income countries. This approach will foster a comprehensive understanding of HDIs, ultimately contributing to safer and more effective integrated healthcare practices worldwide.

PB-54: Toxicological Aspects of Botanicals

Investigating the Pharmacodynamic Interactions of Açaí Extracts and Anticancer Drugs

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Açaí, *Euterpe oleracea* Mart., is a fruit native to Central and South America that has many bioactive compounds that contribute to many beneficial health effects in humans. Açaí is on the list of the top 40 botanicals currently used in the US. To study the pharmacodynamic interactions between açaí botanical dietary supplements (BDS) and anticancer agents, açaí extracts were tested in combination with methotrexate and tamoxifen to determine synergism, antagonism, or additive effect from concomitant use. Açaí fruit powder and two botanical dietary supplement capsule formulations were extracted using an aqueous, ethanolic, methanolic, and acidic methanolic solution, respectively. Preliminary dose-response experiments on three cell lines, MCF-7, MDA-MB-231, and MCF-10A were completed using Tamoxifen, Methotrexate, and seven standardized açaí extracts. These extracts, based on cyanidin-3-glucoside (C3G) content, were tested against the three cell lines in a range of 10 pg/mL to 1000 ng/mL which includes the human equivalent dose (HED) of 2.321 ng/mL of C3G. All the açaí extracts caused slight toxicity in the normal breast cell line, MCF-10A, at high concentrations (1000 ng/mL C3G). There was no toxicity from the extracts in the two breast cancer cell lines. A combinatorial screening was conducted across all three cell lines by testing a range of the anticancer drug and a HED concentration of the respective extract. The combinations with the most statistically significant ($p < 0.05$) differences in each comparative dose were chosen for a full combinatorial assay which was performed using a 7x7 combination matrix of anticancer drug and selected açaí extract. The data obtained was analyzed via SynergyFinder+. All combinations exhibited an additive or synergistic effect with one or both anticancer drugs on all three cell lines. This study aims to highlight the pharmacodynamic interactions of açaí extracts and anticancer drugs to determine safety and efficacy of concomitant use.

PB-55: Toxicological Aspects of Botanicals

Case Studies Comparing *In vitro* and *In vivo* Toxicological Data to Inform Botanical Safety Assessment

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Evaluation of botanical ingredient safety has traditionally relied upon either a history of safe use or toxicological data from animal studies. Challenges in incorporating data from New Approach Methods (NAMs; i.e., *in silico*, *in vitro*, and alternative animal tools) in botanical safety assessments include addressing complex botanical chemistries; accounting for absorption, distribution, metabolism, and excretion; and distinguishing bioactivity from adversity in sensitive systems. To build confidence in the application of NAMs, case studies are needed to compare traditional human and animal data to NAM based data. Orthogonal datasets containing *in vivo* rodent data and *in vitro* data will be discussed for botanicals including black cohosh (*Actaea acemose*), *Ginkgo biloba*, ephedra (*Ephedra sinica*), and wormwood (*Artemisia absinthium*). In each case, botanical ingredients were evaluated using *in vitro* studies that mimic the biological target identified in *in vivo* toxicological studies. For example, *Ginkgo biloba* extract was found to be hepatotoxic and carcinogenic in the liver of mice and rats with mechanistic studies implicating constitutive androstane receptor (CAR) and pregnane X receptor (PXR) pathways. A human-cell–based liver model measuring metabolizing enzymes reflective of CAR and PXR activity was then used to evaluate multiple *Ginkgo biloba* extracts. Correlation of findings in human cell-based systems confirmed that the mechanism of action was conserved, although species differences in receptor activity indicated that similar outcomes would not be anticipated in humans. Additional in vivo targets in case studies include cardiotoxicity (ephedra), genotoxicity (black cohosh), and neurobehavioral effects (wormwood). Careful evaluation from case studies will be useful in assessing the utility of NAM assays for botanical safety and identifying biological targets that require additional methods development.

PB-56: Toxicological Aspects of Botanicals

Nature's Weaponry: A Study on the Molluscicidal Potential of Saponin-rich Root Extract of Aleppo Milk-vetch collected from the Jordan Rift Valley

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The digenetic trematode *Bolbophorus damnificus* (formerly *B. confusus*) has been associated with high catfish mortality and poor economic returns from catfish ponds in Mississippi, Arkansas and Louisiana. These qualitative and quantitative losses exceeded $1,236/ha in moderate to severely affected ponds (>34% of the fish infected). Ramshorn and Biomphlaria are the intermediate hosts of these trematodes in the catfish pond. One of the strategies is to control the intermediate host using copper sulfate ($CuSO_4$), but, being non-selective, it is equally toxic to fish and to the environment as well. Active alternatives may be identified from the GRAS list. As part of collaborative project to search for selective and eco-friendly solutions, we tested the root saponins from *Astragalus aleppicus*. Saponins are one of the groups that are used to control snails, earthworms, etc. In basic screening, the sample induced 100% mortality in Ramshorn (3 d exposure) and 53% mortality in Biomphlaria (1 d exposure) at 100ppm.

PB-57: *Regulatory* Aspects of Botanicals

Empowering the Healthcare Ecosystem with Budsinfo™ and Cerium™ for Comprehensive Data Collection

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This research project addresses a critical gap in healthcare practitioner (HCP) knowledge, attitudes, and documentation concerning adverse events associated with non-FDA approved products. National pharmacovigilance guidelines predominantly focus on FDA-approved substances, leaving non-FDA approved products devoid of proper AE reporting guidance for HCPs. This deficiency is amplified by existing challenges HCPs face in reporting AEs across various approved drugs and consumer products. AEs for non-FDA approved products remains notoriously underreported in the US and throughout the world. This issue is especially concerning regarding cannabis & hemp-derived stuffs, amid their widespread market presence. Our innovative proposal empowers patients and HCPs to initiate AE reporting incidents, overcoming barriers, enhancing data collection, and fostering a more comprehensive understanding of adverse events related to non-FDA approved products in the healthcare ecosystem.

We conducted a pilot study featuring a K&A survey and an education intervention for HCPs. The intervention, Cerium education, includes modules on essential topics like pharmacology, dosing, safety, drug interactions, data collection, and AE reporting. It offers interactive features such as education blogs and one-on-one discussions to address specific concerns. In response to these AE reporting limitations, we also developed Budsinfo, a platform for consumers to report adverse events linked to non-FDA approved products, for example, cannabis and hemp-derived products. Budsinfo and Cerium together provide a comprehensive solution bridging critical gaps in adverse event reporting, enabling data-driven decision-making for regulatory agencies and empowering healthcare professionals and the public. Budsinfo streamlines adverse event reporting, creating a user-friendly environment, while Cerium equips healthcare professionals with knowledge and skills to contribute to data collection.

PB-58: Regulatory Aspects of Botanicals

Budsinfo: A User-Friendly Platform for Reporting the Effects of Cannabis and Cannabis-Derived (hemp) Products

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A significant gap exists in systematic data collection for cannabis and cannabis-derived (hemp) products (CCDP) limiting the public's ability to assess their risks and benefits. Products like tetrahydrocannabinol isomers (e.g., D8-THC) derived from hemp are increasingly available in stores and boutiques. Despite their accessibility, the assumption of safety often differs from the reality of potential risks. A publication using data from the Food and Drug Association adverse event system (FAERS) highlighted potential health concerns associated with D8-THC.1 In response, an adverse event reporting tool (AERT) was developed to better inform individuals about the risks associated with CCDP products. This anonymous questionnaire, accessible through a QR code or website link, was distributed at conferences, online webinars, registry newsletters, music festivals, and medical offices. Presented here are preliminary data from the pilot of the CCDP tool on the Budsinfo platform. Currently there are over 350 scans. Overall, 51 reports have been submitted to date. Of the respondents who experienced adverse effects, most were between 50 and 65 years old (32%) and female (66%). Regarding their experiences, 12% reported "getting too high"; 56% obtained their product from legal dispensaries. Most adverse effects were of moderate intensity, lasting 1 to 6 hours, and resolved with self-care at home. The most common reactions were dizziness and anxiety. Inhalation was the most common administration method. These preliminary findings from the CCDP AERT indicate that consumers are willing to share their experiences using regulated and unregulated products. This tool provides an important avenue for individuals to report and access information about the effects of CCDP. The tool can be accessed at www.budsinfo.com. Further analysis and expansion of this tool could significantly contribute to our understanding of the safety profile of these products.

PB-59: Regulatory Aspects of Botanicals

Selling with Science – An Epidemiological Assessment of Herbal Supplementation

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What is the evidence to support the use of herbal supplements and how does that impact the sales of certain products? For example, the use of elderberry syrup to shorten the duration or lessen symptoms of a respiratory infection. A recent article published in HerbalGram reports the sales of herbal supplementation in 2022 as $12 billion. The aim of this study is to understand the strength of the evidence for top selling products. Using the published HerbalGram sales data as a proxy to exposure, we estimated the cost of products using Amazon marketplace, total volume / purchase and computed the cost per dose, and cost for a year supply. This allowed an estimate of per year exposure values. We performed a PubMed and CFSAN Adverse Event Reporting System (CAERS) search using the following terms: psyllium, elderberry, turmeric, apple cider vinegar, ashwagandha, cannabidiol, and cranberry. These top selling herbal products in 2022 contributed to over $1.0 billion in sales. Product recall, and label claim searches were also performed. The data was used to determine potential factors associated with driving or affecting sales. The table shows that apple cider vinegar had the highest ratio of publications the year prior to record sales being achieved. The overall proportion of adverse event reports by product compared to prior years was highest for cannabidiol followed by turmeric. A few notable publications were published in 2021. 1-4 These data may be prone to several biases. Cognitive biases may be driving sales. Notoriety bias, a type of selection bias, may be affecting the number of events reported in CAERS. There are a number of limitations: Social media could be an avenue that drives sales and product advertisements with false claims. In summary, consumers should be aware of herbal supplement product recalls and evidence supporting any claims, and ultimately be aware they are consuming many products, which have an undisclosed level of risk.

PC-1: Chemistry Aspects of Botanicals

O-Aroyl-β-aminopropioamidoximes; Synthesis and Biological Evaluations

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O-Aroyl-β-aminopro-pioamidoxime compounds are insufficiently studied despite their importance in drug research and being potential pharmacophores and building blocks in medicinal chemistry. The synthesis has been conducted according to Kayukova L. et al. (ISRN Organic Chemistry, 2012, pp.945893-945893). Several leads have shown promising *in vitro* antitubercular activity with MIC values of 1–100 µg/mL, which inspired further evaluations. Several analogs have been synthesized given the previously addressed structure-activity relationship (SAR), and the chemical structures of the newly synthesized compounds have been established via NMR and LC/MS analysis. Moreover, amidoximes functionality suggests potential herbicidal activity, as they are known for their coordination abilities with metal ions and form metal complexes that can disrupt plant enzyme functions, leading to growth inhibition. The synthesized analogs are undergoing in-depth evaluation for their antimicrobial and herbicidal activities.

PC-2: Chemistry Aspects of Botanicals

Development of PBD Small Molecules for the Treatment of Proinflammatory Pain States

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Chronic pain affects more than 116 million adults, with a cost of $636 billion annually in the United States. However, currently, available pharmacotherapy can control pain in only a fraction of patients. This clinical reality drives an urgent need to develop safe and effective analgesics. One approach is to identify novel analgesic drug targets. Agonists of cannabinoid receptor 1 (CB1) are non-optimal given their psychoactive effects; however, CB2 agonists are devoid of these liabilities and can sharply reduce inflammatory and neuropathic pain in rodents. Using structure-based rational drug design, we have synthesized CB2-selective pyrrolo[2,1-c][1,4]benzodiazepine (PBD) analogs with outstanding oral bioavailability, biodistribution to the central nervous system and good efficacy in a rodent model.

A strong preliminary data package has been catalyzed by NIGMS COBRE funding and has revealed at least one lead compound (4k) that is stable (up to 240 min at physiological pH), has a positive pharmacokinetic profile (t1/2 in brain ~23 h following oral administration), is efficacious against acute visceral pain (equipotent to oxycodone in an acetic acid writhing assay). Our lead compounds have been evaluated at the NIMH Psychoactive Drug Screening Program (PDSP) against a battery of drug targets, and the lead compounds (4k) do not show appreciable binding affinity on any of these targets. Further, our in vivo data in the conditioned-place preference paradigm suggests the absence of rewarding effects. PBD analogs could be transformative for the treatment of visceral pain and other pro-inflammatory pain states.

PC-3: Chemistry Aspects of Botanicals

Resveratrol and Piperine Loaded Cubosomal Drug Delivery: A Novel Approach for the Management of Melanoma

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Melanoma is one of the most aggressive types of skin cancer, with a high incidence rate and mortality throughout the world. The present study aimed to develop, characterize, and evaluate the resveratrol and piperine-loaded cubosomes (RPC) for targeting melanoma. A novel, self-assembled RPC nanoformulation was developed using glycerol monooleate and Pluronic F-127 by homogenization technique. Optimization of RPC using 2-factor 3-level factorial design indicated cubic-shaped structures, having a mean particle size and zeta potential of 110 ± 3.30 nm and -34.3 ± 1.04 mV, respectively. The entrapment efficiency of resveratrol (RV) and piperine (PI) entrapped inside the RPC was 88.12 ± 1.54 and 85.17 ± 0.25%, respectively. *In vitro* drug release of optimized RPC demonstrated biphasic drug release with diffusion-controlled release of resveratrol (RV; 92.7 ± 2.08%) and piperine (PI; 72.48 ± 3.36%). Optimized RPC was further formulated into cubosomal gel (RPC-Gel) by using carbopol (1.5% w/v), and the gel was evaluated for ex-vivo permeation and deposition, which shows better drug permeation and deposition in mice skin layers in comparison to resveratrol and piperine gel (RP-Gel). Biocompatibility of optimized RPC was observed towards L929 (mouse fibroblast), with better anticancer activity against A375 (human melanoma) cell lines compared to pure RV and PI. A stability study showed the ability of RPC to maintain stability at room temperature. The composition of RPC-Gel has been proven non-irritant to the skin of mice. An in vivo local bioavailability study depicted the potential of RPC-Gel for skin localization compared to resveratrol and piperine gel (RP-Gel). Topical application of RPC-Gel into melanoma-bearing BALB/c mice for up to six weeks resulted in tumor regression, thereby proposing the RPC-Gel as a promising drug delivery system through transdermal application for melanoma treatment.

PC-4: Chemistry Aspects of Botanicals

Development and Evaluation of a Novel Quercetin-loaded Nanostructured Liquid Crystalline Dispersion (NLCD) for Targeting Skin Cancer.

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Melanoma is the most aggressive and lethal kind of skin cancer, with an increased rate of incidence and around 75% of all skin cancer-related deaths every year. Quercetin, a naturally occurring glycoside, exerts its potential activity in melanoma skin cancer. Owing to its poor pharmacokinetic properties, a novel topical nano-formulation is required, which has more potential to cross the skin's stratum corneum barrier. A novel nanostructured liquid crystalline dispersion (NLCD) has been used in the current study to improve the applicability of quercetin. Quercetin-loaded NLCD was prepared using a top-down approach followed by high-speed homogenization and probe sonication technique. The optimized NLCD (QNLCD5) contains cubic-shaped nanoparticles with a particle size and zeta potential of 123.6 nm and -36.7 mV, respectively. The entrapment efficiency of quercetin entrapped inside the NLCD was 95.06%. Quercetin was estimated in developed QNLCD using the developed RP-HPLC technique. *In vitro* drug release of optimized QNLCD5 demonstrated biphasic drug release with diffusion-controlled release of quercetin 71.63%. Cell compatibility studies of free quercetin and QNLCD5 were evaluated against a mouse fibroblast cell line (L929), which showed no significant difference in cell viability, which confirms its cytocompatibility. *In vitro* cytotoxicity studies in the human melanoma cell line (A375) demonstrated an improved anticancer activity of QNLCD5 in comparison with free quercetin. The results of an *in vitro* anti-melanoma study demonstrated QNLCD to be a promising drug delivery system for melanoma treatment and could be a promising topical nanocarrier for melanoma treatment.

PC-5: Chemistry Aspects of Botanicals

Semi-synthetic Modification of Labdane Diterpenoid Andrographolide Isolated from *Andrographis paniculata* for PPAR-γ Agonism

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Owing to the important pharmacological activities and structural flexibility, andrographolide suffers serious limitations of water solubility and low bioavailability; therefore, an attempt has been made to synthesize novel derivatives of the natural product by incorporation of the spiro-isoxazoline ring via 1,3 dipolar cycloaddition to give candidates with better lipophilicity and bioavailability for the treatment of diabetes mellitus as PPAR-γ agonists. In this work, novel isoxazolyl analogs of natural product andrographolide have been synthesized, out of which four compounds, 5b, 5c, 5d, and 5e, exhibited significant *in vivo* blood-glucose-lowering comparable to standard drug Rosiglitazone. These compounds also exhibited significant *in vitro* PPAR-γ transactivation activity. Compounds 5b, 5c, 5d, and 5e recovered the activity of serum AST, ALT, and ALP and did not cause any damage to the liver. Compounds 5e significantly affect PPAR-γ gene expression as it increases the PPAR-γ expression by 2.0-fold, compared to standard drugs Rosiglitazone (1.0-fold) and Pioglitazone (1.5-fold). Moreover, the semi-synthetic analogs displayed better lipophilicity than andrographolide, as observed from octanol-partition coefficient data. It can be concluded that the semi-synthetic analogs 5b, 5c, 5d, and 5e may be considered potential candidates for developing new antidiabetic agents.

PC-6: Chemistry Aspects of Botanicals

Harnessing Nature's Defense: Mediterranean Flora Bioprospecting to Search for Potential Antimalarial Compounds in Plant Extracts

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Malaria is a parasitic disease that represents a major public health problem worldwide, particularly in developing and African countries. According to the World Health Organization (WHO) in 2022, there were an estimated 249 million malaria cases and 608 thousand death cases in 85 countries. *Plasmodium* is the parasite genus responsible for malarial infection involving five *Plasmodium* species. The most significant are *P. vivax* and *P. falciparum*, where the latter is the most clinically relevant. In this study we have screened a selected library of fifteen plant extracts against two clones of drug-resistant strains of *P. falciparum* parasite D-6 and W-2. The in vitro test is done in parasitized whole blood using the parasite lactate dehydrogenase assay. The polar ethanolic extract of *Inula viscosa*, *Salvia dominica*, and *Bellevalia zohari* showed antiprotozoal activity. These extracts showed more activity against W-2 strain. The IC50 against W-2 strain were 14, 15, and 35 µg/mL respectively, compared to 30, 21, 40 µg/mL against D-6 strain. *Bellevalia zohari* and *Salvia dominica* are reported here for the first time. Further investigation of fractions and unique compounds from *Bellevalia zohari* will be presented.

PC-7: Chemistry Aspects of Botanicals

Deciphering Triglyceride Complexity in Wild Eastern Mediterranean Echium Seed Oil via Application of Paterno-Büchi Modulated Lipidomics Profiling

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There is a growing emphasis on unraveling the intricacies of the fabric of triglycerides (TG) found in oils, specifically, those rich in ω-3 polyunsaturated fatty acids (PUFA). While conventional methods such as gas chromatography are capable of detecting fatty acid classes, it is inadequate to specify the double bond position in these fatty acids. Echium oil, rich in α-linolenic acid (ALA, 18:3ω-3) and stearidonic acid (SDA, 18:4ω-3), surpasses many other plant-resourced seed oils due to their anti-inflammatory and cardio-protective effects. In this study, a pseudo-targeted approach was implemented to profile the fatty acid and triglycerides found into two echium species *Echium glomeratum* and *Echium judaeum*. The TG characterization is feasible with high resolution mass coupled to high performance liquid chromatography (HRMS-HPLC). Additionally, the positioning of double bonds in these fatty acids can be accessed via Paterno-Büchi acetyl pyridine derivatization of TG. This study shows that *Echium glomeratum* and *Echium judaeum* have different fatty acid and triglycerides (TG) profiles, with (ω-3: ω-6) ratio being 3.5 and 1.5 respectively, corresponding to higher ALA (45.50%) and SDA (12.59%) in *E. glomeratum*. TGs comprise 93% of lipids in echium oil. The most abundant TGs (50-60 carbons) were profiled in both species with comprehensive and simultaneous assignment of the double bond structure. The superior *E. glomeratum* oil profile, was further assayed for its cytotoxicity, cell migration assay, and antioxidant activity.

PC-8: Chemistry Aspects of Botanicals

Molecular and Structural Insights of Boswellic Acid Analogues as Potent Anti-diabetic, Antioxidant, Anti-hyperlipidemic and α-Glucosidase Inhibitors

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Boswellic acid and its derivatives are known for their potential anticancer, anti-inflammatory and α-glucosidase inhibitory activities. Olibanum has been reported for the presence of *β*-Boswellic acid (*β*-BA) and 11-keto-*β*-boswellic acid (*β*-KBA). Here in this study chemical composition of oleo gum resin was investigated by 1D and 2D NMR techniques viz., 1H, 13C, DEPT, HSQC, HMBC, and COSY, and NEOSY, ESI-MS. Moreover, the isolated chemical components were evaluated for their anti-diabetic, *in silico* pharmacokinetic and α-glucosidase inhibition activities. Chemical analysis revealed the presence of one new triterpene, called 3α-hydroxyurs-5:19-diene (1) with 12 reported components such as 8 triterpenoids (2–9), 2 diterpenoids (10 and 11) and 2 straight chain alkanes (12 and 13). Likewise, 10 reported components were isolated from *B. sacra.* Moreover, *β*-BA and *β*-KBA were isolated from gum resin of *B. sacra.* Our findings showed that *β*-BA and *β*-KBA demonstrated anti-diabetic, antioxidant, and anti-hyperlipidemic effects recommending these components as potent candidates for diabetes. Moreover, other isolated components such as 1, 3, 10, 11, 15, and 17–19 showed significant effects against α-glucosidase. It is obvious from our overall results that these chemical components had an efficient therapeutic effect on the blood glucose level and blood biochemistry.

PC-9: Chemistry Aspects of Botanicals

Fatimanols Y and Z: Two Neo-clerodane Diterpenoids from *Teucrium yemense*

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Teucrium L. (Lamiaceae), commonly known as germander, is a cosmopolitan genus of about 300 species mainly distributed in South and Central America, Southern Asia, and the Middle East but predominantly prevalent in the Mediterranean basin. The *Teucrium* genus is a rich source of diterpenoids, particularly neo-clerodanes which are used as chemotaxonomic markers for *Teucrium* species. *Teucrium* species have been used traditionally as diuretic, diaphoretic, antipyretic, and antiseptic agents for centuries in many parts of the world. *Teucrium yemense* (Defl.), a medicinal plant, grows in Yemen and Saudi Arabia and is also referred to as Reehal Fatima. The plant has a long history of use in these regions for the treatment of diabetes, rheumatism, and renal conditions. Phytochemical investigation of the aerial parts of *T. yemense* yielded two previously undescribed neo-clerodane diterpenoids, namely fatimanols Y and Z (1 and 2) along with the known teulepicephin, 8-acetylharpagide and teucardosid. Structure elucidation was accomplished from their 1D and 2D NMR and MS characteristics as well as by comparing them to related reported compounds. The new molecules expand understanding of secondary metabolites of this genus.

PC-10: Chemistry Aspects of Botanicals

New Bisprenylated Benzoic Acids from Yerba Santa (*Eriodictyon californicum*)

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Eriodictyon californicum, commonly known as Yerba Santa, is a plant native to western North America. Its leaves have traditionally been used to treat asthma, upper respiratory infections, allergic rhinitis, and other disorders by indigenous people. The diverse array of medicinal properties, including anti-inflammation, antioxidation, bronchodilation, immune modulation, and wound healing, makes it a subject of interest in herbal medicine and scientific research. The plant is rich in bioactive compounds such as flavanones, flavones, and phenolic acids, contributing to its therapeutic potential. To explore the healthcare function and to fully utilize this plant, a chemical investigation was carried out, and 22 bisprenylated benzoic acids were isolated and identified from the plant. Among them, 17 were new compounds that had not been previously reported in the literature.

PC-11: Chemistry Aspects of Botanicals

Alkamides from the Roots of *Echinacea angustifolia* and Their Anti-inflammatory Effects Compared to Alkamides from *E. purpurea*, Evidence of Synergism

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Echinacea products are among the most commonly used herbal supplements in the U.S. They are medicinally useful mainly for their immunostimulatory and anti-inflammatory effects. The anti-inflammatory effects of Echinacea have been attributed to their content of alkamides, while the alkamides contribute in part, beside other constituents to the immunostimulation. The medicinally used *Echinacea* species include *E. purpurea*, *E. angustifolia*, and E. *pallida*.

In a previous study, we isolated ten alkamides including a novel lkamidesated lkamides from *E. purpurea*. Herein, the isolation methodology and structural determination of five alkamides (1-5), from a commercial sample of *E. angustifolia* roots, are presented. Of these are two previously unreported alkamides. The isolation was carried out employing multiple chromatographic techniques. The isolates were identified based on 1D and 2D NMR, HRESIMS, and FTIR spectral data. In addition, the anti-inflammatory effect of these alkamides and others isolated from *E. purpurea* in our laboratory, hexanes, DCM, and ethanol extracts from both species of *Echinacea*, and a partially purified fraction belonging to *E. angustifolia* were assessed using the *in vitro* assay that measures the level of inhibition of iNOS in LPS induced macrophages.

The IC50 of iNOS for the pure alkamides isolated from *E. angustifolia* and *E. purpurea* are close to each other with a range of 18.0–25.0 µM. The IC50 of the hexanes extracts of *E. angustifolia* and *E. purpurea* were 8.0 and 14.0 µM, respectively. The IC50 of the ethanol extract of *E. purpurea* was 44.0 µM, while the ethanol extract of *E. angustifolia* was inactive. Further, a column chromatography fraction of *E. angustifolia* exhibited the most potent inhibition of iNOS with IC50 5.65 µM. The anti-inflammatory activity observed in the current study is presumably a result of synergistic effect of multiple alkamides of the extract or the partially purified fraction rather than contribution of the individual compounds.

PC-12: Chemistry Aspects of Botanicals

Hyperforin-like Polycyclic polyprenylated acylphloroglucinols from *Garcinia gummi-gutta*

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Garcinia gummi-gutta is a well-known plant belonging to the family Clusiaceae and is considered native to Southeastern Asia and India. *G. gummi-gutta* fruit extracts or its botanical dietary supplements, have gained immense popularity in the United States as a remedy for weight loss. As a result, its supplements are available over the counter with attractive slimming aids and a substantial human population consumes them with the notion that being of natural origin they have no side effects. Parallelly, the cases of *G. gummi-gutta*–associated hepatotoxicity have markedly increased. In response, the United States Food and Drug Administration (US FDA) and the Centers for Disease Control and Prevention warned the consumers and manufacturers of these products (e.g., Hydroxycut and OxyElite Pro). The rind of *G. gummi-gutta* fruit has several phytochemicals including organic acids, polyisoprenylated benzophenones, and polyprenylated acylphloroglucinols (PPAPs), but hydroxy citric acid is thought to be the essential constituent responsible for weight loss effects and liver toxicity.

PPAPs possess various pharmacological activities and they fascinated us as they structurally resembled hyperforin, a potent agonist of human pregnane X receptor (hPXR) that leads to herb-drug interaction (HDIs), especially in chronic pathophysiology. Subsequently, studies also suggested that overactivated PXR significantly contributes to liver toxicity. Recently, we revised the chemical structure of guttiferone J which has substantial potency for PXR activation. Herein, we present the isolation and characterization of eight PPAPs, including four previously undescribed ones, from *G. gummi-gutta* and their PXR activation potential.

PC-13: Chemistry Aspects of Botanicals

Molecular Docking of Kratom Alkaloids Including their Metabolites at μ-Opioid Receptor: A Systematic Computational Study

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Mitragyna speciosa, a plant grown in Asia and Africa's tropical and subtropical regions and commonly known as kratom, has traditionally been used for medicinal treatments for centuries. Kratom's growing civilian popularity has also generated increased scientific interest as a potential opioid withdrawal treatment or as a potential analgesic due to its purported pain-relieving effects. Concern in the forensics and healthcare community has subsequently escalated due to the potential addictive properties associated with kratom's euphoric effects. Over 40 Kratom alkaloids have been determined to be primary contributors to the plant's psychoactive effects. Despite its potential therapeutic value, kratom use has been scrutinized for many adverse effects, including multiorgan toxicity and cardiotoxicity. However, no experimental information is available at the molecular level on the binding mechanism or putative binding site of kratom alkaloids and their metabolites at the μ-opioid receptor (MOR) (PDB ID: 5C1M). In the present study, we investigated the possibilities of MOR activation by kratom alkaloids and their metabolites by studying their binding mechanism and interaction profiles at the active-state MOR X-ray crystal structure, in concert with molecular docking, binding free-energy calculations, and all-atom molecular dynamics (MD) simulations. The results of the docking studies have proposed unique binding poses for the kratom alkaloids compared to traditional opioids, and MD studies are in progress. Identification of key interactions between kratom alkaloids, their metabolites, and the MOR will help to elucidate the relationship between kratom compounds and the MOR, as well as allow for the design of new analogs.

PC-14: Chemistry Aspects of Botanicals

Phytochemical and Biological Investigation of *Peucedanum guvenianum* from West Anatolia

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Peaucedanum is represented by more than 120 species in the Apiaceae family, widely distributed in Europe, Asia, and Africa. Various extracts of aerial and underground parts of several *Peucedanum* species well-known traditional medicine and have been used in folk medicine for treatment of various conditions, such as cough, cramps, pain, rheumatism, asthma and angina. Phytochemical and pharmacological studies have shown that several secondary metabolites, including coumarins, phenolic acids, flavonoids, phenylpropanoids, chromones, fatty acids, steroids, and a number of volatile oils (monoterpenoids, sesquiterpenoids) have been identified from the *Peucedanum* species. The major constituents of this plant are furanocoumarin and pyranocoumarins and these compounds have various beneficial effects such as anti-inflammatory, anti-asthma, chemopreventive, smooth muscle relaxan, cytotoxic, antiplatet, neuroprotective, and anti-osteoclastogenic properties. *Peucedanum guvenianum* Yıldırım & H.Duman is a newly described species endemic to the West Anatolia region of Türkiye. Herein, we report the isolation and characterization of ten coumarin derivatives from the roots of *P. guvenianum* and their biological activities assessment.

PC-15: Chemistry Aspects of Botanicals

Discovery of Potential hNaV1.1 Inhibitors for the Treatment of Drug-resistant Epilepsy

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Epilepsy is a neurological illness characterized by recurrent spontaneous epileptic seizures. Epileptic adverse effects include cognitive, psychological, social, and neurodegenerative consequences. More than 65 million people around the world and about 3.4 million people in the United States have active epilepsy. Many epilepsy patients do not respond to conventional antiepileptic drugs and therapies. Recently, the FDA has approved cannabidiol (CBD) (Epidiolex) as a treatment for two rare forms of childhood epilepsy that are resistant to current drugs; namely Dravet syndrome (DS) and Lennox Gastaut syndrome (LGS). Many researchers have tested CBD and its derivatives in rat and mouse models of epilepsy and have found anticonvulsant effects with differing levels of potency. CBD showed promising effects in patients with drug-resistant epilepsy. However, the major disadvantages of CBD are poor solubility, chemical instability, excessive metabolic instability, low oral bioavailability (approximately 5-6%), fast accumulation in fatty organs such as the brain, and very high plasma protein binding (>99%). Given these findings, we started our journey to identify novel CBD analogs or other minor constituents of cannabis with superior and sufficient efficacy and enhanced drug-like properties (such as water solubility, low lipophilicity, chemical, and metabolic stability as key properties). The identified hits may have a substantial impact on the treatment of epilepsy. There is no CBD bound hNav1.1 complex available either NMR or X-ray crystal structure; therefore, we first constructed and validated CBD bound hNav1.1 complex using computational approaches such as docking, Prime-MMGBSA calculations and all-atom molecular dynamics simulations. In addition, we identified potential hits from minor constituents of cannabis and CBD analogs by docking them at hNav1.1 channel. The *in vitro* results are pending, and potential results will be presented.

PC-16: Chemistry Aspects of Botanicals

Application of Molecular Dynamics to Assess the Binding Orientation of CBD within hNav1.1 Receptor

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Epilepsy is a devastating disease with severe adverse effects and pprox.. 65 million people around the world and about 3.4 million people in the United States have active epilepsy. CBD showed promising effects in patients with drug-resistant epilepsy. Previously, molecular modeling and site-directed mutagenesis studies have been employed to investigate the mechanism of Nav1.1–CBD binding interactions; however, the precise orientation of CBD within hNav1.1 is not well established. In the present study, we explored the potential binding-site orientation and interactions of CBD within Nav1.1 using computational approaches including docking, Prime-MMGBSA and molecular dynamics. To gain insight regarding potential binding orientations of CBD within Nav1.1, we performed Induced-Fit Docking of CBD on cryo-EM structure of the human Nav1.1 (hNav1.1) (PDB ID:7DTD). We evaluated two orientations of CBD within Nav1.1: one in which the terpene moiety is directed towards the extracellular region and another in which the terpene moiety is directed towards the key residue Phe1772. In addition, we performed 500 nanosecond molecular dynamics simulations of the commencing from the best docked pose for each of the two orientations for CBD within Nav1.1, including POPC membrane, water and appropriate ions. The molecular dynamic is in progress and results will be presented in the conference. The results of this work may be helpful for the design of novel CBD analogs that fit optimally into the Nav1.1 binding pocket.

PC-17: Chemistry Aspects of Botanicals

Phytochemical Profile of *Elephantorrhiza elephantina* Rhizome

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Elephantorrhiza elephantina (Burch) Skeels (Fabaceae), commonly known as elephant root, is widely used by southern African indigenous people as a source of food, medicine, and tanning /dyeing materials. The tuberous red rhizome is the most commonly used part of the plant in traditional medicine for the management of various metabolic and infectious diseases in both humans and animals. Twenty-two compounds mainly catechin, taxifolin, and proanthocyanidin derivatives were isolated and characterized from methanolic extract of *E. elephantina* rhizome. Elephantinaside A-F were found to be undescribed compounds. Structure elucidation was mainly based on 1D- and 2D-NMR and HRESIMS data. The absolute configuration of isolates was determined via NOESY NMR and experimental and calculated ECD data analyses.

PC-18: Chemistry, biology, and safety of volatile organics from aromatic and medicinal plants

Design and Synthesis of Structurally Novel Acylphloroglucinols Against *Cryptococcus* Species

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Cryptococcus neoformans is an invasive fungus, transmitted through the inhalation of spores and causes cryptococcosis, an infection commonly associated with immunosuppressed patients with AIDS, cancer, or organ transplants. Naturally occurring acylphloroglucinols provide a large range of structurally diverse compounds. A number of compounds within this structural class are known to be active against various fungal and bacterial pathogens, but only a few compounds were reported to show activity against *Cryptococcus* spp. The synthetic compound 2-methyl-1-(2,4,6-trihydroxy-3-(4-isopropylbenzyl)-phenyl)propan-1-one, classified as an acylphloroglucinol, has exhibited potent in vitro antifungal efficacy against two strains of *C. neoformans* (ATCC 90113 and H99) and one strain of *C. gattii* (ATCC 32609). This promising activity suggests its potential as a lead compound for subsequent structure and activity optimization. In our approach, we strategically designed and synthesized acylphloroglucinols, mimicking a lead compound. This involved the incorporation of n-butyryl and n-pentanoyl groups into the phloroglucinol core, coupled with the introduction of a heterocyclic aromatic ring in the side chain. These designed compounds are anticipated to exhibit reduced lipophilicity, increased antifungal efficacy, and decreased cytotoxicity. Our work presents a collection of potential compounds characterized by potent antifungal activity and enhanced chemical stability against *Cryptococcus* species.

PC-19: Chemistry, biology, and safety of volatile organics from aromatic and medicinal plants

Design, Synthesis and Biological Evaluation of Coumarin Based TRKA Activators as Anticancer Agents

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Coumarins are an important class of natural plant metabolites that offer a variety of biological activities. The coumarin scaffold is widely used for the development of highly effective anticancer agents with minimum side effects. The tropomyosin receptor kinase (TRK) family of receptors are encoded by neurotrophic tropomyosin receptor kinase (NTRK) genes and plays a role in the development and normal functioning of the nervous system. The TRK receptor family comprises three members (TrKA, B, & C) encoded by the genes NTRK1, 2, & 3, respectively. NTRK gene fusions have shown different rates of occurrence in rare cancers (frequency >80%), and common cancers (frequency <25%). Following its prevalence in both rare and common cancers, NTRK gene fusions have emerged as a viable target for anticancer drug therapy. Several of the currently used chemotherapeutics induce autophagic cell death. Activation of TrkA in human glioblastomas might be beneficial therapeutically.

Seven compounds were investigated with possible activity on the NGF/TrkA pathway with a coumarin-scaffold (5c-g, 5j, and 5k) that exhibited significant growth inhibition in the glioma SF539 cell line. Their effect was investigated on TrkA activation at three doses (10 µM, 5 µM and 2.5 µM) in the Indigo Bioscience, Human Tropomyosin Receptor Kinase A reporter assay system (TrkA). All samples except 5k showed activation on TrkA at either 10 uM or 5 uM. 5c and 5d were able to cause ~2-fold induction of TrkA (~100% increase in activity). Moreover, 5e, 5f, 5g, and 5j showed a parabolic effect, with the highest activity at the middle concentration 5 µM. These data suggest that the glioblastoma growth inhibition detected by the coumarin-scaffold derivatives investigated in this study could be at least in part working through TrkA activation.

PC-20: Chemistry Aspects of Botanicals

Butanolides from the Twigs of *Casearia grewiifolia*

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Casearia grewiifolia Vent. (Salicaceae) is a shrub or small tree that grows in tropical and subtropical areas such as Southeast Asia and some Pacific islands. The extracts of this plant have been used traditionally to treat diarrhea, fever, and itching. Previous investigations for this plant have reported the presence of amides, clerodane-type diterpenoids, lignans, other phenols, and steroids as chemical constituents, and bioactivities such as antimycobacterial, antimalarial, and cytotoxic effects. To explore new chemical constituents from this plant, the twigs of *Casearia grewiifolia* were investigated and as a result, three butanolides derivatives (1–3) were isolated. The chemical structures and absolute configurations were established by detailed NMR spectroscopic data including 2D 13C–13C Incredible Natural Abundance DoublE QUAntum Transfer Experiment (13C–13C INADEQUATE), mass spectra, hetero half-filtered TOCSY (HETLOC), Mosher esterification procedure, and electronic circular dichroism (ECD).

PC-21: Chemistry Aspects of Botanicals

Revisiting *Mandragora officinarum* L.: From Magic to Phytochemistry and Bioactivities

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Mandragora officinarum L. (mandrake) is a perennial herbaceous plant from the nightshade (Solanaceae) family, native to the Mediterranean region. The plant has a rich history of uses from ancient times for its healing and psychotropic properties. However, its wider use has declined due to the presence of tropane alkaloids, which can lead to poisoning, if used improperly. In general, the number of publications on *M. officinarum* chemical composition and bioactivities is rather scarce. The majority of the previously performed studies were focusing on its alkaloids. The aim of this study was to fractionate oven and freeze-dried *M. officinarum* fruits (berries) and roots in to the lipophilic and higher polarity fractions by using consecutive extraction with supercritical CO2 and pressurized liquids using the increasing polarity solvents and to evaluate the phytochemical composition and antioxidant potential of the fractions obtained. First of all, proximate composition of *M. officinarum* berries and roots was evaluated by using standard methods

	Proteins, %	Fats, %	Insoluble fibers, %	Minerals, %	Moisture, %
Roots	10,18 ± 0,41	4,7 ± 0,6	8,33 ± 0,05	4,35 ± 0,01	9,7 ± 0,35
Dried at 60°C fruits	13,27 ± 0,51	13,63 ± 0,31	21,77 ± 0,2	5,35 ± 0,02	6,24 ± 0,18
Freeze-dried fruits	13,67 ± 1,55	13,26 ± 0,4	22,47 ± 1,3	4,92 ± 0,02	8,27 ± 0,13

The content of lipophilic fractions isolated at 350 Mpa pressure and 50°C temperatures was approximately 0,5% for the roots and 8% for fruits. Additionally, the total amount of phenolic compounds and ABTS radical scavenging activity were also determined in the roots, fruits and leaves of this plant. The same assays were also carried out not only with dry raw material but also with various extracts: CO_2, ethanolic, acetone and water.

PE-1: Chemistry, biology, and safety of volatile organics from aromatic and medicinal plants

Research on Natural Sunscreens Based on Medicinal Plants.

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Research on natural solar filters (sun creams for albinos) based on medicinal plants. Protecting the skin from the sun is a public health issue. Due of the growing awareness of the risks of skin cancer due to damage from UV-A and UV-B rays, UV filters are now found in many beauty products applied daily. Our skin cells have essential fatty acid barriers, and when UV arrive, they penetrate this barrier by manufacturing oxygen molecules called “singlet oxygen”, which can be harmful to the cell and to the cell nucleus and attack DNA.

Thus, the sun is responsible for sunburn and premature aging of the skin as well as the appearance of skin cancers. "At a certain time, when their DNA has been frequently attacked, cells end up having abnormal DNA, which can then give rise to a cancer cell", notes Dr. Gaucher. Outside of cancer, cells that are repeatedly damaged age faster. A solution: protect yourself from the sun by applying protection solar filters.

This involves verifying the natural sun filter effect based on medicinal plants. In presence of significant activity certain medicinal phytochemicals such as flavonoids could be used in the production of natural sun protection (plant-based sunscreen) in accordance with WHO guidelines according to traditional medicine and the European Union cosmetics directive. The study of toxicity *in silico*, *in vitro* and/or *in vivo* make it possible to cover all the potential problems linked to the molecule, such as the identification of its potential irritant, sensitizer and/or photo irritant and/or sensitizing effects as well as its embryotoxic and reprotoxic potential and finally carcinogenicity and genotoxicity.

PE-2: Chemistry, biology, and safety of volatile organics from aromatic and medicinal plants

Sustainable Cultivation of Spicy and Aromatic Plants Applying Green Manure

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Understanding the effects of green manure on the development and quality of plants can contribute to the promotion of more sustainable agricultural practices and the maximization of the economic and medicinal potential of species such as *Ocimum basilicum* L. and *Origanum majorana* L. The objective was to evaluate the potential of *Crotalaria juncea* and *Cajanus cajan* as green manure in the production of biomass and in the essential oil metabolism of *O. basilicum* and *O. majorana*. Six treatments and four replications were used, each containing five plants per replication, totaling 120 plants. The treatments consisted of five green manure doses (0, 3, 6, 9, and 12 kg/m²) and a chemical control. After 120 days of cultivation, the dry weight of leaves, stem, roots, and total plant, as well as the oil content and yield, and the chemical composition of the essential oil were analyzed. The use of *C. juncea* and *C. cajan* as green manure demonstrated a positive and significant impact on the production of dry weight in vegetative organs, as well as on the yield and chemical composition of the essential oil. The green manure management exerted a positive influence on the essential oil production, showing contents and yields comparable to chemical fertilization. α-Terpineol stood out as the major compound in the essential oil of *O. majorana*, reaching higher concentrations in plants treated with 12 kg/m² of *C. cajan*. In conclusion, the application of the dose of 12 kg/m² of *C. juncea* or *C. cajan* resulted in the best performances in terms of growth and nutrient accumulation. It also affirmed the effectiveness of sunn hemp and pigeon peas as a fundamental green manure strategy for the sustainable production of aromatic plants.

PE-3: Chemistry, biology, and safety of volatile organics from aromatic and medicinal plants

Quality Assessment and Authentication of Lavender Essential Oil

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[1]Young Living Essential Oils

The pleasant aroma of lavender (*Lavandula angustifolia*) essential oil, combined with its purported health benefits, makes it a popular choice for aromatherapy, cosmetic, flavor, and fragrance applications. The widespread use of lavender essential oil lends itself to potential adulteration. To assess the quality and authenticity of lavender essential oil, it is vital to also understand which factors influence the essential oil profile and which analytical techniques are optimal for analysis.
To investigate authentication of lavender essential oil, samples (n = 41) were procured directly from farmers/distillers and used as reference materials. These essential oils were analyzed to determine profiles and related data by GC, enantioselective GC, and GC/IRMS. Said analysis resulted in the identification of 43 authentic marker compounds, enantiomeric ranges for 15 compounds, and stable isotope ranges for four prominent compounds in authentic lavender essential oil. This dataset was used to assess the quality of commercially available lavender essential oil samples (n = 12) purchased from online retailers. Nine of the twelve (75%) commercial samples studied were adulterated, and 17 adulteration marker compounds were identified from these commercially available samples. These studies stress the importance of understanding factors contributing to natural variation and establish the utility and importance of using a multifaceted analytical approach to differentiate quality and determine authenticity of lavender essential oil.

PE-4: Chemistry, biology, and safety of volatile organics from aromatic and medicinal plants

Characterization of Cannabis Smoke Condensate and its Inhibitory Effects on CYPs in Human Lung

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Smoked cannabis flower is the most commonly utilized form of cannabis for medical and recreational purposes. Cannabis is typically used in the treatment of various chronic conditions and diseases including amyotrophic lateral sclerosis, cancer, Crohn's disease, epilepsy, glaucoma, and others. These conditions typically require the use of one or more conventional medications including lung therapeutics concurrently which leads to concerns over botanical-drug interactions (BDIs). Combusted cannabis leads to the formation of thousands of compounds and this study focuses on the collection and characterization of cannabis smoke condensate (CSC) and an assessment of its in vitro inhibitory effects on six major cytochrome P450 enzymes (CYP) in the lung. Three standardized cannabis cigarettes sourced from the US National Institute of Drug Abuse Drug Supply Program were consecutively combusted in an enclosed smoke exposure system. Generated smoke was routed through an ultra-cold condenser permitting the collection of CSC. CSC was weighed and analyzed for the presence of 8 major cannabinoids (CBs) via LC-MS/MS. *In vitro* enzyme inhibition studies were conducted/ongoing using human lung S9 to evaluate the potential inhibitory effect of CSC on CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2B6, and CYP2E1. A total of 14.6 mg (4.87 mg per cigarette) of CSC was collected, containing 0.022% CBD, 1.163% CBN, 9.317% THC, 0.193% CBG, and 0.008% 11-OH-THC of the total weight. The half-maximal inhibitory concentration (IC50) of CSC for CYP3A inhibition was determined to be 17.85 µM with Δ9-tetrahydrocannabinol (THC) as the index substrate. The study revealed that the relative CB content of cannabis smoke is substantially different from that of non-combusted cannabis flowers. Furthermore, CSC produced a mild inhibitory effect on CYP3A activity. Further investigations are ongoing to elucidate the inhibition mechanisms and assess the inhibitory effects on other CYPs and drug metabolizing enzymes.

PE-5: Chemistry, biology, and safety of volatile organics from aromatic and medicinal plants

Discovery of Selective Cannabinoid Receptor Type II Ligands from Sandalwood Oil

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Bioassay-guided fractionation of the essential oil of *Santalum album* led to the identification of α-santalol and β-santalol as a new chemotype of cannabinoid receptor type II (CB2) ligands with Ki values of 10.49 and 8.19 μM, respectively. Nine structurally new α-santalol derivatives were synthesized to identify more selective and potent CB2 ligands. The synthetic compound with a piperazine structural moiety demonstrated a Ki value of 0.99 μM against CB2 receptor and did not show binding activity against cannabinoid receptor type I (CB1) at 10 μM. α-Santalol, β-santalol, and the synthetic α-santalol derivative increased intracellular calcium influx in SH-SY5Y human neuroblastoma cells that were attenuated by CB2 antagonism or inverse agonism, supporting the results that these compounds are CB2 agonists. Molecular docking showed that α-santalol and the synthetic α-santalol derivative had similar binding poses exhibiting a unique interaction with Thr114 within the CB2 receptor, and that the piperazine structural moiety is required for the binding affinity of this synthetic compound. A 200 ns molecular dynamics simulation of CB2 complexed with the synthetic α-santalol derivative confirmed the stability of the complex. This structural insight lays a foundation to further design and synthesize more potent and selective α-santalol-based CB2 ligands for drug discovery.

PE-6: Chemistry, biology, and safety of volatile organics from aromatic and medicinal plants

Micro-Morphology Characterization and HS-SPME-GC-MS Analysis of Floral Part of *Quararibea funebris* (La Llave) Vischer, Traditionally Known as Rosita de Cacao

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The flowers of *Quararibea funebris* (La Llave) Vischer are used to make a traditional drink called tejate, to which it adds aroma, flavor, and consistency. The aim of this work is to describe the morphoanatomy of the flower of *Q. funebris* and analyze the change of its volatile chemical composition during the drying process from 0 to 180 days using headspace solid-phase microextraction (HS-SPME)-gas chromatography-mass spectrometry (GC-MS). The cross-section of the calyx, corolla, androecium, and gynoecium, the presence non-glandular fused stellate trichomes, calcium oxalate crystals and large secretory ducts are very characteristic. The chemical analysis revealed that the most abundant compounds of the essential oil (yielding 0.04%) were trans-farnesol and geraniol. HS-SPME analysis revealed a more complex composition in the fresh flower than the dry flower. A total of 31 components were identified, □□-ocimene, linalool, citronellol, geraniol, methyl geranate, and trans-farnesol with the highest relative abundance. Nonanal and geranyl acetone as distinctive components in the 180- day-old, dried flowers. These results can help in identification and volatile chemical profiling of dry flowers and also be used as quality control parameters to confirm the raw materials sold commercially in the name of Rosita de Cacao.

PE-7: Chemistry, biology, and safety of volatile organics from aromatic and medicinal plants

Chemical Composition and Biological Activities of *Eugenia sellowiana* DC. (Myrtaceae) Essential Oil

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Myrtaceae is an important and promising class of plants with sources of plant bioactives. This family is widely distributed in Brazil's Cerrado biome, a vast ecoregion of tropical savanna in eastern Brazil. While some species in this biome are well-characterized, others, like *Eugenia sellowiana*, remain less known. This research aims to investigate the biological potential of *E. sellowiana* leaf essential oil as an insecticide and antifungal agent and to conduct a toxicological screening for hemolytic potential. *E. sellowiana* leaves were collected from the Cerrado area in Hidrolândia, Goiás (16°54'02" S and 49°15'34" W), dehydrated, and the sample powdered, then submitted to hydro-distillation with a Clevenger apparatus for 2 hours. Gas chromatography-mass spectrometry (GC/MS) elucidated the chemical composition. Larvicidal activity against third-instar *Aedes aegypti* larvae was investigated. The potential antifungal effect was examined by microdilution in broth against ten fungal strains. Potential hemolysis was assessed using commercially obtained sheep erythrocytes. The extraction process yielded 0.36% of essential oil (OE) comprised of 25 substances, with limonene, z-caryophyllene, germacrene D, and bicyclo germacrene showing better percentages. Larvicidal activity was promising, with significant lethality in *Aedes aegypti* larvae (LC_{50} 100 µg/mL). A substantial antifungal effect was observed against various strains (MIC/MFC 32/1024 µg/mL). In evaluating hemolytic potential, the essential oil of *E. sellowiana* demonstrated a hemolysis rate of less than 9% at the highest concentration tested (244 µg/mL), suggesting the material is non-toxic. The results indicate that the biopotential of *E. sellowiana* is promising and warrants further investigation. Additionally, the study underscores the importance of preserving plant species like *E. sellowiana* in the Cerrado biome.

PE-8: Chemistry, biology, and safety of volatile organics from aromatic and medicinal plants

Dereplication of The *Aaronsohnia factorovsky* Phytochemicals and Essential Oil: A Thriving Daisy in The Heart of Salty Moab Mountains-Eastern Dead Sea.

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Aaronsohnia factorovskyi Warb. & Eig (AF) is a rarely investigated herb that belongs to the daisy family Asteraceae. It has been traditionally used as an herbal tea with a medicinal value. In particular, it is used for kidney stones, toothache, anemia, cancer, and diabetes. AF has a strong and pleasant aroma and desirable hot stinging taste. In this study, we have explored the chemical composition of the plant employing various analytical techniques. The chemical composition of the essential oil was analyzed by gas chromatography-mass spectroscopy (GC-MS), and the secondary metabolites of the polar methanolic extract were analyzed with liquid chromatography-high resolution electrospray ionization mass spectrometry (LC-HRESIMS). The cytotoxicity of the essential oil was carried out by MTT assay in T47D, and A549 cell lines for ductal cell carcinoma and non-small cell lung cancer. Our results demonstrated a cytotoxic effect of the volatile oil at 2.517 µl/mL, and 1.844 µl/mL respectively. Myrcene was the major compound identified in the hydro distilled volatile oils, with concentration of 26.65%; and together with ocimene <(Z)-β-> and capillene, they accounted for about half of the oil. Furthermore, the untargeted profiling of the AF methanolic extract has detected the alkamide thienyl-hexadien-isobutylamide as the major compound. Comprehensive chemical composition of AF oil and polar extract will be presented.

PE-9: Chemistry, biology, and safety of volatile organics from aromatic and medicinal plants

Electrophysiological Assessment of the Essential Oil of *Murica gale*, an Irish Wetland Plant on Seizure Activity

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The literature has reported on many plant metabolites, volatile extracts and whole fresh material exhibiting anti-seizure activity, with evidence of administering these directly to tissue having anti-convulsant effects [1]. Seizure activity can be reduced via the inhalation of certain essential oils (EOs) [1,2]. Myrica gale (MG) is a native Irish bogland shrub and has been known as a natural remedy for fits in dogs [3]. This study aimed to assess the acute anti-epileptic potential of 2 Irish wild MG EOs (EO1M, EO15C) and 1 commercial Canadian EO (COM) using in vitro electrophysiology. Extracellular local field potential (LFP) recordings measured ictal-like events (ILEs), in adult Lister hooded rat brain slices (400ym). ILEs were elicited using the proconvulsant 4-aminopyridine (26.7mM). Following establishment of baseline ictal activity (5 ictal events), the EOs were individually applied to slice via perfusate (0.5%v/v). LFP recordings of ILE activity were recorded from the superficial layers (II-III) of the medial entorhinal cortex (mEC). The effects of each EO on ictal duration (ID), inter-ictal duration (IID), first spike amplitude (FSA), spectral power density (SPD) and number of seizures (NoS) were analysed. Acute application of EO1M significantly reduced ID ($n=9$, $P < 0.01$), IID ($P < 0.01$), NoS ($P < 0.01$), PSD ($P < 0.01$) and FSA ($P < 0.05$). EO15C, significantly reduced ID ($n=9$, $P < 0.01$), IID ($P < 0.01$) and NoS ($P < 0.01$). COM did not demonstrate any significant activity for all parameters measured, ID, IID, NoS, PSD, and FSA ($n=9$, $P > 0.01$).

We demonstrate that EOs extracted from Irish MG samples, can reduce seizure like activity in this model. The variance in plant metabolites for each sample are assumed to bear some significance in the variation of activity assessed. Future work aims to reveal the mechanistic nature of individual terpenes and/or complex mixture of EOs on seizure activity in brain tissue.

PE-10: Chemistry, biology, and safety of volatile organics from aromatic and medicinal plants

Quality Evaluation of Peppermint Oils and Commercial Products: An Integrated Approach Using Conventional and Chiral GC/MS Combined with Chemometrics

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The herb peppermint (*Mentha* x *piperita* L) is a perennial aromatic herb which is a natural hybrid of *Mentha aquatica* L. (water mint) and *Mentha spicata* L. (spearmint). Peppermint essential oil (EO) has a multitude of applications such as a fragrance in cosmetics, personal care, and industrial products; or as a flavoring ingredient in food and beverages. Despite its popularity and economic significance, peppermint EO is often adulterated in order to reduce production costs and to increase profits. Peppermint EO can be adulterated by one or a combination of four methods: 1) the addition of synthetic compounds which are not naturally present in the EO; 2) the addition of synthetic compounds which are present in natural EO; 3) the addition of natural compounds from other sources (i.e. plants) or enzymatic production; and/or 4) the addition of EO fractions or oil which have a similar composition to all or part of natural peppermint EO. Although ISO standards for peppermint EO using conventional GC techniques exist, detecting sophisticated forms of adulteration remains challenging. In order to address these issues, the goal of our investigation was to develop and utilize both conventional and chiral GC/MS techniques to analyze samples of known and unknown provenance. Next, the data obtained from the chiral analysis of known provenance samples was averaged and used to establish a point of comparison for unknown samples. In addition, data obtained from the GC/MS analysis was also subjected to chemometric analysis in order to detect outliers.

PE-11: Chemistry, biology, and safety of volatile organics from aromatic and medicinal plants

Unlocking the Aromatic Symphony: Terpenes and Furanocoumarins in Grapefruit Essential Oil Orchestrate Metabolic Harmony

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The essential oils (EOs) of *Citrus* (Rutaceae) fruits have been utilized for centuries and remain popular due to their numerous beneficial health effects. Particularly, grapefruit EO has been touted for topical and internal administration due to the abundance of limonene, which some scientific literature has identified to modulate metabolism. While the major constituents of grapefruit EO are monoterpenes, sesquiterpenes have been identified in minor quantities. Indeed, many botanicals have been demonstrated to affect metabolism through the pregnane X receptor (PXR). Activation of PXR can result in the increased transcription of metabolic enzymes, such as cytochrome P450s (CYP450s). Additionally, grapefruit isolates have been shown to contain a wealth of oxygenated heterocyclic compounds (OHCs) with scaffolds similar to the prototypical bergamottin. Such furanocoumarins are heavily implicated or have been demonstrated in modulating inhibitory activity at CYP450s, principally CYP450 3A4. Considering not only the concomitant administration of both terpenoids and furanocoumarins in grapefruit EO but also the interplay between PXR and CYP450s, it is critical to assess the potential effects on the metabolism of the phytoconstituents. In the context of metabolism, the harmful effects of grapefruit furanocoumarins may be mitigated by the upregulation of PXR from volatile grapefruit compounds in the matrix of essential oil. A computational approach was implemented to assess the plethora of grapefruit EO volatile organic compounds (VOCs and OHCs) to support this hypothesis. Parent grapefruit EO VOCs and furanocoumarins were docked with Schrödinger Glide to select PXR and CYP450s 1A1, 2C9, 2D6, and 3A4 crystal structures. The findings suggest that activation of PXR and inhibition of CYP450s may happen concurrently. This necessitates additional validation through experimental techniques that involve coincubation of both prioritized grapefruit volatile and furanocoumarin compounds.

PE-12: Chemistry, biology, and safety of volatile organics from aromatic and medicinal plants

Chemical Analysis and Antibacterial Efficacy of *Cinnamomum camphora* (L.) Nees et Eberm. Essential Oil Against *Klebsiella pneumoniae*: *In vitro* and *In silico* Study

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The essential oil of *Cinnamomum camphora* (Campor) stands out in the literature for its antimicrobial, insecticide and antioxidant properties, but it has high volatilization and low stability, hence to overcome these limitations nanoencapsulation of this oil could be the desired alternative. The objective of this work was to identify the volatile compounds from *Cinnamomum camphora* (L.) Nees et Eberm., leaf essential oil from India and to establish its antibacterial effect against *Klebsiella pneumoniae* (Schorete) Trevisan (MTCC 618). The essential oil was extracted by hydrodistillation and chemical constitutes were identified by using GC-MS. Chitosan based nanoformulations of the essential oil were prepared using ionic gelation method and characterized by UV, SEM, XRD, FTIR, in vitro release and by encapsulation efficiency. The antibacterial activities were evaluated against Gram-negative bacteria *K. pneumonia* by agar well diffusion assay and its mode of action was predicted by *in silico* studies. Major content identified by gas chromatography-mass spectrometry (GC-MS) were Carvone (55.71%), limonene (18.83%), trans-carveol (3.54%), cis-carveol (2.72%), beta-bourbonene (1.94%), and caryophyllene oxide (1.59%). The essential oil displayed antibacterial effects against *K. pneumonia* with IC50 of 0.087 mg/mL. Further, results confirmed the successful encapsulation of the *Cinnamomum camphora* essential oil with 94.7% encapsulation efficiency and 90% of loading capacity. Nanoparticle size analyzer, scanning electron microscope (SEM) showed that CSNPs were spherical particles with a range of 200 to 220 nm. The results of in vitro release study indicated that the release of essential oil was phased, and chitosan encapsulated essential oil had certain sustained-release properties.The *in silico* ADMET and molecular docking studies confirm that the results have a greater affinity with the *in vitro* tests carried out for the selection of new antibacterial products of natural origin.

PE-13: Chemistry, biology, and safety of volatile organics from aromatic and medicinal plants

Antidiabetic Activity of Standardized *Geranium robertianum* and *Geranium subcaulescens* Extracts and their Major Compound Geraniin

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In the last 10 years, diabetes mellitus has risen from 14th to 10th place among global causes of death with a mortality. Although the use of conventional drugs in treating diabetes is widespread, plants have been a therapeutic source in traditional treatment. There are 350 *Geranium* L. species worldwide and 39 in Turkey. *Geranium* species are popular in Turkey, Northern Peru, Morocco and Algeria for andiabetic purposes. In light of this information, the aerial parts of *G. robertianum* and roots of *G. subcaulescens* were investigated for antidiabetic effect. α-Glucosidase, α-amylase for antidiabetic activity, cholesterol esterase, pancreatic lipase enzymes have been studied due to the association between diabetes and obesity. The main compound in the extracts was determined to be geraniin by RP-HPLC, and both extracts were standardized based on geraniin and methylgallate as markers. *G.subcaulescens* showed higher activity than *G.robertianum* in all four enzyme models. When all enzyme inhibitory activity results were compared, it was concluded that both species showed strong inhibitory activities, especially against α-glucosidase and α-amylase enzymes. Standardization of the extracts by RP-HPLC revealed that geraniin (20.90 ± 0.06 mg/100 mg) and methyl gallate (10.65 ± 0.02 mg/100 mg) in *G. subcaulescens* were higher than geraniin (16.33±0.02mg/100 mg) and methyl gallate (0.72 ± 0.07 mg/100 mg) in *G.robertianum*. Geraniin particularly strongly inhibited the enzymes α-glucosidase and α-amylase. On the other hand, it was observed that the activity increased as the amount of methyl gallate increased in the extracts, but it was not effective as a single compound. The results showed that geraniin is the effective compound of *Geranium* species and methylgallate also contributes to the synergistic effect. In light of all these findings, future *in vivo* activity and molecular docking studies on geraniin are planned.

PE-14: Chemistry, biology, and safety of volatile organics from aromatic and medicinal plants

α-Copaene and its Oxidation Products: NMR Analysis and Fire Ant Repellent Activity

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As part of a natural product discovery program for identifying fire ant control agent, the essential oil extracted from Gurjun balsam (*Dipterocarpus turbinatus*) was screened and demonstrated repellent effects against imported fire ants. Bioassay-guided isolation of the oil resulted in the identification of α-copaene (1) as the major phytochemical responsible for the activity. Surprisingly, α-copaene, a highly strained tricyclic sesquiterpene with one double bond, is susceptible to autoxidation in the presence of air, producing a previously undescribed hydroperoxide [5-hydroperoxy-α-copaene (2)] and copa-2-en-4-ol (3). Chemical epoxidation of α-copaene afforded a-copaene epoxide (4), which underwent stereoselective ring opening to generate β-copaen-4α-ol (5). Potassium permanganate mediated oxidation resulted in the formation of copa-3-ol-4-one (6) and copa-3,4-diol (7). The stereochemistry of these oxidation products was unequivocally defined by 2D NMR experiments, which has addressed some confusions in literature. Fire ant assay of the oxidation products demonstrated a promising repellency potential. The repellency activity obtained was corroborated with molecular docking of the active compounds with pheromone-binding protein Gp-9 (Source: Solenopsis invicta x Solenopsis richteri). Site mapping predicted two putative binding sites. The screened compounds gave a docking score in the range of -5.828 to -8.869 Kcal/mol and -1.707 to -3.188 Kcal/mol, respectively in Sites 1 and 2. Overall, these findings highlight the potential role of α-copaene and its oxidation products in fire ant management.