24th Annual OXFORD ICSB

April 20th - 23rd, 2026

The Oxford International Conference on the Science of Botanicals is an annual meeting to discuss:

- Approaches for post market surveillance
- Risk and safety assessment
- Quality controland adverse event reporting (AER) for botanical dietary supplements (BDS) and natural products
- Regulatory aspects with perspectives from government, manufacturers and trade associations

POSTER ABSTRACTS

http://oxfordicsb.org | https://facebook.com/OxfordICSB | icsb@olemiss.edu | Phone: (662) 915-7821 | Fax: (662) 915-7989

POSTER ABSTRACTS

OXFORD ICSB

INTERNATIONAL CONFERENCE ON THE SCIENCE OF BOTANICALS

2026

Regulatory Aspects of Botanicals

PA-1: Prevalence and Patterns of Use of Natural Health Products Among New Zealand Adults: Findings from Two National Cross-Sectional Studies

Lee E, Harrison J, Barnes J

School of Pharmacy, University of Auckland, New Zealand

Natural health products (NHPs) and traditional/complementary/alternative medicine (TCAM) therapies are used for healthcare reasons. Many high-income countries collect national data on NHPs/TCAM use, but limited data are available for New Zealand (NZ). Two surveys using different recruitment methods were undertaken to assess study feasibility and collect preliminary data on the prevalence and patterns of use of NHPs/TCAM among NZ adults: i) online market research (MR) panel survey; ii) survey of a random sample from the NZ electoral roll (ER). Self-reported data on NHP exposures, consultations with TCAM practitioners, and conventional medicines exposures were collected. Descriptive statistics were used to summarise prevalence and patterns of use. Multivariable logistic regression was applied to the MR dataset to examine the impact of sociodemographic variables on use of NHPs/TCAM therapies. In total, 992 participants were recruited via an MR panel; the ER method response rate was 2.6% (129/5000). Participant characteristics were generally comparable across datasets and with the NZ 2018 Census for key sociodemographic variables. Prevalence estimates were similar across datasets: NHP use (past 12 months) was reported by 57.6% (MR) and 61.2% (ER) of respondents; TCAM practitioner consultations (past 12 months) were reported by 22.9% and 24.8%, respectively. Among current NHP users, 71.1% (MR) and 76.4% (ER) also use conventional medicines. Female participants, younger individuals, and conventional medicines' users were more likely to use NHPs/consult TCAM practitioners/use any TCAM. Participants with higher incomes (>$50,000/year) were more likely to consult TCAM practitioners. Findings indicate substantial self-reported exposure to NHPs and TCAM practitioner consultations. A larger, nationally representative dataset, ideally linkable to other health-data collections, is needed to validate these findings.

PA-2: A Look Back at the OTC Botanical Drug Route: How Phytomedicine Became Dietary Supplements

Sherman AC

William Reed, Ltd.

In 1992, Bill Clinton was elected president, Silence of the Lambs swept the Academy Awards, grunge broke into mainstream fashion, and the phrase "surfing the Internet" was coined. It was also the year a group of European and American phytomedicine manufacturers petitioned the Food and Drug Administration to facilitate the entry of products—backed by rigorous science and quality assurance—into the U.S. market as over-the-counter botanical drugs. Europe at the time had a thriving market of standardized, science-backed herbal drugs that were part of sophisticated drug regulatory systems. Phytomedicine in the United States, meanwhile, had suffered since its heyday in the early 1900s, affected by advertising excesses, economic disincentives, the loss of pharmacognosy programs and the lack of patentability. By the 1960s, however, there were indications of a revitalization of pharmaceutical botany and the consumer market.

In 1986, the companies that would make up the European-American Phytomedicines Coalition (EAPC) had their first collective meeting with U.S. manufacturer Nature's Way which was interested in expanding the company's portfolio with products backed by rigorous scientific research and quality assurance. Since the U.S. regulatory environment was not equipped to provide access for OTC botanical drugs from overseas, it was proposed to work with FDA to establish a suitable path to market. Work also began to hatch a parallel route that would protect consumer access to and create a regulatory framework for dietary and herbal supplements in the face of another looming FDA clamp down around claims. Despite ongoing efforts, proposals for an OTC botanical drug route met with inaction by FDA, and botanicals were redefined as dietary supplements with the passage of the Dietary Supplement Health and Education Act (DSHEA) in 1994—

fundamentally changing their legal and commercial status. Further research will explore continued efforts post-DSHEA to establish a new regulatory category for herbs and phytomedicines based on a less stringent procedure and standard of evidence than a new drug application (NDA) yet robust enough to ensure public safety and promote responsible use of these products.

PA-3: From Alerts to Evidence: Advancing the Use of *In Vivo* Genotoxicity Dose-Response Data for Complex Mixtures

Doepker C, Franzen A, Wikoff D, Thompson C

Complex mixtures derived from natural products and thermal processing (e.g., wood pyrolysis derived flavors, roasted coffee/tea, baked goods and botanicals) can contain one or more constituents with genotoxic potential. Such constituents are not intentionally added to mixtures and typically occur at very low levels - so low that they are insufficient to pose a genotoxic hazard in the mixture. Recent regulatory decisions have highlighted these challenges, particularly when constituent-level genotoxicity hazards are identified despite negative whole-mixture *in vivo* genotoxicity data. Emerging literature and scientific discussion support use of *in vivo* genotoxicity data to derive quantitative points of departure for considerations for risk characterization. In some cases, this approach may be more conservative than those based on traditional 2-year carcinogenicity bioassays. Two recent expert workshops (i.e., BfR and EFSA) emphasized the need for additional case studies to refine and validate this evolving paradigm. This poster synthesizes the current state of the science, includes key takeaways from stakeholder perspectives from these workshops, and integrates these insights into a transparent and structured approach for evaluating the genotoxicity of mixtures containing genotoxicants yet producing negative *in vivo* whole mixture results. Opportunities to consolidate and update guidance, reduce interpretative ambiguity, and advance 3R objectives by potentially decreasing reliance on 2-year carcinogenicity bioassays when robust *in vivo* genotoxicity data are available, are discussed. Collectively, the proposed approach provides a pragmatic and health-protective method to reconcile constituent-based hazards with whole mixture evidence when available.

PA-4: The new ICH E11A guideline – advantage for the development of natural health products for children?

Kelber O [1], Abramov-Sommariva D [2], Steinhoff B [3], Köhler S [4], Kraft K [5]

[1] Phyto & Biotics Tech Platform, Phytomedicines Supply and Development Center, Bayer Consumer Health, Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany; [2] Bionorica SE, Neumarkt, Germany; [3] GPT e.V., Bonn, Germany; [4] Dr. Willmar Schwabe GmbH & Co. KG, Karlsruhe, Germany; [5] Center for Internal Medicine of University Medicine Rostock, Rostock, Germany

There is still a shortage of medicines for children, even though the present regulation was supposed to facilitate their development. However, the approval of herbal products for children has rather become more tempting. Today, it has become clear that the aim must be to avoid large, placebo-controlled clinical trials in children wherever possible. With the guideline for pediatric extrapolation (E11A) published in 2024, there is now a binding guideline for the extrapolation from adult to pediatric patients. The new guideline was systematically analyzed regarding the extent to which it can facilitate the approval of herbal products for children.

Three questions are addressed in the guideline: How similar are (1) the disease, (2) the pharmacology of the drug, and (3) the response to therapy in adults and pediatric patients? The pediatric extrapolation is considered as a continuum: Accordingly, if there are major differences between adults and children, more data are required. A pediatric extrapolation plan is then derived, which lists any necessary studies. Here particular emphasis lays on the use of pharmacokinetic data, but also real-world data (RWD) are in scope. While for most HMPs, but also for locally acting preparations and vaccines, no pharmacokinetic data can be collected, refering to the similarities between adults and children can be a way forward. For this, also RWD can be a support. This underlines the importance of initiatives to give more weight to RWD in pediatric extrapolation. Overall, the new guideline may give cause for optimism.

PA-5: Identity Review of New Dietary Ingredients Derived from Algae

Li X, Yakes BJ

Algae can grow in extreme conditions, both in fresh and salt waters, which creates unique morphology, specific expression of proteins, specialized enzymes, and antioxidant pigments. As such, algae have been widely recognized for their nutritional properties, especially in eastern countries. As a nutrient-dense source of vitamins, minerals, proteins, polyunsaturated fatty acids, and antioxidants, algae dietary supplements, as well as isolated ingredients produced by algae, have been expanding within the United States due to increasing consumer demand. Due to this, new dietary ingredient notifications (NDINs) of these products have been continuously submitted to FDA for review, and the subject ingredients include diverse identity properties, such as various source species, components and compositions, and production methods.

NDIN identity review requires evaluation of the ingredient itself as well as the controls in place to ensure a reasonable expectation of safety. This assessment includes evaluating the accuracy of species determination (e.g., visual identification plus a confirmatory method); appropriateness of the Latin binomial name assignment; manufacturing protocol including in-process controls; specifications; rigor of laboratory testing for marker compounds, toxic elements, toxin and/or microbial contamination, constituents (both desired and ancillary), and nutrient determination (e.g., omega-3, protein, pigment, enzyme activity); as well as processing by-products detection (e.g., residual solvents). This poster will highlight the scientific review process used to support identity determination of algal dietary supplements and algal-produced ingredients with the goal of sharing the best practices that can be used to support algae identification in notifications.

Chemistry, Biology, and Safety of Volatile Organics from Aromatic and Medicinal Plants

PA-6: Authentication and Quality Assessment of Frankincense Essential Oils from Five *Boswellia* Species Using GC/QToF and Chemometric Analysis

Lee J [1], Wang M [2], Zhao J [1], and Khan IA [1,3]

[1] National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677. [2] Natural Products Utilization Research Unit, Agricultural Research Service, United States Department of Agriculture, University, MS 38677. [3] Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA

Frankincense essential oil, derived from various *Boswellia* species, is valued for its therapeutic and aromatic properties, yet species authentication remains challenging due to market adulteration. Given the commercial importance and varying market values of different *Boswellia* species, reliable analytical methods for species differentiation are essential to prevent mislabeling and ensure product authenticity. A GC/QToF method was developed to analyze and assess the quality of frankincense (*Boswellia* spp.) essential oil (EO). Authentic samples from five *Boswellia* species were analyzed (*serrata*, *sacra*, *frereana*, *papyrifer*, and *carterii*). By comparing the total ion chromatogram of each species, a distinct pattern for each species can be observed. For example, *B. papyrifer* samples contain large amounts of caprylyl acetate (23.02-29.79%), which is largely absent in samples from other species. *B. serrata* samples contained large amounts of α-thujene (35.53-41.88%), while *B. sacra* contained the greatest amount of alpha-pinene (33.86-38.26%). *B. frereana* contains a greater amount of *p*-cymene (18.25-20.50%) in comparison to other species. When compared to other species, *B. carterii* contained a greater amount of beta-caryophyllene (1.88-3.79%). In addition to qualitative analysis, the data was subjected to chemometric analysis. A principal component analysis (PCA) was conducted to investigate the variation observed between species. The PCA successfully separated samples based upon their respective species group. The greatest variation between species was explained by PC1 (44.83%) with good separation being observed in the PCA plot. With data obtained from the PCA, a sample class prediction model was constructed based on a partial least square-discriminant model. Cross-validation of the model indicated a prediction accuracy greater than 99%. Overall, this method can be used to distinguish the species from which the essential oil was obtained and assess its quality.

PA-7: Differentiation and Quality Assessment of White and Pink Grapefruit Essential Oils Using GC/MS and Chemometric Analysis

Lee J [1], Wang M [2], Zhao J [1], and Khan IA [1,3]

[1] National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677. [2] Natural Products Utilization Research Unit, Agricultural Research Service, United States Department of Agriculture, University, MS 38677. [3] Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA

Grapefruit (*Citrus* × *paradisi*) essential oil is available commercially in both white and pink varieties. To enable quality assessment and variety authentication, a GC/MS method was developed to assess the quality of white and pink grapefruit (*Citrus* × *paradisi*) essential oil (EO). Authenticated white and pink EO samples were analyzed to establish quality standards. Qualitatively, both white and pink EOs were similar. For example, the limonene content of white (72.92-76.21%) and pink (68.72-76.18%) EOs were very similar. The content of other major components were also similar, for example, *α*-pinene, sabinene, *β*-myrcene, and *β*-caryophyllene. Interestingly, on average, white EO contained more nootkatone than pink EO samples (avg. 1.08 vs. 0.41%). In contrast, pink EO samples tended to contain more alpha-terpineol than white samples (avg. 0.79 vs. 0.22%). Analyzing the data using PCA allowed for the separation of white and pink EO samples into their respective groups. The greatest variation between white and pink EO groups was explained by PC1 (46.16%) indicating good separation. The PCA plot shows two distinct groups; however, individual samples within each group are not tightly clustered, indicating variability among samples within each group. This variation was also observed in the qualitative analysis of the samples. The data obtained from the PCA was used to construct a sample class prediction model based on a partial least square-discriminant model. When cross-validation of the prediction model was performed, the model successfully predicted the sample classification with over 99% accuracy. By combining the qualitative method to assess the EO's quality with the chemometric method to predict the sample classification, a comprehensive analysis of unknown "grapefruit EO" products can be performed.

PA-8: Development of an SPME-GC/QToF Method for Quantifying Cinnamaldehyde in Human Serum Following Oral Cinnamon Oil Capsule Administration

Lee J [1], Wang M [2], Zhao J [1], Khan IA [1,3]

[1] National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677. [2] Natural Products Utilization Research Unit, Agricultural Research Service, United States Department of Agriculture, University, MS 38677. [3] Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA

Solid phase microextraction (SPME) is a rapid, sensitive, solvent-free, and economical method of extracting analytes from a variety of matrices by partitioning them from a liquid or gaseous sample into an immobilized stationary phase. SPME coupled with gas chromatography–quadrupole time-of-flight mass spectrometry (GC-QToF) was evaluated for its practicality in quantifying cinnamaldehyde in human serum following oral administration of capsules containing cinnamon essential oil. The method required minimal sample preparation, reducing the potential for human error. SPME parameters, such as extraction time and temperature, were optimized to ensure extraction efficiency. A robust calibration curve (10–1500 ng, $R^2 = 0.995$) demonstrated excellent accuracy and reproducibility. Overall, the approach provided high sensitivity and avoided matrix interferences, making it suitable for trace-level analysis. Although cinnamaldehyde was not detected in any post-dose samples for up to 24 hours (suggesting rapid metabolism or poor absorption from the dosage form), the workflow proved efficient, cost-effective, and adaptable for pharmacokinetic studies of volatile and semi-volatile compounds.

PA-9: NMR-Based Metabolomic Approach for Quality Assessment of Grapefruit Essential Oils

Zhao J [1], Wang M [2], Lee J [1], Khan IA [1]

[1] National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677; [2] Natural Products Utilization Research Unit, ARS. Department of Agriculture, University, Mississippi 38677, USA

Grapefruit (*Citrus paradisi* Macfad.) essential oil (GEO) is extensively used in food, cosmetic, and pharmaceutical products because of its characteristic aroma and reported bioactivities. Its chemical composition is dominated by monoterpenes, particularly limonene, together with numerous minor oxygenated compounds that contribute to sensory quality and functionality. The composition of GEO is influenced by factors such as cultivar, geographical origin, extraction method, and storage conditions. Owing to its relatively high market value, GEO is prone to adulteration, which compromise product authenticity and quality, underscoring the need for reliable analytical methods for differentiation and adulteration detection. In this study, a ^{1}H NMR–based fingerprinting and profiling strategy combined with chemometric analysis was developed for the quality assessment and adulteration detection of GEOs. A total of 38 GEO samples were investigated. Different from conventional gas chromatography–based techniques that mainly target volatile components and may be limited in detecting subtle compositional differences or complex adulteration strategies, the NMR method provided a complementary, non-destructive, and inherently quantitative approach that enables holistic characterization of complex mixtures. When coupled with multivariate statistical analysis, NMR fingerprinting allowed effective sample discrimination, while NMR profiling facilitated identification of metabolites contributing to observed differences. Besides limonene, the characteristic signals and chemical profiles attributed to furanocoumarins and aldehydes were assigned using 2D NMR analysis. In addition, 26 commercial GEO products collected from the market were evaluated for potential adulteration; among them, 10 exhibited distinct spectral fingerprints indicative of adulteration. The study demonstrated the effectiveness of NMR-based metabolomic approaches for authentication and quality control of essential oils.

Trade and Taxonomical Aspects of Botanicals

PA-10: Conservation and Protection Status of United States Pharmacopeial Convention's Compendial Articles of Botanical Origin

Brinckmann JA, Schippmann U, Marles RJ, Sarma ND, Monagas MJ, Clapper G, Brendler T

Compendial species play a vital role in the production of drugs, food ingredients, pharmaceutical excipients, dietary supplements, and herbal medicines that ensure human well-being and support public health systems. According to the 2019 World Health Organization (WHO) Global Report on Traditional and Complementary Medicine, 34 Member States reported that herbal medicines were included in their National Essential Medicine Lists (WHO 2019). Since 1995, environmental concerns have been incorporated into the USP Convention Resolutions. For its 2025–2030 revision cycle, USP has proposed "Resolution V: Help Foster More Environmental Sustainability Across the Pharmaceutical Life Cycle", including, among its Environmental Social Responsibilities, resource stewardship to reduce energy, emissions, and waste; appropriate water management; and biodiversity conservation.

Monographs for botanically derived articles appear in the United States Pharmacopeia (USP) and the National Formulary (NF)—published together as USP–NF—as well as in the Dietary Supplements Compendium (DSC), the Food Chemicals Codex (FCC), and the Herbal Medicines Compendium (HMC). Each compendium applies its own admission criteria, which consider factors such as intended use, regulatory status, and safety profile. Currently, none of the official or legally recognized USP compendia provide information on the conservation or protection status of botanical species. This study identified 355 compendial higher plant species. The inclusion of these species in any of the USP compendia signals the botanical's significance for human health and its role in commercial trade and use both in the United States and globally. This research systematically documents the botanical species listed in these five USP compendia and reports their conservation or protection status based on sources such as: the Appendices of the Convention on International Trade in

Endangered Species (CITES), the International Union for Conservation of Nature (IUCN) Red List of Threatened Species, NatureServe Global and National Ranks, and national Red List assessments from other countries.

As a result, USP proposes steps to inform stakeholders about the conservation status of compendial species and to collaborate with federal and global multilateral organizations to find healthcare solutions.

PA-11: Chemical Fingerprints and Bioactivities of *Piper* spp. Essential Oils

Monteiro LM[1], Santos VLP [2], Tolouei SEL[3], Khan SI [4], Wang M [5], Lee J [5], Farago PV [1], Perera WH [6], Shah FM [5], Ali A [5], Minteguiaga, M , Khan IA [4], Gasparotto Júnior A [3], Manfron J [1]*

[1] Postgraduate Program in Pharmaceutical Sciences, State University of Ponta Grossa, Ponta Grossa, Paraná, Brazil, [2] Uninter University Center, Curitiba, Paraná, Brazil, [3] Pharmacology Department, Federal University of Parana, Curitiba, Parana, Brazil, [4] National Center for Natural Products Research, University of Mississippi, University, Mississippi, USA, [5] Natural Products Utilization Research Unit, Agricultural Research Service, United States Department of Agriculture, University, Mississippi, USA, [6] CAMAG Scientific, Inc, Wilmington, North Carolina, USA, [7] Universidad de la República - UdelaR, Centro Universitario Regional Noreste, Espacio de Ciencia y Tecnología Química - ECTQ, Tacuarembó, Uruguay

Species of *Piper* genus are rich in essential oils with aromatic and medicinal properties and are traditionally used to treat inflammation, pain, digestive and respiratory disorders, and to promote wound healing. This study examined the chemical composition and biological potential of *Piper amalago*, *P. caldense*, *P. grazielae*, and *P. solmsianum*. GC-MS analyses revealed a predominance of monoterpenes and sesquiterpenes, mainly bicyclogermacrene, *β*-caryophyllene, *β*-pinene, and *α*-pinene, while HPTLC confirmed distinct chemical profiles for each species. Biologically, *P. grazielae* exhibited the strongest antiradical activity (DPPH assay), and the remaining species also demonstrated notable bioactive potential. Overall, the samples showed promising activity in assays related to fire ant (*Solenopsis* spp.) control under the tested conditions, while showing low cytotoxicity against melanoma (SK-MEL), ovarian (SK-OV-3), breast (BT-549), and KB cell lines. Safety was additionally assessed in non-tumorigenic renal cell lines (LLC-PK1 and VERO). These findings underscore the value of *Piper* species as sources of bioactive natural compounds with potential pharmacological applications and possible use in pest management, and highlight the need for further chemical, biological, and toxicological studies to ensure their safe and effective use.

PA-12: Proposal to Conserve the Name *Cinnamomum cassia* Nees ex Blume against *C. cassia* (L.) J.Presl/D.Don (Lauraceae)

Külkamp J [1], Chittiboyina AG [1,2], Osman AG [1], Khan IA [1,2]

[1] National Center for Natural Products Research, [2] Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, Mississippi 38677, USA

Cinnamomum cassia Nees ex Blume (1826) is the scientific name for Chinese cassia, the second most traded cinnamon species worldwide, essential to global commerce, traditional medicine, and food industries. Despite its ubiquity, the name is a later homonym and technically illegitimate under the International Code of Nomenclature (ICN). This proposal seeks to conserve C. cassia Nees ex Blume against its earlier homonyms to ensure nomenclatural stability and public safety. The conflict arises from *Laurus cassia* L. (1753), which was based on mixed material and later combined into *Cinnamomum* by Presl and Don in 1825. However, the Linnaean basionym actually refers to what is now known as *Neolitsea cassia* (L.) Kosterm. When Blume published *C. cassia* in 1826, describing the true Chinese cassia, his name became a later heterotypic homonym of the Presl/Don combinations. Under strict priority, the species would have to be called *Cinnamomum aromaticum* Nees (1831), a name far less recognized. The adoption of *C. aromaticum* would cause significant disruption. A survey of literature shows *C. cassia* appearing in 1,080 titles over the last 50 years, compared to only 24 for *C. aromaticum*. Furthermore, *C. cassia* is critically linked to public health information regarding its high coumarin content. A name change would confuse consumers and health professionals, potentially leading to dangerous herb-drug interactions

for patients on anticoagulants. This action preserves a culturally and economically vital name, preventing taxonomic chaos and ensuring the safety of the average consumer by maintaining the established link between the name and its chemical profile. We here designate Blume's collection as the lectotype, selecting it from original syntypes to anchor the name to the correct taxonomic concept. We propose the conservation of *C. cassia* Nees ex Blume under Art. 14 of the ICN to ensure global nomenclatural stability and protect centuries of traditional and commercial knowledge.

PA-13: Diagnostic Morpho-anatomical and Histochemical Traits for Species Delimitation in Native Brazilian *Eugenia*

*Armstrong L[1], da Silveira I [2], Monchak IT [1], Iarmul J [3], Chaves DSA [4], Manfron J [1]**

[1] Postgraduate Program in Pharmaceutical Sciences, State University of Ponta Grossa, Ponta Grossa, PR, Brazil, [2] Postgraduate Program in Health Sciences, State University of Ponta Grossa, Ponta Grossa, PR, Brazil, [3] Postgraduate Program in Botany, Curitiba, PR, Brazil, [4]Department of Pharmaceutical Science, Federal Rural University of Rio de Janeiro, Rio de Janeiro, Brazil

Eugenia (Myrtaceae) is a species-rich genus in Brazil with recognized medicinal, nutritional, and economic value. This study comparatively characterized eight native species (*E. brasiliensis, E. involucrata, E. longipedunculata, E. myrcianthes, E. neoverrucosa, E. punicifolia, E. pyriformis, and E. uniflora*) by integrating morpho-anatomical, micromorphological, and histochemical markers. Morphological and anatomical analyses revealed diagnostic differences in leaf blade dimensions, petiole architecture, margin traits, epidermal cell patterns, and cuticular ornamentation. Non-glandular trichomes were observed in *E. myrcianthes*, *E. punicifolia*, and *E. pyriformis*, and sand crystals were exclusive to *E. myrcianthes*. All species had a uniseriate epidermis, whereas only *E. myrcianthes* presented a hypodermis. Secretory cavities varied in distribution among organs, and stem anatomy showed brachysclereids in most species, with fibers in *E. involucrata* and *E. myrcianthes*. Histochemical assays detected lipids, lignin, phenolics, and starch in all species, while alkaloids were restricted to *E. longipedunculata* and *E. neoverrucosa*. Multiple calcium oxalate crystal types were recorded, including platy aggregate crystals described here for E. pyriformis. These integrated structural and histochemical markers provide robust diagnostic characters for species delimitation and support future chemotaxonomic and quality control studies involving Eugenia raw materials.

PA-14: Establishing a Fit-for-Purpose DNA Identity Test for Reishi

Leafworks

Accurate botanical identity testing is foundational to quality, safety, and regulatory compliance in the natural products industry. For medicinal fungi like reishi, identity determination is complicated by extensive taxonomic revision, inconsistent nomenclature, and variable morphology, contributing to ambiguity around what constitutes "reishi" in commercial and regulatory contexts. This presentation describes the development of a DNA-based identity test for reishi by LeafWorks, with an emphasis on defining the target identity that reflects both scientific and commercial realities. Although reishi historically has been labeled *Ganoderma lucidum*, modern taxonomy recognizes multiple distinct, medicinal species that have been marketed under this name. Consequently, identity testing methods limited to *G. lucidum sensu stricto* may fail to capture the diversity of materials present in the marketplace.

To address this challenge, LeafWorks developed a test framework that explicitly incorporates genetic, taxonomic, regulatory, and commercial perspectives. We define reishi as an inclusive group of medicinally used *Ganoderma* species recognized across authoritative sources, including *G. lucidum*, *G. lingzhi*, and *G. sinense*, as listed in Herbs of Commerce version 3 and the USP monograph, together with their accepted taxonomic synonyms. This operational definition, referred to here as *Ganoderma lucidum sensu lato*, does not seek to resolve taxonomic debates, but rather to transparently reflect how reishi is defined, sourced, and regulated in commerce. The presentation outlines how authenticated botanical reference materials, curated taxonomy, and representative genetic sampling were used to design a DNA test. This work illustrates the importance of transparent identity definitions and well-documented reference materials in the

development of fit-for-purpose DNA methods and offers a model for addressing similar challenges in other complex botanical and fungal ingredients.

PA-15: Propagation of Hemp for Important Minor Cannabinoids - THCV, CBDV and CBG

Lata H [1,2], Chandra S [1,2], Majumdar CG [1], Radwan MM [1,2], Khan IA [1,2], ElSohly MA [1,2,3]

[1] National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA; [2] Department of Biomolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA; [3] Department of Pharmaceutics and Drug Delivery, School of Pharmacy, University of Mississippi, University, MS 38677, USA

The growing scientific and commercial interest in hemp-derived cannabinoids has expanded beyond the major compounds Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) to include several pharmacologically significant minor cannabinoids such as tetrahydrocannabivarin (THCV), cannabidivarin (CBDV), and cannabigerol (CBG). These compounds are attracting considerable attention due to their potential therapeutic roles in metabolic disorders, neurological diseases, epilepsy, and inflammatory conditions. However, their natural abundance in most hemp cultivars is typically low, posing challenges for consistent large-scale production. Consequently, the development of efficient propagation strategies is essential to generate plant material with stable chemotypes enriched in these minor cannabinoids while maintaining agronomic performance and regulatory compliance for hemp cultivation. In this study, based on GC-FID analysis, elite high-yielding female plants were selected from seed-raised plants of different chemovars, namely THCV, CBDV, and CBG. These selected plants served as mother plants for further vegetative propagation and micropropagation. Plants grown through vegetative propagation and micropropagation were compared with their respective mother plants for cannabinoid profile and content using GC-FID analysis at maturity. Our results show that plants produced through vegetative propagation and micropropagation were highly comparable in cannabinoid profile and content to their respective mother plants. The study demonstrates that the biotechnological methods followed can be used for the mass propagation of elite chemotypes with desirable cannabinoid profiles for the production of biomass with consistent cannabinoid composition. This study plays an important role in ensuring reliable production of minor cannabinoids to support pharmaceutical research, nutraceutical development, and the expanding hemp-based bioeconomy.

PA-16: Indoor Propagation of Medicinal Mushrooms for the Production of Psilocybin - a Controlled Drug Substance

Lata H [1,2], Chandra S [1,2] Khan IA [1,2]

[1] National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA; [2] Department of Biomolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA

Psilocybin, a naturally occurring indole alkaloid produced by several medicinal mushroom species, has gained increasing scientific interest due to its emerging therapeutic potential in the treatment of neuropsychiatric disorders. However, psilocybin is classified as a controlled drug substance in many countries. In the United States, it is designated as a Schedule I controlled substance by the Drug Enforcement Administration (DEA), and therefore its production must follow standardized and regulated procedures. This study explores propagation strategies for psilocybin-producing medicinal mushrooms to achieve controlled and reproducible biomass production for research purposes.

Different propagation techniques, including spore germination, mycelial culture, and substrate-based cultivation, were evaluated to optimize growth conditions and ensure consistent fungal development. Three strains of *Psilocybe cubensis* named B+, Hillbilly, and Golden Halo, were selected for the study. Particular attention was given to cultivation parameters that support healthy growth and stable metabolite production. The propagation process involved several stages, including selection of commercial spores, spawn preparation, substrate preparation, optimization of fruiting conditions, and harvesting. Biomass produced under optimized conditions will be utilized for natural products research at NCNPR. All cultivation activities are conducted in compliance with DEA regulations (License #18185/8.2), supporting future research on standardized fungal-derived therapeutics under an appropriate regulatory framework.

PA-17: Gamma Tubulin Gene Region as Molecular Marker for the Identification of Various Species of the Family Rosaceae

Techen N [1], Parveen I [1], Chittiboyina AG [1], Khan IA [1,2]

[1] National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences; [2] Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, MS 38677, USA

Identification of plant DNA that is present in processed plant material provides a challenge as the DNA is often fragmented into pieces of <300 bp. Often the PCR amplification of accepted barcode regions (ITS) from fragmented DNA is without success due to the need of DNA >300 bp as template. The identification of small informative genomic regions, called DNA mini-barcodes helpful for species identification, are therefore desired.

The nuclear low copy Gamma Tubulin gene consists of 9+ exons and introns of various sizes. It was tested for their species discrimination ability to distinguish between various species of genera of the family Rosaceae. Species discriminatory power of various areas of the gamma tubulin gene were tested from the genera *Malus* (apple), *Prunus* (cherry, peach, almond, plum), *Fragaria* (strawberry), *Rosa*, and *Rubus* (raspberry, blackberry). Several locations were identified that were effective DNA mini-barcodes helpful for identification of these species. These DNA mini-barcodes have been applied to processed plant material found in juice, fruit preserve, and yogurt with fruit with varying success.

PA-18: Unravelling *N*-Acetylcysteine's Natural Origins (NAC)-A Critical Examination of Prior Scientific Literature

Osman AG [1], Avula B [1], Khan IA [1,2], Chittiboyina AG [,1,2]*

[1] National Center for Natural Products Research, University of Mississippi, University, Mississippi 38677, United States; [2] Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, Mississippi 38677, USA

N-acetyl-L-cysteine (NAC) is a constituent of many dietary supplements, advertised as an immune support, liver health support, antioxidant, and as an enhancer for glutathione levels. It is a highly researched substance, extensively cited in research articles, reviews, books, patents, and clinical trials. It was originally discovered as a synthetic compound that was proven to be a clinically effective mucolytic agent for muco-pulmonary disorders in 1963. Later it was recognized as an antidote for acetaminophen (Tylenol®) overdose and related hepatotoxicity. NAC has been widely and erroneously reported in the scientific literature as a constituent of *Allium* species, primarily garlic (*Allium sativum*) and onion (*Allium cepa*), and hence frequently cited as "dietary *N*-acetylcysteine". Thus, the controversial status of *N*-acetylcysteine (NAC) as a currently advertised dietary supplements has renewed interest in its origin. In this literature study, a large quantity of research articles, reviews, patents, and book chapters were thoroughly examined to investigate whether NAC is a naturally occurring compound, with a particular focus on its potential presence in plants. Thus, the primary objective of this opinion was to verify the origin of NAC. The methodologies implemented in establishing NAC's putative presence in plant or herbal sources, with a specific focus on *Allium* species, often touted to contain NAC were rigorously evaluated. By analyzing the strengths and weaknesses of prior research, we were able to clarify the evidence supporting the origin of NAC as a synthetic compound, with no existence in plants.

PA-19: Natural Product-Based Colorants as Substitutes for Petroleum-Based Synthetic Food Dyes: Sources, Chemistry, and Characteristics

Neal WM [1], Osman AG [1], Khan IA [1,2], Chittiboyina AG [1,2]

[1]National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, Mississippi, 38677, United States; [2] Department of Bio-Molecular Sciences, School of Pharmacy, The University of Mississippi, Mississippi, 38677, USA

Worldwide, consumers are becoming increasingly conscious of the ingredients used in food products. Rising safety concerns, especially regarding the behavioral effects and carcinogenicity of petroleum-based synthetic food dyes, have driven recent regulatory actions mandating their withdrawal. However, these food colors cannot simply be discontinued, as they serve more functions beyond mere aesthetics. With a burgeoning global demand for new substitutes becoming apparent, naturally derived ingredients are poised to fill the void. A review of scientific literature explored the current climate surrounding food colors by critically analyzing petroleum-based synthetic food dyes alongside their associated safety concerns and by summarizing the applicability of several natural food colors as substitutes. These natural colors include betalains, anthocyanins, carotenoids, iridoids (as precursors to gardenia (genipin) blue color), phycocyanins, curcuminoids, and chlorophylls. Chemical and biological properties, natural sources, and inherent challenges (including stability or safety concerns) are discussed for each natural pigment, offering compelling opportunities for seamless integration within the food industry.

Quality Aspects of Botanicals

PA-20: Chromatographic and Phytochemical Differentiation of Ginseng Types Through Analytical Testing

Tran K, Fast P

Eurofins Botanical Testing Laboratory, CA 92821

This study compares the phytochemical and chromatographical differences of white, red, and black ginseng through qualitative identification testing and quantitative assay analysis. Results highlight distinct ginsenoside transformations and bioactive compound variations driven by processing methods, underscoring their implications for pharmacological potency and quality control.

PA-21: Value Assignment of Triterpene Glycosides in Black Cohosh Reference Materials (RMs) with Mass Spectrometry Detection

Coskun SH [1,2], Rimmer CA [2], Hayes H[2], Kuszak AJ [3]

[1] Kelly Government Solutions Contractor in support of the Office of Dietary Supplements, National Institutes of Health, Bethesda, MD, USA, [2] National Institute of Standards and Technology, Gaithersburg, MD, USA, [3] Office of Dietary Supplements, National Institutes of Health, Bethesda, MD, USA.

Black cohosh (*Actaea racemosa* L. Nutt.) is a widely used herbal dietary supplement; however, quality concerns persist due to mislabeling and adulteration with other *Actaea* species. To support reliable characterization and authentication, this study reports the assignment of values for key triterpene glycosides in candidate black cohosh Reference Materials (RMs) [RM 8656 *Actaea racemosa* (Black Cohosh) Rhizomes, RM 8657 *Actaea racemosa* (Black Cohosh) Leaves, RM 8658 Black Cohosh Rhizome Extract, and RM 8659 Black Cohosh Solid Oral Dosage Form (SODF)]. These matrices were selected to represent powdered botanical materials, non-target plant parts that may be associated with misidentification or adulteration, processed extracts, and finished commercial dosage forms commonly encountered in the dietary supplement supply chain.

A previously developed liquid chromatography–mass spectrometry (LC-MS) method was optimized through extraction and matrix-effect evaluations and applied to four candidate materials: black cohosh rhizomes, leaves, rhizome extract, and a solid oral dosage form. Seven triterpene glycosides (27-deoxyactein, cimigenol 3-*β*-D-xyloside, cimiracemosides C and D, 23-epi-26-deoxycimicifugoside, and cimicifugosides H-1 and H-2) were successfully separated and quantified. The method also enabled the detection of cimifugin, a marker for adulteration with Asian Actaea species. Quantitation was achieved using ginsenoside Rh2 as a chemically similar internal standard. Although minor integration challenges were observed for select analytes in specific matrices, overall analytical performance demonstrated reproducible separation and measurement. These results provide values for triterpene glycosides in black cohosh RMs and strengthen analytical tools for dietary supplement quality assessment and adulteration detection.

PA-22: Development of a Novel Non-Targeted Approach for Authentication of American Ginseng Using NMR and LC-QTOF-MS

Coskun SH [1,2], Place BJ [2], Berkkan A [3], Sheen DA [2], Kuszak AJ [4], Rimmer CA [2], Hayes H [2], Urbas AA [2]

[1] Kelly Government Solutions Contractor in support of the Office of Dietary Supplements, National Institutes of Health, Bethesda, MD, USA, [2] National Institute of Standards and Technology, Gaithersburg, MD, USA, [3] Gazi University Faculty of Pharmacy, Ankara, Türkiye, [4] Office of Dietary Supplements, National Institutes of Health, Bethesda, MD, USA

The National Institute of Standards and Technology (NIST) and the National Institutes of Health, Office of Dietary Supplements (NIH ODS), collaborate to advance tools for analytical characterization of dietary supplements. Historically, most efforts have targeted specific analytes in defined matrices, whereas non-targeted analysis (NTA) may offer a more comprehensive approach for material authentication and detection of adulteration. Adulteration of ginseng products remains a persistent concern that varies by species, product form, and geographic origin, underscoring the need for improved quality-control approaches.

In this pilot study, the chemical compositions of authenticated ginseng materials were evaluated using nuclear magnetic resonance (NMR) spectroscopy and liquid chromatography–quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) coupled with chemometric analysis. *Panax quinquefolius* (American ginseng) was selected as the primary target species, with more than 50 root samples used to develop and validate a statistical classification model. *Panax ginseng* and *Panax notoginseng* were included as closely related test species. Multivariate analysis of NMR and LC-MS data successfully differentiated American ginseng from the test groups. Next steps include challenging the model with closely related samples and materials spiked with known adulterants to evaluate robustness in NTA. In parallel, an interlaboratory study has been designed under the Dietary Supplement Quality Assurance Program (DSQAP) to assess the utility and feasibility of candidate reference materials for the authentication of American ginseng using non-targeted methods. Participating laboratories will apply their own analytical and chemometric approaches to a common set of curated materials, enabling evaluation of both material fitness-for-purpose and variability in laboratory-specific NTA workflows.

PA-23: Determination of Kratom Alkaloids in Botanical Products Using Multi-Criteria LC–MS Analysis

Sheehan D [1], Meckler E [1], Li Y [1], Upton R [2], Zhang M [1]

[1] Department of Chemistry and Biochemistry, Ohio University, Athens, OH, 45701; [2] American Herbal Pharmacopoeia, Scotts Valley, CA 95066

Kratom (*Mitragyna speciosa*) is a botanical product widely sold in the United States as powders, capsules, tablets, gummies, and beverages. Natural kratom leaves primarily contain mitragynine (MG) as the dominant alkaloid. However, an increasing number of commercial products contain elevated levels of the more potent opioid alkaloid 7-hydroxymitragynine (7-OH) and the semi-synthetic compound mitragynine pseudoindoxyl (MGP). Reliable analytical identification of these compounds is critical for dietary supplement testing, regulatory monitoring, and public health

protection. However, kratom matrices contain numerous structurally related alkaloids and isomeric compounds that complicate mass spectrometric identification and may lead to false positive assignments.

The objective of this research is to develop a robust LC-HRMS workflow capable of confidently distinguishing naturally occurring kratom alkaloids from semi-synthetic or enriched derivatives in commercial products. A total of 38 commercial kratom products, representing multiple formulations, were analyzed and compared against a botanically authenticated kratom voucher specimen (AHP-Verified) using ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS). Chromatographic separation, accurate mass measurement, and targeted MS/MS fragmentation were integrated to differentiate MG, 7-OH, MGP, and related alkaloid isomers.
Results demonstrated that reliance solely on accurate mass detection or commonly used multiple reaction monitoring (MRM) transitions produced overlapping peaks on chromatograms, highlighting a significant risk of false identification of 7-OH. Incorporating retention-time matching and diagnostic fragment ions enabled confident differentiation of 7-OH and MGP from co-eluting alkaloids. Among the surveyed products, MG was detected in all samples, while 7-OH and MGP were primarily observed in certain tablet and capsule formulations. This work provides a rigorous analytical framework for reliable kratom alkaloid identification, supporting improved testing of botanical products and advancing analytical methods relevant to dietary supplement safety and regulatory science.

PA-24: Development of USP Monographs for Dandelion Root and *Taraxacum* Species Whole Plant

Ma C

Dandelion Root consists of the dried root of *Taraxacum officinale* F.H.Wigg. (Fam. Asteraceae) to be used in traditional medicines for its potential health benefits, including aiding digestion, acting as a natural diuretic, supporting liver health and helping lower blood and cholesterol levels. The whole plants of closely related species of *Taraxacum mongolicium* and *Taraxacum sinicum* are also called dandelion to be used as traditional herbal medicines in Asian countries. Typically, T. officinale root and leaf are harvested and used separately, while *T. mongolicium* and *T. sinicum* are harvested with root and leaf together as the whole plant to be used for herbal medicine.

Both dandelion root and *Taraxacum* species whole plant are popular dietary supplements. To help protect from adulteration and support high quality products, USP is developing monographs for Dandelion Root and *Taraxacum* species whole plant. The monographs contain identification methods utilizing UHPLC/HPLC, HPTLC and botanical characteristics, and UHPLC/HPLC assay method to quantify phenolic acids. *Taraxacum* species whole plant monograph also contains a GC identification method testing for triterpenes. The presence of flavonoids in the leaf can differentiate dandelion root from dandelion leaf and Taraxacum species whole plant.

PA-25: Validated HPTLC Method for the Authentication of Witch Hazel Distilled Fractions Combined with Conventional and Non-Conventional Methods for the Purification of Chemical Markers

Diaz AV, Perera W

Hamamelis virginiana L. (Hamamelidaceae), commonly known as Witch Hazel (WH), is a deciduous shrub or small tree native to the damp woods of the eastern zones of North America and Canada. The plant's barks and leaves are rich in polyphenols — notably hydrolyzable and condensed tannins (including the characteristic hamamelitannin and oligomeric proanthocyanidins)—which are widely regarded as the main bioactive constituents responsible for its astringent, anti-inflammatory and antioxidant properties. Despite the well-established cosmetic and pharmaceutical applications of Witch Hazel distillates, there is currently no HPTLC method previously published to authenticate the distilled fraction; in addition, few reports are available that describe the detailed chemical composition of steam-distilled extracts from Witch Hazel bark. In this study, an orthogonal HPTLC method was developed and validated to authenticate bark raw material and Witch Hazel steam-distilled extracts. Although some important chemical markers in barks were detected by HPTLC, they are not present in the distilled fraction; therefore, a semi-preparative HPLC method was also developed for purifying other

components. Due to the low yield of distilled extract fraction per gallon, bark material was subjected to solvent maceration using a series of solvents of increasing polarity as another strategy to purify chemical markers. Extracts and fractions coming from column chromatography were monitored by HPTLC.

PA-26: HPTLC Identity Verification of *Mitragyna speciosa* (Kratom) in Commercially Available Dosage Forms

Perera, WH [1,2], Upton R [3]

[1] CAMAG Scientific, Inc. 515 Cornelius Harnett Drive Wilmington, NC 28401. [2] HPTLC Association Dianastrasse 10 CH-4310 Rheinfelden. Switzerland. [3] American Herbal Pharmacopeia, PO Box 66809 Scotts Valley, CA 95067

Mitragyna speciosa Korth. (Kratom) is a tropical tree of the Rubiaceae family native to Southeast Asia and its leaves are commercially available in a wide range of dosage forms, including powder, capsules, tablets and extracts. Although U.S Food and Drug Administration has not recognized kratom as a safe or effective treatment for any condition yet, pain management is the most common use reported at higher doses while at low doses is its stimulant effect. The indole alkaloids, especially mitragynine and 7-hydroxymitragynine are the main active constituents. This study describes an analytical approach for the identity verification of kratom in commercially available dosage forms using HPTLC fingerprinting. The main method was based on the analysis of the indole alkaloids and representative kratom products were analyzed. Chemical profiles were evaluated for consistency with authenticated reference materials, with emphasis on mitragynine and 7-hydroxymitragynine. A multi-ingredient herb was also analyzed and the method performed well by identifying kratom and detecting kava root, the limit of detection in a spiking test of kava in kratom was 1%. A second method with a more polar developing solvent was used to analyze kratom extracts and detect other metabolites. All these results support the use of chromatographic fingerprinting as a robust tool for identity testing and contribute to the development of standardized analytical methods for kratom.

PA-27: Extent Similarity assessment for a Quantitative Fingerprinting Approach in the Quality Control of Natural Health Products

Wu X [1], Bauer A [1], Kelber O [1], Bauer R [2]

[1] Phyto and Biotics Tech Platform, Bayer Consumer Health, Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany. [2] Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens-University Graz, Graz, Austria

Analysis of natural health products presents unique challenges due to their complex nature and the often-unknown identity of their active constituents. Regulatory frameworks, such as the one provided by the European Medicines Agency (EMA/HMPC/541422/2017 Corr.), requires a comprehensive/holistic and robust analytical method. To go beyond the limited scope of traditional analytical markers, Fingerprinting techniques, such as Correlation Coefficient or Euclidean Distance have been applied to evaluate the quality of HMPs. One approach allowing sensitive and selective quantitative analysis of all constituents, Extent Similarity has emerged as a promising method for the quantitative evaluation of fingerprinting data, that offers a comprehensive view of natural health products quality, especially for natural health products categorized as “other extracts” as no active constituents are defined but only proxy analytical markers are monitored.

The aim of this study was to develop and validate a quantitative fingerprinting method via HPLC-DAD in combination with a standardized quantitative chemometric approach, specifically utilizing the Extent Similarity method in the quality control of natural health products containing licorice root fluid extract. The fingerprinting methods were applied to an exemplary herbal extract, with a focus on capturing the holistic quality of complex mixture. The method involves chromatographic techniques coupled with fingerprinting analysis to evaluate the quality of multi-component systems of natural health products.
The Extent Similarity method enables us to perform a full analysis of an extract like that of licorice root to detect changes within the product. The Fingerprinting results assessed by the Extent Similarity method proved to be suitable for the

quantitative quality control of natural health products. It demonstrated accuracy, sensitivity, and reliability in capturing the full spectrum of phytochemical constituents. This method has the potential to enhance regulatory assessments and ensure the consistent quality of natural health products.

PA-28: Enantiopurity and Quantification of Huperzine A in *Huperzia serrata* Dietary Supplements Using UPC²-MS Combined with LC-QToF-MS Untargeted Metabolomics

Katragunta K, Avula B, Tatapudi KK, Wang YH, Chittiboyina AG, Khan IA
[1]National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, Mississippi 38677, USA.
[2] Department of Biomolecular Sciences, School of Pharmacy, The University of Mississippi, University, Mississippi 38677, USA

Huperzia serrata, commonly known as toothed club moss, is used in dietary supplements for memory enhancement and cognitive support. It contains the bioactive alkaloid huperzine A, a potent, reversible acetylcholinesterase inhibitor. Huperzine A can increase central acetylcholine levels, a neurotransmitter critical for learning and memory. Few *H. serrata* dietary supplements showed considerable variability in labeled *vs*. measured (−)-huperzine A content, and few products contained a synthetic racemic form of (±)-huperzine A rather than the natural enantiomer isolated from the plant. This study aimed to develop and validate an ultra-performance convergence chromatography–mass spectrometry (UPC²-SQD-MS; i.e., supercritical fluid chromatography–mass spectrometry, SFC-MS) method for the accurate quantification of (−)-huperzine A and (±)-huperzine A in *H. serrata* raw materials and commercial supplements. Additionally, LC-QToF-MS-based untargeted metabolomics was applied to characterize the alkaloid profile of *H. serrata* and support authenticity assessment. Huperzine A was quantified in authenticated *H. serrata* plant materials and a panel of marketed supplements. Method validation included assessments of linearity, sensitivity, precision, accuracy, and recovery over a working range of 0.05–25 µg/mL. Untargeted LC-QToF-MS analysis, combined with accurate mass measurement and database-assisted annotation, enabled tentative identification of more than 70 secondary metabolites, including lycopodium alkaloids in substantial amounts, and multiple huperzine analogs, in H. serrata extracts. Comparison of plant metabolomic fingerprints with those of dietary supplements allowed differentiation between products containing authentic *H. serrata* with (−)-huperzine A or naturally derived (±)-huperzine A, and those likely formulated using synthetic racemic procedures. This approach revealed potential adulteration and underscores the need for robust chromatographic–mass spectrometric tools in the quality control of huperzine A–containing dietary supplements.

PA-29: Quantitative Analysis and Simultaneous Characterization of Triterpenoids and Phenolics in *Inonotus obliquus* (Chaga) using LC-UV-ELSD and LC-UV-QToF

Avula B, Katragunta K, Tatapudi KK, Khan IA
[1]National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, Mississippi 38677, USA.
[2] Department of Biomolecular Sciences, School of Pharmacy, The University of Mississippi, University, Mississippi 38677, USA

Inonotus obliquus is widely recognized as the Chaga mushroom. Chaga contains various bioactive compounds, including polysaccharides, triterpenoids, polyphenols, and melanin. To address the characterization and quantitative analysis of triterpenoids and phenolics in Chaga, a multi-analytical approach combining LC-UV-ELSD and LC-UV-QToF has been developed. These methods were designed to quantify 11 compounds, comprising seven triterpenoids and four fatty acids, using LC-UV-ELSD, and four phenolics using the LC-UV-QToF method. Calibration curves for these compounds demonstrated excellent linearity within the tested range. The methods exhibited high precision, with intra- and inter-day relative standard deviations below 3% and recoveries ranging from 91% to 104%. The validated methods were applied to analyze eleven sclerotia samples, one mycelium sample, three grain-based samples, and eighteen dietary supplements. Results revealed that eight of the eighteen supplements (44%) contained ground mycelium, which primarily shows the presence of fatty acids but lacks detectable levels of triterpenoid and phenolic markers characteristic of Chaga. Triterpenoids and hispidin, identified as key bioactive compounds, were detected in eight (44%) of the eighteen supplements; however, these products also showed the presence of fatty acids and/or betulin. Two (11%) of the 18 supplements showed the presence of phenolic compounds only; no triterpenoids were detected. Additionally, untargeted metabolomic screening using LC-UV-QToF tentatively identified 103 compounds from diverse chemical groups, including

nine reference compounds. These findings provide valuable insights for the quality assessment of dietary or food supplements marketed as containing Chaga.

PA-30: Single-Dose Human Pharmacokinetics of Cinnamic Acid from Cinnamon Capsules Using a Sensitive UHPLC–MS/MS Method

Zahiruddin S [1], Wang Y-H [1], Kothapalli HB [1], Chittiboyina AG [1,2], Stevens N [3], Tuilevuka C [3], Osguthorpe RJ [3], Khan IA [1,2]

[1] National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA. [2] Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA. [3]doTERRA International, Pleasant Grove, UT 84062, USA

Cinnamon is widely consumed for its potential health benefits, with cinnamaldehyde recognized as its primary bioactive constituent. In humans, cinnamaldehyde undergoes rapid oxidative metabolism to cinnamic acid, a key intermediate that is subsequently converted to benzoic acid and hippuric acid [1, 2]. Despite this, human pharmacokinetic data describing systemic exposure to cinnamic acid following cinnamon consumption remain limited. The present study investigated the single-dose pharmacokinetics of cinnamic acid in healthy human volunteers after oral administration of a cinnamon-containing capsule, using a newly validated UHPLC–MS/MS method.

Nine healthy adults received a single oral dose of a 200 mg cinnamon-containing capsule, with trans-cinnamaldehyde as the main constituent. Plasma concentrations of cinnamic acid were quantified using a sensitive and selective UHPLC–MS/MS assay with the limit of detection (LOD) of 2.5 ng/mL, the limit of quantification (LOQ) of 8.0 ng/mL, and excellent linearity over the calibration range (R^2 = 0.999). The analytical method demonstrated robust performance and was well-suited for pharmacokinetic evaluation. Following oral administration, cinnamic acid was rapidly detected in plasma, with a mean time to maximum concentration (Tmax) of 1.11 ± 0.69 h. The mean maximum plasma concentration (Cmax) was 644.30 ± 350.92 ng/mL. The apparent elimination half-life (t½) of cinnamic acid was 1.63 ± 0.62 h, and the systemic exposure (AUC0–∞) was 1305 ± 444.63 h.ng/mL. In conclusion, cinnamaldehyde is rapidly metabolized as cinnamic acid in humans after oral intake of a cinnamon-containing capsule, resulting in measurable systemic exposure. These results establish cinnamic acid as a key metabolic intermediate after dietary cinnamon intake and provide essential pharmacokinetic insight.

PA-31: Analysis of Thirteen Cannabinoids in Cosmetic Products by UHPLC–MS/MS and LC-UV- HRMS

Zahiruddin S[1], Wang Y-H[1], Avula B[1], Chittiboyina AG[1,2], Khan IA[1,2]

[1]National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, Mississippi 38677, USA. [2] Department of Biomolecular Sciences, School of Pharmacy, The University of Mississippi, University, Mississippi 38677, USA

There has been a rapid growth of cannabinoid-infused products, including cosmetics. However, quality and safety issues have raised significant concerns [1,2]. This study reported the development and validation of a sensitive and robust ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) method for the simultaneous quantification of a broad panel of cannabinoids in cosmetic formulations.

The method targets 13 cannabinoids: CBDA, CBGA, CBG, CBD, CBDV, CBN, CBC, THCAA, Δ9-THCV, Δ9-THC, Δ8-THC, 9S-HHC, and 9R-HHC. Sample preparation procedures were systematically optimized to ensure efficient extraction across diverse cosmetic matrices while minimizing matrix-related interferences. Chromatographic separation was achieved using a UPLC C18 CORTECS column (1.6 µm, 2.1 x 100 mm) with gradient elution, and analytes were detected using multiple reaction monitoring (MRM) under optimized electrospray ionization conditions. A complementary LC-UV-HRMS method was employed when analyte concentrations exceeded the upper calibration limits of the UHPLC–MS/MS method.

Method validation conducted in accordance with FDA guidelines demonstrated excellent analytical performance. All

analytes exhibit strong linearity ($R^2 > 0.99$), low limits of detection (0.005-0.025 µg-g-1), and low limits of quantification (0.01-0.04 µg-g-1). Accuracy was within acceptable limits, and intra- and inter-day precision values were below 4% relative standard deviation, confirming the method's robustness and reliability.

Application of the validated method to 88 commercially available cosmetic products revealed substantial variability in cannabinoid content. Overall, the validated method provides a reliable and practical analytical approach for assessing the safety of cannabinoid-containing cosmetic products.

PA-32: UHPLC/Q-ToF Analysis of Glucosinolates in Fresh *Brassica* Species and Dietary Supplements

Lee J [1], Wang M [2], Barickman C [2], Avula B [1], and Khan IA [1,3]

[1] National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677,USA. [2] Natural Products Utilization Research Unit, Agricultural Research Service, United States Department of Agriculture, University, MS 38677, USA. [3] Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677,USA

Glucosinolates, which are sulfur-containing metabolites in *Brassica* species, are associated with health-promoting effects, including antioxidant and anticancer activity. Despite extensive characterization in fresh plant material, limited data exists for *Brassica*-based dietary supplements. In addition, estimated daily dietary intake data for the United States does not exist. Current ISO guidelines for the analysis of glucosinolates recommend the use of HPLC/UV, which could lead to the possibility of compound misidentification. To address these issues, this study developed a UHPLC/Q-ToF method for the quantification of 15 glucosinolates (iberin, progoitrin, epi-progoitrin, sinigrin, raphanin, sinalbin, napin, sibarin, erucin, glucobrassicin, nasturtiin, 4-methoxyglucobrassicin, neoglucobrassin, 4-hydroxyglucobrassicin, and glucobarbarin). A total of 38 authentic samples consisting of *Brassica* species (6 kale, 9 broccoli, 10 cauliflower, 8 brussels sprouts, and 5 turnip) and 8 samples from other commonly used glucosinolate containing plants (2 horseradish, 3 wasabi, and 3 watercress) were analyzed. In addition to authentic samples, glucosinolates from 69 dietary supplements from these groups were also quantified. Glucosinolate content varied among authentic samples, for instance; sinigrin ranged from 35.573 to 76.294 mg/100 g in cauliflower. However, dietary supplements showed greater variability, with sinigrin concentration in cauliflower-based products ranging from 0.051 to 53.547 mg/100 g. These findings demonstrate the need for improved manufacturing practices and standardization of glucosinolate-containing dietary supplements.

PA-33: Reference Material Development for *Hericium erinaceus* and Other Mushrooms

Spelman K [1,2]

[1]Nammex (North American Medicinal Mushroom Extracts) Box 1780, Gibsons, British Columbia, Canada V0N 1V0. [2] Massachusetts College of Pharmacy and Health Sciences179 Longwood Avenue, Boston, MA 02115

The lack of species-specific analytical standards has historically limited reliable authentication of medicinal mushrooms in commerce. This poster describes the development of authenticated *Hericium erinaceus* (Lion's Mane) and other mushroom, and selected mycelia, reference materials by Nammex using a structured species identification protocol designed to support analytical method validation and standards development, including ongoing work with AOAC INTERNATIONAL.
Voucher specimens sourced from production partners undergo macroscopic and morphological authentication to confirm diagnostic characteristics of *H. erinaceus* and are retained as reference specimens. Species identity is further confirmed using next-generation DNA sequencing for voucher specimens and 1:1 extracts. Chemical identity is verified using High-Performance Thin-Layer Chromatography (HPTLC) and HPLC with chromatographic alignment to authenticated references, and consistent detection of marker compounds such as hericenone C, hericene A, and ergosterol.

Independent Nuclear Magnetic Resonance (NMR) spectroscopy conducted by a third-party laboratory provides ongoing

verification through comparative analysis against authenticated voucher materials. Together, these orthogonal methods underpin Nammex's authenticated *H. erinaceus* reference materials and contribute to its advisory role in AOAC's Botanical Identity and Dietary Supplements Integrity (BIDSI) program, including the development of a species-specific Standard Method Performance Requirement (SMPR) for *H. erinaceus* materials. Authenticated *H. erinaceus* and other fungal reference materials, including selected mycelia, are available from Nammex upon request.

PA-34: Multidisciplinary Approaches to Medicinal Plant Authentication and Quality Control

Zafar M

Department of Plant Sciences, Quaid-i-Azam University, Islamabad, 45320, Pakistan.

The key component of the process involved in developing botanical-based products to produce pharmaceuticals, fragrances and cosmetics in a sustainable manner is the proper authentication, standard growth, responsible harvesting and efficient post-harvest management of the plant resources. This approach was combined plant taxonomy, melissopalynology, ethnobotany, and curation of data stored in herbariums to highlight the major issues involved in producing high-quality and safe plant material to be used in industry and pre-clinical purposes. Taxonomic authentication underpinned by classical morphology, pollen micromorphology and reference herbarium specimens is the basis to eradicate the problem of misidentification and adulteration in species, which continue to be significant limitations to the botanical supply chain. Melissopalynological is another tool of quality assurance to track the sources of flora, confirm the geographical origin and the purity of the ecology of products of plant origin. Ethnobotanical records of medicinal plants, wild fruit and vegetables give important clues to botanical drug discovery, which connects traditional with modern pharmacological targets. The development of new techniques on cultivation, sustainable harvesting and post-harvest processing are cited in terms of maintaining bioactive compounds and reducing chemical destruction. The chemical profiling, toxicity screening and pre-clinical evaluation approaches are emphasized as the key tools of certifying botanical extracts safety, efficacy, and regulatory compliance. Also provided in the abstract are the applicability of the existing policies on fragrances and cosmetics with a statement of why harmonized standards based on scientific authentication and traceability are required. The value of digitized herbaria collections and managed biodiversity databases is emphasized as a strategic source of facilitating regulatory frameworks, conservation and botanical drug discovery innovation. This multidisciplinary approach, as a whole, shows how integrative plant sciences can be used to mediate between traditional knowledge and modern quality evaluation to discover new targets of safe, effective, and sustainable botanical products.

PA-35: From Traditional Use to Supplement Safety: History of Safe Use Assessment of *Jabuticaba* Fruit

Jureziz A [1], Cousineau S [1], McGuire C [1], Jewell P [1,2], Vincent-Hall T [1]

[1] NSF, Ann Arbor, MI; [2] University of Michigan School of Public Health Environmental Health Sciences Graduate Program, Ann Arbor, MI

Growing consumer demand for certified botanical products underscores the need for a consistent, transparent pathway to evaluate the safety of botanical dietary ingredients (DIs). Many botanicals lack authoritative upper safety limits (USLs) or conventional toxicology datasets, limiting the applicability of standard safety evaluation procedures and necessitating alternative approaches that can reliably establish safe use. Among these is a structured history of safe use (HoSU) assessment, which critically reviews published and grey literature to characterize human exposure to the botanical DI in foods and traditional preparations and to determine whether such use is relevant to contemporary dietary supplement applications. Using established peer reviewed frameworks, defined parameters are evaluated to determine whether historical consumption supports a safe intake. Key parameters include botanical identity, production process, and compositional and stability data. Additionally, experience of continued use is assessed based on the extent of use, characteristics of consuming populations, the botanical's typical role in the diet, and common handling or preparation methods. Human data, including adverse event signals, observational evidence, and other relevant toxicity information such as *in vitro* or *in vivo* studies are also considered. When anchored based on ingredient characterization, a HoSU

assessment provides a rigorous, human relevant alternative in the absence of authoritative or data derived USLs. This approach was applied to evaluate the history of safe use of *jabuticaba* fruit. *Jabuticaba* (*Myrciaria cauliflora*) is a Brazilian fruit commonly consumed as a fresh fruit or in foods such as jams, juices, and wines. The cultivation, market availability, and routine dietary inclusion across diverse populations are well documented, particularly in the Midwest and Southeast regions of Brazil where most production occurs. Reported serving sizes are approximately 100 grams (~12 fruits), and national dietary surveys indicate consumption across age, sex, urban/rural, and socioeconomic strata, demonstrating broad population exposure. Additional qualitative and empirical observations highlight its role as a seasonal snack food, its use in household and commercial preparations, and its cultural presence spanning several centuries. No adverse event signals were identified in national reporting systems or in human studies of *jabuticaba* fruit juice consumption. In Canada, whole or minimally processed *jabuticaba* fruit is classified as a non-novel food ingredient based on a documented history of safe use as a food, further supporting its established dietary role. Taken together, its longstanding inclusion in the diet, widespread consumption among diverse demographic groups, and the absence of reported adverse effects support the use of compositionally comparable fruit preparations in dietary supplements at intake levels consistent with customary food consumption.

Biological Aspects of Botanicals

PB-1: Coumarin Bioactivity Is Modulated by the Gut Microbiome *via α,β*-Unsaturated Lactone Reduction

Mingolelli G, Bashatwah RM, Jurek MJ, Riley AP, Burdette JE, Henke M

Department of Pharmaceutical Sciences, Retzky College of Pharmacy, University of Illinois Chicago, Chicago, IL, 60607 USA

Phytochemicals found in diet and dietary supplements are ingested and can be metabolized by the human gut microbiome. Microbial metabolism can alter bioactivity and bioavailability and may impact host health. Yet, many phytochemicals within herbal supplements have uncharacterized gut microbial metabolism pathways. This includes coumarins, which are abundant bioactive compounds in supplements, food, and cosmetics. To expand upon our recent characterization of coumarin (1,2-benzopyrone) metabolism to 3,4-dihydrocoumarin by the microbiome, we set out to test whether this pathway extends to other coumarins and evaluate impacts on bioactivity. Using LC-MS/MS metabolomics and *ex vivo* cultures of human feces, we explore the scope of *α,β*-unsaturated lactone reduction in a screen of 12 structurally diverse bioactive coumarins. Through this pathway, the culturable gut microbiome can metabolize isocoumarin, simple, furano, pyrano, and prenylated coumarins with an unsubstituted 3,4-alkene bond. In a screen of monocultured microbiome isolates, we determined that this pathway is shared by 12 species and enables metabolism of multiple coumarins by 11 species. In *E. coli*, a putative coumarin reductase, NemA, is necessary for furanocoumarin and pyranocoumarin metabolism. Through comparing viability of MDA-MB-435 melanoma cells treated with methoxsalen or its microbial metabolites, we determined that microbiome metabolism diminished furanocoumarin cytotoxicity. In summary, we characterized a metabolic pathway from the human gut microbiome which is shared across multiple species to modulate the bioactivity of a variety of coumarins. Continued study of *α,β*-unsaturated lactone reduction using *in vivo* models will establish if microbial metabolism of methoxsalen contributes to treatment efficacy, and its relevance to patient outcomes.

PB-2: Evaluation of Carotol and its Microbial Metabolites as Potential CYP450 Enzyme Inhibitors and Antioxidant Agents

Orabi KY, Al-Yousef MW, Alrushaid S, Sary HG

Natural products have long been used as medicines and continue to represent an important source of modern therapeutics. In recent years, interest in natural products has increased due to their favorable safety profiles and environmental compatibility. Essential oils derived from plants contain numerous bioactive constituents, particularly terpenoids, which are known to exhibit antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. Carrot

seed essential oil is a rich source of carotol, a sesquiterpene alcohol with potential biological activity. Moreover, several terpenoids are known to inhibit cytochrome P450 (CYP450) metabolic enzymes, suggesting that carotol may exert similar effects.

Carotol was extracted from carrot seed essential oil and purified using preparative high-performance liquid chromatography. In addition, three microbial metabolites of carotol were obtained using Absidia coerulea ATCC 6647 cultures. The identities of carotol and its metabolites were confirmed using various spectral techniques, including IR, MS, and 1D and 2D NMR. The purified carotol and its metabolites were evaluated *in vitro* for antioxidant activity and CYP450 enzyme inhibition. Both carotol and its metabolites exhibited mild antioxidant activity in the micromolar range and are anticipated to possess potential inhibitory effects on CYP450 enzymes. Overall, carotol emerges as a promising natural compound with antioxidant and enzyme inhibitory properties. These findings support its potential relevance in natural medicine and contribute to a better understanding of terpenoid-based bioactive compounds.

PB-3: Dihydrocapsiate, a Non-Pungent Constituent of *Capsicum annuum* Inhibits Lipid Accumulation *via* Activation of PPARα and NRF2

*Chae H-S [1], Ahmed N [1], Dale OR [1], Chittiboyina AG [1,2], Cantrell CL[3], Khan IA [1,2], Khan SI [1,2] **

[1] National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, Mississippi 38677, United States. [2] Department of Biomolecular Sciences, School of Pharmacy, The University of Mississippi, University, Mississippi 38677, United States. [3] Natural Products Utilization Research Unit, Agricultural Research Service, United States Department of Agriculture, University, MS 38677, United States

Dihydrocapsiate is a key nonpungent ingredient of *Capsicum annuum* along with capsiate. We previously reported the effects of a capsiate rich fraction of *C. annuum* on nuclear receptors, glucose uptake, and adipogenesis. In this study, we investigated the effects of dihydrocapsiate on activation of PPARα, PPARγ, LXR and NRF2 in hepatic (HepG2) cells by luciferase assays and regulation of adipogenesis in differentiated adipocytes by Oil red O assay. Dihydrocapsiate caused a 2.49-fold activation of PPARα and 5.23-fold activation of NRF2 at 100 µM and 300 µM in comparison to vehicle control in hepatic cells. To further explore the potential of dihydrocapsiate against obesity and metabolic disorder, its effect on adipogenesis was determined in adipocytes (3T3-L1 cells). Dihydrocapsiate (100 µM) inhibited lipid accummulation (20.0%) in differentiated adipocytes. These results indicate the potential utility of dihydrocapsiate in alleviating the symptoms of metabolic syndrome via reducing lipid accumulation in adipose tissue. Results also supported that dihydrocapsiate contributes to the anti-obesity effects of *C. annuum* along with other constituents such as capsiate and capsaicin.

PB-4: An Integrated Approach for Evaluating the Safety of Cosmetic Botanical Ingredients Using NAMs Designed to Screen the Skin Sensitization Potential

Chae H [1], Dale OR [1], Gurley CM [1], Chittiboyina AG [1,2], Khan IA [1,2], Khan SI [1,2]

[1] National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, MS 38677, USA. [2] Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA

Botanicals or herbal supplements have been used in cosmetics for a long time especially for skin nutrition, elasticity, moisturizer and rejuvenation. Cosmetic products with herbal ingredients are not always safe and may often cause skin sensitization or skin reactivity which could sometimes end in serious adverse outcomes such as allergic contact dermatitis. We have set up a series of non-animal *in chemico* and *in vitro* methods (NAMs) to evaluate the safety of cosmetic ingredients which include Direct Peptide Reactivity Assay (DPRA), KeratinoSens™, and the human Cell Line Activation Test (hCLAT). These methods are based on activation of key events in the adverse outcome pathway of skin sensitization and cannot be used alone for risk assessment. They are used in combination with each other according to a defined approach

established by OECD guidelines and decision is made based on weight of evidence (WoE). Another high throughput Dansyl Cysteamine Assay (DCYA) is used for screening the chemical reactivity of complex ingredients, such as essential oils or botanical extracts. We have screened fragrance ingredients and determined their skin sensitization potential before and after chemical degradation. A thorough investigation was also performed for the constituents of German chamomile (*Matricaria chamomilla*) which is a popular cosmetic ingredient. We also evaluated Oakmoss absolute which is one of the most popular natural fragrance ingredients. Based on WoE, Oakmoss extract as well as orcinol, ethyl orsellinate, and usnic acid were classified as skin sensitizers along with previously known atranol and chloroatranol.

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

PB-5: Skin Sensitization Potential of Cold-Pressed Grapefruit Essential Oil in Relation to Its Coumarin Constituents

*Chae H-S [1], Zahiruddin S [1], Dale OR [1], Gurley C [1], Wang Y-H [1], Khan IA[1,2], KhanSI [1,2 *]*

[1]National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, Mississippi 38677, USA.
[2] Department of Biomolecular Sciences, School of Pharmacy, The University of Mississippi, University, Mississippi 38677, USA

In this study, we investigated the skin reactivity potential of grapefruit essential oil using two OECD recommended methods (KeratinoSens™ and h-CLAT) and analyzed its compositional profile in terms of coumarin content to assess its safety for topical applications. Several coumarins were found to activate the NRF2 pathway in human keratinocytes with bergamottin being most potent (EC1.5 = 0.83 µM) followed by bergapten (EC1.5 = 1.3 µM) and citropten (EC1.5 = 1.7 µM). None of the tested coumarins showed an increase of CD54 or CD85 expression and thus considered inactive in HCLAT assay. UHPLC-MS/MS analysis revealed that bergamottin was one of the most abundant coumarin in the oil (1197.1 µg/g). However, all tested samples of the grapefruit essential oil showed negative results for skin sensitizing activity in both methods up to the highest tested concentration of 200 µg/mL. These results indicate that while individual coumarins are potent NRF2 activators, the overall composition of cold-pressed grapefruit essential oil presents a favorable safety profile. Therefore, it could be a promising and safe natural ingredient suitable for topical applications.

PB-6: Phytochemical Profiling and Assessment of Antibacterial, Antidiabetic Potential of *Syzyium cumini*

Gulia V [1], Rani J [1]*

[1] Department of Botany, Chaudhary Devi Lal University, Sirsa, Haryana, India.

Herbal based medicines continue to play an essential role in complementary and traditional medicine system, particularly in developing regions, offering more culturally accepted and cost-effective healing options. *Syzygium cumini* (L.) Skeels, widely known for its ethnomedicinal value, was investigated for its phytochemical richness and therapeutic potential against diabetes and bacterial infections. This study evaluated methanolic, hydro-ethanolic, and aqueous leaf extracts for their total phenolic (TPC) and flavonoid (TFC) contents, functional group characterization via FTIR, and compound profiling through LC-MS. Methanolic extract exhibited the highest TPC and TFC values, suggesting superior antioxidant potential. Antibacterial assays using well diffusion method demonstrated effective inhibition of both Gram- negative and Gram-positive bacteria, with the methanolic extract exhibiting the greatest activity. LC-MS analysis identified diverse bioactive compounds, notably flavonoids, triterpenoids, and triterpenes, which are linked to antidiabetic and antimicrobial actions. Enzyme inhibition assays revealed significant α-amylase and α-glucosidase inhibitory effects of methanolic extract (IC_{50}: 53.73 µg/mL and 2.479µg/mL, respectively), whereas DPP-IV activity was not inhibited. Glucose uptake studies on 3T3-L1 cells indicated dose-dependent enhancement, and insulin quantification showed increased secretion upon extract treatment. Cytotoxicity evaluation via MTT assay confirmed the extracts biocompatibility up to 1000 µg/mL. These findings collectively support the antidiabetic and antibacterial potential of *S. cumini* leaf extract, validating its traditional usage and indicating its promise as a phytotherapeutic agent. Bioactivities and chemical profile support its ethnomedicinal and

traditional uses and suggest its potential as a natural therapeutic font aligned with sustainable development goals. Further isolation and mechanistic studies are necessary to authenticate its pharmacological potential.

PB-7: Integrative Ethnopharmacology: Combining *In Silico* Molecular Docking and AI-Driven Analysis to Predict Anti-Cancer Mechanisms of Endemic *Veronica* and *Prangos* Species

Rostami S[1], Taghipour M [2], Aslim B [3]

[1] Department of Management Information Systems, Ankara Bilim University, Ankara, Turkey. [2] Department of Surgery, The Dudley Group NHS Foundation Trust, UK. [3] Department of Biology, Faculty of Science, Gazi University, Ankara, Turkey.

Traditional screening of medicinal plants is often resource-intensive. Previous *in vitro* studies by the authors have established the anti-proliferative potential of endemic species, specifically *Veronica lycica* and *Prangos platychloena*, on human colon cancer cell lines (Caco-2). However, identifying the specific molecular interactions between the plant's major bioactive metabolites and oncogenic targets remains a challenge.

This study aims to perform an *in silico* drug screening study to elucidate the molecular mechanisms of these endemic plants. We propose a hybrid framework that integrates molecular docking simulations with Artificial Intelligence (AI) to prioritize potent anti-cancer compounds. The study utilizes a multi-step computational approach. First, the major active metabolites identified in the *Veronica* and *Prangos* extracts (from previous thesis data) are selected as ligands. Second, Molecular Docking simulations are performed to analyze the binding affinity and interaction modes of these metabolites against key colorectal cancer-associated proteins (e.g., EGFR, COX-2). Finally, the resulting docking scores and physicochemical parameters are fed into Machine Learning (ML) algorithms (Random Forest and Neural Networks) to predict biological activity and filter the most promising lead compounds for future development. By combining experimental thesis data with *in silico* docking and AI analysis, this research provides a cost-effective roadmap for drug discovery. It transforms traditional herbal knowledge into precise, data-driven therapeutic candidates.

PB-8: *In Vitro* and *In Silico* Evaluation of Metabolic Enzyme Inhibition by Turkish Medicinal Plants: Quinic and Chlorogenic Acids as Key Bioactives in *Stachys cretica*

Pekacar S[*], Özüpek B, Güverti ÖF, Yılmaz MA, Çakir O, Tarhan A, Öner E, Tugay O, Deliorman Orhan D

Traditional medicinal plants are widely used for disease prevention and treatment; in Turkey, ethnopharmacological surveys report medicinal uses for ~1,546 plant species. Because metabolic disorders are increasing globally, we screened nine medicinal plants collected in Ankara and Tokat (*Vicia articulata*, *Securigera varia, Stachys cretica, Globularia orientalis, Astragalus inflatus, Onosma mollis, Fibigia eriocarpa, Achillea arabica, and Artedia squamata*) for *in vitro* antidiabetic (*α*-glucosidase, *α*-amylase), anti-obesity (pancreatic lipase), anti-cholesterol (pancreatic cholesterol esterase) and antioxidant activities (DPPH radical scavenging, metal chelating capacity, ferric reducing power). *S. cretica* showed the highest *α*-glucosidase and pancreatic cholesterol esterase inhibition (73.27 ± 1.51% and 46.93 ± 1.48%), whereas the strongest *α*-amylase inhibition was observed for *O. mollis* (59.46 ± 1.86%), followed by *S. cretica* (50.43 ± 3.53%); none of the extracts inhibited pancreatic lipase. In parallel, S. cretica displayed strong DPPH radical scavenging and ferric reducing activity but weak metal chelation. LC-MS/MS of S. cretica identified quinic acid (68.567 mg/g) and chlorogenic acid (33.999 mg/g) as major constituents. Consistent with prior work on *S. cretica* subsp. anatolica reporting potent *α*-glucosidase inhibition (IC50 24.18 ± 1.44 µg/mL) and quinic-acid-rich extracts (55.708 mg/g) supported by computational binding, our in silico docking suggested favorable interactions of quinic and chlorogenic acids with *α*-glucosidase and *α*-amylase. Docking ranked chlorogenic acid > acarbose ≈ quinic acid for *α*-glucosidase and acarbose ≈ chlorogenic acid > quinic acid for *α*-amylase. Overall, these findings support *S. cretica* as a promising source of antidiabetic constituents and extend the literature by providing comparative screening and evidence of pancreatic cholesterol esterase inhibition.

PB-9: Screening for Bioactive Molecules and Peptides from *Strychnos spinosa*, Its *In-Silico* Inhibitory Molecular Reactive Indices against Tyrosinase, and All-Inclusive *In-Vitro* Anti-Oxidative Investigations.

Alaribe SC, Blessing E, Titilayo BE, Miralles-Robledillo JM, Allen G, Omotoyosi O, Oyintonbra OP, Okpaka P, Oloro D, Abdulsalam Y, Pire C, Townley GG, Nwaokorie F, Martinez-Espinosa RM

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, College of Medicine Campus, University of Lagos, Nigeria; Multidisciplinary Institute for Environmental Studies, Ramon Margalef (IMEM), Applied Biochemistry Research Group, Universidad de Alicante, Spain; Department of Chemistry, Nelson Mandela University, Gqeberha, 6031, South Africa; Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Alicante, Carretera de San Vicente, Spain; Department of Medical Laboratory Science, College of Medicine, University of Lagos, Lagos, Nigeria.

Oxidation is a natural biochemical process in living organisms that is important for energy production and other metabolic functions. However, excessive oxidation (oxidative stress) has been implicated in both the onset and progression of numerous chronic diseases, including cancer, neurodegenerative disorders, cardiovascular conditions, and premature ageing (Korovesis et al., 2023). Strychnos spinosa, a spiny deciduous tree of Sub-Saharan African origin, is well-known in traditional medicine. Investigations on four of its bioactive molecules, Ursolic acid, Linoleic acid, 24-Hydroperoxy-24-vinylcholesterol, and Saringosterol, through comprehensive *in silico* analyses, *in vitro* antioxidant assays, reactivity indices, binding affinities, molecular interactions, and pharmacokinetic predictions, and their antioxidant evaluation were carried out. By analyzing their electronic properties, docking scores, and binding energies against three protein targets, 2Y9X for tyrosinase inhibition, 1M17 for tyrosine kinase inhibition, and 5L38 for oxidative stress activities, significant correlations were discovered between the chemical reactivity, interaction mechanisms, and bioavailability of the compounds. Linoleic acid exhibited exceptional binding affinity across all targets, displaying the highest binding energies. It also showed extensive metal coordination and salt bridge interactions, contributing to its potency as a tyrosinase and oxidative stress inhibitor. Saringosterol demonstrated strong inhibitory activity against multiple protein targets, supported by its high binding affinities and robust pharmacokinetic properties, showing high intestinal permeability (QPPCaco: 1939.636 nm/sec), indicating favorable oral bioavailability and permeability, although its solubility is low. Despite the stability and solubility challenges of 24-Hydroperoxy-24-vinylcholesterol, it exhibits the highest tyrosine kinase inhibition activity (ΔGBind: −24.94 kcal/mol), likely due to its ability to form strong, short-distance hydrogen bonds with the target protein.Ursolic acid provides a well-balanced range of moderate activities, excellent bioavailability, and strong, stable binding interactions, specifically demonstrating superior chemical reactivity with the lowest HOMO-LUMO gap (6.143eV) and highest chemical softness (0.326 eV^{-1}). Complementing these computational insights, proteomic analysis identified the peptide VVDALGNPIDGKGPIK as exhibiting high stability (instability index: −23.04) and favorable bioactivity (Peptide Ranker score: 0.42941). These results were corroborated by *in vitro* antioxidant assays, where among the 2 extracts tested, ethyl acetate exhibited the strongest antioxidant activity across DPPH, CUPRAC, ABTS, and Phenanthroline assays. These findings establish Strychnos spinosa as a valuable source of natural antioxidants and enzyme(tyrosinase)inhibitors for potential applications in cosmeceuticals and nutraceuticals.

PB-10: Repellency and Toxicity of Maleimide-Derived Compounds Against Imported Fire Ants

George G [1], Ali A [1], Shah FM [1], Chen J [2], Khan IA [1,3], Li X-C [1,3]

[1] National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, MS 38677, USA. [2] Biological Control of Pests Research Unit, USDA-ARS, Stoneville, MS 38776, USA. [3] Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA

Maleimides represent an important class of compounds exhibiting a wide range of biological activities. Owing to their moderate hydrophobicity and ability to cross cell membranes, several members of this class (e.g., fluoroimide and farinomalein) have been explored as agricultural fungicides. Despite their promising applications, the potential of maleimides against imported fire ants remains unexplored. In this study, a series of substituted maleimide derivatives—featuring substitutions at the imide nitrogen and on the maleimide core —were evaluated for fire ant repellency and

toxicity. N-Substituted maleimide derivatives demonstrated higher toxicity, whereas core-substituted derivatives (e.g., phthalimides) exhibited superior repellency. Specifically, N-substitutions bearing aromatic functional groups resulted in significantly higher toxicity (LC_{90} values of 7.8, 14.7, and 15.6 ppm for benzyl, α-methylbenzyl, and p-chlorobenzyl derivatives, respectively) against hybrid imported fire ants, compared with N-substitutions containing simple alkyl chains, which displayed only marginal toxicity (LC_{90} values of 100 – 125 ppm). Among the phthalimide derivatives, N-alkyl substitutions showed greater repellency than aryl substitutions. The allyl-substituted phthalimide exhibited the lowest minimum repellent effective dose (MERD) of 7.8 ppm, followed by the methyl- and ethyl-substituted analogues (all with MERDs of 15.6 ppm). In summary, substitutions bearing aromatic functional groups on the imide nitrogen of the maleimide scaffold enhance toxicity, whereas allyl and alkyl substitutions on the nitrogen of the phthalimide core improve repellency, providing valuable insights for further structural optimization. Overall, these findings highlight the potential of maleimide-derived compounds as promising candidates for fire ant management.

PB-11: Patchouli Essential Oil-Derived Compounds as Potential Natural Repellents and Insecticides for Fire Ant Control

Rahman Z [1,2], Ali A [1], Khan IA [1,2], Li X-C [1,2]

[1] National Center for Natural Products Research, The University of Mississippi, University, MS 38677, USA. [2] Department of Bio Molecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA

Imported fire ants (*Solenopsis* spp.) continue to pose significant ecological and economic challenges, with current control practices relying predominantly on synthetic insecticides. Concerns regarding environmental persistence, non-target effects, and resistance development have driven interest in alternative, plant-based pest management strategies. Essential oils offer chemically complex mixtures with diverse biological activities, making them attractive candidates for insect control. Patchouli essential oil, derived from *Pogostemon cablin* (Lamiaceae), is enriched in sesquiterpenes but has not been systematically investigated against imported fire ants. In this study, the repellency and toxicity of patchouli oil were assessed against hybrid (*S. invicta* × *S. richteri*), black (*S. richteri*), and red (*S. invicta*) imported fire ants using a bioassay-guided approach. Chemical profiling by gas chromatography-mass spectrometry (GC-MS) combined with silica gel chromatography identified patchouli alcohol as the principal bioactive constituent. Digging bioassays demonstrated strong, concentration-dependent repellency, while contact toxicity assays confirmed insecticidal activity across all three fire ant species. To explore additional bioactive constituents and enable future optimization, pogostone, another major component of patchouli oil, was isolated. A series of analogues was synthesized and submitted for biological evaluation against fire ants. Ongoing bioassays will determine the insecticidal and repellent potential of these derivatives. Collectively, this work highlights patchouli essential oil as a promising source of environmentally benign compounds, establishing a foundation for the development of natural-product-based fire ant control agents.

PB-12: A Screening Approach to Evaluate Essential Oil Induced Glucose Uptake in Skeletal Muscle Cells

Pallangod AA [1,2] Chae H [1], Khan IA [1,2], Khan SI [1,2]

[1] National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, MS 38677, USA. [2] Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA

Impaired glucose uptake and metabolism play a central role in metabolic disorders such as obesity, dyslipidemia, and diabetes mellitus, making the maintenance of glucose homeostasis essential for disease management. Targeting skeletal muscle glucose uptake, primarily mediated by insulin-stimulated GLUT4 translocation, represents a promising therapeutic strategy. In the search for safer alternatives to current antidiabetic therapies, 13 essential oils from the NCNPR repository were screened for glucose uptake enhancing activity in differentiated C2C12 muscle cells. Glucose uptake was quantified using a fluorescent glucose analog (2-NBDG), with Hoechst 33342 used for normalization, while cell viability was assessed using the MTT assay. Among the tested oils, Blue Tansy Oil significantly enhanced glucose uptake by 57.00% and 36.58% at 20 and 10 µg/mL, respectively, compared to the untreated control. Rosiglitazone was included as the positive control under similar experimental conditions. Cytotoxicity studies confirmed that Blue Tansy Oil was non-toxic up to the highest

tested concentration of 20 µg/mL. These results suggest that Blue Tansy Oil has the potential to increase muscular glucose uptake, through GLUT4 modulation, and warrants further investigation to understand its efficacy against hyperglycemia.

PB-13: AhR Activation by *Mitragyna speciosa*: Implications for Herb-Drug Interactions

Cheepilla L [1,2], Hussain I [1], Ali Z [1], Chittiboyina AG [1,2], Khan IA [1,2], Khan SI [1,2]

[1] National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, MS 38677, USA; [2] Department of Biomolecular Sciences, School of Pharmacy, The University of Mississippi, MS 38677, USA.

Mitragyna speciosa (Kratom) has been traditionally used for pain, opioid withdrawal, anxiety, depression, fatigue and mood disorders. Kratom leaves contain more than 54 alkaloids, among which mitragynine and 7-hydroxymitragynine are the primary alkaloids acting as partial agonists for µ-opioid receptors. Recently, kratom has gained widespread popularity, resulting in its supplements being increasingly available in the United States, often marketed with attractive claims. Despite its increased consumption, its interaction with prescription drugs is not well understood. Moreover, there is no report available for AhR-mediated herb-drug interactions (HDIs).

In this study, we evaluated the potential of kratom and its alkaloidal constituents to cause AhR-mediated HDIs which could result in altering the pharmacokinetic of the co-consumed drugs. We prepared a kratom methanolic extract, an alkaloid-rich fraction, and an alkaloid-free fraction and subjected them to an AhR activation assay in reporter cells. Cell viability assay confirmed that the tested extracts were nontoxic up to 60 µg/mL. Subsequent analysis at subtoxic concentrations revealed that the methanolic extract and alkaloid-rich fractions induced AhR activity in a dose-dependent manner, whereas the alkaloid-free fraction did not show any effect. These findings indicated that the AhR activation potential of kratom is primarily mediated by its alkaloids. The results suggest that kratom consumption may cause AhR-mediated HDIs and could alter the bioavailability of co-administered drugs which are the substrates of CYP1A1 and 1A2, expression of which is regulated by AhR.

PB-14: *In Vitro* Cytotoxicity Screening for Detection of Toxic Amino Acids/Protein Fractions in Food and Supplement Products

Haron MH [1], Ali Z [1], Zulfiqar F [1], Khan IA [1,2], Pugh ND [1]

[1] National Center for Natural Products Research, [2] Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University MS 38677, USA

The alternative protein and amino acid supplements market is expanding rapidly with many new products. However, some of those products have not been traditionally consumed or used at the label-recommended doses. At the same time, the new 2025-2030 U.S. Dietary Guidelines for Americans encourages increased daily protein intake, recommending 1.2-1.6 g of protein per kg of body weight (a 50-100% increase compared to the previous recommendation of 0.8g per kg). The combination of higher recommended protein consumption and growing availability of diverse new products raises concerns that additional research may be needed to confirm and establish safe dosage levels. This potential safety concern was previously demonstrated with tara flour, a new plant-based protein ingredient used in a Daily Harvest food product that was subsequently recalled due to adverse human health effects. Using a mouse model, our group identified baikiain (a non-protein amino acid within tara flour) as a possible agent responsible for these adverse health events. A major challenge in identifying baikiain was not knowing its mechanism of action and not having an *in vitro* screen to detect toxicity. Therefore, we have identified an *in vitro* screen for detection of amino acids and protein fractions with potential adverse health effects, the cell-based MTT assay, since mechanism of action is not always known. To detect toxicity, samples need to be evaluated at high concentrations between 0.3 – 20mg/ml. Evaluation of commercially available pure compounds indicated that some non-protein amino-acids exhibit toxicity when compared to protein amino-acids. In the current work, we optimized an extraction procedure to obtain amino acid fractions to enable evaluation of complex food

matrices in the MTT assay. Our screening of the amino acid fraction from 50 commercial food products demonstrated that some exhibited toxicity. Collectively, these data indicate that initial *in vitro* screening of commercial protein food products and supplements is valuable and can guide subsequent animal studies to confirm product safety and ultimately help prevent harmful products from entering the marketplace.

PB-15: Xenobiotic Receptor Mediated Herb–Drug Interaction Potential of *Pausinystalia yohimbe*

Husain I [1], Dale OR [1], Annam SCVAR [1], Ali Z [1], Chittiboyina AG [1,2], Khan IA [1,2], Khan SI [1,2]

[1] National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, MS 38677, USA. [2] Department of Biomolecular Sciences, School of Pharmacy, The University of Mississippi, MS 38677, USA.

P. yohimbe (syn. *Corynanthe yohimbe*) is an evergreen tree belonging to the family Rubiaceae. The bark of this plant is widely used as an herbal supplement for the management of male sexual dysfunction. Owing to its perceived aphrodisiac and stimulant properties, yohimbe has been extensively marketed in over-the-counter formulations, including products promoted for physical performance enhancement. Indole alkaloids represent the principal bioactive constituents of yohimbe, with yohimbine and its structural analogues being primarily responsible for its pharmacological effects. Despite its widespread use, systematic safety evaluations remain limited. To date, no comprehensive studies have addressed its effect on xenobiotic receptors (XRs), particularly the aryl hydrocarbon receptor (AhR) mediated herb–drug interactions (HDI). Nevertheless, the concurrent use of yohimbe-containing supplements with conventional medications may pose a clinically relevant risk of HDI through alterations in the pharmacokinetics of co-administered drugs, a concern of particular significance for patients receiving long-term pharmacotherapy. In the present study, a methanolic extract prepared from P. yohimbe bark and its alkaloids were evaluated for cytotoxicity in AhR-responsive cell lines. Subsequently, their effect on AhR activity was assessed using HepG2-AhR reporter cells. The tested extract and isolated alkaloids were found to significantly enhance AhR activity in a concentration-dependent manner. To determine whether AhR activation resulted in downstream transcriptional responses, total RNA was isolated, and the mRNA expression levels of canonical AhR-regulated cytochrome P-450 enzymes, including CYP1A1, CYP1A2, and CYP1B1, were quantified. The results demonstrated that the extract and the selected alkaloids induced a dose-dependent upregulation of these genes. In conclusion, these results indicate that P. yohimbe can modulate AhR signaling and may contribute to clinically relevant herb–drug interactions. Nevertheless, further mechanistic and translational studies are warranted.

PB-16: Natural Product-Driven Antibacterial and Antifungal Screening Program at NCNPR

Shamim K,[1] Bennett M,[1] Khan SI,[1,2] Khan IA[1,2]

[1] National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA; [2] Division of Pharmacognosy, Department of Biomolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA.

The increasing prevalence of antimicrobial resistance and growing concerns regarding the safety of synthetic preservatives have intensified the search for new antimicrobial agents from natural sources. Natural products represent an important reservoir of chemically diverse bioactive compounds with potential antibacterial and antifungal properties. In this ongoing research program at NCNPR, natural compounds and extracts are being systematically evaluated for their antimicrobial activity against clinically and industrially relevant microorganisms. Antimicrobial susceptibility testing is being performed using the broth microdilution method following the guidelines of the Clinical and Laboratory Standards Institute (CLSI), with minor modifications. Antifungal assays are conducted according to CLSI documents M27 and M38, while antibacterial activity is evaluated following CLSI M07 protocols. The assays are based on microbial growth inhibition determined by measuring optical density using a microplate reader following incubation under appropriate growth conditions. For anaerobic bacterial strains, assays are being conducted in an anaerobic chamber to maintain oxygen-free conditions required for optimal growth.

The screening program includes broad antimicrobial evaluation against representative bacterial and fungal species, followed by targeted investigations relevant to food safety and human health. Microorganisms currently being tested include fungal pathogens such as *Candida* spp., *Cryptococcus* spp., and *Aspergillus fumigatus*, as well as bacterial species including *Staphylococcus* spp., *Escherichia coli*, *Pseudomonas* spp., *Klebsiella* spp., and *Enterococcus* spp. Additional targeted studies are being conducted against foodborne pathogens (*E. coli, Salmonella Typhi, and Listeria monocytogenes*) and clinically relevant microorganisms such as *Candida* spp., methicillin-resistant *Staphylococcus aureus* (MRSA), and *Cutibacterium acnes*. This integrated screening effort aims to identify promising natural products with antimicrobial activity and support the development for therapeutic applications as well as natural preservatives that can be used for food and cosmetic products.

PB-17: Investigating the Role of the Gut Microbiome in Immulina®-Mediated Antiviral Immune Responses

Shamim K, Vaughn S, Mir TM, Tripathi S, Zhang J, Khan IA, Marshall GD, Jackson CR, Ashfaq MK, Pugh ND

In our recently published study, Immulina®, an extract of *Limnospira fusiformis* (commercial name spirulina), orally administered during the prodromal phase of influenza infection reduced disease progression and enhanced antiviral immune responses in mice. Our working hypothesis is that Immulina® acts through gut-associated immune pathways. The objective of this study was to investigate the involvement of the gut microbiome in mediating Immulina's antiviral immune responses.
Male mice infected with influenza A (H1N1) virus were treated orally with Immulina® (25, 50 and 100 mg/kg) or vehicle control using the prodromal dosing regimen. Large intestinal contents were collected on days 3, 5, 7, and 10 post-infection. Gut microbiome composition was assessed by 16S rRNA gene sequencing. Community-level differences were analyzed using Bray-Curtis dissimilarity and PERMANOVA, and associations between microbiota and cytokine responses were evaluated using correlation analyses.

Immulina treatment accounted for 6% of bacterial community variation, with 25 and 100mg/kg doses exhibiting significant changes from infected controls. Bacterial taxa that showed differences in abundance between treatment groups included Desulfobacterota (phyla level) and Tannerellaceae and Muribaculaceae (family level). In the 25 mg/kg group, Bray-Curtis dissimilarity scores were statistical significance with levels of IL-6 (Mantel correlation (r) = 0.20), IFN-γ (r = 0.30) in lung tissue. Immulina® was associated with a minor alteration of the gut microbiome variation and composition changes correlated with lung cytokine levels. Whether these effects are clinically significance in mediating Immulina's antiviral immune responses remains to be determined.

PB-18: Evaluating Whether Andrographolide Fully Accounts for the *In Vitro* Immunomodulatory Activity of *Andrographis paniculata*

Zhang J [1], Ahmed NH [1], Khan SI [1,2], Shamim K [1], Burnett GC [3], Marshall GD [4,5], Bates JT [3,4,5] Chittiboyina AC [1,2,6], Khan IA [1,2], Pugh ND [1]

[1] National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University MS 38677, USA; [2] Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University MS 38677, USA; [3] Cell and Molecular Biology, University of Mississippi Medical Center, Jackson MS 39216, USA; [4] Department of Medicine, University of Mississippi Medical Center, Jackson MS 39216, USA; [5] Center for Immunology and Microbial Research, University of Mississippi Medical Center, Jackson MS 39216, USA; [6] Department of Chemistry and Biochemistry, University of Mississippi, University MS, 38677, USA.

Ethnobotanically, *Andrographis paniculata* has been widely used as a medicinal plant for its broad-spectrum antiviral properties. However, a major knowledge gap exists – not all bioactives in this botanical have been identified. Previous studies suggest that its antiviral activity cannot be fully explained by andrographolide and its major derivatives alone. A commercial extract of *A. paniculata* manufactured from aerial parts that was standardized to contain ≥10% andrographolides, as well as a hydroethanolic extract of *A. paniculata* botanical reference material, were evaluated *in*

vitro for NF-κB inhibition in THP-1 monocytes, iNOS inhibition in RAW 264.7 macrophages and Nrf2 activation in HepG2 cells.

Both the commercial *A. paniculata* extract and the botanical reference material inhibited NF-κB and iNOS activity while activating Nrf2 signaling. In the NF-κB assay, andrographolide exhibited an IC_{50} value of 13 µg/mL, compared with 55.5 µg/mL for the commercial extract. Based on its content in the extract, the predicted IC_{50} value of andrographolide was 6.0 µg/mL, indicating that andrographolide accounts for approximately 45% of the NF-κB inhibitory activity of the extract. In the Nrf2 assay, maximal activation was achieved at 50 µg/mL for the commercial extract (24-fold increase over control), which was 1.5-fold higher than that of andrographolide, reaching a 16-fold induction at 4.4 µg/mL. In the iNOS inhibition assay, the hydroethanolic extract of the botanical reference material showed an IC_{50} value of 4.9 µg/mL, which is comparable to that of andrographolide (4.4 µg/mL). In addition, a translationally relevant mouse model of antiviral resilience has been established for future evaluation of newly identified active phytochemicals. Overall, the *in vitro* data across multiple cellular targets in this study supports a working model in which the immunomodulatory activities of *A. paniculata* cannot be attributed to andrographolide alone, but instead arise, at least in part, from the combined contributions of andrographolide and additional unidentified immunologically active phytochemical constituents.

PB-19: Cannabinoids as Sleep Aids against Alzheimer's Disease?

Hammond N, Thomas L, Leonette S, Zheng HA

Department of Pharmaceutical Sciences, School of Pharmacy, Albany College of Pharmacy and Health Sciences, 106 New Scotland Ave, Albany, New York, 12208

Alzheimer's disease (AD) affects millions of patients' lives and families, with symptoms such as memory loss, confusion, agitation, as well as sleeping disorders. AD exhibits a bidirectional relationship with sleep, where disease progression is associated with a decline in sleep quality and duration. On the other hand, emerging literature indicated that chronic low sleep quality may be a main cause for AD. Upon literature and preliminary clinical findings, we hypothesize that selected cannabinoids and personalized pharmacotherapy protocol can improve the quality of sleep in AD patients and even slow the disease progression. Cannabinoids like CBG, CBN, and CBD have shown promise in improving sleep. For example, clinical trials have shown promises to treat sleep disorders using CBN in combination with CBG. Cannabinoids' therapeutic potential of anti-inflammatory and neuroprotection can also be beneficial for AD patients. Therefore, we propose to use cannabinoids as an intervention to mitigate sleep disturbances associated with AD. Further translational research to develop optimized formula and dosing, cannabinoids may be good sleep aid for AD population, as a solution to mitigate AD symptoms and progression.

PB-20: Investigating the Impact of Medicinal Cannabis Use on the Metabolism of Antidepressant Drugs: An *In Vitro* Preclinical Study

Kotwal P [1], Husain I [1], Tatapudi KK [1], Avula B [1], Radwan M [1,2], Chandra S [1,2], Wanas A[1], Welch R [3], Chittiboyina AG [1,2], Khan IA [1,2], Khan SI [1,2]

[1] National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, MS 38677, USA; [2] Department of Bio-Molecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA; [3] National Center for Cannabis Research and Education, Research Institute of Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi, MS 38677, USA

Prolonged use of medical cannabis has raised concerns regarding its potential interactions with conventional medications. In this study, we investigated the effect of medical cannabis on the metabolism of antidepressant drugs if taken simultaneously, with a specific focus on CYP1A2-mediated metabolism which is regulated by aryl hydrocarbon receptor (AhR). Hydroethanolic extracts of cannabis (extract-A, -B, and -C) standardized for CBD and THC contents and cannabis-based market products (product-A, -B, and -C) were screened for their effects on AhR activation and CYP1A2 mRNA

expression in an AhR specific cell line and CYP1A2 enzyme activity in human primary hepatocytes. Additionally, the metabolism of clozapine, a CYP1A2 substrate antidepressant drug, was evaluated in hepatocytes following exposure to cannabis extracts and products. Results showed that both cannabis extracts and market products induced AhR transcriptional activity (4-10-fold at 10 µg/mL) in a dose-dependent manner, and increased CYP1A2 gene expression by several folds. Additionally, in hepatocytes upon treatment with the cannabis extracts, CYP1A2 enzyme activity was increased (2-3-fold) and metabolism of clozapine was increased by (1.5-2.4-fold). On the other hand, in baculosomes assay CYP1A2 enzyme activity was significantly inhibited in a concentration dependent manner with IC_{50} values of 0.6-1.3 µg/mL for extracts and 1.9-3.9 µg/mL for products. Collectively, these outcomes demonstrate that medicinal cannabis can significantly modulate AhR signaling and CYP1A2 enzyme activity, which may alter the hepatic metabolism of its substrate drugs such as antidepressants indicating a potential risk of herb-drug interactions. The overall implications of these findings require more in-depth mechanistic studies to establish clinical correlation and understand translational relevance.

Acknowledgements: This research is funded by the Cannabis Science Research & Education Award (CSREA), under award number 100211605G. Ms. Olivia Dale is acknowledged for her unwavering technical support during this study.

PB-21: Screening the Cardiotoxicity Potential of Medicinal Plants Used as Ingredients in Top Selling Dietary Supplements in the US Market

Ahmed N [1], Nabi Z [1], Chittiboyina AG [1,2], Khan IA [1,2], Khan SI [1,2]

[1] National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, Mississippi, 38677, United States; [2] Department of Bio-Molecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA

Cardiotoxicity is a significant health concern derived from various factors such as certain medications (chemotherapeutic agents), exposure to environmental toxins (e.g. heavy metals, PFAS), and chronic alcohol consumption. There is an increased interest in using herbal supplements as an alternative or in combination with medical therapies for alleviating the symptoms of various health conditions. In general, herbal supplements are considered safe due to their natural origin from plants. However, several medicinal plants have been reported to cause liver, kidney or cardiac toxicity if consumed improperly. As part of our continuous efforts to study the safety of herbal dietary supplements commonly available in the US market, we screened the hydroethanolic extracts of 35 medicinal plants for their cardiotoxic potential.

H9c2 cells derived from embryonic rat heart tissue were used as an *in vitro* cardiomyocyte model due to their ability to respond to cardiac stimuli. Cells were treated with various concentrations of plant extracts and cell viability was determined by a formazan dye assay based on cellular metabolic activity. The intracellular generation of excessive reactive oxygen species (ROS) has been associated with cardiomyocyte damage. Increase in ROS production by the extracts was determined by DCFH-DA method. Out of the selected 35 plants for this study, most showed no or minimal toxicity to H9c2 cells up to a concentration of 100 µg/mL, while a few exhibited toxicities to a certain extent. *Withania sommifera* leaf showed toxicity with an IC_{50} of 25 µg/mL while the root was not toxic. A few plants were also found to increase oxidative stress in H9c2 cells such as *Glycyrrhiza glabra* root and *Hydrastasis canadensis* root showing >2-fold increase in ROS at 100µg/mL. Doxorubicin was included as a control drug known for cardiotoxicity and ABAP was included as an inducer of oxidative stress.

Acknowledgments: This research is supported in part by “Discovery & Development of Natural Products for Pharmaceutical & Agrochemical Applications” funded by the USDA, Specific Cooperative Agreement No. 58-6060-6-015, and “Science-Based Authentication of Botanical Ingredients” funded by the CFSAN US FDA grant number 5U01FD004246. Ms. Olivia Dale and Ms. Nisha Mishra are acknowledged for excellent technical support during this study.

PB-22: Marshmallow Cough Syrup – Strong Protective Effect in Irritative Cough

Kelber O [1], Seibel J [2]

[1] Phyto & Biotics Tech Platform, Phytomedicines Supply and Development Center, Bayer Consumer Health, Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany. [2] Medical Affairs Consumer Health, Bayer Vital GmbH, Leverkusen, Germany

The use of the aqueous extract of marshmallow root for respiratory symptoms is well established since many centuries. STW 42, a cough syrup based on this extract, has been characterized by analytics, as well as by studies of its anti-inflammatory and physical effects, which are key for its fast and strong action in irritative cough. To characterize these, physical parameters were studied in comparison to 5 other commercially available liquid cough preparations.

STW 42 and the other liquid cough preparations (coded 1-5) were studied by rotational viscosimetry and regarding their retention on a mucosal surface (EpiOral resp. *ex vivo*) after labelling with [14C]-galactose and repeated rinsing, mimicking the effect of swallowing saliva on the pharyngeal mucosa. The viscosity of the preparations tested was in a range of 482-26 mPas, ranking in order of decreasing viscosity STW 42 > 2 > 1 > 3 > 4. The order of retention was (in decreasing order) STW 42 > 4 > 1 > 5 > 2 > 3. Comparison of the viscosity values with epithelial retention data indicated that viscosity alone did not account for the retention on epithelial tissues. The results confirm the unique physical properties of marshmallow cough syrup and so underline its usefulness in irritative cough which has been confirmed by a huge body of evidence.

PB-23: Integrative Anatomical and Chemical Markers for the Characterization and Differentiation of *Bidens* Species (Asteraceae)

Sabedotti C [1], da Silveira I [2], Migacz IP [1], Cruz LS [3], Ventura ACT [1], Farago PV [1,2], Mondin CA [4], Raman V [5], Brito ES [6], Ribeiro PRV [7], Beltrame FL[1], Manfron J [1,2]

[1] Postgraduate Program in Pharmaceutical Sciences, State University of Ponta Grossa, Ponta Grossa, PR, Brazil, [2] Postgraduate Program in Health Sciences, State University of Ponta Grossa, Ponta Grossa, PR, Brazil, [3] Department of Pharmaceutical Science, Midwest State University, Guarapuava, PR, Brazil, [4] Environmental Connect Institute, Higienópolis, Porto Alegre, RS, Brazil, [5] National Identification Services, U.S. Department of Agriculture, Washington, DC, USA, [6] Postgraduate Program in Chemical Engineering, Federal University of Ceará, Fortaleza, CE, Brazil, [7] Embrapa Tropical Agroindustry, Fortaleza, CE, Brazil.

Bidens pilosa, *B. alba*, and *B. subalternans* are frequently misidentified because of their marked morphological similarity. This study comparatively evaluated anatomical, histochemical, and chemical markers to characterize and differentiate these species. Light microscopy, field-emission scanning electron microscopy, and energy-dispersive X-ray spectroscopy were used to assess diagnostic morpho-anatomical traits. Histochemical assays and LC-MS profiling were applied to obtain chemical information from the extracts, and chemometric analysis was used to classify species-specific chemical patterns. The combined morpho-anatomical and histochemical analyses enabled reliable differentiation of the three species, while LC-MS revealed phenolic compounds as the major constituents of the extracts. Chemometric tools further discriminated the species based on their chemical profiles. Altogether, the integration of anatomical, histochemical, and chemical fingerprinting approaches proved to be a robust strategy for Bidens species authentication and differentiation, supporting the quality assessment of botanical raw materials.

PB-24: Metabolic and Anti-inflammatory Effects of Gastro-resistant Curcumin–Piperine Microparticles in an Experimental Model of Metabolic Syndrome

Marcon MV [1], Justus B [1], Nadal JM [1], Borges CL [1], Farago PV [1], Manfron J [1], Lívero FAR [2]

[1] Postgraduate Program in Pharmaceutical Sciences, State University of Ponta Grossa, PR, Brazil. [2] Postgraduate Program in Pharmacology, Federal University of Paraná, Curitiba, PR, Brazil.

Metabolic syndrome is a multifactorial disorder characterized by adiposity, dyslipidemia, hyperglycemia, chronic low-grade inflammation, and progressive hepatorenal dysfunction. Curcumin has relevant metabolic and anti-inflammatory properties, but its oral efficacy is limited by poor bioavailability. This study evaluated the metabolic and anti-inflammatory effects of gastro-resistant curcumin–piperine polymeric microparticles in an experimental model of metabolic syndrome. Microparticles were prepared by spray drying using Eudragit S100 and showed high encapsulation efficiency for both curcumin and piperine. Metabolic syndrome was induced in spontaneously hypertensive rats by dyslipidemia induction and fructose consumption, followed by treatment with curcumin–piperine microparticles (25, 75, or 225 mg/kg) or conventional pharmacotherapy. Anthropometric, glycemic, lipid, hepatic, renal, and inflammatory parameters were evaluated after chronic treatment. The untreated group exhibited increased epididymal fat mass, abdominal obesity index, fasting glucose, total cholesterol, triglycerides, VLDL-c, LDL-c, hepatic enzymes (γGT, AST, ALT), urea, creatinine, and pro-inflammatory cytokines (TNF-α and IL-6), with reduced HDL-c. Treatment with curcumin–piperine microparticles promoted dose-dependent improvements in these parameters. The highest dose (225 mg/kg) normalized fasting glucose, visceral adiposity, lipid profile, hepatic and renal biomarkers, and reduced TNF-α and IL-6 to levels comparable to basal animals, with IL-6 normalization observed only at this dose. These findings demonstrate that the gastro-resistant curcumin–piperine formulation exerts robust metabolic and anti-inflammatory effects in metabolic syndrome, supporting pharmacotechnical optimization as a key strategy to enhance the biological efficacy of curcumin as an adjuvant intervention.

PB-25: Computational Validation of ABC's HerbClip Citations Using the RetractionWatch Database

Neal WM [1], Rickett CD [2], Sukumar SR [2], Khan IA [1,3], Chittiboyina AG[1,3,4]

[1] National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, MS 38677, USA; [2] Hewlett Packard Enterprise, Spring, TX 77373, USA; [3] Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA. [4] Department of Chemistry and Biochemistry, University of Mississippi, University, MS 38677, USA

As researchers often face pressure to publish, the growing number of publications does not necessarily correlate with the quality and/or significance of the reported research. In perceived or real publish-or-perish environments, scientists may feel compelled to resort to dishonest practices to inflate their portfolio, enhance their reputation, and/or increase their chances for research support. The recent increase in retractions of inappropriate publications that disseminated unjustified conclusions based on fabricated data indicates the validity of these concerns for science in general. One concern is that certain journals and/or publishers might disseminate unreliable material at elevated rates. In response to this growing problem, the American Botanical Council (ABC) sought to ensure that its HerbClip Educational Service—a twice-monthly series of summaries and critical reviews of recently published scientific and clinical herbal literature—is based on reliable information. HerbClip's commitment to accurate sources helps enable readers to make informed, responsible assessments and decisions regarding research, development, and potential therapeutic use of herbs, phytomedicinal ingredients, and related products.

Using artificial intelligence and data-mining techniques, the HerbClip digital archive was searched for retracted articles. Comparing against the Retraction Watch database through computational resources from Hewlett Packard Enterprise, a knowledge graph (a method of representing large and complex datasets in a network-like format that allows computers to visualize and calculate connections between the data) of retracted articles across all scientific disciplines was created. Additionally, Orange Data Mining was used to evaluate the prevalence of herbal medicine in retracted

scientific literature. The analysis found no matches between HerbClip citations and retracted literature. Overall, less than 2% of the articles from the Retraction Watch database were related to herbal medicine. As a result of this work, a method has been developed for evaluating the reliability of scientific journals based on the quality of their citations.

PB-26: Vitamin D Variants as PXR Activators: Integrating Computational and *In Vitro* Approaches for Mechanistic Insight

Larson Jr G [1], Neal WM [2], Khan SI [1,2], Gurley BJ [2], Khan, IA [1,2], Chittiboyina AG[1,2]

[1]Department of Bio-Molecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA;[2] National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, MS 38677, USA

Vitamin D−a fat-soluble secosteroid−is essential for calcium homeostasis, skeletal integrity, immune modulation, and muscle function. The increasing prevalence of indoor, sedentary lifestyles combined with widespread supplementation and growing prescription use has heightened concern over maintaining optimal vitamin D levels. According to updated Endocrine Society guidelines, vitamin D insufficiency is defined as serum levels below 20 ng/mL (50 nmol/L) and sufficient levels range from 30 to 50 ng/mL. Endogenous synthesis is initiated in the skin, where ultraviolet B radiation converts 7-dehydrocholesterol to cholecalciferol (vitamin D_3), which is subsequently hydroxylated in the liver and then kidneys to form calcitriol (1,25-dihydroxyvitamin D_3), the biologically active metabolite. Beyond its established signaling through the vitamin D receptor (VDR), emerging evidence indicates that vitamin D and its variants may also interact with other nuclear receptors, including the pregnane X receptor (PXR)−a key regulator of xenobiotic metabolism. Given the structural and functional similarity between VDR and PXR (e.g. the large, promiscuous ligand binding domain (LBD) of PXR), we hypothesized that PXR may serve as an off-target mediator of vitamin D interactions. These interactions could activate downstream pathways, inducing cytochrome P450 enzymes and transporters that modulate drug metabolism. This raises concerns about exposure to the risk of clinically significant drug-drug interactions (DDIs), particularly in patients receiving concomitant medications. Computational docking studies using an agonist bound PXR structure (PDB ID: 1NRL) in the Schrödinger Glide program demonstrated that calcitriol exhibited favorable binding within the PXR LBD. This was supported by GlideScore and binding free energy analyses. These findings provide a mechanistic framework linking vitamin D to PXR-mediated xenobiotic regulation and suggest a potential basis for increased DDI risk. To validate computational predictions, *in vitro* PXR activation assays were conducted using vitamin D and its variants. By integrating *in vitro* and *in silico* data, a more comprehensive evaluation of vitamin D/PXR interactions was constructed. Further *in vitro* experiments and randomized/controlled clinical studies are required for validation of the hypothesis and will help to clarify its physiological and clinical relevance.

Acknowledgments: This research in part was partially supported by “Science-Based Authentication of Botanical Ingredients” funded by the Center for Food Safety and Applied Nutrition, US FDA grant number 5U01FD004246.

PB-27: Discovery of Cannabidiol Analogs as Dual 5-HT_4 Receptor and Voltage-Gated Sodium Channel Antagonists

Chaturvedi K [1,#], Osman AG [2,#], Pandey P [2], Annam SCVAR [2], Elhendawy M [1], Radwan MM [2], Godfrey M [1], Khan IA [2,3], Chittiboyina AC [1,2,3]*

[1] Department of Chemistry and Biochemistry, University of Mississippi, University MS 38677, United States; [2]National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University MS 38677, United States; [3] Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University MS 38677, United States.

Cannabidiol (CBD) is a non-intoxicating compound with effects that extend beyond cannabinoid receptor signaling, including interactions with serotonin pathways. While its activity at the 5-HT_1A receptor is established, its engagement with other serotonin receptor subtypes remains less studied. To explore this, we synthesized a set of CBD analogs and evaluated their activity across multiple serotonin receptors. Screening identified selective interaction at the 5-HT_4 receptor for several compounds. Among these, H4-CBD showed improved receptor interaction compared to CBD and demonstrated antagonist behavior in follow-up assays. Given the link between serotonin signaling and neuronal excitability, selected

compounds were further tested for their effects on voltage-gated sodium channel 1.1 (Nav1.1). Both CBD and H4-CBD showed strong inhibition of Nav1.1, with the analog retaining substantial activity. Overall, structural modification of the CBD scaffold enabled enhanced interaction with 5-HT_4 while maintaining sodium channel inhibition. These findings support further exploration of CBD-derived analogs as modulators of pathways involved in neuronal hyperexcitability.

Acknowledgments: This research was supported by several grants: National Institute of General Medical Sciences (NIGMS) grant P30GM122733-01A1 and acknowledge the research support from the Mississippi Medical Cannabis Research Program at the National Center for Cannabis Research and Education, University of Mississippi.

PB-28: Cannabidiphorol (CBDP) as a Dual-Site Negative Allosteric Modulator of the Cannabinoid CB1 Receptor

Pandey P [1], Zagzoog AH [2], Zaharia T [2], Chatterjee S [1], Khan IA [1,3], Paris JJ [3], Laprairie RB [2],[2] Chittiboyina AG [1,3*]*

[1] National Center for Natural Products Research, University of Mississippi, University, Mississippi 38677, United States; [2] College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, Saskatchewan S7N5E5, Canada; [3] Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, Mississippi 38677, United States.

Δ^9-Tetrahydrocannabinol (THC) is widely recognized for its analgesic properties; however, its psychoactive effects continue to limit broader therapeutic application. The identification of cannabidiol (CBD) as a negative allosteric modulator (NAM) of the cannabinoid receptor 1 (CB1R) has highlighted allosteric regulation as a promising strategy to fine-tune cannabinoid signaling. Cannabidiphorol (CBDP), a naturally occurring homolog of CBD isolated from a *Cannabis sativa* chemovar (FM2), shares structural similarity with CBD, yet its pharmacological profile remains unexplored. In this study, we evaluated the allosteric activity of CBDP at CB1R. A combination of site-directed mutagenesis, *in vitro* functional assays, and molecular dynamics simulations demonstrated that CBDP behaves as a negative allosteric modulator by interacting with two distinct receptor regions. These include the previously characterized ORG27569 binding pocket and a secondary intracellular site formed by transmembrane helices 1, 2, 6, and 7 along with helix 8. *In vivo* assessment using tetrad behavioral assays and novel object recognition (NOR) testing in male C57BL/6NHsd mice indicated that co-administration of CBDP with THC produced modest effects on cognitive performance compared to THC alone, while maintaining THC-induced antinociception. Overall, these findings suggest that CBDP functions as a dual-site CB1R NAM and represents a potentially useful scaffold for modulating cannabinoid receptor signaling with improved therapeutic profiles.

Acknowledgments: This research was supported by several grants: National Institute of General Medical Sciences (NIGMS) grant P30GM122733-01A1 awarded to P.P., and A.G.C.; GlaxoSmithKline Research Chair in Drug Discovery and Development endowment awarded to R.B.L.; National Institutes of Health (NIH) National Center for Research Resources grant C06RR14503 (facility construction) awarded to School of Pharmacy, University of Mississippi; College of Pharmacy and Nutrition, University of Saskatchewan scholarships awarded to A.H.Z. and T.Z.; CbdMD to study 'Biology and Chemistry of Minor Cannabinoids' awarded to A.G.C. The authors, P.P., and A.G.C. acknowledge the research support from the Mississippi Medical Cannabis Research Program at the National Center for Cannabis Research and Education, University of Mississippi.

PB-29: Challenges in Sea Lice, *Lepeophtheirus salmonis* Management with Natural Compounds: A Joint Approach

Pietrak MR [1], Rehman JU [2], Nanayakkara NPD [2], Khan IA [2] and Polinski M [1]

[1] National Coldwater Marine Aquaculture Center, USDA-Agriculture Research Service, 25 Salmon Farm Road, Franklin, ME 04634. [2] National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677

Sea lice have been a persistent pest for the salmon farming industry for the past 50 years, and economic estimates suggest a total cost to producers of approximately 9% farm gate value in recent years. The USDA National Coldwater Marine Aquaculture Center is in the process of developing integrated pest management strategies that involve selective breeding, cleaner fish, and novel therapeutics to help the industry manage sea lice. To broaden these research efforts, a partnership with the National Center for Natural Products Research at the University of Mississippi has been established to screen natural compounds for their potential to kill larval sea lice.

Salmon is farmed at low temperatures in the ocean, and the biggest challenge is the solubility of the natural compounds in cold saline water. These conditions may reduce compound availability and impact. The extreme tides experienced at the mouth of the Bay of Fundy in Maine and the logistics of treating modern net pen aquaculture cages presents challenges in delivery of natural compounds to freely swimming sea lice copepodids seeking host and adults attached to fish body. Considering these, 15 compounds (A-O) were selected belonging to different chemical groups. These compounds are from plants listed in GRAS list. Compounds were evaluated for toxicity at 20ppm in triplicate setting with 10 copepodids/replicate and mortality was recorded 1hr after exposure. Eleven compounds showed toxicity to copepodid in an hour and confirmed 12hr post exposure data. Five compounds induced 100% mortality in copepodids and rest were partially toxic. Two of the compounds were insoluble. Our baseline studies exhibited potential of natural products in killing sea lice copepodids. Our focus will be enhancing solubility of natural compounds and potentially seeking low split dosages for greater impact in shorter exposure times.

PB-30: Molluscicidal Activity of Cedarwood Oil (*Cedrus deodara*) and Patchouli (*Pogostemon cablin*) against Ramshorn Snails (*Planorbella trivolvis*)

Sehrish, A [1], Rehman JU [1], Nanayakkara NPD [1], Herath HTB [1], Khan IA [1], Griffin MJ [2], Mischke CC [3], Richardson BM [4], Wise DJ [3], Waldbieser GC [4]

[1]National Center for Natural Products Research, University of Mississippi, 1558 University Circle, Oxford, Mississippi 38677, USA. [2] Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Thad Cochran National Warmwater Aquaculture Center, Delta Research and Extension Center, Mississippi State University, 127 Experiment Station Road, Stoneville, Mississippi 38776, USA. [3] Mississippi Agriculture and Forestry Experiment Station, Thad Cochran National Warmwater Aquaculture Center, Delta Research and Extension Center, Mississippi State University, 127 Experiment Station Road, Stoneville, Mississippi, 38776, USA. [4] USDA, Agricultural Research Service, Warmwater Aquaculture Research Unit, Thad Cochran National Warmwater Aquaculture Center, PO Box 38, 141 Experiment Station Road Stoneville, MS 38776.

Biomphalaria havanensis (Family: Planorbidae) snails serve as intermediate hosts for *Bolbophorus damnificus*, a trematode parasite responsible for significant losses in catfish aquaculture in the southeastern United States. Current snail control in catfish ponds relies heavily on copper sulfate ($CuSO_4$), a broad-spectrum, non-selective molluscicide that poses risks to fish health and aquatic ecosystems. Consequently, environmentally benign alternatives are needed. Plant-derived natural products represent promising eco-friendly molluscicides due to their selective toxicity, reduced environmental persistence, and minimal impact on non-target organisms. In collaboration with the National Center for Natural Products Research (NCNPR), the University of Mississippi, Mississippi State University College of Veterinary Medicine, the Mississippi Agricultural and Forestry Experiment Station, and the USDA Warmwater Aquaculture Research Unit, this study evaluated the molluscicidal potential of essential oils of cedarwood (*Juniperus virginiana*) and Patchouli (*Pogostemon cablin*).

Adult *Biomphalaria havanensis* snails reared at the National Center for Natural Products Research (NCNPR) were subjected to lethality bioassays to determine dose- and time-dependent toxicity. Commercially sourced cedarwood (*Cedrus deodara*) and patchouli (*Pogostemon cablin*) essential oils exhibited strong molluscicidal activity at concentrations of 10, 20, and 50 ppm following a 72-hour exposure period. Bioassay-guided fractionation of cedarwood oil identified α-atlantone and trans-α-atlantone as the active compounds, with 93.33% and 100% mortality at 10 ppm, respectively. Similarly, bioassay-guided fractionation of patchouli oil identified patchouli alcohol as the active compound, 100% mortality at 25 ppm. Overall, these essential oils demonstrated promising molluscicidal activity at relatively low doses against *B. havanensis* (Family: Planorbidae) snails.

PB-31: Clinical Pharmacokinetics of Single and Multiple Oral Doses of Feel Free Classic (a Kava/Kratom Beverage) in Healthy Adult Participants: PK Modeling and Steady-State Determination

Ceniceros F [1], Berthold E [2], Crosswhite M [1], Atallah R [3]

[1] Consulticx Sciences Corp., Statesville, NC; [2] Planted in Science LLC, Albuquerque, NM; [3] Botanic Tonics, Tulsa, OK.

Kava (*Piper methysticum*) is a perennial shrub native to Melanesia, Micronesia, and Polynesia, with primary bioactive constituents comprising kavalactones, including kavain, dihydrokavain, dihydromethysticin, methysticin, yangonin, and demethoxyyangonin. Kratom (*Mitragyna speciosa*) is a tropical tree native to Southeast Asia; its principal bioactive constituents are indole alkaloids, led by mitragynine, which is metabolized to the more potent active metabolite 7-hydroxymitragynine. Together with additional kratom alkaloids, these compounds contribute to the overall neuroactive (CNS) pharmacologic profile of kratom-containing products.

Here, we report pharmacokinetic (PK) results from three studies conducted in healthy adult participants: (1) a 72-hour single-dose PK characterization following kratom-only administration; (2) a 72-hour single-dose PK characterization following kava-only administration; (3) a single-dose PK characterization following co-administered kava and kratom and (4) a 6-day repeated-dose study under co-administration conditions to achieve and characterize daily trough concentrations and steady state. Single-dose and repeated-dose PK datasets were integrated using a standard modeling approach to develop and evaluate a long-term exposure model capable of predicting PK profiles across a broad range of kava and kratom constituents under extended-use conditions. Comparative analyses were also performed to assess whether clinically meaningful differences in PK parameters exist between single-agent and co-administration conditions.

Chemistry Aspects of Botanicals

PC-1: Design and Synthesis of Structurally Novel Acylphlorolgucinols against *Cryptococcus* Species.

Saad Y [1,2], George G [1], Khan IA [1,2], Li X-C [1,2]

[1]National Center for Natural Products Research, The University of Mississippi, University, MS 38677, USA; [2]Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA.

Cryptococcus neoformans is an invasive fungus−transmitted through the inhalation of spores−that causes cryptococcosis, an infection commonly associated with immunosuppressive patients with AIDS, cancer, and organ transplants. Increased drug resistance to this pathogen and limited antifungal drugs for effective management of this difficult-to-treat fungal disease highlights the need to discover new lead compounds for drug development in this therapeutic area. 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. In the search of antifungal compounds against this clinically important pathogen, the synthetic acylphloroglucinol 2-methyl-1-(2,4,6-trihydroxy-3-(4-isopropylbenzyl)phenyl)-propan-1-one was identified to show potent *in vitro* antifungal activities against two *C. neoformans* strains and one *Cryptococcus gattii* strain (ATCC 32609). Guided by this scaffold, we strategically designed and synthesized a series of analogues using Friedel–Crafts acylation of phloroglucinol with acyl bromides, followed by alkylation to incorporate diverse acyl and aromatic substituents. The goal was to probe the influence of acyl chain modifications and the aromatic substitutions of the side-chain on antifungal activity. Structure–activity relationship (SAR) analysis showed that antifungal activity was sensitive to modifications on the acyl chain and aromatic ring. The isobutyryl group proved essential for potency, with certain alkyl and halogen substitutions maintaining or enhancing activity. In contrast, heteroatom insertion typically reduced efficacy. Among the synthesized analogues, 2-methyl-1-(2,4,6-trihydroxy-3-(naphthalen-2-ylmethyl)phenyl)propan-1-one emerged as the most potent, with an MIC of 1.2 µg/mL against *C. neoformans*. Collectively, these findings validate acylphloroglucinols as a valuable scaffold for antifungal drug discovery, highlight structural features

that contribute to enhanced antifungal activity, and provide a framework for future optimization toward potent therapeutic agents for the treatment of cryptococcosis.

PC-2: Isolation and Characterization of Chemical Markers in *Pausinystalia yohimbe* Bark

Ospanov M [1], Ali Z [1], Khan IA [1]

[1] National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, MS 38677, USA

Pausinystalia yohimbe (Rubiaceae) is primarily native to the tropical regions of the west coast of Africa. The bark of this plant has long been used by indigenous African communities for its aphrodisiac properties and for treating various conditions such as fever, leprosy, and cough. In modern times, yohimbe is widely available in supplement form, including capsules, standardized extracts, tinctures, and yohimbine HCl products. Popular brands such as Nutricost, Horbaach, Primaforce, NutraBio, GNC, and Amazon Basics offer these supplements for sale. *P. yohimbe* is well-known for its use in addressing sexual health issues in men. Additionally, it is recognized for its stimulant, hallucinogenic, hypotensive, and tonic effects. Traditionally, it has been employed to help manage blood pressure, relieve chest pain, combat fatigue and depression, and act as a local pain reliever. Athletes have also used it as a performance-enhancing aid. Yohimbine, the active compound in yohimbe, is also prescribed for specific medical conditions. However, the use of yohimbe as an unregulated dietary supplement raises safety concerns, and as a result, the FDA placed it on its "unsafe herb" list in March 1997 following numerous reports of adverse side effects associated with its use. In terms of its chemical composition, *P. yohimbe* contains several alkaloids, primarily yohimbine and its isomers, which belong to the indole alkaloid class. These compounds exhibit a variety of biological activities. Research into the plant has led to the isolation and identification of 11 distinct indole alkaloids: yohimbine, *β*-yohimbine, *α*-yohimbine, 19,20-dehydro-17-*α*-yohimbine, alloyohimbine, (16*R*)-dihydrositsirikine, tetrahydroalstonine, corynanthine, corynantheidine, and 3-epiyohimbine (pseudo-*β*-yohimbine). These compounds have been characterized through mass spectrometry (MS) and nuclear magnetic resonance (NMR) techniques, with their properties compared to existing literature for validation.

PC-3: Cyclopeptides from *Annona muricata* Fruits and Their Cytotoxicity Evaluation

Aydoğan F [1], Fronczek FR [2], Khan SI [1,3], Avula B [1], Tatapudi KK [1], Khan IA [1,3], Ali Z [1]

[1]National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University MS 38677, USA; [2]Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70803, USA; [3] Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University MS 38677, USA.

Annona muricata L., commonly known as soursop, graviola, or paw-paw, belongs to the Annonaceae family, which comprises approximately 130 genera and 2,300 species. Native to the warmest tropical regions of South and North America, *A. muricata* has been traditionally used in various cultures as herbal remedies for a wide range of ailments, including fever, diarrhea, dysentery, hematuria, urethritis, asthma, and parasitic and hepatic disorders. The fruit pulp (soursop) is widely consumed either fresh or processed into commercial food products such as juices, ice cream, jams, and yogurts due to its unique flavor and aroma. Preliminary phytochemical studies on *A. muricata* have revealed a diverse array of secondary metabolites, including acetogenins, cyclopeptides, alkaloids, essential oils, phenolic compounds, amino acids, pigments, and vitamins. In this study, we present the detailed isolation and structure elucidation of cyclopeptide derivatives, along with various metabolites from the fruits of *A. muricata* and conduct cytotoxic evaluations of pure compounds.

PC-4: Design and Evaluation of VU730-Based Heterocyclic Analogues: Synthesis, Insecticidal Activity, and SAR Insights for Managing Citrus Psyllid Infestation

Annam, SCVAR [1], Cremades, A[2], Swale, DR[2], Chittiboyina, AG [1,3]

[1]National Center for Natural Products Research; 2 Emerging Pathogens Institute, Department of Entomology and Nematology, University of Florida, FL 32610, United States. [3]Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, United States.

Citrus greening disease is a major threat to global citrus production and is primarily transmitted by the Asian citrus psyllid (*Diaphorina citri*). To identify new chemical agents for psyllid control, a series of novel heterocyclic derivatives was designed and synthesized based on the previously reported active compound VU730. The VU730 core scaffold was assembled via *N*-alkylation, followed by a coupling transformation to introduce structurally diverse substituents, affording a library of twelve analogues that were synthesized, purified, and fully characterized. Single-dose bioassays revealed distinct time-dependent insecticidal effects among the synthesized compounds. At 24 h, compound **4** exhibited the highest early efficacy (~75–80%), comparable to VU730 (~70–75%), indicating a rapid onset of action. By 72 h, selected analogues **3**, **4** and **9** demonstrated pronounced activity (~75–90%), characterized by initial moribundity followed by increased mortality, suggesting sustained and potentially irreversible biological effects. While compound **1** displayed relatively low activity at 24 h but significantly enhanced efficacy at 72 h, indicating a delayed toxic response. The remaining derivatives showed moderate to low activity. Preliminary structure–activity relationship (SAR) analysis indicated that both the identity and positional placement of substituents on the heterocyclic scaffold play critical roles in modulating biological performance. These findings identify promising lead candidates for further optimization and dose–response evaluation and provide a foundation for the development of new chemical agents for managing psyllid infestation in citrus agriculture.

Acknowledgments: This research is supported in part by "Discovery & Development of Natural Products for Pharmaceutical & Agrochemical Applications" funded by the USDA, Specific Cooperative Agreement No. 58-6060-6-015, and by the United States Department of Agriculture - Agriculture and Food Research Initiative awards (2025-70029-44026, PI: Swale). The USDA is an equal opportunity provider and employer.

PC-5: Stereochemistry and Linker Design in Cabazitaxel Derivatives: Targeting *P*-Glycoprotein-Mediated Drug Resistance in Mia PaCa-2 and PC-3 Models.

Annam, SCVAR [1], Pandey P [1], Kirven IA [2], Husain, I [1], Khan, SI [1], Claudio PP [2], Chittiboyina AG [1,3]*

[1]National Center for Natural Products Research, University of Mississippi, University, Mississippi 38677, United States; [2] Cancer Center and Research Institute, University of Mississippi Medical Center, 2500 North State St., Jackson, MS 39216, United States; [3] Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA

P-glycoprotein (*P*-gp)-mediated drug efflux is a major contributor to multidrug resistance (MDR) in cancer, significantly limiting the intracellular retention and efficacy of chemotherapeutic agents. In this study, a series of Cabazitaxel (CBZ) derivatives were synthesized and evaluated for their ability to inhibit *P*-gp activity using Madin-Darby canine kidney (MDCK) and MDR1-MDCK cell models, representing basal and overexpressed transporter conditions, respectively. Several CBZ derivatives exhibited concentration-dependent inhibition of *P*-gp, with (*S*)-configured analogues demonstrating the highest potency, while (*R*)-enantiomers and related derivatives showed moderate to low activity depending on their structural features. Racemic CBZ derivatives demonstrated variable inhibitory profiles, suggesting potential enantiomeric interference effects. Importantly, most compounds showed minimal activity in MDCK cells but significant inhibition in MDR1-MDCK cells, indicating selectivity toward *P*-gp-overexpressing systems associated with drug-resistant tumors. This selective inhibition highlights their potential as targeted modulators of efflux-mediated resistance. Based on these findings, selected CBZ derivatives were further evaluated in pancreatic (Mia PaCa-2) and prostate (PC-3) cancer cell lines to assess their ability to overcome *P*-gp-mediated chemoresistance, where they exhibited moderate antiproliferative activity. Overall, this study demonstrates that structural modification and stereochemical configuration of CBZ derivatives

play a critical role in *P*-gp inhibition and identifies promising candidates for improving therapeutic outcomes in drug-resistant pancreatic and prostate cancers.

PC-6: Synthesis and Applicability of Novel Chemical Probes for the Characterization of Amino Acids

Naqvi A [1], Avula B [2], Khan IA [1,2], Chittiboyina AG [1,2]

[1] National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, MS 38677, USA; [2] Department of Biomolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA

Amino acids are fundamental to biology and medicine, and their importance is amplified by the growing interest in alternative proteins. The 2022 Daily Harvest French Leek Crumble incident, where over 500 consumers reported gastrointestinal issues, including hospitalizations, highlights the potential risks associated with novel plant-based protein sources. The suspected culprit, baikiain, is a non-proteinogenic amino acid (NPAA) found in tara flour. While NPAAs serve various functions in plants, some, like baikiain, hypoglycin A (found in unripe ackee fruit), and L-canavanine (found in alfalfa), can be toxic to humans. Current analytical methods lack the sensitivity and selectivity needed to effectively characterize NPAAs within complex biological matrices. Developing a chiral, fluorescent, halogen-containing probe offers a promising solution. Such a probe would enable separation of D- and L-amino acid derivatives, enhance sensitivity, and minimize false positives in analytical techniques like LC-QToF-MS, ultimately improving NPAA identification.

To address this critical need, we have designed two novel molecular probes, one incorporating a dansyl fluorophore and the other a fluorenyl group. We will detail the successful synthesis and characterization of these probes. Subsequently, we will conduct chemical reactions involving these chiral, fluorophoric probes with all 20 natural amino acids to establish their respective retention indices and characteristic fragmentation patterns for proteogenic amino acids and expanded to several other known NPAAs. Finally, we will apply the resulting mass spectral data to analyze legume-based extracts and novel plant-protein products, enabling the profiling and identification of potentially harmful NPAAs.

PC-7: Triterpenoid Saponins with Rare N-Acetylglucosamine and Sulphoquinovose Units from *Massularia acuminata* Stem Bark

Zulfiqar F [1], Ali Z [1], Avula B [1], Zhao J [1], Chittiboyina AG [1,2], Khan IA [1,2]

[1] National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, MS 38677, USA; [2] Department of Biomolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA.

Massularia acuminata (G. Don) Bullock ex Holy (Rubiaceae) is a tropical plant native to western Africa and locally known as *pako ijebu* or *orin ijebu* in southern Nigeria. The stem of the plant is traditionally used as a chewing stick for oral hygiene, while its decoction or infusion is employed as an aphrodisiac. Preliminary phytochemical screening of the aqueous stem bark extract revealed the presence of alkaloids, saponins, anthraquinones, phenolics, flavonoids, and tannins. However, despite its extensive traditional use and the commercial availability of supplements claiming aphrodisiac and testosterone-enhancing effects, detailed phytochemical investigations of *M. acuminata* remain limited, and its bioactive constituents have not been fully characterized.

In addition to its reputed aphrodisiac properties, previous studies have demonstrated antimicrobial and anti-inflammatory activities of the plant. As part of our ongoing efforts to explore the phytochemical constituents of medicinal herbs, we investigated the stem bark of *M. acuminata*. This study reports the isolation and structural elucidation of 20 triterpenoid glycosides, including 10 previously undescribed compounds. These isolates may serve as valuable chemical and biological markers for the species. Structural characterization of the compounds was achieved using comprehensive spectroscopic analyses, including advanced nuclear magnetic resonance (NMR) spectroscopy and high-resolution electrospray ionization

mass spectrometry (HRESIMS). This work significantly expands the known chemical diversity of *M. acuminata* and underscores the structural diversity and potential chemotaxonomic relevance of secondary metabolites within the genus.

PC-8: The Natural Products Magnetic Resonance Database (NP-MRD): An Essential Database Resource for the Natural Products Community

Abstract