ICSB and ISE 2025 – Joint Meeting

Poster Abstracts

Authenticity, Agronomy, Analytical and Taxonomical Aspects of Botanicals

PA-1

Could wildfires be a threat to plants?

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Wildfire particulates (soot) contain carcinogenic contaminants such as polycyclic aromatic hydrocarbons (PAH) and toxic elements. When wildfire particulates deposit on plants, the contaminants in them could be taken up by plants, making them a potential danger for human consumption and or use. Hence, studying the impact of such particulates on plants is of significant importance. Using tomatoes as a case study, we have exposed plants to soot during a 2- to 3-month period through foliar and soil exposures at 100 and 500 mg/mL levels. Biomass data collected after exposure revealed an increase in leaves, stems and root weights, compared to the control treatment. Besides, elemental analysis using inductively coupled plasma optical electron microscopy performed on soot and plants exposed to the soot showed high levels of toxic elements, namely, aluminum, cadmium, chromium, lead, and titanium, with values respectively at 127.2; 3.7; 41.1. 493.4, 85.8 and 4 ppm. The data from foliar and soil exposures also showed a decrease in the content of nutrient elements such as copper, zinc, calcium, sulfur, iron, potassium, in comparison to the control, with a significant correlation with the levels of exposure. Furthermore, a GC-MS method was developed to identify and quantify PAHs in the soot and the exposed plants. Preliminary data showed high levels of PAHs. These results indicate the possible contamination of plants grown in open nurseries, gardens or farms and used as botanicals, medicinal products and food, due to wildfire events. Previous research has also sown the change in plants chemistry due to soot. Hence, there is an urgent need for solutions to prevent or minimize wildfire-induced decline in the plants safe consumption or use by humans.

PA-2

Misidentification remains common in selected historically substituted Traditional Chinese Medicine herbs

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Various studies have observed that some minimally processed botanicals sold for use in Traditional Chinese Medicine are adulterated or substituted with species having similar appearance and/or traditional uses. In this study, commercial samples of several plants for which aboveground parts are used, and for which Leon & Lin's (2017) reference work reported known substitutes, were examined. All samples of Ma Chi Xian (Portulaca oleracea), Yi Mu Cao (Leonurus japonicus), Ze Lan (*Lycopus lucidus*), and Zi Hua De Ding (*Viola philippica*) were at least mostly consistent with the stated identities, though some contained contaminants. Three of nine samples of Xi Xian Cao (*Sigesbeckia orientalis*) were substituted with species of Lamiaceae and two others were heavily contaminated, one with Asteraceae species. Nine of 11 samples of Jin Qian Cao (*Lysimachia christinae*) were substituted with *Desmodium styracifolium* (Guan Jin Qian Cao), and a tenth was a multispecies mixture. Four of nine samples of Sang Ji Sheng (*Taxillus chinensis*) appeared consistent with that identity, but three contained only stems; two were substituted with probable *Viscum coloratum* (Hu Ji Sheng), one was a mixture of the two genera, and two contained sliced larger woody branches, one labeled Morus. Contrarily, a mixed sample sold as Hu Ji Sheng contained mostly Taxillus and little or no *V. coloratum*. Two samples of Xiang Ru (*Mosla chinensis*) were unidentifiable but were possibly contaminated with Elsholtzia, while one labeled E. cristata possibly contained Mosla. Also, two samples of a large flower, Ling Xiao Hua (Campsis), were seen, both substituted with Paulownia. Mislabeling was common for some examined herbs, and perhaps underestimated in others because products are sold in a condition that makes morphological observation of minor admixtures very difficult.

PA-3

Novel nuclear DNA marker to distinguish between species of the Family Rosaceae

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The nuclear internal transcribed spacer region (ITS) has been frequently used to distinguish between plant species. This marker region has been helpful when analyzed from material, such as fresh tissue, resulting in high quality DNA. In contrast to that, fragmented DNA, often present in processed or aged material, is not a suitable target for this marker region. That is either due to the inability of amplifying PCR products larger than 250 bp from fragmented DNA or due to the overpowering presence of fungal DNA, such as mold, which is often the unwanted target from older plant samples. To overcome the disadvantages of the ITS PCR amplification, alternative nuclear genomic regions are needed to help with species identification. The nuclear gamma tubulin (GT) gene region has been investigated to serve as a useful DNA marker region for (fragmented) plant DNA. Focus was to result in small PCR amplicons >300 bp useful as DNA mini barcodes. The investigated species Malus spp., Prunus spp., Rubus spp., and Fragaria spp. are from the Family Rosaceae.

PA-4

Quantification of adulteration in traded ayurvedic raw drugs employing machine learning approaches with DNA barcode database

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Overexploitation and unscientific extraction of medicinal plants owing to huge market demand has resulted in the depletion of the existing natural resources which demand immediate conservation/ restoration efforts. The Convention on Biological Diversity and the Convention on International Trade of Endangered Species have been established for sustainable conservation by regulating trade in threatened species. Since medicinal plants are usually traded as dried, shredded or powdered plant parts, it is hard to trace the exact identity of endangered species involved in trade, which consequently result in the extinction of wild resources. DNA barcoding offers a novel prospective for taxonomists and a reliable alternative to morphological identification which has greatly transformed the species identification process. Recently, amalgamation of DNA barcoding and Machine Learning Algorithm (MLA) has been reported as precise means for species authentication. In this background, a case study has been conducted to track the illegal trade and chain of custody validation of medicinal plants endemic to the Western Ghats of India. Original as well as traded raw drugs were collected, CBOL recommended standard plastid barcode gene regions and nuclear gene region, were employed for developing the reference library of barcode sequence database. In MLA, DNA barcoding analysis is performed with a reference library sequence of known species (BRM) and query sequence of traded samples. Different supervised learning methods were tested on DNA barcodes with 10-fold cross validation. The best classifier with 100 % accuracy was further utilized for the authenticity of traded samples. Our study could identify illegal trade and rampant adulteration of raw drugs in herbal market and herbal industries. Therefore, besides species authentication, restoration, cultivation and conservation measures have to be initiated to enhance the quality of ayurvedic formulations and to reduce the decline.

PA-5

Truth in the roots: an investigation into Ginseng adulteration 2015-2025

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Ginseng is one of the most widely consumed and researched herbal medicines worldwide. In 2023, retail sales of ginseng dietary supplements grew by 12.6%, reaching $8 million in the mainstream channel in the US (1). The main commercially used Panax species include *P. ginseng* (Asian ginseng), *P. quinquefolius* (American ginseng), and *P. notoginseng* (tienchi ginseng). The prices of ginseng products vary significantly based on the species, age, growth conditions (cultivated or harvested from wild), quality, and provenance. The high demand and taxonomic similarities contribute to both unintentional and economically motivated adulteration. Ginseng adulteration occurs in many ways, such as the marketing of other plant materials as ginseng, substitution of lower-cost species within the Panax genus, adulteration with undeclared aerial parts of ginseng, and admixing previously extracted waste plant material to unextracted root materials (2). As part of the ABC-AHP-NCNPR Botanical Adulterants Prevention Program, this study presents ginseng adulteration data from 2015 to 2025 based on a review of published authentication studies across global markets. Ginseng adulteration has a long history, with most reports dating back to the late 20th century and more recently, but this study focuses on the past ten years to provide a contemporary assessment within the constraints of this presentation. The prevalence of adulteration and the main adulterants in commercial ginseng products are assessed using data from genetic, chromatographic, and spectroscopic analyses. Findings show regional differences in adulteration rates and highlight the importance of appropriate regulations, adequate regulatory enforcement, and fit-for-purpose testing methods to ensure product authenticity.

PA-6

From cultivation to standardization - Improving *Hericium erinaceus* quality control with a research-based approach

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Mushroom-based dietary supplements, especially those derived from *Hericium erinaceus*, or Lion's Mane, are gaining popularity. However, product standardization and quality testing are falling behind; most mushroom supplements are standardized to β-glucan content which has several limitations. Mushrooms are rich in β-glucan, but so are common adulterants such as yeast or growth substrates, meaning fraudulent or low-quality products may pass quality testing. Additionally, β-glucan levels provide minimal insight into the bioactive potential of a supplement. Thus, we developed the first Lion's Mane supplement standardized to a species-specific bioactive compound, Erinacine A. Erinacine A has been linked to improved neurological function in several clinical trials, however until recently a validated standard was unavailable. Since Erinacine A is concentrated in Lion's Mane mycelium, we optimized liquid mycelium cultures to maximize its production. From these cultures, we created a highly purified analytical standard (90%), enabling us to standardize future products to this bioactive compound. We further developed robust UPLC and HPTLC methods for quantifying Erinacine A and identifying *H. erinaceus*, respectively. To validate these methods, parallel validation studies were conducted in the United States and Taiwan. This multi-site verification process demonstrated the analytical procedures' reliability across different laboratory environments. Using these methods, we assessed commercially available products marketed as meeting β-glucan quality standards. Notably, marketed Lion's Mane mycelium products exhibited consistently low concentrations of Erinacine A, frequently falling below levels observed in products labeled as containing only the fruiting body. This study lays the foundation for dietary supplement ingredient optimization, with a focus on mushroom cultivation, extractions, and standardization to enhance bioactive compound content in the final products.

PA-7

Authentication of commercial *Croton tiglium* L. oils used as raw material for peeling formulas

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Croton tiglium L. oil (CO) is a natural matrix with a long history of use in dermatology (aesthetic procedures), as component of different formulas (e.g. Hetter´s, Baker & Gordon), for promoting cellular renewal, and stimulating collagen synthesis. This stimulant activity in the skin can be related to its chemical composition (croton oleic acid, crotonic acid, crotonyl alcohol, crotonyl alcohol bisester, crotonyl alcohol trisester, phorbol esters and others). Authentic samples of CO are often quite expensive. However, recently, several commercial brands can be found in stores and on the internet, raising concerns about quality and authenticity of these commercial products. Thus, development of authentication protocol analysis is important to ensure the authenticity of this raw material, and safety and efficacy of the dermatologic preparations used for peeling procedures. High-performance thin-layer chromatography (HPTLC), and Nuclear Magnetic Resonance (NMR) are robust analytical tools used to probe the chemical profile of natural products material and can be applied in quality control evaluations. Therefore, the objective of this study was to utilize these complementary techniques to study both validated samples and commercial products marketed as containing “pure CO”. Phorbol 12-myristate 13-acetate and Sigma-Aldrich® CO were used as reference standards, and 4 commercial samples were evaluated in parallel. Analyses indicate that some commercial CO products do not present the same chemical profile as the standard. The use of these brands could result in undesirable outcomes during chemical peel procedures. This study demonstrated that the application of analytical techniques could be used to generate a rapid QC protocol for the evaluation of this important natural matrix used as a raw material in dermocosmetics formulas used in aesthetics offices around the world.

PA-8

Botanical products -adulteration causing serious risk to the public health

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In the UAE coronary heart disease (CHD) has emerged as the leading cause of mortality over a 20-year period of rapid socioeconomic development. CHD risk factors of non- insulin-dependent diabetes mellitus (NIDDM), obesity, and hypertension are the most commonly prevalent ailments. In the present scenario, the demand for botanical products is growing exponentially throughout the world during the last few decades. However, the purity of these herbal products exposes the human population to multiple risks and creates major concerns for various health agencies on both national and international levels. Adulteration of botanical products with undeclared synthetic drugs or by mixing the analogs of prescription drugs that are created by replacing or adding functional groups to the original chemical are the recent major problems since they may cause adverse side effects. The illegally added adulterants are frequently anorexic, anxiolytic, and antidepressant pharmaceuticals. As a result, the World Health Organization (WHO); the European Union (EU), and the U.S. Food and Drug Administration (FDA) are expanding their alert to consumers about tainted phytopharmaceuticals that contain undeclared, active pharmaceutical ingredients. The adulterants included prescription medications such as sildenafil and fluoxetine, withdrawn medications including sibutramine and phenolphthalein, and unapproved drugs including dapoxetine and designer steroids. Twenty percent of the adulterated supplements contained 2 or more undeclared drugs, for example, weight loss supplements containing both an anorectic and a laxative. Most supplements adulterated with drugs were marketed as weight loss, sexual enhancement, or sports supplements. Consumers may unknowingly take products laced with varying quantities of approved prescription drug ingredients, controlled substances, and untested and unstudied pharmaceutically active ingredients.

PA-9

In vitro propagation of hemp chemovars

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Whether cannabis is one species or more, is a long-standing topic of debate within the research community. However, according to the current scientific consensus, cannabis is considered as a single but highly variable species, Cannabis sativa L. On the other hand, cannabis can also be classified based on its cannabinoid profile, which is more relevant when the goal is to produce biomass for research and cannabis-based phytopharmaceuticals. Based on the cannabinoids profile cannabis chemovars can be classified in three distinct groups, drug type chemotypes (THC > 0.3%), Intermediate chemovars (THC~CBD), and non-drug type (hemp) chemovars (THC < 0.3%). However, cannabis can also be selectively bred for chemotypes high in other cannabinoids such as Tetrahydrocannabivarin (THCV), Cannabidivarin (CBDV), Cannabichromene (CBC), and Cannabigerol (CBG). According to laws of the USA, drug type chemovars can only be propagated under DEA regulations whereas, hemp can be cultivated under a USDA license, as a commodity. In the present study, screened and selected (based on GC-FID analysis) female plants of high CBD, CBDV and CBG chemovars were mass-propagated using nodal segments containing axillary buds. Disinfected explants were inoculated on Murashige and Skoog's medium supplemented with various concentrations of Thidiazuron (TDZ, a cytokinin) and Indole-3-butyric acid (IBA, an auxin) and compared with metatopolin (mT). Regenerated plants were successfully acclimatized and grown indoor until they achieved the desired vegetative growth then transplanted outdoors in the field alongside with their mother plants. Furthermore, in vitro propagated plants were evaluated for their gas and water vapour exchange characteristics using Li-COR photosynthetic system. Our results show that in vitro propagated plants of C. sativa were morphologically as well as functionally comparable to each other and to their respective mother plants.

PA-10

Analysis of cannabinoids and terpenes in tissue culture raised hemp plants for quality control

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Cannabis is a source of unique group of compounds called cannabinoids. So far, more than 550 constituents have been isolated from cannabis, of which 129 are phytocannabinoids. Among phytocannabinoids, D9-Tetrahydrocannabinol (D9-THC) is reported to be the most psychoactive compound with a wide spectrum of therapeutic potential. On the other hand, cannabidiol (CBD), a non-psychoactive compound is reported to possess promising pharmacological activities such as antiepileptic, particularly for the treatment of intractable pediatric epilepsy. As a plant, cannabis is a dioecious and wind-pollinated species. A significant plant-to-plant variation in its cannabinoids profile and content is observed within a single cannabis variety. For the production of a biomass product with consistent phytocannabinoids profile, sinsemilla (seedless female) plants are preferred. To obtain sinsemilla, male plants are removed from the cultivation site as soon as they appear, female plants with desirable profiles are screened and selected as mother plants for future preparation of cuttings. Selected mother plants are then multiplied by using vegetative propagation or through the use of biotechnological tools including micropropagation. In the present study, screened and selected female plants of CBD, CBDV and CBG chemovars were mass-propagated using nodal segments containing axillary buds. Well rooted plants were acclimatized indoors and eventually transplanted outdoors in the field and cultivated along with mother plants. These plants were successfully grown and harvested at maturity. Biomass samples of each chemovar were collected and analyzed for their cannabinoids and terpenes content using HPLC and GC/MS techniques, respectively. Our results demonstrate that the cannabinoid and terpene profiles of tissue culture-derived plants were consistent among the individual plants and were found to be comparable to those of their respective mother plants.

PA-11

Cannabis sativa production, a regulatory overview of DEA and USDA regulations

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In December of 2018, the 2018 Farm Bill was signed into law. It removed hemp, defined as cannabis plant and derivatives of cannabis sativa with extremely low concentrations of Delta-9 tetrahydrocannabinol (Delta-9 THC, no more than 0.3 percent Delta-9 THC on a dry weight basis), from the definition of controlled substances under the Controlled Substances Act of 1970 (CSA). The 2018 Farm Bill also directed the United States Department of Agriculture (USDA) to establish a national regulatory framework for hemp production in the United States. USDA published a final rule on January 19, 2021, that provides regulations for hemp production and was made effective on March 22, 2021, under the U.S. Domestic Hemp Production Program (DHPP). The State of Mississippi enacted Senate Bill 2725, also known as the Mississippi Hemp Cultivation Act, on June 29, 2020, to allow hemp cultivation in the state by registrants with the USDA. The University of Mississippi has been growing cannabis plants under DEA Registration for many decades and recently started growing hemp under a USDA license. This presentation highlights the lessons learned to comply with these new rules.

PA-12

Micromorphological, histochemical, and elemental characterization of *Bersama lucens* (Hochst.) Szyszyl traditionally used in folk medicine with neuro modulation

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Scientific interest in the traditional use of medicinal plants for managing antidepressant-like condition has driven extensive research into their morphological, histochemical, and elemental properties. This study focuses on Bersama lucens (Hochst.) Szyszyl, commonly employed in folk medicine for their potential mood-enhancing properties in South Africa. Micromorphological analysis using Scanning electron microscope revealed distinct anatomical features, such as trichomes, stomatal structures, and epidermal patterns, which can serve as key taxonomic markers. Histochemical assays using standard methods detected bioactive compounds, including alkaloids, flavonoids, and phenolic compounds, localized in specific tissues, supporting their traditional uses. Elemental analysis using energy-dispersive X-ray Spectrometry (EDX) was identified essential micronutrients and trace elements, such as magnesium, potassium and silicon, known to influence neurological function. These findings collectively offer a strong basis for verifying these plants and comprehending their pharmacological significance, opening the door for additional research into their potential as therapeutic potential in antidepressant-like conditions.

PA-13

Beyond the Label claim: Demystifying Steroidal Lactones to Counter RRT-Driven Adulteration and Assay Inflation in Ashwagandha Root Extracts

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Ashwagandha (Indian Ginseng, *Withania somnifera* (L.) Dunal, Fam. Solanaceae) has been quite popular in the past six years, particularly since COVID-19, because of its adaptogenic properties, which are backed up by numerous scientific studies. According to recent sales data from mainstream or natural channels in the United States, ashwagandha is among the top five herbal supplements. Its efficacy revolves around the withanolides, as an active ingredient which comes under the class of steroidal lactones and their respective glycosides, which ultimately helps manufacturer to standardize their extracts. Only roots are covered in several pharmacopoeia monographs; none of them discuss aerial components in-depth. Because of their comparable chemistry, it provides an additional edge for manufacturer to adulterate the root extract with aerial extract for their own economic benefits. Our current findings, manifests mere way to detect aerial part's presence in root extract even after post treatments which typically eliminates aerial markers. This identification is possible because of a unique marker that is predominantly found in aerials parts but trace amounts only, in roots. However, according to the USP-NF/PF Ashwagandha root dry extract method, this new molecule, unidentified and co-elutes with 12-deoxywithastramonolide(12-DW)(RRT: 0.96), it leads to inflated assays, when we analyze these tainted extracts on the RRT concept. To prevent this there is a potential for creating a monograph on ashwagandha aerial components independently, making it easier to distinguish between the aerial and root portions or revising the chromatographic conditions which elutes the unique peak separately from 12-DW for accuracy in standardization. We intend to discuss our findings and thoughts on the stated observations in more detail during the presentation. We anticipate that our research will spark additional discussion on the subject to improve our knowledge of ashwagandha's root quality.

PA-14

Dietary supplement laboratory quality assurance program (DSQAP) consortium at NIST

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The National Institute of Standards and Technology (NIST), in collaboration with the National Institutes of Health, Office of Dietary Supplements (NIH ODS), initiated the Dietary Supplement Laboratory Quality Assurance Program (DSQAP) in 2007 to provide opportunities for DS testing laboratories to participate in interlaboratory comparison studies aimed at improving analytical measurements of chemical constituents in DS ingredients and products. DSQAP exercises are designed to evaluate methodological advancements and measurement challenges associated with nutritional and toxic elements, fat- and water-soluble vitamins, fatty acids, active and/or marker compounds, and contaminants in samples distributed by NIST. As part of a revamp of NIST QAPs, NIST and ODS are revitalizing DSQAP into a consortium. The consortium's goal is to bring together stakeholders and participants in a more collaborative approach to aid in designing future DSQAP interlaboratory studies that will identify and address measurement and reference material needs. This poster will highlight the future design of the consortium and its benefit to the dietary supplement testing community.

PA-15

Beyond the superfoods of the enslaved

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As part of an ongoing exploration into the trans-Atlantic exchange of tropical ethnobotanical knowledge, we look beyond the foods that were introduced to the Americas with the aid of enslaved African expertise. This review identifies intersections between the pharmacognosy of people forcibly translocated from Africa with the Indigenous peoples and European colonists in the harnessing of neotropical species of pharmaceutical, nutraceutical or cosmeceutical interest. While the medicinal knowledge of the enslaved was both feared and valued by the plantations, their individual contributions were seldom recorded. Outstanding examples can be found by tracing the histories of discovery of the anti-malarial bitterwoods. Perhaps the most celebrated of the these is Quassie Bitters (*Quassia amara*, Simaroubaceae) a native wild plant was used medicinally by Africans in the Guianas, until it was introduced to European colonists (1730s). It was ascribed to Kwasi, a healer from the maroon communities, i.e. is fugitive Africans living alongside Indigenous populations in the forested highlands of the interior. This valuable plant was soon named in his honor by the Dutch. Less well-remembered is Majoe Bitters or Macary Bitters from Picramnia antidesma, another member of the family. It was named for the enslaved woman who made it known to science in Jamaica (1790s). By the same token, a number of therapeutically valuable species must be attributed to anonymous healers of the indigenous people of the Americas. Arriving on the West African coast, sometimes unintentionally, several of these herbs were incorporated into local medicine, but in ways that not come to the attention of the colonial authorities of the day. Other indigenous neotropical remedies have attained global significance, notably the Jesuit Bark from *Cinchona* sp. of Peru, the source of quinine.

PA-16

Rooting out the truth: analysis of Ashwagandha root and leaf extracts

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Ashwagandha (*Withania somnifera*) has been traditionally used in Ayurvedic medicine for its numerous health benefits, such as heart health, sleep aid, and reducing stress and anxiety. The roots of ashwagandha are primarily used and responsible for these health benefits, in particular for the 'active' withanolide compounds. In order to confirm the quality of ashwagandha root raw materials, a study has been underway in differentiating ashwagandha root from leaf, in particular developing the analytical techniques for botanical identification and quantitation of marker compounds, determining the limit of detection (LOD) of leaf, as well as identifying and quantitating the withanolides present, to help rule out adulteration.

PA-17

Americans' perspectives on botanical drugs: insights from a national survey

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To assess American adults' familiarity with and preference for FDA-regulated botanical drugs compared to synthetic or biologic drugs for chronic disease treatment, a nationwide survey was conducted. This online survey was administered from July 18-20, 2023 by Harris Poll to 2,078 U.S. adults aged 18 and older on behalf of Legacy Healthcare, a Swiss biopharmaceutical company developing a botanical drug for the treatment of Alopecia Areata, an autoimmune disease. The survey employed a Bayesian credible interval to ensure data accuracy, with a margin of error of ±2.7 percentage points at a 95% confidence level. Respondents received detailed background information on botanical drugs, including their FDA approval requirements, prior to answering questions about their preferences. The survey revealed that only 27% of respondents were familiar with FDA-regulated botanical drugs while 43% had never heard of FDA-regulated botanical drugs. However, when provided with background information on botanical drugs and presented with the choice of treatment for a chronic illness, 47% expressed a preference for botanical drugs, compared to just 18% who favored synthetic or biologic drugs. Although awareness significantly influenced preferences: 53% of those familiar with botanical drugs preferred them, compared to 38% of those unfamiliar; preference for botanical drug was always two to three-fold higher than synthetic and biological drugs. Additionally, parents expressed a strong preference for botanical drugs for their children, with 50% favoring them versus 14% for synthetic or biologic drugs. Despite the current low level of awareness, Americans demonstrate a clear preference for botanical drugs over synthetic and biological drugs, assuming both are considered safe, effective, and equally priced.

PA-18

Unlocking unculturable bacterial diversity to fight human and agricultural challenges.

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Microbes represent a significant resource and are a reservoir of untapped chemicals that can be repurposed for human needs. However, only 1% of microbes can independently grow in laboratory conditions to be extracted and screened for biological activity. The remaining 99% of species found in nature can only survive in communities or specific conditions otherwise not reproduced by standard laboratory methods.Tapping into the chemical diversity produced by unculturable bacteria can unlock new potential solutions to some of the world's most urgent problems, such as drug-resistant human and agricultural pathogens.

At Bactobio, our mission is to discover new bioactive metabolites from previously uncultured microbes. Our state-of-the-art proprietary platform combines engineering biology, next-generation sequencing, and machine learning to provide access to this vast unexplored biological resource. The integration of advanced computational and genomic strategies with robotic in vitro screening capabilities and high-resolution analytical techniques allows us to rapidly identify, isolate and characterize new candidates for commercial development. With over 4,000 genetically divergent bacteria, we now have the largest collection of novel bacterial species in the world, and novel hit and lead compounds for further development. Here, we provide an overview of our platform to take microbes from soil to laboratory production, isolation and structure characterization novel bioactive compounds.

PA-19

Study registration practices on PROSPERO for medicinal plant extracts and herbal medicines: An Evaluation focusing on the description of the botanical drugs

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PROSPERO is an international prospective registry for systematic reviews of health-related outcomes, reducing duplication and reporting bias in studies. The number of studies evaluating the pharmacological effects, (clinical) efficacy or toxicity of medicinal plant extracts is constantly increasing; however, reporting quality and reproducibility of these studies remain unsatisfactory. The Consensus-based guidelines for the Phytochemical characterization of Medicinal Plant extracts (ConPhyMP), including the open access tool (1, 2), facilitate transparent reporting, reproducibility, and interpretations of studies. Using adapted ConPhyMP guidelines, we assess the reporting quality of 1727 registered studies involving medicinal plant extracts/herbal medicines on PROSPERO from inception 2011 to January 2024 using PRISMA guidelines for search protocols (3) (Figure 1). Out of 65 studies in our detailed analysis, only five report the minimum requirements (i.e., adhering to ConPhyMP guidelines), including reporting of study materials and phytochemical analysis of plant extracts/herbal medicines under investigation. Within 3-5 years of registration on PROSPERO, 83% of the studies remain under review, 13% are discontinued reviews, and only 4% reach publications. For specific medicinal plants, we found that Cannabis sativa L., Curcuma longa L., Zingiber officinale Roscoe, Panax ginseng C.A.Mey., and Gingko biloba L. have the highest number of registered studies on PROSPERO (n= 952, 362, 210, 176, 92, respectively), with n= 92, 32, 17, 2, 4, respectively reaching publications. Our findings underscore the necessity for rigorous assessment and scrutiny during the registration of medical and health-related research involving medicinal plant extracts, particularly for studies with clinical outcomes or the potential to generate evidence-based information. Enhancing the registration process could boost publication rates while improving research quality, reporting and interpretations.

PA-20

USP admission evaluation for European Elder berry ingredients: a new cautionary labeling statement

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European Elder berry, *Sambucus nigra* L. fruit has a long history of traditional use as a cold and flu remedy. The United States Pharmacopeia (USP) has recently published a family of quality monographs including specifications for identity, composition, purity and limits of contaminants for different ingredients derived from the elder berry fruit (i.e., juice concentrate, dry juice, liquid and dry extracts and fruit powder). The USP Dietary Supplements Admission Evaluation and Labeling Expert Committee (DSAEL EC) reviewed reports indicating that type I allergy to elder berry is caused by an allergen with significant homology to ribosomal inactivating proteins. Assessment report on *S.nigra*, fructus by European Medicines Agency (EMA) cited an article mentioning lectins are able to promote the release of histamine from basophils and mast cells. S. nigra agglutinin can cause allergic reactions because it is able to release interleukins IL-4 and IL-13 from human basophils and mast cells. Further, the EMA report identified several adverse events reported to the World Health Organization Uppsala Monitoring Centre (WHO-UMC). Additionally, the Health Canada monograph for *S. nigra* recommends including a cautionary label to warn users to discontinue use if hypersensitivity or allergic reactions occur. Considering this evidence, the DSAEL EC decided to introduce a cautionary statement regarding allergic reactions that reads: “Elder berry may cause allergic reactions. Individuals at high risk of allergic reactions due to preexisting food allergies or other health conditions should consult their healthcare providers before using elder berry products. Discontinue use immediately if hypersensitivity or an allergic reaction occurs.” This presentation will

include a summary of this evaluation and the rationale for the proposal of a new cautionary labeling statement for dosage forms derived from elder berry ingredients.

PA-21

Chemical profiling of mushrooms using integrated analytical techniques

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The standardization of functional mushroom products requires robust analytical frameworks to ensure authenticity and quality. Our research employs an integrated analytical approach—including high-performance thin-layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), iodine–starch colorimetric test, and Megazyme β-glucan assays—to identify and quantify key bioactive markers such as polysaccharides, terpenoids, sterols, phenolics, nucleosides, and amino acids in a range of functional mushrooms. Some of the target species include *Hericium erinaceus* (lion's mane), Ganoderma lingzhi (reishi), *Cordyceps militaris* (cordyceps), and *Inonotus obliquus* sterile conk (chaga). Building on this foundation, we apply the same orthogonal analytical approach to guide the development of stable psilocybin mushroom cultivars. By systematically monitoring chemical profiles–particularly indole alkaloids–under varying cultivation and post harvest conditions, we aim to achieve greater stability and reproducibility in psilocybin mushroom cultivars. This comprehensive approach, combined with the evaluation of multiple cultivation batches and post harvest processing conditions, allows us to establish a detailed reference profile that captures the inherent compositional variability of these species.

PA-22

Ergothioneine—A unique active compound primarily in mushrooms

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Decades of research have shown that functional mushrooms contain antioxidants and immune-boosting compounds. Ergothioneine (ERGO), a sulfur containing betaine amino acid found in mushrooms, has shown health benefit potentials. Humans do not produce ERGO; it is absorbed from the diet via a transporter system known as OCTN1, which is present in high concentrations in tissues with high oxidative stress. It is estimated that 95% of ERGO in the human diet comes from mushrooms.

Mushrooms contain significant amounts of ERGO. Its content is assessed over several samples of six species to determine an average ERGO content for each. Mushroom species that have been previously evaluated for ERGO content include golden oyster, reishi, shiitake, maitake, lion's mane, cordyceps, chaga, tremella, turkey tail, enoki, and porcini.

According to the current research, wild porcini mushrooms are the highest source of ERGO while functional mushrooms, like golden oyster, lion's mane, and cordyceps, contain significant amounts of ERGO themselves. The presence and amount of ERGO is an important marker that can be used with other key fungal constituents to create chemical profiles for different mushroom species.

PA-23

Strategies for the authentication of Mushroom powders by high-performance thin-layer chromatography

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Mushrooms have been widely recognized for their nutritional and medicinal properties. They are rich in various bioactive compounds that contribute to their health benefits. Among these metabolites, β-glucans, dietary fibers, triterpenoids, micronutrients and sterols can be found. Several mushroom species have been identified for their antioxidant, antidiabetic, antibacterial and antiviral potential, and the incorporation of the mushrooms into the diet contributes to the overall well-being. Mushrooms are being consumed in different forms e.g. fresh and dried fruit body, and as a powder supplement among others. The identification of the mushrooms sold as powder form requires the use of molecular, spectroscopic or chemical techniques. High-Performance Thin-Layer Chromatography (HPTLC) stands as one of the most used analytical techniques for botanicals ID testing. Herein, it's described the strategies used to authenticate more than 10 of the most common mushrooms fruiting body including varieties by using advanced HPTLC analysis. Cordyceps species were studied in detail and distinguished using chemometric analysis while Cordyceps militaris was investigated beyond identity and a feasibility study gathering samples from the market was also included. The aim of this work is to demonstrate that qualitative and quantitative HPTLC analysis combined with suitable data evaluation it's likely an unsurpassable tool for routine QC.

PA-24

Fingerprinting *Hammamelis virginiana* L. (American Witch Hazel) distillate fractions by HPLC and HPTLC

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Hamamelis virginiana L. (Hamamelidaceae), commonly known as witch hazel, is a small tree native to the damp woods of eastern North America and Canada. Widely used by the pharmaceutical industry, witch hazel is a key ingredient in skin care products and dermatological treatments for conditions such as sunburn, irritated skin, and hemorrhoids. Despite the numerous reports on the medicinal properties of witch hazel formulations, there is a limited number of studies on the chemical fingerprinting and compound analysis composition of this plant. In this study, a comparative analysis was performed on the solid-phase extraction (SPE) of witch hazel solids distillate using three different SPE cartridges: Superclean Envi Carb PSA, Strata C18, and Strata C8. A gradient of water: methanol was used, ranging from 100% water to 100% methanol (21 fractions). For each SPE cartridge, the fractions were analyzed using High-Performance Thin-Layer Chromatography (HPTLC) to obtain and compare their fingerprint profiles. Also, it was obtained the fingerprint chromatogram of witch hazel solids distillate using HPLC-DAD and compare it with HPTLC profiles for each SPE extraction. Additionally, the fingerprint profiles of three different final witch hazel distillate products were obtained and compared.

PA-25

Integrative approach to quality control of the Brazilian herb 'Carqueja' (Baccharis) using microscopy and HPTLC

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This study explores the anatomical and chemical characteristics of six Baccharis species named *B. articulata*, *B. myriocephala*, *B. pentaptera*, *B. riograndensis*, *B. sagittalis*, and *B. trimera*. Frequently referred to “as carquejas”, these species possess winged, aerial photosynthetic stems (cladodes) and are traditionally used to address digestive and diuretic ailments. These species are very similar morphologically and identification poses a problem for taxonomists. The research aimed to provide identification of these commonly misclassified species through detailed microscopic and HPTLC analysis. Techniques such as light microscopy, scanning electron microscopy and HPTLC were employed. An HPTLC method based on the TLC one described in the Brazilian Pharmacopeia with some modifications, was developed. The method is not only specific for B. trimera but also discriminate related species. A feasibility study was performed using samples sold as carqueja from Brazil stores and the online US market. Results will be discussed in detail. Key anatomical characters, including trichomes, the presence or absence of oil bodies, and a subepidermal collenchyma layer at the wing margin, were identified as critical markers for species differentiation.

PA-26

Light and scanning electron microscopy of leaves and stems of *Erythroxylum microphyllum* A St.-Hil.

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The genus Erythroxylum P. Browne, the largest within the family Erythroxylaceae Kunth, comprises approximately 240 species. Erythroxylum microphyllum A. St.-Hil., popularly known as coca, cocão and fruta-de-tucano, is a species native to Brazil and found predominantly in the southern and southeastern regions of the country. This study aims to investigate the morpho-anatomical characteristics of E. microphyllum using light and scanning electron microscopy. E. microphyllum is a shrub that can reach up to 80 cm in height. The leaves are diminutive, measuring 4.8 mm ± 0.7 in length and 2.1 mm ± 0.16 in width, with the petiole measuring 1 mm ± 0.0 in length. Microscopically, the leaves are hypostomatic, exhibiting a dorsiventral mesophyll structure, consisting of 3-4 layers of palisade parenchyma and 5-6 layers of spongy parenchyma. Both epidermal layers are uniseriate and covered by a thick cuticle. The midrib, in cross-section, is convex to straight, with a single collateral vascular bundle arranged in an open arc, surrounded by sclerenchymatous fiber caps on both sides. The petiole has an irregular shape, with its vascular bundle exhibiting a similar structure to that of the midrib. The stem displays small, bright dots along its entire length macroscopically. In cross-section, it is rounded, with lenticels present above the epidermis. Sclereids are distributed between the cortex and the vascular bundle, as well as within the phloem. Starch grains are observed throughout the parenchymatic rays and the pith.

PA-27

Applications of trichome morphology and microscopy in authentication and differentiation of three morphologically similar Solidago species (Asteraceae)

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Correct species identification of plants is critical for ensuring the safety of plant raw materials. Microscopy, as an accessible and reliable technique, plays a key role in the identification, authentication and quality control of botanicals. Within the Solidago genus (Asteraceae), identifying closely related species such as S. canadensis L., S. chilensis Meyen, and S. microglossa DC., is challenging due to their similar morphologies. This study aimed to identify anatomical markers that can effectively distinguish and characterize these species. Aerial vegetative organs were analyzed using standard histological and microscopic techniques. This study identified six distinct types of trichomes, namely i) Simple and multicellular non-glandular trichomes, ii) Non-glandular trichomes with an enlarged base and elongated, tapered apical cell, iii) Uniseriate non-glandular trichomes, iv) Biseriate non-glandular trichomes, v) Flagelliform glandular trichomes, and vi) Capitate glandular trichomes. Of these, S. canadensis exhibited trichomes of types i, ii and v, while S. chilensis displayed types i, ii, v, and occasionally vi. In contrast, S. microglossa possessed all six types of trichomes. Additionally, the presence of secretory ducts in the stem pith was only observed in S. canadensis, providing another distinguishing feature. Therefore, the combination of trichome types and the presence or absence of secretory ducts can be used effectively to differentiate between these morphologically similar species.

PA-28

Purification of lectins from different legume seeds (Fabaceae) by PEG 600–ammonium-sulfate aqueous two-phase system and their characterization using mass spectrometry

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Lectins are glycoproteins that can bind reversibly to different carbohydrates and hence used as defense mechanism against pathogens and pests in plants. Due to their sugar binding properties lectins have been explored in various physiological processes and found to have insecticidal action, antibacterial effects and antifungal properties, anti-tumorous, and analgesic properties in humans. Legumes are the major source of lectins among plants. However, they cause anti-nutritional effects like gastro-intestinal problems if ingested unprocessed or undercooked. Food safety is a major concern these days and therefore, the legume lectins are being studied in detail. Traditionally lectins were purified using various protein purification methods and chromatography techniques that are both labor and cost intensive and time consuming. In the present work, lectins were purified from different species of legumes using a two-step method called PEG 600–Ammonium-Sulfate Aqueous Two-Phase System (ATPS) and were characterized by Mass Spectrometry (LC-MS/MS). These techniques provide a valuable tool for rapid purification and identification of anti-nutritional factors such as lectins from commonly consumed foods and may help in investigating food poisoning or outbreaks related to legume or plant protein products.

PA-29

High-throughput quantitative analysis of purine alkaloids in species and hybrids of Ilex by UHPLC-QTOF MS

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In the monogenetic Aquifoliaceae (holly family), Ilex is a speciose angiosperm genus with roughly 600 species and a fossil-pollen record suggesting a cosmopolitan distribution since the late Cretaceous. Ilex species have been used for medicinal applications in Chinese pharmacopeia and consumed traditionally as teas worldwide. Caffeine has been reported in several Ilex species. Little is known about the secondary-metabolite diversity of purine alkaloids within and among species I. guayusa, I. paraguariensis, and I. vomitoria.

To determine the content of purine alkaloids in the leaf and stem parts of I. guayusa, I. paraguariensis, I. vomitoria, and I. vomitoria × paraguariensis hybrids, a sensitive Ultra-High-Performance Liquid Chromatography coupled with Quadrupole Time-of-flight Mass Spectrometer (UHPLC-QTof MS) method was developed to identify and quantify purine alkaloids, namely theobromine, theophylline, theacrine, caffeine, and methylliberine. Caffeine and theobromine were present in the leaves and stems of I. guayusa, respectively, as the highest quantity at 965 and 849 µg/g. Theophylline and theacrine were detected in all species in low quantities. Methylliberine was not detected in any of the species.

PA-30

HRAM-LCMS identification of Coconut (*Cocos nucifera*) and detection of adulterated Coconut water powders in commercially available products

Plowman S and Krzeszowiec M

NOW Foods

Commercially available coconut water powders were analyzed by Q – Exactive Orbitrap MS in positive mode using Full MS-AIF to determine authenticity. A compound with m/z of 1103.40876 was detected with good abundance and was determined to be a suitable marker compound of genuine coconut water. This compound was identified in a different work as a major coconut cytokine, 14-O-{3-O-[β-D-galactopyranosyl-(1->2)-α-D-galactopyranosyl-(1->3)-α-L-arabinofuranosyl]-4-O-(α-L-arabinofuranosyl)-β-D-galactopyranosyl}-trans-zeatin riboside (G3A2-ZR or “gazer”). Water from a fresh coconut was used as standard reference material and bottled coconut water as control; detection of this compound in bottled coconut water control samples served as evidence of stability during pasteurization and would be expected to withstand freeze drying into powder form. This complex and synthetically unavailable molecule could thus identify cases of economically motived adulteration where coconut water has been diluted with water and inexpensive sugars or where coconut water was altogether absent and instead substituted with mixtures of potassium chloride and sugar. Based on a market survey of eleven commercially available samples, 63.6% of coconut water powders were determined to be free of gazer and therefore adulterated.

PA-31

Quantitative LC-QToF-MS analysis of mycochemicals in *Amanita muscaria*, Psilocybe spp., and consumer products

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The psychedelic mushroom market has expanded rapidly due to changing regulations and increasing consumer demand. Product diversity now extends beyond traditional capsules and tablets to include gummies, powders, and confectionery items, complicating quality control efforts. To assess the quality and potential adulteration of Amanita muscaria and Psilocybe cubensis-based products, a validated LC-QToF-MS method was developed. This method focused on five characteristic compounds: ibotenic acid, muscimol, muscarine, psilocin, and psilocybin which are constituents of A. muscaria and P. cubensis mushrooms. Method validation demonstrated satisfactory linearity, precision, and recovery of all five analytes. Psilocin and psilocybin levels ranged from 0.001–1.6% and 9.9–19.3%, respectively, in five Psilocybe species samples, while ibotenic acid, muscimol, and muscarine levels in two samples of Amanita muscaria were 0.03–0.04, 0.01–0.02, and 0.01–0.02%, respectively. By comparing commercial products to authentic samples, we evaluated the overall quality of 27 across various formulations. Our analysis included 14 gummies, three chocolates, six capsules, one tablet, and three powders. While 11 of 14 gummies claimed to contain Amanita mushroom extracts, only muscimol and muscarine were detected, without ibotenic acid. Interestingly, one gummy product indicated the presence of psilocin and psilocybin despite the labeling that claimed, “no psilocybin.” Eleven products contained psilocin and psilocybin as anticipated, but five products lacked all target compounds. These findings underscore the need for standardized product specifications. Nevertheless, the established LC-QToF-MS approach could serve as a valuable tool for evaluating the quality of magic mushroom-based consumer products.

PA-32

Comprehensive profiling of free protein and nonprotein amino acids in common legumes Using LC-QToF: Targeted and non-targeted approaches

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Legumes, a dietary staple for centuries, have seen an influx of conventional and unconventional varieties to cater to human care-conscious consumers. These legumes often undergo pre-treatments like baking, soaking, or boiling to mitigate the presence of nonprotein amino acids (NPAAs) and reduce associated health risks. The recent tara flour health scare, linked to the NPAA baikiain, emphasizes the need for robust analytical methods to ensure the safety and quality of both traditional and novel plant-based protein alternatives. While traditional techniques provide insights into protein and nonprotein amino acid profiles, modern liquid chromatography-mass spectrome-try (LC-MS) offers superior sensitivity and specificity for NPAA detection. This study employed an LC-QToF method with MS/MS analysis to comprehensively map the distribution of free NPAAs and PAAs (as their AQC derivatives) in various legume samples. A total of 47 NPAAs and 20 PAAs were identified across the legume samples, with at least 7–13 NPAAs detected in each sample. Sulfur-containing NPAAs, such as S-methyl-L-cysteine, γ-glutamyl-S-methyl cysteine, and S-methyl homoglutathione, were predominantly found in Phaseolus and Vigna species. Cysteine and methionine were the sulfur-containing PAAs identified. Gel electrophoresis and protein quantification were also conducted to understand legume protein composition holistically. This orthogonal approach provides a valuable tool for ensuring the overall quality of plant-based proteins and may aid in investigating food poisoning or outbreaks related to such products.

PA-33

Chemical profiling, characterization, and quantitative analysis of phenolic acids, diterpenoids, and flavonoids in aerial parts of *Andrographis paniculata* using LC-PDA and LC-QToF-MS

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Andrographis paniculata, a popular botanical supplement known as "King of Bitters," has seen increased demand post-COVID-19 due to its potential immune-boosting properties. However, concerns about product quality and authenticity have arisen, as adulteration with inferior materials or single-marker, andrographolide-based quality assurance can compromise the efficacy and safety of these supplements. To address these issues, a comprehensive analytical method using UHPLC-PDA and LC-QToF-MS was developed to quantify and identify diterpenoid lactones, corresponding glycosides, and flavonoid glycosides in plant samples and dietary supplements. The method was validated for precision, accuracy, linearity range, limits of detection (LOD), and limits of quantification (LOQ). The LOD and LOQ were 10–300 ng/mL and 25–1000 ng/mL, respectively. Using both MS ionization modes, along with diagnostic fragment ions and neutral losses produced by collision-induced dissociation, 103 compounds were identified in the A. paniculata samples. These compounds included diterpenoids, flavonoids, corresponding glycosides, and phenolic acids, with fourteen of them being standard compounds. Plant samples, including botanically verified reference material, were analyzed using 14 chemical reference standards to quantify total flavonoid glycosides (compounds 6, 10, and 11; 0.74 ± 0.8 mg/g), diterpenoid lactones (compounds 7, 13, and 14; 14.5 ± 0.5 mg/g), and diterpenoid lactone glycosides (compounds 5, 8, 9, and 12; 5.4 ± 0.9 mg/g). In contrast, supplement products exhibited significant variability in these bioactive compounds. Additionally, a similar discrepancy was observed for the diterpenoid lactone andrographolide (compound 7), a common quality assurance marker, with levels of 0.4–23 mg/g in plants versus 0–278 mg/serving size in supplements. This inconsistency emphasizes the critical need for rigorous quality control and comprehensive analytical methodologies to ensure efficacy and quality.

PA-34

DNA testing of “100% hemp-derived" tetrahydrocannabinol (THC) products

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In 2022, the state of Minnesota enacted a law permitting the sale of adult-use Cannabis products containing THC (tetrahydrocannabinol) provided that the THC is derived from “industrial hemp”. These products circumvent marijuana law by claiming that the plant source meets the statutory definition of “industrial hemp” in having less than 0.3% THC. We previously developed a DNA test to discriminate between predominantly THC-producing Cannabis and predominantly CBD-producing “industrial hemp”. Our work has shown that highly potent CBD-producing Cannabis is genetically not industrial hemp but rather a hemp x marijuana hybrid. We have yet to apply our technology to adult-use Cannabis products where quality assurance and regulatory compliance are questionable. With support from the Cannabis Research Center at the University of Minnesota School of Public Health, we propose to develop protocols and technology capable of evaluating Cannabis product claims.

Biological Aspects of Botanicals

PB-1

Harnessing big data for stroke management: Real-world evidence from Taiwan's national databases

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Stroke, or cerebrovascular accident, is a leading global cause of mortality and long-term disability. In Taiwan, traditional medicine (TM) is widely integrated into stroke management and is covered by the National Health Insurance (NHI) program. The advent of big data from the National Health Insurance Research Database (NHIRD) and the Taiwan Stroke Registry (TSR) provides an unparalleled opportunity to assess the effectiveness of herbal therapies in stroke management. This study leverages these robust databases to evaluate the potential benefits of TM for stroke patients.

We conducted a nationwide, population-based cohort analysis using data from the NHIRD, encompassing detailed claims records for over 23 million individuals insured under Taiwan's NHI program. Additionally, the NHIRD was linked to the TSR, enhancing the depth and accuracy of the analysis. The integration of TM in stroke care demonstrated significant benefits. Patients receiving TM treatments exhibited a lower mortality risk compared to non-TM users. Among TM modalities, acupuncture combined with traditional herbal medicine yielded the most substantial reduction in mortality risk, followed by traditional herbal medicine alone. Acupuncture alone showed a relatively higher risk. The most frequently used herbal formula was Bu-Yang-Huan-Wu-Tang, while Shi Chang Pu (Rhizoma Acori gramini) and Dan Shen (Radix *Salviae Miltiorrhizae*) emerged as the most prescribed single herbs. Notably, among stroke patients with dysphagia, those receiving TM treatment exhibited a significantly lower risk of aspiration pneumonia. In summary, this analysis exemplifies the power of big data in advancing evidence-based herbal medicine studies, highlighting promising traditional herbal prescriptions for future drug development.

PB-2

Inhibition of bacteria and virus-mediated inflammation in the respiratory tract by botanical extracts

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Bacterial and viral pathogens are the major causes of infections and complications in the respiratory system. Bacterial pharyngitis (sore throat) caused by Streptococcus pyogenes is an example of inflammation of the upper respiratory tract (URT) infections. Also, exaggerated inflammatory responses are major underlying causes of viral pneumonia severity and morbidity. As part of our effort to provide pharmacological evidence for the use of botanicals in treating infection-mediated exaggerated inflammation in the respiratory tract, we evaluated dried extracts of selected botanicals on the release of pro-inflammatory mediators in human lung BEAS-2B and human tonsil epithelial (HTEpiC) cells challenged with Streptococcus pyogenes lipoteichoic acid (LTA). Extracts were also investigated for effects on inflammation induced by the synthetic double-stranded RNA (dsRNA), Poly(I:C) in BEAS-2B and (HTEpiC) cells. This presentation will discuss results from studies showing potential benefits of botanicals such as *Garcinia kola* (bitter kola), *Rosmarinus officinalis* (rosemary) *Andrographis paniculata* (andrographis) and *Zingiber officinale* (ginger) in reducing infection-mediated inflammation in lung and tonsil epithelial cells.

PB-3

Evaluating potential anticancer natural compounds for their cardiotoxicity

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Cardiotoxicity induced by chemotherapeutic agents, including certain natural products, is a common adverse effect that increases the risk of morbidity and mortality of patients undergoing chemotherapy. Assessing cardiotoxicity early in drug discovering is essential to ensure the safety of potential anticancer agents and to avoid costly failures in subsequent investigations. In the present study, we aimed to investigate the cardiotoxicity of four cytotoxic secondary metabolites as potential anticancer agents, representing diverse classes of chemicals, including isoliquiritigenin, corchorusoside C, 5,3'-dihydroxy-3,6,7,8,4'-pentamethoxyflavone, and murralongin. The heart-derived H9c2 cells, which exhibit many characteristics of cardiomyocytes, were used to assess the impact of these compounds on the cell viability and mitochondrial functions. The cell viability assay revealed that, at the doses tested, none of these compounds significantly reduced cell viability after 72 hours of treatment, while paclitaxel showed a substantial reduction in cell viability. The observation on cell viability was further supported by the evidence that both the mitochondrial membrane potential (MMP) and superoxide levels in mitochondria were not significantly altered by the test compounds at the tested concentrations. In contrast, carbonyl cyanide m-chlorophenyl hydrazone (CCCP) and antimycin A, which served as positive controls, showed significant changes in the MMP and mitochondrial superoxide levels, respectively. Our studies suggest that these compounds, which demonstrate promising efficacy against cancer cells, exhibit minimal toxicity in H9c2 cells. The results represent a promising avenue for anticancer therapy with reduced risk of cardiotoxicity, warranting further investigation into their mechanism of action and therapeutic potential.

PB-4

Extracts of tolsi and nôti leaves in termites control in Reunion island

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All legislation tends to reduce the use of synthetize biocide in pest control. It's in this context that the craftsman work in pest-control must find new solutions to fight against pests. In particular, the "termidor" synthetic biocide has been prohibited since March 2024 in France and Europe for termites control. However, no alternative solution exists since today. Therefore, ORLAT has conducted a first study to provide leads for a natural biocide from secondary metabolites produced by plants. Two plants in particular were tested: the tolsi (Ocimum tenuiflorum, Lamiaceae) and the nôti (Vitex negundo, Lamiaceae). These two plants were chosen because of their biocidal potential. The tolsi and the nôti have green and purple specimens and the two specimens were studied. A water/ethnaol extracts of both plants were obtained using an Accelerated Solvent Extraction (ASE). These extracts were then subjected to contact tests with three species of termites very present in Reunion's Island: Coptotermes gestroi, Prorhinotermes canalifrons and Crytotermes brevis.

PB-5

The effect of acetone and water solvents on the appearance of metabolites in the organs of Lannea discolor and the evaluation of their *in vitro* antioxidant, and antibacterial activities

Rambau U, and Masevhe NA

Lannea discolor is a widely used medicinal plant in southern Africa, valued for its health-promoting properties due to its phytochemical composition. These bioactive compounds, including hydrolysable tannins, alkaloids, triterpenoids, and flavonoids, contribute to its therapeutic potential. However, scientific research on the plant faces limitations, such as concerns about the safety and efficacy of inorganic solvents and the bioavailability of extracted phytometabolites. The study analyzed Lannea discolor using conventional phytochemical screening, Liquid Chromatography-Mass Spectrometry (LC-MS), and spectrophotometric assays (Folin-Ciocalteu for Total Phenolic Content [TPC] and Aluminium Chloride for Total Flavonoid Content [TFC]). The investigation identified 27 metabolites from leaves, 10 from stems, and 20 from roots using water and acetone extracts. These metabolites included phenolic acid derivatives (e.g., gallic acid, p-coumaric acid glucoside, digalloyl glucose) and flavonoids (e.g., quercetin-3-glucuronide, kaempferol derivatives, procyanidins, and epicatechin). The TPC ranged from 11.31–243.90 mg GAE/g, while the TFC ranged from 31.07–326.76 mg QE/g, varying across plant parts and solvent types. Acetone extracts generally exhibited higher phenolic and flavonoid content than water extracts. In the DPPH antioxidant assay, the most active extract demonstrated an IC50 value of 0.001541 µg/ml, outperforming ascorbic acid (2.5733 µg/ml). Antibacterial assays revealed varying susceptibility among bacteria, with acetone extracts showing superior minimum inhibitory concentrations (MICs) compared to water extracts. This study highlights the potential of Lannea discolor for pharmacological applications, emphasizing the need to explore non-toxic solvents for metabolite extraction and validate the plant's traditional medicinal uses through its chemical profile.

PB-6

Effects of matrix oil on hemp-mediated inhibition of cytochrome P450 2C19 activity: can purified CBD be used as a surrogate for the complex mixture?

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Hemp (*Cannabis sativa*) by definition contains ≤0.3% of THC. Unlike THC, CBD is non-psychotoxic, which contributed to passage of the Farm Bill in 2018. Since then, hemp and other CBD-containing products have become widely available over the counter. CBD is a potent inhibitor of multiple cytochrome P450 (CYP) enzymes in vitro. Whether purified CBD (CBDp) can serve as a surrogate for the complex mixture (hemp) remains unknown. This work aimed to compare the inhibitory effects of CBD in a hemp product (CBDh) with CBDp as an inhibitor of CYP activity in human liver microsomes (HLM). A hemp oil, standardized to CBD (0.45, 4.5, 45 µM), was screened as an inhibitor of CYP1A2, 2C9, 2C19, 2D6, and 3A4 activity in HLM (0.1 mg/mL). CYP2C19 was the most sensitive, prompting a comparison of the inhibition kinetics of CBDh and CBDp against CYP2C19 activity (S-mephenytoin 4-hydroxylation). Based on differences in kinetic parameters between CBDh and CBDp, effects of the matrix oil (MCT) on CYP2C19 activity were tested. Partitioning of CBD into HLM was next tested in oil of different compositions. The mixed inhibition model best described the velocity vs. inhibitor concentration profiles. Vmax of S-mephenytoin and Ki of CBD were similar between CBDh and CBDp conditions (44 pmol/min/mg and 0.14 vs. 0.12 µM, respectively), whereas Km of S-mephenytoin and Kii of CBD were different (6.2 vs. 3.7 µM and 3.81 vs. 1.07 µM, respectively). MCT alone inhibited CYP2C19 activity by 13-21%. Partitioning of CBD into HLM in the MCT environment was 10% lower compared to control. Differences between kinetic parameters under CBDh and CBDp conditions suggest that CBDp may not be a suitable surrogate for CBDh and potentially other oil-based hemp products. These observations may extend to additional oil-based botanical products and may apply to clinically relevant scenarios, including the effects of high-fat meals and fatty liver disease on xenobiotic disposition.

PB-7

Traditional medicine: An indispensable source of bioactive entities

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Traditional Medicine can play important role in our modern Healthcare system and Pharmaceutical industry. Intensive research was done and still needed for verification of the claimed therapeutic potential of plants used Traditionally. The active secondary metabolites present in such plants could serve as drugs or lead compounds. Study of traditional plants could also demonstrate new activities of known compounds. Our investigation of selected Traditional plants in the last decade led to some interesting discoveries. Our phytochemical investigation of Tephrosia purpurea directed by ex-vivo bronchodilator activity using Guinea Pig tracheal muscles resulted in the isolation of seven active compounds including four new compounds. The mechanism of the bronchodilator effect was explored. One of the isolated compounds showed promising anticonvulsant effect. A dose-dependent alleviation of pilocarpine-induced epilepsy was demonstrated with improvement of the mice's locomotor activities. In contrast, the standard drug diazepam significantly augmented the suppression of the mice's locomotor activities induced by pilocarpine. Tephrosia purpurea and one of the active components alleviated pancreatic and lung injuries resulted from Ischemia-reperfusion that offers an attractive therapeutic option for severe acute pancreatitis and its associated acute lung injury.

PB-8

Utilisation of plants for ethnoveterinary needs in Africa: An assessment of research trends and status from 2001-2024

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This study provides a bibliometric analysis of academic publications on medicinal plants in ethnoveterinary practices across Africa from 2001 to 2024. A systematic search of Scopus, Web of Science, and Dimensions identified 190 relevant documents, revealing an annual growth rate of 5.92% in publications, with peaks in 2012 and 2022. Citation trends indicate a decline post-2014, reflecting potential shifts in research priorities or ageing foundational works. The Journal of Ethnopharmacology, Veterinary Parasitology, and Tropical Animal Health and Production accounted for approximately 40% of the journals for the literature publication. Key contributions include Yineger H (2007) as the most cited document, while Eloff JN, Masika PJ, and McGaw LJ emerged as leading authors. The thematic analysis highlighted a focus on traditional veterinary practices, pharmacological properties of plants, and cultural contexts in livestock health. Reference spectroscopy traced the earliest citations to 1738, underscoring the historical depth of the field. Institutions such as the University of Pretoria and Ahmadu Bello University were identified as ethnoveterinary research leaders in Africa, with South Africa, Ethiopia, and Nigeria dominating geographical outputs. Interestingly, Belgium and the Czech Republic were the most cited countries overall, while Nigeria and South Africa led citations within Africa. Limited international co-authorship suggests untapped opportunities for global collaboration. This review offers a comprehensive understanding of African ethnoveterinary research, highlighting its evolution and potential for integrating traditional knowledge into contemporary veterinary practices.

PB-9

Cardiorenal effects of *Baccharis milleflora* in normotensive rats

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Baccharis milleflora (Less.) DC. is a Brazilian native plant used in popular medicine for its diuretic and antihypertensive properties. However, the pharmacological mechanisms underlying these effects continue insufficiently characterized. This study aims to investigate the cardiorenal effects of the ethanol-soluble fraction (ESBM) of *B. milleflora* in normotensive rats. The ESBM were evaluated in both acute and prolonged treatments in normotensive rats. Mechanistic studies were performed to explore the involvement of renal Na+/K+/ATPase, angiotensin-converting enzyme, erythrocyte carbonic anhydrase, as well as the potential roles of bradykinin, prostaglandins, and nitric oxide. LC-DAD-MS analysis identified 33 chemical compounds, including chlorogenic acids, glycosylated phenolic derivatives, C-glycosylated flavones, and O-glycosylated flavonols. No acute toxicity was observed in the treated rats. ESBM confirmed significant diuretic, natriuretic, and potassium-sparing effects. Treatment with ESBM reduced serum creatinine and malondialdehyde levels, while increasing nitrite concentrations, a marker of nitric oxide bioavailability. Notably, pre-treatment with L-NAME, a nitric oxide synthase inhibitor, eliminated the diuretic effects of ESBM. The ESBM exhibited significant diuretic and natriuretic effects, with a potassium-sparing action in both acute and prolonged treatments. These effects are likely mediated by the activation of the nitric oxide-cyclic GMP pathway. This research supports the potential therapeutic use of B. milleflora in clinical settings requiring diuretic intervention.

PB-10

Nutrify Genie: From ideation to clinical validation - revolutionizing ethnobotanical approaches for GI symptoms including irritable bowel syndrome (IBS)

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Nutraceuticals are being increasingly recognized as essential complements to pharmaceutical therapies. Traditional plant-based medicines have long been used, yet their integration into evidence-based treatments has been limited due to a lack of clinical validation and standardized formulations. Nutrify Genie (NG-AI), an advanced artificial intelligence (AI) platform, bridges this gap by transforming ethnobotanical knowledge into scientifically validated, precision-targeted solutions. NG-AI uses a Retrieval-Augmented Generation (RAG) framework that synthesizes ethnobotanical knowledge, clinical research, and pharmacological insights to develop tailored formulations. Key steps in the development process include: 1) Keyword Identification and Mapping: NG-AI identifies a wide range of ethnobotanical sources and maps disease biomarkers to plant compounds with historical efficacy. 2) Clinical Evidence Compilation: The platform compiles data from 100+ scientific sources to evaluate plant-based ingredients based on their documented benefits. 3) Synergy and Pharmacological Analysis: NG-AI assesses ingredient interactions, considering bioavailability (ADME), safety, and pharmacological activity through Structure-Activity Relationship (SAR) analysis. 4) Formulation Optimization: NG-AI suggests optimal dosages and ratios for each ingredient based on disease pathology and clinical data, ensuring efficacy and safety. A clinical trial (CTRI/2021/04/032735) validated the NG-AI-derived IBS formulation, showing: 64.23% improvement in GSRS-IBS scores, 57.2% increase in IBS-QOL scores, and complete resolution of GI symptoms (diarrhea, constipation, bloating, pain, and nausea) in several participants. No adverse effects were reported. By aligning with clinical evidence, pharmacological insights (ADME, SAR), and optimized dosages, NG-AI is pioneering the transformation of ethnobotanical solutions into clinically validated therapeutics.

PB-11

Use of pomegranate extract for improving effects of photoaging

Wu H and Sivamani R

Determine the efficacy of Pomegranate extract (PE) for improving effects of photoaging in healthy women. A 4 week - randomized, double-blind, placebo-controlled clinical study was conducted on healthy subjects, females ages 30 to 70 years with Fitzpatrick skin type I-IV. Eighteen subjects were screened, enrolled, and randomized into 3 groups: 5% PE cream (n=6), 1% PE cream (n=6) and vehicle-placebo (n=6). The 1% PE cream group shows minimal change, while the PE 5% group demonstrates a slight reduction in erythema. Hyperpigmentation decreases in all treatment groups, notably in the PE 5% group. Lightness parameter and wrinkle images remain stable over 4 weeks. Results elucidate the potential of PE 1% and 5% in improving the health of photodamaged skin, specifically targeting hyperpigmentation and erythema. It is suggested that upcoming research efforts consider increasing the number of participants in order to enhance the statistical significance of the results. Subjects reported yellow-brown staining of skin and linens. There was not development of others skin reactions.

PB-12

Integrating network pharmacology, metabolomics and *in vitro* assays to investigate the ethnopharmacological relevance of *Ageratum conyzoides* L.

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Ageratum conyzoides (Family: Asteraceae) is an annual herb considered a weed in India and is used as indigenous medicine in many regions of Asia, South America, and Africa. This study aims to scientifically validate the traditional claims and explore the medicinal importance of *A. conyzoides* L. by identifying the bioactive compounds in the leaf extract (ACLE) through metabolomics and their pharmacological targets through in vitro assays and a network pharmacology-based approach. A total of 48 metabolites were identified by GC-MS-based untargeted metabolomics. Apart from precocene-II, several other compounds of biological relevance, such as Caryophyllene, n-Hexadecanoic acid and phytol, along with a few previously unreported metabolites, were also identified, thus corroborating the use of the plant in traditional medicine. In vitro studies suggested that ACLE not only showed potent antioxidant activity but also demonstrated significant protease and anticoagulant properties. ACLE showed α-fibrinogenolytic activity and additionally led to prothrombin degradation under physiological conditions, suggesting its anti-thrombotic nature. ACLE also showed better antiplatelet activity in comparison to Aspirin at the tested dose under physiological conditions. Further, ACLE also showed the ability to lyse normal as well as denatured mammalian clots in vitro, suggesting its thrombolytic potency. Network pharmacology-based analyses identified 10 bioactive components in ACLE, and 13 action target genes were predicted, a few of which potentially participate in processes including coagulation cascades, platelet activation, and haemostasis. This study provides insight into the pharmacological relevance of *A. conyzoides* L. and its potential ethnomedicinal relevance in treating various haemostasis-associated ailments.

PB-13

Combination therapy: synergism between the constituent plants of selected antimalarial polyherbals improves therapeutic selectivity

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The increasing emergence of resistance to antimalarial drugs and their low structural diversity underscores the need to explore new therapeutic strategies. Combination therapy improves efficacy by synergistic effects that improve therapeutic selectivity and slows down parasite resistance [1]. Hence, the potential of combining plant extracts and/or introducing these into conventional treatment regimens are tools to be systematically explored. In this study, the in vitro antiplasmodial and synergistic interactions between the constituent plant aqueous and ethanol extracts of six selected polyherbals (Nefang, PFC, PFH, PFA, PFT, PFS) were evaluated with the view to assessing the modulating role on their therapeutic selectivity. *In vitro* antiplasmodial activities of polyherbal constituents were evaluated on multidrug resistant P. falciparum strain [2], followed by cytotoxicity screening [3]. Extract interactions were analyzed using an equipotency ratio drug combination approach [4]. The 50% fractional inhibitory concentration (FIC50) and combination indices (CI) were calculated from determined EC50 values. Out of 96 extracts 18 exhibited good antiplasmodial activities (SI > 250). Exhibited fold increases in activity of polyherbal extracts (aqueous; ethanol): Nefang (5; 3), PFC (8; 0.8), PFH (6; 39), PFA (4; 0.3), PFT (1.3; 12.6), PFS (5.7; 0.2), indicating improved therapeutic selectivity, potential efficacy and safety for fold increases ≥ 4. Out of 120 paired extracts, 21 aqueous and 16 ethanol exhibited synergism (CI < 0.8). In summary, observed synergism between constituent plants improved the therapeutic selectivity of the polyherbals, which could be as a result of chemical modifications of bioactives in nature through increased hydrophobicity and reduced toxicity. Metabolomics analysis of the polyherbals will enable the identification of potential bioactive metabolites and inform strategies for prioritization.

PB-14

Influence of phytohormones on the tannins accumulation in *Emilia coccinea* (asteraceae) on Gamborg b5 medium by *in vitro* culture

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Plants constitute a natural reservoir of active substances but are found in low doses. The nature of the substrate and its phytohormone content can contribute to increasing the content of desired metabolites in vitro. In vitro cultures constitute a pathway for high accumulation of secondary metabolites in plant cells. This study aims to optimize in vitro culture conditions for a strong accumulation of tannins in the calluses of Emilia coccinea under the influence of five phytohormones: 3-indole acetic acid (IAA), indole butyric acid (AIB), naphthalene acetic acid (ANA), 6- benzylaminopurine (BAP), 2,4-dichlorophenoxyacetic acid (2,4-D) on Gambourg B5 medium. The leaves were sterilized and the explants were obtained from these leaves using a cutter then inoculated into Petri containers each containing the Gamborg B5 solid culture medium enriched with these phytohormones at concentrations and various combinations. The following parameters were taken during in vitro culture: percentage of infection, percentage of living explant, percentage of callus formation during tillage, size and mass of calluses at the end of culture. The culture duration was 21 days. The analysis of tannin contents was evaluated by dosing with a UV-visible

spectrophotometer in the different media treatments. This analysis showed that, the supply of phytohormones stimulates the production of tannins, the dosage of these tannins made it possible to obtain the highest tannin contents in the M18 media at a rate of 21.20mg/g of MF with a BAP at 0.030mg/L and AIB at 0.1mg/L, M11 at a rate of 15.31mg/g MF in Gamborg B5 medium, while the intial explant had a tannin content of 6.86mg/g of MF. Generally, the tannin contents of calluses from Gamborg B5 medium supplemented with phytohormornes are higher than those produced in nature (p-value < 0.05). This method can therefore be used to boost tannin accumulation in Emilia coccinea using in vitro leaf culture. These results may provide a solid basis for treating cultivated plants to improve their physicochemical performance and bioactivity, which could be of economic interest for their exploitation.

PB-15

Daphne jejudoenosis protects kidney proximal tubular cells against hydrogen peroxide-induced oxidative stress

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Reactive oxygen species (ROS), particularly hydrogen peroxide (H2O2), are significant contributors to oxidative stress and kidney tubular injury. Daphne jejudoensis (DJ), a plant native to Jeju Island, is recognized for its anti-inflammatory properties; however, its effects on kidney diseases have not been extensively studied. This research investigated the impact of DJ extract on H2O2-induced oxidative injury and ROS production in kidney proximal tubular HK-2 cells. Extracts from various parts of the DJ plant significantly improved cell viability in H2O2-exposed cells, with the leaf extract exhibiting a particularly strong protective effect. The DJ leaf extract notably reduced levels of superoxide anions and hydroxyl radicals, thereby mitigating oxidative damage. It also prevented the decrease in superoxide dismutase activity, suggesting an enhancement of the antioxidant defense system. Furthermore, fractions obtained from the 70% ethanol DJ leaf extract, particularly the ethyl acetate (EA) fraction, demonstrated protective effects against H2O2-induced injury. The EA fraction was especially effective in enhancing the activity of key antioxidant enzymes, underscoring its potential for protecting kidney cells from oxidative stress. These findings suggest that *Daphne jejudoensis*, particularly its leaf extract and EA fraction, may serve as a promising natural therapeutic agent for oxidative kidney injury.

PB-16

Toxicity and repellency of (E/Z)-3-butylidenephthalide: A natural compound isolated from *Ligusticum porteri* root extract evaluated against imported Fire Ants (Hymenoptera: Formicidae)

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Imported fire ants (Hymenoptera: Formicidae) are pests of significant urban, agricultural, and medical importance. As a part of our ongoing natural product screening program, we screened (E/Z)-3-butylidenephthalide, a natural compound that was isolated from the ethanolic extract of Ligusticum porteri roots, for its toxicity and repellency against red, black, and, hybrid imported fire ants, using high throughput toxicity and repellency bioassays. (E/Z)-3-butylidenephthalide showed toxicity and repellency against these fire ants. The residual repellency of (E/Z)-3-butylidenephthalide lasted up to 4 weeks. Complete data will be presented in the poster.

PB-17

Anti-inflammatory effects of Tormentil (*Potentilla erecta* (L.) Raeusch., rhizoma) *in vitro* and in an *in vivo* colitis model

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Potentilla erecta (L.) Raeusch., commonly known as Tormentil, is a flowering plant of the Rosaceae family with native distribution across Europe and Western Asia. It has a long history of use for its medicinal properties and preparations from the underground parts were traditionally used to rinse oral cavity ulceration, for toothache and for stomach problems. In Europe today, Tormentil rhizome is used in traditional herbal medicinal products for the symptomatic treatment of mild diarrhoea or minor inflammations of the oral mucosa, exclusively based on long-standing use. A methanol extract of Irish Tormentil rhizome, prepared by Soxhlet extraction, was screened for effects on lipopolysaccharide (LPS) induced cytokine release in phorbol 12-myristate 13-acetate differentiated THP-1 cells. Tormentil extract (100 µg/mL), and its hydrolysable tannin component agrimoniin (100 µM), reduced the production of TNF-α, IL-1β, IL-6 and RANTES. The pharmacological effects of Tormentil are often attributed to the high tannin content of the rhizome. Mechanistically, Tormentil extract treatment and agrimoniin treatment reduced LPS-induced NF-κB phosphorylation while agrimoniin could significantly reduce LPS-induced phosphorylation of JNK and P-38 MAPK in western blot analysis. The Tormentil extract was assessed for disease-modifying effects in a dextran sodium sulfate (DSS) mouse recovery model of colitis. Disease Activity Index (DAI) scores were significantly lower for Tormentil extract (400 mg/Kg) treated mice compared to mice treated with vehicle. The primary goal in IBD management is to induce and maintain remission. This work demonstrates that Tormentil rhizome extract can ameliorate symptoms and aid recovery in a DSS-induced colitis model, an effect likely due to attenuation of gastrointestinal inflammation, and suggests a new avenue for complementary therapy investigation.

PB-18

Studies on the anti-inflammatory properties of diarylnonanoids from *Erica cinera*

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Erica cinerea is noted for its diverse secondary metabolites, including terpenoids, coumarin glycosides, flavonoids and diarylnonanoid glycosides. While some studies indicate its use as a urinary antiseptic and diuretic, there is limited information regarding its traditional use for the treatment of inflammatory conditions. The current study assesses the anti-inflammatory potential of E. cinerea with a focus on identifying the active principals responsible for its effects using a systematic bioactivity-guided approach. At the outset of the study, a crude methanolic extract of E. cinera was prepared and analysed for cell viability and its effects on IL-6, Rantes, TNF-α, and IL-1β using the THP-1 cell line stimulated with LPS which yielded promising results. The extract was then fractionated using various chromatographic techniques, and the resulting fractions were evaluated for cell viability and anti-inflammatory activity. The most active fraction, after extensive chromatography, yielded seven compounds, which were structurally characterised by NMR and mass spectrometry and identified as being coumarin glycosides and diarylnonanoid glycosides. Of these, the diarylnonanoid glycosides significantly suppressed LPS, PAM3CSK4, and IL-1β induced cytokine release, while coumarin glycosides showed minimal activity. Mechanistic studies indicated that the diarylnonanoid glycosides inhibited LPS induced NF-KB phosphorylation, suppressed NF-KB reporter activity, downregulated LPS-induced C/EBPβ expression, and reduced JNK and P38-MAPKs phosphorylation. This research underscores the potential

of the diarylnonanoid class of compounds in E. cinerea as potential therapeutic candidates for the treatment of inflammatory conditions.

PB-19

Vachellia gummifera (Willd.) Kyal. & Boatwr. mitigates UVA-induced photoaging in keratinocytes by inhibiting the p38 MAPK pathway

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Vachellia gummifera (Willd.) Kyal. & Boatwr. is a medicinal plant endemic to Morocco with potential antioxidant properties. The characterization of the aqueous extract of *V. gummifera* using HPLC-MS/MS revealed that the extract is rich in simple phenolics and flavonoid mono-glycosides. We evaluated the aqueous extract of *V. gummifera* using the immortalized human skin keratinocyte cell line (HaCaT) to obtain an insight into its ability to mitigate UVA-induced oxidative stress and the underlying signaling pathway involved in the process. The extract was tested in HaCaT cells with progressively increasing concentrations up to 100 µg/mL, demonstrating no signs of toxicity and confirming its biocompatibility. In addition, the pre-treatment of HaCaT cells with *V. gummifera* extract was able to mitigate the deleterious effect induced by UVA light. Accordingly, the extract at 25 µg/mL was able to reduce intracellular Reactive oxygen species (ROS) levels and counter the depletion of GSH produced by UVA, as determined using DCFDA and DTNB assays, respectively. Later, the *V. gummifera* extract showed the capacity to modulate mitogen-activated protein kinase (MAPK) signaling pathways by reducing the phosphorylation of p38.

PB-20

Advancing wound care: Anti-inflammatory and angiogenic mechanisms of Ya-Samarn-Phlae (YaSP) gauze dressing for diabetic and chronic wound healing

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In Thai medicine, a traditional preparation of Ya-Samarn-Phlae (T-YaSP) is used as an infused oil to treat chronic and diabetic wounds. It is reputed for its antibacterial, antioxidant, and wound-healing properties. Even with its long-standing application, scientific evidence supporting the mechanisms of diabetic wound healing is still scarce. This study aims to develop a novel gauze dressing impregnated with T-YaSP (YaSP) to enhance its practical application and elucidate its mechanisms of action in promoting wound healing in both non-diabetic and type 2 diabetic wounds. Throughout the six-month storage period, the alpha-mangostin content in YaSP remained stable, while curcumin levels experienced a notable decline. Topical application of YaSP exhibited significant anti-inflammatory effects, reducing oxidative stress and inflammation markers. It facilitated wound closure in both diabetic and non-diabetic models. On the seventh day, TGF-β1 and VEGF levels rose, signifying enhanced angiogenesis and granulation tissue formation during the proliferation phase. However, TGF-β1 levels decreased by the eleventh day, consistent with reduced inflammation and improved remodeling. The treatment effectively regulated collagen synthesis, increasing type III collagen in the initial stages and type I collagen later in the healing process. Histological evaluations confirmed decreased inflammation, increased neovascularization, and enhanced collagen production. YaSP serves as a gauze dressing

offering an effective approach for managing diabetic wounds, demonstrating remarkable wound-healing capabilities by controlling excessive inflammation, fostering angiogenesis, and balancing collagen synthesis throughout the remodeling phase.

PB-21

Herb-Drug Interaction Potential of Cannabis

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The *Cannabis sativa* L. (cannabis) has long been used in traditional medicines around the world for treating various conditions. Nowadays, medical cannabis is considered a promising option for the treatment of certain diseases, such as, epilepsy, and chronic pain, etc. The consumption of cannabis for therapeutic purposes has surged globally, imposing an urgent need for understanding its interactions with conventional medications if consumed concurrently. Cannabis contains cannabinoids such as tetrahydrocannabinol (THC) and cannabidiol (CBD), which are known for their pharmacological properties and may significantly affect drug metabolism and transport mechanisms. This study aimed to investigate the herb-drug interaction potential of the extracts of cannabis (CE-1 and CE-2), particularly in terms of their effects on xenobiotic nuclear receptors, such as the pregnane X receptor (PXR) and aryl hydrocarbon receptor (AhR) which are the key regulators of drug metabolizing enzymes and transporters. Additionally, we examined the influence of cannabis extracts on cytochrome P450 (CYP) enzymes, including both CYP inhibition and CYP activation, as well as the impact on membrane transporters like P-glycoprotein (P-gp). Both tested extracts (CE-1 and CE-2) demonstrated considerable activation of PXR and AhR, which could lead to altered expression of drug-metabolizing CYP enzymes and transporters (e.g., P-gp). This may result in pharmacokinetic herb-drug interactions that affect drug efficacy and increase the risk of adverse reactions, highlighting the importance of careful co-administration of cannabis with other medications. Our findings highlight the need for increased awareness among consumers, healthcare providers, and patients regarding the potential herb-drug interactions associated with cannabis use, ensuring safe therapeutic practices. Further research is warranted to establish clinical correlation.

PB-22

Exploring the CYP inhibitory potential of selected medicinal plants: Implications for herb-drug interactions

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Medicinal plants have been traditionally used for therapeutic purposes. In recent years, their consumption has surged in the form of nutraceuticals, botanical drugs, and dietary supplements alongside prescription and non-prescription medications. This trend has raised significant health concerns, primarily due to the possibility of herb-drug interactions (HDI) that may occur when herbs or phytochemicals inhibit cytochrome P450 (CYP) enzymes, leading to altered pharmacokinetics and potential drug toxicity. While a few medicinal herbs, such as Hypericum perforatum, Glycyrrhiza glabra, Camellia sinensis, etc. are known as HDI perpetrators, many others remain unexplored.In our continued quest to ensure the safety of medicinal plants, we have systematically screened hydroethanolic extracts from an extensive collection at NCNPR. This study focuses on evaluating their

interactions with two key drug-metabolizing enzymes CYP2D6 and CYP2C9 by using in vitro enzyme assays. The results indicate that several plant extracts exhibit significant inhibition of CYP2C9 posing a risk of HDI when co-administered with conventional drugs metabolized by this enzyme. Interestingly, the activity of CYP2D6 was not inhibited by most of the tested extracts. Our findings underscore the need for further exploration of HDI, especially for patients using both herbal treatments and conventional medications. It is crucial for healthcare professionals to be aware of these interactions to prevent potential adverse effects in polypharmacy scenarios. This research contributes to deeper understanding of the interactions between medicinal plants and pharmaceutical drugs, further highlighting the importance of integrative approaches in clinical settings to mitigate adverse effects and improve patient safety in the use of combined therapies. Further research on the potential candidates that could be future perpetrators of HDI is warranted.

PB-23

Phytochemistry and bioactivity of two species of aphid-induced Tajikistani *Pistacia vera* L. (pistachio) galls and comparative fruit, gall, leaf, and resin materials

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In Central Asia leaf galls from Pistacia vera (pistachio) have been reported to accumulate tannins to extremely high levels (up to ~50% or more), have been employed traditionally in cardiac and respiratory illnesses, and have been used in natural dyeing for woven rug textiles. An aphid-induced gall (Baizongia pistaciae) of the closely related Pistacia integerrima (syn. Pistacia chinensis ssp. integerrima) is one of the only galls available in international commerce, as an Ayurvedic drug (karkatshringi) used for digestive and respiratory disorders; in cases of diarrhea and vomiting; as a styptic for nosebleeds and as an expectorant; in diabetes mellitus, blood and liver disorders, and cancers; and for snakebites. The high tannin, polyphenol (flavonoid, etc.), and terpenoid content of these galls explains their traditional uses. While a body of research on aphid galls in Pistacia spp. exists, studies of P. vera galls, particularly from Tajikistan and Central Asia, remain limited. This study provides a prima facie phytochemical and bioactivity profile of immature and mature stages for two wild-collected galls (Forda riccobonii and Geoica utricularia) from Tajikistani P. vera, including comparative samples. High-performance thin-layer chromatography (HPTLC), high-resolution LC-MS-MS, and GC-MS confirmed the presence of hydrolysable tannins, flavonoids, and terpenoids, with notable differences between fruit, gall, leaf, and resin samples and even in between gall types. MALDI mass spectrometry imaging of dried gall tissue slices showed spatial localization of hydrolysable tannins and polyphenols. Colorimetric assays were used to measure antioxidant capacity of galls, sample extracts, and their components, and in vitro bioassays with rodent adipocyte and skeletal muscle cell lines were performed to explore their potential for diabetes mellitus. Ongoing studies aim to characterize specific antioxidant and bioactive compounds in Pistacia vera galls and related materials.

PB-24

C. elegans-Based Assays to identify the health benefits and safe doses of food ingredients

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The dual requirements of safety & efficacy testing present significant challenges for advancing the science of food ingredients and functional foods. Traditional approaches to nutritional profiling rely on chemical analysis techniques like mass spectrometry, which cannot directly assess the biological activities of food products in animals. Conventional mammalian testing methods are slow, costly, and increasingly regulated. To address these gaps, we have developed an innovative C. elegans-based high-throughput testing platform, leveraging AI-assisted analysis of high-content imaging data. C. elegans has a short life cycle, high genetic homology with humans, shares many toxicology-relevant cellular pathways, and can be cultured rapidly, but assays have been held back by slow and labor-intensive analysis methods. Our platform evaluates developmental and reproductive toxicity (DART) by quantifying developmental parameters such as length, area, and body volume, and reproductive endpoints such as the number and stages of in utero embryos. Beyond toxicity, our system also measures the efficacy of ingredients, assessing their impacts on lipid metabolism, gut permeability, oxidative stress resilience, and motility. As a proof of concept, we validated our oxidative stress resilience assay using epigallocatechin gallate (EGCG), a well-known antioxidant derived from green tea. The assay showed that EGCG mitigates induced oxidative stress. We also demonstrate the quantification of intestinal permeability and changes to lipid storage. By integrating advanced imaging, AI analysis, and a biologically relevant model organism, our technology offers a cost-effective and scalable solution for rapidly identifying both the health benefits and potential adverse effects of novel food products. This platform has the potential to accelerate the development of safe and effective foods, contributing to the long-term goals of improving human health through innovative dietary solutions.

PB-25

Proximate composition, nutritional analysis, and antilipoxidant and immunostimulatory activities of selected bananas cultivated in Hainan, China

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Banana fruit is well known for its high nutritional value and as a rich source of bioactive compounds. Fenjiao, Plantain, Red banana and Haigongjiao are four selected banana varieties widely cultivated in Hainan, China. Our aim was to investigate their proximate composition, vitamins C and B6, amino acids, minerals, bioactive compounds, anti-lipoxidation endproducts (ALEs) and immunostimulatory activities. Each variety was collected from two different counties in Hainan. Results showed Haigongjiao had the highest level of soluble sugars, proteins, and vitamin C. Ten essential amino acids were present in all the varieties. The ratio of essential to total and to non-essential amino acids in Red banana and Haigongjiao met the WHO/FAO criteria for an ideal protein. K and Mg were the most abundant macro-elements in all samples, Red banana contained much higher Zn and Mn levels than other varieties. The highest levels of carotenoids were detected in Haigongjiao and Red banana, which also presented a much higher level of flavonoids, serotonin and rutin. Red banana exhibited stronger ALEs and immunostimulatory activities, compared to other varieties. These findings demonstrated that Red banana and Haigongjiao varieties possess a better nutritional and health value.

PB-26

Evaluation of glyceroglycolipids for *in vitro* antiviral activity against influenza A (H1N1)

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The University of Mississippi Botanical Dietary Supplements Research Center is focused on evaluating the potential use of Limnospira (formally Arthrospira) to enhance resilience against influenza viral infection. Previously we reported that the fatty acid content on this botanical was significantly correlated with in vitro immune-enhancing activity [Toll-like receptor (TLR)2/TLR1 activation]. Since glyceroglycolipids are the major source of fatty acids in Limnospira, the objective of this study was to evaluate the in vitro antiviral activity of sixteen compounds. Seven of these were isolated from Limnospira [representing sulfoquinovosyldiacylglycerol (SQDG), sulfoquinovosylmonoacylglycerol (SQMG), monogalactosylmonoacylglycerol (MGMG) and sulfoquinovosylglycerol (SQ-glycerol) classes), while nine were commercially obtained [representing monogalactosyldilinolenoylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and SQDG classes]. Antiviral activity was measured by assessing the inhibition of influenza A (H1N1) cytopathic effects on Madin-Darby canine kidney cells, with Ribavirin and Oseltamivir Acid as positive controls. Two compounds exhibited selective antiviral activity and belonged to the SQDG class (compound A exhibited an EC50 = 0.51µg/mL with a selective index > 200, compound B exhibited an EC50 = 6.5µg/mL with a selective index > 15).

PB-27

Cytoprotective effect by mucoadhesivity of *Quararibea funebris* flower mucilage

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The flowers of Q. funebris are known as “Cacahuaxochitl” or “Rosita de cacao” and are used to prepare the pre-Hispanic drink tejate. The flower gives the drink its characteristic flavor and aroma, as well as its viscosity, due to the mucilage content, making it a key ingredient of the drink. The flowers have 6.2 % mucilage. Previous experiments have reported antiulcer properties of some mucilage. In the present work, the gastroprotective effect of mucilage isolated from Q. funebris flowers was evaluated in an animal model of indomethacin-induced damage. Gastric mucosal lesions were assessed by macroscopic alterations. Gastric levels of prostaglandin E2 (PGE2), tumor necrosis factor-alpha (TNF-α), leukotriene B4 (LTB4), glutathione, nitrites/nitrates, and H2S were investigated. Lipid peroxidation and catalase enzymatic activity were also determined. These results suggest that the mucilage has an antioxidant effect and acts as a protective barrier of the gastric mucosa through a mucoadhesive effect that coincided with the rheological studies of this mucilage. The rheological experiments showed that the mucilage of Q. funebris had a mucoadhesive effect with mucin at pH 1.6 and 6.5, presenting a greater mucoadhesive effect at pH 1.6. From this study, it can be concluded that the mucilage of Q. funebris has a potent cytoprotective effect against damage induced by indomethacin, mainly by acting as an antioxidant agent and by forming a protective barrier of the gastric mucosa by mucoadhesion.

PB-28

Cardiovascular and renal benefits of *Baccharis milleflora* (caqueja) in hypertension: molecular mechanisms and therapeutic implications

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Cardiovascular diseases are the leading cause of morbidity and mortality globally, prompting increased interest in complementary therapies, such as medicinal plants, as adjunctive treatments. This study aims to evaluate the cardioprotective effects of the ethanol-soluble fraction derived from the vegetative aerial parts of Baccharis milleflora (ESBM) in hypertensive rats. The plant's cladodes were harvested, and an aqueous extract was prepared via infusion and then treated with ethanol to yield the ethanol-soluble fraction (ESBM). ESBM was analyzed by liquid chromatography coupled with diode array detection and mass spectrometry. Data obtained from mass spectrometry (MS and MS/MS) were processed using the GNPS platform to construct molecular networks, identifying three predominant clusters. Wistar-Kyoto and spontaneously hypertensive rats were assigned to different experimental groups: naive, control (vehicle), hydrochlorothiazide (25 mg/kg), and ESBM (30, 100, and 300 mg/kg). After 28 days of treatment, we assessed renal function, electrocardiographic profiles, blood pressure, mesenteric vascular bed reactivity, biochemical markers, and histopathological changes. Additionally, the molecular pathways involved in the pharmacological actions of ESBM was investigated. Treatment with ESBM at 30 mg/kg resulted in significant antioxidant, diuretic, and antihypertensive effects, as well as the reversal of endothelial dysfunction and left ventricular hypertrophy associated with hypertension. Moreover, the antihypertensive effects of ESBM were abolished when animals were treated with a non-selective nitric oxide synthase inhibitor, a cGMP inhibitor, or a non-selective K+ channel blocker. These findings demonstrate that B. milleflora exhibits both cardioprotective and renoprotective properties when administered chronically to hypertensive rats.

PB-29

Chemical and biological evaluation of nanoencapsulated Brazilian green propolis from the Caatinga biome: antinociceptive and anti-inflammatory effects

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Propolis is a natural product renowned for its medicinal properties and widespread use in both Brazil and the international market. Among the diverse types of Brazilian propolis, green propolis from the Caatinga biome stands out due to its rich biological activities, positioning it as a promising candidate for pharmaceutical development. However, its resinous nature and the low solubility of its components lead to challenges for effective utilization. To address these limitations, nanotechnology offers a powerful tool to enhance its bioavailability and therapeutic potential. Twelve compounds were isolated through different chromatographic techniques, including flavanones (naringenin, 7-O-methyleriodictyol, sakuranetin), flavones (hispidulin, cirsimaritin), flavonols (quercetin, quercetin-3-methyl ether, kaempferol, 6-methoxykaempferol, viscosine, penduletin), and one

chalcone (kukulkanin B). The analgesic and anti-inflammatory properties of green propolis from the Caatinga were evaluated in both its free form (GP) and nanoencapsulated form (GPN). A water-in-oil nanoemulsion was developed using a Span 80 and Tween 80, resulting in a formulation with a particle size of approximately 200 nm and a polydispersity index <0.3. The analgesic activity of GP and GPN was assessed using the formalin test in mice. Anti-inflammatory effects were evaluated via the paw edema test in rats. GPN demonstrated significant analgesic activity in the neurogenic phase of the formalin test at doses of 9 and 27 mg/kg in mice. Furthermore, GPN reduced paw edema from the third hour of observation, exhibiting a comparable effect to the highest tested concentration of GP (90 mg/kg). These findings underscore the potential of Caatinga green propolis, particularly in nanoemulsion form, as a valuable agent in antinociceptive and anti-inflammatory therapies, paving the way for its incorporation into innovative pharmaceutical applications.

PB-30

HepG2 and HEK293 cytotoxicity assays: potential *in vitro* screening tool to detect toxic non-protein amino acids in food products

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New products continue to emerge in the alternative protein and amino acid supplement markets. However, since some of the new products have not traditionally been widely used and/or previously consumed at the label-recommended doses, there may be safety concerns. This potential safety issue was recently exemplified with tara flour, a new plant-based protein ingredient within a food product from Daily Harvest that was recalled due to adverse health events. Tara flour was found to contain baikiain (a non-protein amino acid) and this compound was hypothesized to be a causative agent for the reported adverse health events based in vivo toxicity detected in a mouse model. A major challenge in identifying baikiain was the inability to screen for toxicity because no toxic effects were detected for tara-derived extracts and compounds using various in vitro bioassays. Therefore, the objective of the current work was to develop an in vitro screen for detection of amino acids with potential adverse health effects. Our approach focused on three cell-based assays (MTT assay with HepG2 cells, MTT assay with HEK 293 cells, and glutathione deletion with HepG2 cells) since mechanism of action is not always known. Results indicate that the MTT assays are valuable screening tools, but samples need to be tested at high concentrations (100µg/ml to 10mg/ml). The cytotoxicity IC50 of baikiain (893µg/ml for HepG2 and 62µg/ml for HEK) was about one order of magnitude lower than values observed for the protein amino acids. Not all non-protein amino acids exhibited toxicity (e.g., the IC50 values of the dietary supplement L-theanine was within the range of protein amino acids). Collectively, these data indicate the MTT assays are a suitable in vitro safety screen for testing extracts/fractions from alternative protein food products and amino acid supplements to determine if additional animal studies are needed and potentially help prevent harmful products from entering the marketplace.

PB-31

Identification of LXR Agonists from *Massularia accuminata*

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Phytochemical investigation evaluated the activation of Liver X Receptor (LXR) by compounds derived from *Massularia accuminata* using reporter gene assays in HepG2 human hepatoma cells. HepG2 cells were treated with isolated compounds. Luciferase activity, indicative of LXR activation, was measured after 24 hours. 6 compounds (2, 3, 9, 10, 11, and 13) exhibited LXR agonistic activity (fold change > 2). While compound 1 demonstrated decreased cell viability (IC50 2.79 μM), compound 10 showed comparable LXR activation to known agonists. These findings suggest that compound 10 from *M. accuminata* possesses LXR agonistic properties and may hold promise for the treatment of atherosclerosis.

PB-32

A tri-stage preclinical research on anti-Toxoplasma gondii candidates from *Daphne koreana Nakai*-based Traditional Chinese Medicine

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Toxoplasma gondii (T. gondii), the protozoan that causes toxoplasmosis, is a global public health concern, necessitating the exploration of safer and more potent alternatives to current treatments for T. gondii infection. Submicron emulsions, which are renowned for their ability to enhance drug solubility, hold promise as modern drug delivery systems for enhancing drug safety and effectiveness. Daphne koreana Nakai (D. koreana), an herbal plant that grows in the Changbai Mountain area of China, is used to make traditional Chinese medicines (TCMs) with diverse biological activities, including anti-parasitic activity with unknown effects on T. gondii. In this study, we isolated and identified 28 D. koreana compounds then evaluated their toxicity via MTT assays and screened them for biological activity and druggability using network pharmacology and molecular docking analyses. Notably, daphnetin (DAP) emerged as a promising compound with superior pharmacological properties and druggability, despite its poor solubility in water. To enhance its solubility, DAP was encapsulated within a submicron emulsion-based drug delivery system (DAP@seDDS). Rigorous in vitro prescription screening, quality assessments, and in vivo pharmacological evaluations in a murine model of T. gondii infection demonstrated superior efficacy of DAP@seDDS against T. gondii as compared to unencapsulated DAP monomer. This comprehensive study provides valuable insights into the discovery, druggability assessment, and pharmacological evaluation of submicron emulsion-encapsulated drugs, paving the way for the clinical development of natural drugs derived from TCMs. Our work presents a collection of potential plant derived compounds characterized by potent antifungal activity and enhanced chemical stability against Cryptococcus species.

PB-33

Antifungal Potential of Metabolites from Phytopathogenic fungi *Curvularia* spp.

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The widespread use of pesticides in agriculture has raised concerns due to their environmental impact, development of resistant pest strains, and potential health risks. As a result, interest in safer alternative compounds for pest management is increasing. Fungal secondary metabolites, renowned for their structural diversity and biological activities, have emerged as promising alternatives to synthetic pesticides. The utilization of these natural compounds offers a sustainable approach to managing agricultural pests. We investigate secondary metabolites' chemical composition and biological activities from a Curvularia spp. isolated from an infected apple tree leaf, focusing on their phytotoxic and antifungal properties. Seven compounds tyrosol, phenethyl alcohol, 4-hydroxybenzaldehyde, 3-(4-hydroxyphenyl)propionic acid, 4-(3-hydroxypropyl)phenol, tyramine, and N-(4-hydroxyphenethyl)acetamide—were isolated from the ethyl acetate extract of the fungus, marking the first report of these metabolites in Curvularia species. The ethyl acetate extract demonstrated significant phytotoxicity against Agrostis stolonifera, a monocot, with tyrosol and 4-hydroxybenzaldehyde identified as the active compounds. Additionally, phenethyl alcohol and N-(4-hydroxyphenethyl)acetamide exhibited antifungal activity against Colletotrichum fragariae, the causative agent of strawberry anthracnose. These findings support the potential of Curvularia spp. as a source of bioactive compounds with applications in agriculture and plant disease management for sustainable crop protection strategies.

PB-34

Clinical evidence of the impacts of oligosaccharides versus procyanidins in preventing urinary tract infection (UTI)

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The increasing public interest in cranberry products for UTI prevention, particularly their role in hindering bacterial adhesion to the urinary tract lining, merits scientific scrutiny. Despite the uncertainty surrounding the effectiveness of oligosaccharides or procyanidins in UTI prevention, recent studies highlight the potential of cranberry oligosaccharides in reducing bacterial adherence linked to UTIs. Yet, the detection of these oligosaccharides in urine post-consumption of cranberry juice rich in oligosaccharides remains unverified. To address this, a pilot study in humans was conducted over three days, with the initial day serving as a control and cranberry juice intake on the subsequent days. This study employed chromatographic enrichment and high-resolution mass spectrometry/nuclear magnetic resonance (HRMS/NMR) analysis of urine samples and evaluated their impact on polymicrobial biofilm formation by (Candida albicans, Escherichia coli, and Enterococcus faecalis). Notably, certain oligosaccharides, including a specific octasaccharide (m/z 1218.25), were detected in human urine within 2-4 hours of juice consumption, corroborating earlier findings in pig studies (Coleman, Ferreira et al, J. Nat. Prod. 2019, 82, 589−605). Furthermore, our findings revealed that cranberry juice oligosaccharides selectively impacted E. coli, while procyanidins were

effective against E. faecalis in a pre-formed polymicrobial biofilm. However, neither metabolite showed significant effects on C. albicans. These preliminary results pave the way for a comprehensive investigation into the therapeutic potential and specific UTI-preventive activities of these identified oligosaccharides.

PB-35

Quality assessment and RP-HPLC analysis of phenolic compounds in elderberry flower teas: a comparison of marketed and organically grown samples based on European Pharmacopoeia criteria

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The flowers of the European elder (*Sambucus nigra* L., Viburnaceae) are widely used as herbal tea in Turkey due to their health benefits. In a recent study, quality control analyses were conducted on tea samples marketed as elderflower. Elderflower is included in the positive list in the "Plant List" published by the Ministry of Agriculture and Forestry of the Republic of Turkey. In the study, quality controls of nine samples obtained from herbalists and online sources, as well as one organically grown sample, were examined according to the European Pharmacopoeia 10.0 criteria. The samples were evaluated in terms of quality criteria in the European Pharmacopoeia 10.0 (morphological and microscopic analysis, foreign matter content, loss on drying, total ash content, and thin layer chromatography (TLC)). In addition, the phenolic acid (chlorogenic and caffeic acid) and flavonoid (rutin and hyperoside) contents of the samples were determined qualitatively and quantitatively by the High-performance liquid chromatography (HPLC) technique. Microscopic investigation revealed that not all tissue elements were present in all samples, but elements from several plants and/or organs were discovered. In terms of total ash content and loss on drying, the samples met the pharmacopoeia standards. In half of the samples, microscopic examination revealed the presence of foreign matter (such as multicellular trichomes, dwarf elder (Sambucus ebulus) pollens, and sclerenchyma bundles). TLC and HPLC analysis findings showed that all samples contained chlorogenic acid, but not rutin, hyperoside, or caffeic acid. In summary, the results revealed that all ten samples did not meet the European Pharmacopoeia 10.0 standards. It was concluded that since these teas used for medicinal purposes did not meet the European Pharmacopoeia quality criteria, this situation should be evaluated in terms of public health, and the relevant control mechanisms should be activated.

PB-36

Evaluating the effects of different doses of X-ray -irradiation on bioburden, cannabinoid, terpene, and moisture content of cannabis biomass

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Cannabis has gained recognition not only as a medicinal product for treating various diseases but also as a versatile industrial crop with multiple applications. As an agricultural product, cannabis is susceptible to contamination by environmental pathogens, such as aerobic bacteria, yeast, and mold. It is essential to implement strict controls on the levels of these microorganisms in herbal cannabis products to ensure they meet safety guidelines for human use. To eliminate these risks, X-ray irradiation is increasingly being studied and used as a remediation process. It's a non-thermal process for pathogen control to ensure microbial safety and preserve the quality and potency of the cannabis product, including its chemical and physical properties (e.g., cannabinoids, terpenes, moisture content). X-rays penetrate the plant tissue and disrupt the DNA of any microorganisms present, effectively killing or rendering them harmless. This study was designed to determine the effect of X-ray irradiation specifically looking at how escalating doses of irradiation affect the microbial contamination as well as on the chemical and

physical profiles of the cannabis biomass, particularly cannabinoids, terpenes, and moisture content. Analyses were conducted on the samples before and after irradiation applied at six different doses of X-ray (1000, 1500, 2000, 2500, 3000 and 4000 GRAY). Total Aerobic Microbial Count (TAMC) and Total Yeast and Mold Count (TYMC) met the USP limits for inhalation use at 3000 GRAY. The Bile tolerant Gram-Negative Bacteria was absent at only 4000 Gray. The full cannabinoid profile was measured by GC/FID and HPLC analysis, while terpenes profile and moisture content were determined using GC/MS and Loss on Drying (LOD) methods, respectively.

PB-37

An *in vitro* evaluation of common botanical extracts on carboxylesterase 1 (CES1) catalytic activity

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Carboxylesterase 1 (CES1) is an abundant hepatic drug metabolizing enzyme whose substrates include endogenous compounds and medications from a variety of therapeutic classes. CES1 is a serine hydrolase that hydrolyzes the cleavage of amides, esters, and thioesters. Concerns have been raised over botanical-drug interactions (BDIs) and their potential to impair enzymatic function resulting in therapeutic failures or adverse effects. BDIs have been extensively investigated with the cytochrome P450 family of enzymes; however, CES1 BDIs have remained largely uninvestigated. This study assessed the CES1 in vitro inhibitory effects of 18 commonly used botanical extracts and their major constituents using an established incubation assay and LC-MS/MS analysis. Extracts of ashwagandha, cannabis, saw palmetto, St. John's wort, turmeric, and yohimbe all demonstrated significant reversible inhibition of CES1 metabolism. Individual phytoconstituents (i.e., cannabigerol, curcumin, lauric acid, linoleic acid, hypericin, hyperforin, kaempferol, and tetrahydrocannabinolic acid (THCA)) demonstrated significant reversible inhibition of CES1. THCA was the most potent inhibitor of CES1 and displayed a complete mixed competitive-noncompetitive type inhibition. Cannabis, St. John's wort, and turmeric extracts all displayed inhibition potencies (Ki) of < 1 μM relative to their most abundant constituent. Additionally, curcumin was found to be most stable in 50:50 methanol:water (1% formic acid) and least stable in a phosphate buffer solution (pH = 7.4). Using available pharmacokinetic data, it is anticipated that most extracts and constituents will impact the clearance of CES1 substrate medications. However, further in vitro and clinical studies must be conducted to fully elucidate BDI risk through CES1 impairment.

PB-38

Assessment of Caribbean Medicinal Plants in Primary Health Care

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Caribbean medicinal plants have been used for centuries by indigenous populations, African descendants, and other cultural groups. Many communities rely on these plants for their therapeutic properties playing a key impact in public health.

Research has been carried on to validate scientifically the medicinal properties of many Caribbean plants. The Program of Applied Research to Popular Medicine in the Caribbean (TRAMIL) has played a significant role in the detection, validation, and diffusion of the uses of medicinal plants. These studies involve their pharmacological potential, safety, efficacy, and proper dosage guidelines. TRAMIL is an interdisciplinary program applied to traditional medicine in Caribbean countries that contributes to the rescue of popular knowledge and the protection of plants biodiversity. The primary tool of TRAMIL research is based on the participative ethnopharmacological survey. Its starting point is not in the plants but in the symptoms or health problems, and in the perception of these symptoms by targeted groups. The survey focusses on popular knowledge about medicinal plants. Besides, botanical identification is ensured through collection and submission to appropriate herbaria and generation of vouchers. The plants remedies with significant levels of use are selected and investigated throughout scientific literature review, pharmacological, chemical and clinical tests. More than 422 plants are currently listed in the Caribbean pharmacopeia.

Panama has been part of TRAMIL network since 1982 integrating researchers and health personnel convinced of the usefulness of Caribbean medicinal plants. Ethnopharmacological inventory of medicinal plants has been conducted to Amerindians: Ngäbe-Buglé, Africans from Caribbean Cost (Colon and Bocas del Toro) and mestizos located in Panamanian land.

PB-39

Advancing dermal toxicity assessment for botanicals: Progress from the Botanical Safety Consortium's Dermal Working Group

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The Dermal Working Group of the Botanical Safety Consortium, established in 2023, is focused on evaluating in vitro tools to screen for dermal toxicity of botanicals, including skin irritation, skin sensitization, and phototoxicity. To do this, the team is considering assays that are already available for these endpoints for single chemicals and use data-rich botanicals as case studies to evaluate the suitability of these assays for botanicals. Case studies include poison ivy for skin sensitization, essential oils such as bergamot and cinnamon for skin irritation and phototoxicity, and parsley for multiple endpoints. Negative controls, such as hops and ginseng, are also included to evaluate assay specificity. These botanicals represent a diverse range of chemical constituents and provide a robust test set for assay evaluation. The group is utilizing established in vitro assays, such as the OECD 439 test for skin irritation on reconstructed human epidermis and the OECD 442 series (442C-E) for skin sensitization, which assesses activation of dendritic cells, to determine their suitability for botanical mixtures. Additional testing includes assays like the OECD 432 NRU phototoxicity test and UV absorption analysis. Chemical analyses of the botanicals will be performed to support the identity, and authenticity, and quantify key constituents. By evaluating these assays using the botanical case studies, the working group aims to establish a reliable screening framework for dermal toxicity. These efforts will support regulatory and consumer confidence in the safety of botanical products while advancing the scientific understanding of their composition and toxicity profile.

PB-40

Development of safety evaluation procedures for dietary ingredients used in nutrition and wellness products: addressing the challenges of botanical safety assessment

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The global herbal supplements market has seen astonishing development in recent decades, with forecasts projecting significant growth over the next few years. To ensure that any herbal dietary ingredients (DIs) intentionally added to dietary supplements do not pose a significant risk to public health, a global certification scheme for DI safety assessments is proposed. The initial review includes characterization of the ingredient considering source, manufacturing process, composition and ingredient specifications. Consistent with the ingredient characterization, safety may be demonstrated through identification of an upper safety limit (USL) for ingestion of the DI from any competent global authority. In the absence of an authoritative value, an USL may be derived using core safety data for the DI. However, standard safety evaluation procedures cannot be directly applied to many botanical DIs due to the lack of empirical toxicology data and alternative approaches to safety assessment are required. Detailed compositional information for the DI that would allow for constituents analysis may be considered. Additionally, a history of safe use (HoSU) approach may be considered. HoSU may be established by select competent authorities or substantiated by sufficient history of human ingestion to a significant and diverse population without evidence of adverse effects. This HoSU approach would leverage existing peer-reviewed frameworks that outline the key parameters critical to establishing a HoSU for foods and botanical DIs. If all assessment options have been exhausted due to insufficient data for the subject DI, the proposal of a related DI with sufficient data may be considered provided compositional equivalence is demonstrated. The application of this safety evaluation approach within a global certification decision matrix will address the many challenges of botanical safety assessment to support safe use of botanical DIs in certified dietary supplements.

PB-41

In vitro approaches to predict botanical-induced human liver injury and botanical-drug interactions (BDI): Milk thistle, Yohimbe, and Kava as case studies

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The Botanical Safety Consortium (BSC) aims to improve botanical safety by evaluating the suitability of in silico and in vitro tools to study botanicals. The BSC hepatotoxicity and ADME working groups are exploring tools for safety screening and mechanistic studies. Results from in vitro studies including cytotoxicity, CYP inhibition and induction, transcriptomics, and cell painting using liver microsomes, and 2D and 3D hepatocyte models are presented as case studies. The botanicals studied include Milk thistle, Yohimbe, and Kava. Treatment with Milk thistle did not result in CYP3A4 inhibition or induction, but incubation with or without human liver microsomes resulted in formation of glutathione conjugates with the natural product taxifolin (control). Treatment with Yohimbe did not result in CYP3A4 inhibition or reactive metabolite formation; however, a 3-fold CYP3A4 induction and ~30-fold induction of CYP1A1/1A2 was observed. Kava did not result in CYP3A4 inhibition but multiple reactive metabolites of its major constituent kavalactones were formed and trapped as glutathione conjugates. Treatment of 2D human hepatocytes with Kava extract resulted in a 15-fold induction in CYP3A4 mRNA. Inhibition of CYP3A4 enzyme activity was modeled using an

approach proficient for sub-chronic exposure studies. Milk thistle did not decrease CYP3A4 metabolic activity except when coincident with LDH leakage (cytotoxicity). Yohimbe and Kava extracts inhibited CYP3A4 metabolism at sub-cytotoxic concentrations consistent with scientific literature. This is the first report of the potential for BDI (CYP3A4 and CYP1A2) and aryl hydrocarbon activation associated with Yohimbe. Kava resulted in massive induction of CYP3A4 mRNA in both hepatocyte models, which does not correlate with data from previous clinical studies. Metabolic activation data indicated that Kava forms multiple and abundant electrophilic metabolites with dose-dependent potential to be cytotoxic.

PB-42

Study of the mechanism of drug-induced toxicity produced by açaí-anticancer drug interactions

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Açaí, Euterpe oleracea Mart (Arecaceae), fruit native to the Amazon region has also been shown to have antioxidant and antiproliferative activity. An analysis of the FDA Adverse Event Reporting System (FAERS) suggests a prevalence of the possible interaction of açaí-containing botanical dietary supplements to produce adverse events when taken concomitantly with non-CYP3A4 interactive anticancer drugs. Consequently, our work investigated the pharmacodynamic interactions between açaí botanical dietary supplements (BDS) extracts and non-CYP3A4 interactive anticancer drugs by testing açaí extracts in combination with either methotrexate (MTX) or tamoxifen (TAM) to determine synergy. Cell viability of TAM, MTX, and açaí BDS extract treatments were measured individually across breast cancer cells MCF-7 and MDA-MB-23 and normal breast cell model MCF-10A via MTT assay. Two acidic methanol extracts of BDS, F4AC, and F3AC, along with a methanol extract of açaí pulp, MRME, significantly increased anticancer drug toxicity in combinatorial assays via SynergyFinder Plus. The combination of the acidic methanol extract of one of the BDS (F4AC) showed the highest MTX-induced toxicity via synergistic interactions, specifically with the MCF-10A cell line. This called for further evaluation of the toxicity of the combination of açaí and anticancer drugs. Immunofluorescence microscopy was used with DAPI, Annexin V, and NucFix Red to determine whether the combinations of F4AC and MTX induced apoptosis or caused nuclear condensation of MCF-10A and/ or MCF-7 cells. The images obtained showed an increase in apoptotic and necrotic bodies of MCF-10A cells in the combination. Flow cytometry using Annexin V (apoptosis) and NucFix Red (necrosis) also confirmed that there was a significant increase in apoptotic/late apoptotic and necrotic MCF-10A cells with the combination of F4AC and MTX. Studies are underway to determine the açaí compounds responsible for MTX-induced toxicity in MCF-10A cells.

PB-43

Safety and toxicological evaluation of a standardized Boswellia serrata extract (Wokvel®)

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Boswellia serrata, is a resinous tree native to India. The resin extracted from the Boswellia serrata tree has been used for centuries in traditional medicine, particularly in Ayurveda, for its potential health benefits. The resin contains active compounds known as boswellic acids, which are believed to have anti-inflammatory and pain-relieving properties. The objective of the present study was to investigate potential adverse effects, if any, of a standardized Boswellia Serrata extract (BSE) in rats

following acute and sub chronic administration. The extract was standardized to 44.30 %Total Boswellic acids. The oral LD50 of the extract in Wistar strain rats was found to be greater than 2000 mg/kg. 50 male and 50 female Wistar rats were divided into 6 different groups during randomization. There were 10 animals per sex in groups I to IV (treatment group) and 5 animals per sex in groups V and VI (reversal groups). Groups I and V were dosed with vehicle corn oil as vehicle control (0 mg/kg). Groups II, III, IV & VI were dosed with Boswellia serrata extract (BSE) at different doses (100 mg/kg bw, 300 mg/kg bw, 600 mg/kg bw & 600 mg/kg bw respectively), for 90 days, followed by 28 days recovery phase, without any treatment. All animals survived throughout the observation period without any treatment-related clinical signs, except minimal to moderate salivation in BSE treated groups which was of a shorter duration of 2 to 3 hours without adverse impact on animal health. There were no adverse changes related to test article administration on body weight and feed consumption. The No Observed Adverse Effect Level (NOAEL) of Wokvel®, when administered daily to Wistar rats by oral route for 90 days is the highest dose tested 600 mg/kg body weight. Conclusion: This investigation on Wistar strain rats found that Wokvel® was well tolerated and safe with no adverse effects observed during treatments up to the highest dose tested, 600 mg/kg bw.

PB-44

Protective Effects and Mechanisms of Mongolian Medicine Zhachong Shisanwei Pill Against H_2O_2-Induced PC12 Cell Injury

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Zhachong Shisanwei Pill (ZSP) is a classical Mongolian formula that combines 13 types of Chinese medicinal materials and has been used for treating ischemic stroke (IS) for centuries. However, the underlying molecular mechanisms have yet to be fully elucidated. The aim of this study is to explore potential mechanism of ZSP on nerve cells in cerebral ischemic injury. To simulate the pathological process of oxidative stress following IS, an injury model using PC12 cells was induced with hydrogen peroxide (H_2O_2). Afterward, PC12 cells were treated with ZSP medicated serum at low, medium, and high doses. Various assays were conducted to assess cell viability and oxidative stress indicators, including lactate dehydrogenase (LDH), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), reactive oxygen species (ROS), and mitochondrial membrane potential (MMP). Cell apoptosis was evaluated through morphological assessment and flow cytometry. Additionally, the expression levels of apoptosis-related proteins (Bcl-2, Bax, Caspase-9, Caspase-3, PARP) and signaling pathway proteins (JNK, phosphorylated JNK, ERK, phosphorylated ERK, p38, and phosphorylated p38) were measured using automated western blotting. Our findings indicate that ZSP medicated serum preconditioning improves the condition of PC12 cells injured by H_2O_2. Specifically, it increased cell survival rates and reduced LDH release. Additionally, ZSP treatment decreased ROS levels and MDA content, while enhancing the activity of SOD and CAT in the injured PC12 cells. ZSP also reversed the depolarization of mitochondrial membrane potential and protected cells from apoptosis by modulating the expression of apoptosis-related proteins, including Bcl-2, Bax, Caspase-9, Caspase-3, and PARP. Furthermore, the overactivation of the MAPK signaling pathway due to H_2O_2-induced injury was inhibited, as evidenced by the downregulation of phosphorylated JNK, ERK, and p38 levels.

Mongolian medicine ZSP demonstrates protective effects against H_2O_2-induced oxidative stress and apoptosis in PC12 cells. The underlying mechanism may involve the inhibition of the MAPK signaling pathway, enhancement of antioxidant enzyme activity, reduction of intracellular peroxidation levels, and suppression of intrinsic apoptosis pathways.

Chemical Aspects of Botanicals

PC-1

Eritadenine as a regulator of anxiety disorders: An experimental and docking approach

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Uncertainty persists regarding the specific chemical causal factors and their corresponding behavioral effects in anxiety disorders. Commonly employed first-line treatments for anxiety target G protein-coupled receptors (GPCRs), including inhibitors of monoaminergic systems. Alternatively, emerging natural bioactive strategies offer potential for mitigating adverse effects. Recent investigations have implicated adenosine in anxiety-triggering mechanisms, while eritadenine, an adenosine analog derived from Shiitake mushroom, has displayed promising attributes. This study explores eritadenine's potential as a bioactive substance for anxiety disorders in mice, employing behavioral tests, pentobarbital-sleep induction, and molecular docking. Behavioral test results reveal a pronounced anxiolytic and sedative-hypnotic pharmacological effect of eritadenine. Our findings suggest that eritadenine may modulate locomotor functions mediated by adenosine receptors, with a stronger affinity for binding to A2AAR over A1AR, thus eliciting these effects.

PC-2

Development of proton spin network fingerprints as a tool for dereplication and standardization of the botanical supplement *Centella asiatica*

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Centella asiatica (Apiaceae) has been utilized for centuries in traditional medicine systems in Southeast Asia and Southern Africa, including Madagascar. The pennywort shows preclinical evidence of its therapeutic potential in models of Alzheimer's Disease and other dementias, but the lack of consistency in Centella formulations limits the validation of its medicinal benefits in human trials. Caffeoylquinic acids (CQAs) are pharmacologically relevant metabolites believed to contribute to cognitive enhancement and the neuroprotective effects of *C. asiatica*. Available LC-MS methods are however unable to differentiate the positional isomers of CQAs that are abundant in the botanical extract. Ongoing isolation efforts on C. asiatica plant material provided by the BENFRA Botanical Dietary Supplements Research Center has yielded pentacyclic triterpenes together with two new and five known CQAs. To assist mass spectrometry-based characterization of metabolites, proton Spin Network Fingerprints (pSNFs) have been recorded using selective one-dimensional TOCSY NMR spectroscopy. Results show that individual CQAs have unique pSNFs which can be used to distinguish between CQA regioisomers. A preliminary pSNF library has been created to facilitate the identification of single chemical entities within complex mixtures and extracts. This in-house spin-network fingerprint library is currently being expanded and coupled with mass spectrometric data to complement standardization and quality control efforts towards the development of botanical samples that are suitable for clinical studies.

PC-3

Medicinal Plant and plant compound identification using Artificial Intelligence

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Identifying medicinal plants is a vital and foundational step in the development of products within the field of phytomedicine. Plant identification accuracy is essential, as it not only saves time but also enhances the efficiency of research. When researchers access existing studies and documentation on a specific medicinal plant, it significantly shortens the time required to gather relevant information and insights, allowing for a more focused approach to product development. In recent years, machine learning algorithms have emerged as powerful tools for identifying medicinal plants. These algorithms analyse comprehensive datasets encompassing various medicinal plant characteristics, such as morphology, colour, and texture. Among the different machine learning techniques, deep learning models, especially Convolutional Neural Networks (CNNs), have demonstrated exceptional performance in image recognition tasks. To create a robust identification system, diverse datasets are necessary for training the CNNs. Five medicinal plants were part of the data set to be collected using a cell phone to capture images at different angles. In this context, images of the plants were systematically collected from their natural habitats during different times of day specifically, morning, midday, and afternoon. This approach allows the model to learn how the appearance of plants can change due to variations in light and shadow, enhancing its ability to accurately recognise them in real-world conditions. Moreover, images were captured from multiple angles—including 30%, 60%, and 90% perspectives ensuring that the dataset encompasses a wide array of visual representations for each plant species as displayed in figure 1 below. A design science methodological approach was utilised in system design and machine learning approaches.

PC-4

Microbial metabolism of the monoterpene indole alkaloid vincamine

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Microbial cultures are effective biocatalysts, capable of producing diverse derivatives that are often difficult to obtain from mammals or synthetic methods. This approach has advanced the study of xenobiotic metabolism. Vincamine (VCN), a monoterpene indole alkaloid isolated from Vinca minor L., exhibits various biological activities, including antioxidant, anti-inflammatory, cerebral vasodilatory, and neuroprotective effects. Recent research has also highlighted its potential anticancer properties. This study aimed to isolate and identify vincamine metabolites produced by *Cunninghamella echinulata* ATCC 1382. Initial screening and preparative-scale experiments followed a standardized two-stage protocol. VCN, prepared as a 10% solution in DMF, was introduced into 24-hour-old stage II cultures at a final concentration of 0.1 mg/mL. Substrate and culture controls were included for comparison. After a two-week incubation, the cultures were harvested, and the metabolites were isolated and purified using silica gel column chromatography. Their structures were identified through NMR spectroscopy and X-ray diffraction analysis. The pure metabolites identified included 14-epi-vincamine, 20-hydroxyvincamine, vincamine N-oxide, and 14-epi-vincamine N-oxide. This work underscores the potential of microbial biotransformation as a valuable tool for generating and studying drug metabolites, offering insights into vincamine's metabolic pathways and possible applications in therapeutic development.

PC-5

Macromolecules matter: exploring the potential of plant-based peptides for human health through computational tools

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Plant-based macromolecules, such as proteins and peptides, have been largely overlooked in traditional pharmacognostic investigations. Various factors, including challenging isolation and difficult characterization, have biased scientific focus toward small molecules like alkaloids, terpenoids, etc. With the advent of biologics, the medical paradigm has shifted to consider the value of these once neglected phytoconstituents, particularly concerning human health. For example, a recent study identified a macrocyclic peptide−vodo-C1−as a full agonist of CB2R through in vitro assessment of a Viola odorata extract. Optimization of the peptide structure towards CB2R by chemical synthesis led to vodo-C1-inspired bicyclic loop peptides (vBCLs). Functional assays determined that the vBCLs acted as either negative allosteric modulators (NAMs) or neutral antagonists of CB2R. Since these bicyclic peptides could not be unequivocally established as allosteric or orthosteric CB2R ligands, recent advancements in computational tools afforded a rational approach to predicting the most favorable binding domain for vBCLs at CB2R, in turn elucidating their modulatory properties. First, five binding sites were predicted at the active-state CB2R with Schrödinger SiteMap. Next, using Schrödinger PIPER, vBCLs were favorably predicted to interact with CB2R at the CB2R-G protein binding interface, located at the intracellular surface of CB2R. This region, comprising portions of transmembrane helices 2, 3, 5, and 6, as well as segments of intracellular loops 2 and 3, is critical for inhibitory G protein (Gi) coupling and activation, which leads to downstream signaling. This work proposes that vBCLs bind as NAMs to interfere with CB2R-Gi contacts to prevent activation of the Gi α subunit, preventing the inhibition of several cellular signaling cascades.

PC-6

Synthesis and applicability of novel chemical probes for the characterization of amino acids

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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

Design and synthesis of structurally novel olivetols against *Cryptococcus* species

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Cryptococcus neoformans, a globally distributed pathogenic yeast, is a leading cause of cryptococcal meningitis in individuals with advanced HIV, contributing significantly to AIDS-related mortality despite advancements in care. Olivetol, also known as 5-pentylresorcinol, is a naturally occurring compound derived from lichens and grains like buckwheat, with diverse pharmacological activities such as antioxidant, anti-inflammatory, and antiseptic properties. However, the antifungal activity of olivetol has yet to be explored, especially against Cryptococcus spp. A study on the antibacterial properties of cannabinoids, specifically cannabigerol (CBG), revealed that these compounds exhibit notable antibiotic activity. Studies suggest they may possess promising antifungal properties. Therefore, our work involves mimicking the cannabinoids structure to synthesize cannabigerol like compounds. In our approach, we strategically designed and synthesized olivetol analogs. This involved the incorporation of acyl chains with increasing number of carbons into the olivetol core to access the effect on the activity. This study investigates the structure-activity relationships (SAR) of modified compounds to optimize their biological activity. The analysis focuses on three key aspects: the impact of specific positional modifications on activity, the comparative effectiveness of different acyl groups, and the influence of carbon chain length on steric and lipophilic properties. These findings aim to guide the identification of structural features that enhance the compounds' effectiveness and potential therapeutic applications. These designed compounds are anticipated to exhibit reduced lipophilicity, increased antifungal efficacy, and decreased cytotoxicity.

PC-8

Unraveling the role of vitamin D3 metabolites in PXR activation: A systematic computational analysis

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Vitamin D plays a crucial role in calcium homeostasis, bone health, immune modulation, and muscle function. The increasing prevalence of indoor, sedentary lifestyles, along with widespread vitamin D supplementation and prescriptions, has raised concerns about optimal vitamin D levels, not only due to reduced sunlight exposure but also because of potential drug–drug interactions (DDIs) in patients on prescription medications. According to recent World Health Organization guidelines, vitamin D insufficiency is defined as serum concentrations below 20 ng/mL (50 nmol/L), with normal levels ranging between 30 and 50 ng/mL. Endogenous vitamin D synthesis begins in the skin, where ultraviolet B (UVB) irradiation converts 7-dehydrocholesterol into cholecalciferol (vitamin D_3), which undergoes sequential hydroxylation in the liver and kidneys to produce calcitriol (1,25-dihydroxyvitamin D_3), its biologically active form. Previous experiments have equivocally indicated that vitamin D_3 and its metabolites could differentially interact with nuclear receptors beyond the classical vitamin D receptor (VDR), including the pregnane xenobiotic receptor (PXR), a key regulator of xenobiotic detoxification. To explore these interactions, docking studies were performed using the agonist-bound X-ray crystal structure of PXR (PDB ID:1NRL) with Glide (Schrödinger software). Calcitriol and its variants were analyzed for their binding conformations and affinities using SP Gscore and Prime MM-GBSA binding free energy calculations. Given the promiscuous ligand-binding nature of PXR, we hypothesize that calcitriol may engage PXR, potentially inducing downstream metabolic enzymes such as cytochrome P450 isoforms. This interaction could influence drug metabolism, increasing the risk of clinically significant DDIs and adverse events. Given the promiscuous

ligand binding profile of PXR, it is hypothesized that calcitriol may engage PXR, thereby activating/inducing downstream metabolic enzymes, such as cytochrome P450 isoforms, which could modulate drug metabolism and predispose patients to clinically significant DDIs and adverse events.

PC-9

Studies on the isolation and structure elucidation of berberine, hydrastine and jatrorrhizine from goldenseal root (*Hydrastis canadensis* L.) & Mahonia x media 'Winter Sun' stem bark

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Goldenseal (*Hydrastis canadensis* L., Ranunculaceae) is a traditional North American herbal medicine that has a long history of use for the treatment of a variety of illnesses, primarily as a chemotherapeutic agent for microbial infections. Two of its alkaloid constituents, berberine and hydrastine, have defined pharmacological effects, and research is ongoing. In addition to its presence in goldenseal, berberine (and analogues) are found in many plant families. One such is the barberry family (Berberidaceae), encompassing various medicinal and decorative species, including those of the genus Mahonia (Berberis). This 3-h laboratory experiment has been devised to allow undergraduate students to isolate berberine and hydrastine, from a commercially available root sample of goldenseal, and both berberine and the closely related alkaloid, jatrorrhizine, from the bark of Mahonia x media 'Winter sun'. The isolation procedure is carried out on a miniature, sustainable and environmentally friendly scale using flash column chromatography. A gradient mobile phase system is utilised for the isolation of hydrastine and berberine from goldenseal. A notable feature of the isolation of berberine and jatrorrhizine from Mahonia is the utilisation of silica gel with a 10% w/w loading of sodium carbonate. In this way jatrorrhizine, an acidic alkaloid, is significantly retained while berberine elutes effortlessly from the column. A follow-on spectral assignment workshop is undertaken using 1- and 2-D NMR, MS and IR spectra obtained on the isolated alkaloids. Both formative and summative assessment of student comprehension takes place during the practical, workshop and end of semester college examination. The experiment has been fully validated in the class setting, having been completed by circa 160, 3rd year pharmacy students.

PC-10

Synthesis and Biological Evaluation of Mevalocidin Enantiomers

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The absolute stereostructure of mevalocidin, a prominent bioherbicide derived from fungal sources with a key tertiary carbinol stereocenter, was determined using a strategic aldol reaction. By employing substituted oxazolidinone as a chiral auxiliary, two separable diastereomeric adducts were obtained. Subsequent chemical transformations yielded both enantiomers of mevalocidin. Phytotoxicity assays on *Lemna spp*. confirmed that one enantiomer exhibits significant herbicidal activity, highlighting its potential for organic agriculture. This synthetic approach not only enables the scalable production of mevalocidin but also provides a foundation for designing structurally related herbicides with improved potency and selectivity.

PC-11

Discovery of a novel CBD allosteric binding site at CB1R: Integrating computational and experimental approaches

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The cannabinoid receptor 1 (CB1R) is a potential therapeutic target for conditions such as pain, obesity, emesis, and metabolic syndrome. Unlike orthosteric agonists like THC, cannabidiol (CBD) functions as a negative allosteric modulator (NAM) of CB1R. Structural insights from a 2019 X-ray crystallography study of CB1R in complex with the NAM ORG27569 and the agonist CP55,940 identified a NAM binding site in the extrahelical region within the inner membrane leaflet. However, previous computational models proposed an alternative NAM binding region near transmembrane helices TMH2, TMH6, TMH7, and helix 8, which aligns with known allosteric sites in other GPCRs. Given prior evidence suggesting that CBD may compete with a site overlapping ORG27569's binding region, this study investigated two possible CBD binding sites on CB1R using a combination of computational and experimental techniques. Molecular docking and molecular dynamics simulations supported the likelihood of CBD binding at both the ORG27569-identified extrahelical site and the computationally predicted intracellular site. Site-directed mutagenesis experiments provided further validation, revealing key residues including S401$^{8.47}$ and D403$^{8.49}$, are crucial for NAM activity. Mutations at these positions enhanced [^{3}H]-SR141716A binding, reinforcing their significance in the allosteric modulation induced by CBD. Free-energy calculations combined with metadynamics simulations suggested that CBD binding at the intracellular site induces conformational changes consistent with NAM activity. Collectively, these findings identified six critical residues—Y153$^{2.40}$, I156$^{2.43}$, M337$^{6.29}$, L341$^{6.33}$, S401$^{8.47}$, and D403$^{8.49}$— as essential for the allosteric interaction of CBD with CB1R. This study provides valuable insights that may guide the development of selective and potent CB1R NAMs. Furthermore, identifying this previously uncharacterized allosteric site may help explain the broad pharmacological effects of CBD, given the structural conservation among Class A GPCRs.

PC-12

Synthesis and Chemical Characterization of Delta-10-Tetrahydrocannabinol

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Δ10-Tetrahydrocannabinol (Δ10-THC) presents a fascinating mosaic in the tapestry of cannabinoid science, a positional isomer of tetrahydrocannabinol first discovered in the 1980s. It is a lesser-known cannabinoid with potential therapeutic benefits. Δ10-THC is a slightly different version of tetrahydrocannabinol, with a unique structure that includes a double bond at the 10th position instead of the 9th. Researchers have also identified two epimers with the intricate placement of the 9-methyl group in either the (α) or (β) conformation. Of these, the (α) epimer has a more interesting activity profile, with a potency that is about 30–40% of Δ9-THC's strength due to the double bond moving to the 10th position. While Δ10-THC is not usually found in large amounts in nature, it often appears as a byproduct during the production of synthetic Δ8-THC from CBD or can be synthesized directly from Δ9-THC. The synthesis involves the base conjugated isomerization of Δ9-THC double-bond isomerization. Crystallization employed hexane as the solvent under temperature control to achieve high-purity Δ10-THC crystals. Characterization of the synthesized Δ10-THC was performed using NMR and GC-MS. X-ray crystallography technique was

used to confirm its structure and purity for the first time. The availability of pure Δ10-THC paves the way for the synthesis of both (α) and (β) isomers.

PC-13

Phytochemical investigation of *Astragalus condensatus* and configurational revision of cyclocephagenols using QM/NMR

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Cycloartane-type triterpenoids (CTTs) are unique metabolites produced by photosynthetic eukaryotes, and commonly found in the genus Astragalus L. These compounds feature a distinct 9,19-cyclopropane structure biosynthesized via cycloartenol synthase (CAS). The stereoselective epoxidation with a (24R) configuration leads to intramolecular cyclization, forming the major tetrahydropyran (cyclocephalogenol) or tetrahydrofuran (cycloastragenol) derivatives in Astragalus species. Herein, we report a detailed phytochemical study of Astragalus condensatus which identified the tetrahydropyran and tetrahydrofuran CTTs along with several flavonoid and lignan derivatives. Additionally, quantum chemical calculations revised the absolute configuration of 39 known cyclocephagenol-type triterpenoids from 24S to 24R, a finding supported by X-ray crystallography.

PC-14

Acetogenins from the fruit of *Annona muricata* and evaluation of their cytotoxic activity

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The Annonaceae family (commonly called the custard-apple family) consists of about 130 genera, and 2,300 species most of which are found in tropical and subtropical regions. *Annona muricata* L. (Annonaceae) is a lowland tropical fruit-bearing tree. Various parts of *A. muricata*, such as its fruits, leaves, and bark have been used for medicinal purposes in folk medicine. Over 200 chemical compounds from different parts of Annona species have been identified, including phenolics, acetogenins, terpenoids, cyclopeptides, and alkaloids. Acetogenins are the major components of the genus, consisting of a series of polyketide-derived fatty acid derivatives that possess tetrahydrofuran rings and a methylated γ-lactone, with various hydroxyl and oxo groups along the hydrocarbon chain. They exhibit a broad range of potent biological activities, including cytotoxicity, antitumor, antimalarial, antimicrobial, antifeedant, and pesticidal properties. We present the detailed isolation and structure elucidation of acetogenin derivatives from the fruits of *A. muricata* and the cytotoxic evaluation of extracts and pure compounds.

PC-15

Novel Glyceroglycolipids from Cyanobacteria Arthrospira/Limnospira fusiformis

Abdel-Mawgoud M[1], Annam SCVAR[1], Elbermawi A[1], Huh J[1], Zhang J[1], Ali Z[1], Khan IA[1,2], Pugh ND[1], Chittiboyina AG [1,2,3,]*

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A phytochemical investigation of the methanol extract of *Arthrospira/Limnospira fusiformis* aka Spirulina biomass led to the isolation of two novel compounds, 1-*O*-nonanediooyl-2-*O*-palmitoyl-3-*O*-(6'''-sulfo-α-*D*-quinovopyranosyl)glycerol (**1**) and 1-*O*-(8'-hydroxyoctanooyl)-2-*O*-palmitoyl-3-*O*-(6'''-sulfo-α-D-quinovopyranosyl)glycerol (**2**) together with four known compounds (**3**–**6**). The structures of the isolated compounds were elucidated through the comprehensive analysis of 1D and 2D NMR and HRESIMS data.

PC-16

Phytochemicals from the leaves of *Culcasia parviflora*

Adeboye OM[1,2,3], Zulfiqar F[1], Sowemimo A[2], Sofidiya M[2], Khan IA[1], and Ali Z[1]

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Plants of this genus Culcasia have been used by traditional healers to diagnose and treat various ailments since ancient times. The ethnomedicinal survey showed the use of Culcasia plants in treating pain, inflammation, and cancer and detecting pregnancy. Culcasia parviflora N.E.Br. (Araceae) is traditionally used for pain and inflammation management in Africa and has not been investigated for its metabolites. Nine compounds, belonging to megastigmanes and indoles were isolated from the 95% ethanol extract of the leaves of C. The structures of the isolated compounds were elucidated by 1D and 2D NMR spectroscopy and the data were compared with reported values. Herein, we present their isolation, structure determination, and chemotaxonomic significance.

PC-17

Phytochemicals from the leaves of *Culcasia parviflora*

Adeboye OM[1,2,3], Zulfiqar F[1], Sowemimo A[2], Sofidiya M[2], Khan IA[1], and Ali Z[1]

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Plants of this genus Culcasia have been used by traditional healers to diagnose and treat various ailments since ancient times. The ethnomedicinal survey showed the use of Culcasia plants in treating pain, inflammation, and cancer and detecting pregnancy. Culcasia parviflora N.E.Br. (Araceae) is traditionally used for pain and inflammation management in Africa and has not been investigated for its metabolites. Nine compounds, belonging to megastigmanes and indoles were isolated from the 95% ethanol extract of the leaves of C. The structures of the isolated compounds were elucidated by 1D and 2D NMR

spectroscopy and the data were compared with reported values. Herein, we present their isolation, structure determination, and chemotaxonomic significance.

PC-18

Phytochemical investigation of *Crataegus mixicana* root

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Crataegus is a genus of several hundred species of shrubs and trees belonging to the family Rosaceae, native to temperate regions of the Northern Hemisphere in Europe, Asia, North Africa, and North America. Crataegus mexicana is described as a thorny, deciduous shrub or commonly a tree cultivated for its edible fruits, especially in Mexico, as a fruit crop. Crataegus mexicana has been utilized in different countries as a traditional medicine for treating digestive and cardiovascular illnesses. Additionally, hawthorn extracts have been used as a remedy for congestive heart failure, angina pectoris, and for lowering elevated blood pressure and total plasma cholesterol. It also exhibited antidiabetic and antioxidant effects. Ejocote or Mexican Hawthorn root (Crataegus mexicana) is marketed as a supplement for weight loss. In the current research, a phytochemical investigation was conducted on the root extract of *Crataegus mexicana*, employing multiple chromatographic separation methods followed by analysis by spectroscopic techniques that led to the isolation and structural determination of three triterpenoids (1−3), β-sitosterol and a phenolic compound (epicatechin). This is the first report on the constituents of the roots derived from *Crataegus mexicana*. Moc. & Sesse` ex Dc. In addition, this is the first report on isolating 1 and 2 from the genus Crataegus, while 3 was previously isolated from the Chinese Hawthorn (Crataegus pinnatifida).

PC-19

Structural characterization and anti-inflammatory potential of turmeric (*Curcuma longa* L.) polysaccharides: insights from NMR, SPR, and molecular simulations

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The diverse bioactivities of natural polysaccharides, including antioxidant, anti-inflammatory, and immunomodulatory effects, are increasingly recognized. While *Curcuma longa* L. is renowned for its curcuminoids, its polysaccharide fraction remains an underexplored source of potential bioactives. This project aims to elucidate the structural-functional relationships of these polysaccharides. Specifically, we will: 1) characterize their structural features, including glycosidic linkages and molecular weight distribution, using high-resolution NMR and mass spectrometry; 2) quantify binding interactions with key inflammatory mediators (TNF-α, IL-6) and immune receptors via surface plasmon resonance (SPR); 3) Predict binding affinities and complex stability through molecular docking and dynamics simulations; and 4) Evaluate the synergistic effects of polysaccharides and curcuminoids using co-extraction and in vitro assays. This research will establish a comprehensive structural-activity framework, facilitating the development of novel nutraceutical and anti-inflammatory applications for *C. longa* polysaccharides.

Chemistry, Biology and Safety of Essential Oils and Volatile Organics

PE-1

Bioassay-guided identification and optimization of bioactive compounds from Patchouli essential oil for Fire Ant Control

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Imported fire ants (Solenopsis spp.) are invasive pests and current management relies on synthetic insecticides, which pose environmental and health risks, creating a need for safer, natural-product-based alternatives. Plant-derived repellents and toxicants provide eco-friendly solutions for fire ant control. Patchouli essential oil, extracted from Pogostemon cablin (Lamiaceae), is rich in bioactive sesquiterpenes and widely used in traditional medicine and perfumery. While its insecticidal and repellent properties are well-documented, its potential against fire ants remains unexplored. This study aimed to evaluate the bioactivity of patchouli oil against hybrid (S. invicta x S. richteri), black (S. richteri), and red (S. invicta) imported fire ants using a bioassay-guided approach. Gas chromatography-mass spectrometry (GC-MS) was used to analyze the oil's composition, followed by fractionation via silica gel chromatography. Digging bioassays with six concentrations of the crude oil and its fractions revealed significant repellency and Patchouli alcohol was identified as the active compound. It was further tested for insecticidal activity, exhibiting significant contact toxicity across all three fire ant species. A series of carbamates was synthesized from the alcohol to be assessed for potency enhancement. Our findings highlight the potential of patchouli essential oil as an effective, environmentally friendly solution for fire ant control.

PE-2

Evaluation of skin sensitization potential of essential oil using OECD guideline *in vitro* assays

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This study investigated the skin sensitization potential of Frankincense and Grapefruit Essential Oils using OECD Guideline 442. The KeratinoSens assay, evaluating NRF2 activation in human keratinocytes, and the hCLAT (Human Cell Line Activation Test) assay, assessing the activation of human monocytes by measuring CD54 and CD86 expression, were employed. Of the 7 Frankincense Essential Oil samples tested, 5 samples showed positive activation, suggesting potential for skin sensitization in some cases. Grapefruit Essential Oil did not induce cytotoxicity or significant NRF2 activation in keratinocytes up to 200 µg/mL in the KeratinoSens assay and did not significantly upregulate CD54 or CD86 in monocytes at this concentration in the hCLAT assay. These findings, conducted in accordance with OECD guidelines, suggest a low skin sensitization potential for Grapefruit Essential Oil, while further investigation is warranted for Frankincense Essential Oil.

PE-3

Molluscicidal toxicity of *Leptospermum scoparium* & *Matricaria chamomilla* essential oils against Planorbella trivolvis & *Biomphalaria havanensis*

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Two snails *Planorbella trivolvis* & *Biomphalaria havanensis* (Family: Planorbidae) are vectors of *Bolbophorus damnificus*, an important trematode parasite that can induce significant qualitative and quantitative losses to catfish farms across the southeastern US. Catfish farmers rely on copper sulfate (CuSO4) that is a multi-use, broad-spectrum non-selective chemical to control snails in the pond. It comes with its own risks to fish and ecosystem. Natural products may present an alternative molluscicide with less toxicity to fish. Plant-derived natural products are known to be toxic to insect pests and snails, but unlike other chemicals, they are relatively innocuous to plants and leave no harmful environmental residues. The National Center for Natural Products Research (NCNPR), The University of Mississippi, The Mississippi State University College of Veterinary Medicine, the Mississippi Agriculture and Forestry Experiment Station, and the USDA Warmwater Aquaculture Research Unit teamed up to investigate eco-friendly natural molluscicide alternatives. To study mollsucicidal toxicity of plant-derived natural products, the NCNPR established a rearing facility for both snail species. Subsequently, lethality bioassays were performed against adult snails to determine effects of dose and time. Commercially selected essential oils 'Manuka' *Leptospermum scoparium* and 'German Chamomile' *Matricaria chamomilla* were evaluated and profiled for adulticide toxicity through bioassay guided fractionation. Both oils induced significant toxicity in both species at 50 ppm. Leptospermum induced 100% mortality in both species while Chamomile induced 73.3% and 86.6% mortality against *P. trivolvis* (3 d exposure) & *B. havanensis* (24 hr exposure), respectively. The promising molluscicides will be further investigated for active compounds by bioassay-guided fractionation.

PE-4

Terpenes profiling of cannabis biomass and cannabis essential oils using gas chromatography-mass spectrometry (HS GC-MS)

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With the evolving landscape of medicinal cannabis, reliable terpene analysis becomes fundamental in ensuring quality, enhancing consumer knowledge, and exploring the major terpenes of this versatile plant. This study focuses on applying a headspace coupled with the Gas Chromatography-Mass spectrometry (HS GC-MS) for terpene analysis in Cannabis sativa.

Comparison of the terpene profile using solvent extraction vs non-extracted cannabis plant material was studied, in order to select the process that is more adequate for better recovery and selectivity of the targeted terpenes. Subsequently, a diverse array of cannabis monoterpenes and sesquiterpenes were successfully analyzed in samples from five cannabis chemovars grown at the University of Mississippi, namely, high THC, high CBDV, high THCV, high CBG, and high CBD using the proposed method. Furthermore, comparison is made between the terpenes profile of these plant materials and those of essential oils derived from the hydrodistillation of the respective biomass. This method provides efficient, robust, and reliable qualitative determination of the terpenes profile of cannabis biomass and other cannabis-derived products.

PE-5

UHPLC-MS/MS-based quantification of oxygen heterocyclic compounds and nootkatone in cold-pressed grapefruit essential oils

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Oxygen heterocyclic compounds (OHCs), including polymethoxyflavones (PMFs), coumarins (CMNs), and furanocoumarins (FCMNs), along with the terpene nootkatone (NKT), are key secondary metabolites in cold-pressed grapefruit essential oils (GEOs). While these compounds exhibit a plethora of promising bioactivities (e.g., antioxidant, antiallergic, anti-inflammatory, and antibacterial), potential adverse effects, such as CMN toxicity at high concentrations and FCMN phototoxicity exacerbated by UVA exposure and interactions with certain over-the-counter medications, warrant a sensitive and selective analytical method for determining their levels in GEO-based products intended for topical and oral use.

To address these analytical needs, a rapid 16-minute ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) method was developed for the simultaneous identification and quantification of 25 OHCs (3PMFs, 7CMNs, and 15FCMNs) and NKT in cold-pressed GEOs. The method exhibited excellent linearity (R2> 0.99 for all analytes), low limits of detection (LODs, 6×10-8 to 1.5×10-6 mg/g), and quantification (LOQs 2×10-7 to 5×10-6 mg/g). Intra- and inter-day precision (%RSD) ranged from 1.43 to 3.59% and from 2.16 to 6.90%, respectively. The resulting quantitative data will be essential for establishing safe levels of OHCs in cold-pressed GEOs, thereby minimizing the phototoxicity and drug interaction risks. Furthermore, this validated method can also be applied to various GEO-based products to ensure the quality of raw materials and inform the reduction or elimination of specific OHCs.

PE-6

Differentiation and adulteration detection of cinnamon essential oils by NMR and chemometrics

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Cinnamon essential oil (CEO), extracted from the bark or leaves of the cinnamon tree, typically from the species *Cinnamomum verum* or *C. cassia*, is valued for its rich aroma and numerous health and wellness benefits. The oil's composition and scent are influenced by the source material (bark or leaf) and cinnamon species. Developing efficient methods for authenticating and characterizing CEO is therefore essential. In this study, 95 CEO samples derived from bark, leaves, or a combination of both were investigated for compositional attributes and variation by using NMR techniques. The findings revealed distinctive NMR spectral fingerprints corresponding to different source materials. The characteristic signals and chemical profiles were further identified through 2D NMR analysis. Additionally, 30 commercial CEO samples were evaluated for possible adulteration based on NMR and chemometric analysis. Triethyl citrate, triglycerides (vegetable oil), fatty acids, and synthetic chemicals were

identified in several commercial CEOs. The study demonstrated that NMR combined with chemometric analysis provides a powerful approach for differentiating CEOs and detecting adulteration.

PE-7

Characterization and Identification of Frankincense essential oils via NMR fingerprinting and profiling

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Frankincense essential oil (FEO) has a warm, woody spicy, and slightly sweet aroma and is often used in aromatherapy, skincare, and incense formulations. Extracted from the gum resin of various Boswellia species, including B. sacra (syn. B. carteri), B. frereana, B. serrata, and B. papyrifera, FEO exhibits significant variations in chemical composition despite being marketed under the general term “frankincense oil”. This study employed NMR techniques to characterize the chemical profiles of FEOs derived from different Boswellia species. The analysis uncovered significant compositional differences, as indicated by comparative analyses of NMR spectral fingerprints, alongside the identification of species-specific signals. Furthermore, the samples were effectively classified according to their species through the integration of NMR data and principal component analysis (PCA), and the characteristic NMR signals for β-thujene, α-pinene, p-cymene, and octyl acetate could be used as indicators for differentiation of FEOs derived from different Boswellia species.

PE-8

Are all essential oils created equal? factors affecting the repellent efficacy of Irish *Myrica gale* L. essential oils against yellow fever mosquitos (*Aedes aegypti*)

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Myrica gale L. (MG) has traditionally been used as an insect repellent. Rising concerns over chemical repellents have risen the demand for natural alternatives. The study investigated the effect of extraction techniques–Clevenger (CH) and microwave–assisted hydrodistillations (MAH) on chemical profiles of 6 essential oils (EO) from Irish MG leaf and fruit which in turn influenced their repellency against Aedes aegypti mosquitoes. Commercial EO from MG, Myrtus communis (MC) and Syzygium aromaticum (SA) were compared due to their known insect repellency. Leaves and fruits of MG were collected from Counties–Offaly, Mayo and Kerry and subjected to CH and/or MAH to yield 6 EO. EO were chemically profiled by GC-MS and screened for repellency by in vitro arm-in-cage and Y-tube olfactometer assays. Targeted metabolomics was applied to correlate chemical profile with repellency. 105 compounds were identified in EO from MG, MC and SA with α-pinene (0–64.92%), limonene (0–23.57%), eucalyptol (0–79.86%), eugenol (0–76.28%) and eugenol acetate (0–2.27%) as major components. MAH yielded EO enriched in sesquiterpenes compared to CH, thereby decreasing the monoterpene/sesquiterpene ratio from 10 to 9.2 for same starting material. In repellency against *A. aegypti*, extracted MG EO showed significant variability, with complete protection time ranging from 6.78 – 36.02 min (arm-in-cage) and 0 – >240 min (Y-tube olfactometer). Commercial MG and MC EO had minimal activity, while SA EO was the most effective, with complete protection time of 63.03 min in arm-in-cage assay. Amongst extracted EO, fruit EO (11.45 min) was more active than leaf EO (6.79 min) and in case of fruit oil, CH extracted EO (36.03 min) performed better than MAH extracted EO (11.45 min). Similar trends were observed in Y-tube olfactometer assay. Targeted metabolomics

revealed eugenol, eugenol acetate, α-phellandrene, β-pinene, γ-terpene, camphene, 3-carene and germacrone, components that may contribute to repellency.

PE-9

Genotoxicity evaluation of cinnamon oil using cytokinesis block micronucleus assay

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Cinnamon essential oil has been widely used in daily food spices, medicine, cosmetic substances, and in aromatherapy. Although cinnamon oil has beneficial effects, it contains safrole, a natural substance known for its carcinogenic effects. This study aims to evaluate the cytotoxic and genotoxic effects of cinnamon oil (bark) in Chinese Hamster Ovary (CHO-K1) cells using OEC recommended methods. Cytotoxicity of oil is evaluated by measuring the reduction of MTT to purple formazan crystals (MTT assay) and genotoxicity is carried out by Cytokinesis Block Micronucleus Assay (CBMN) using fluorescent Hoechst 33342 stain to determine Micronuclei formation in 1000 binucleated cells and Nuclear Division Index (NDI) in 500 cells. In the current study, we examined genotoxicity of Cinnamon oil and Safrole with and without metabolic activator (rat liver S9 factor). Data were compared with Cyclophosphamide (10 µg/ml) and Mitomycin C (0.2 µg/ml) as positive controls and DMSO (0.25%) as a vehicle control. The frequency of micronuclei formation in cells treated with cinnamon bark oil (25 - 50 µg/mL), both in the absence and presence of a metabolic activator ranged from 1.5 - 2.6 %, while safrole (0.0275 - 0.055 µg/mL) treatment resulted in 1.7 – 3.8 % micronuclei formation. The NDI results demonstrated no differences between the two treatments and ranged between 1.6 to 1.9. The safrole content of the tested sample of cinnamon oil was 0.11%.

PE-10

Repellency potential of oregano essential oil derived phenolic compounds and their derivatives against imported fire ants

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Imported fire ants are considered one of the most serious medical and agricultural pests across the globe. They mainly include *Solenopsis invicta* Buren (red imported fire ant), *Solenopsis richteri* Forel (black imported fire ant), and the hybrid of these species (S. invicta x S. richteri). As part of a natural product discovery program for identifying fire ant control agents, the essential oil extracted from oregano (Origanum vulgare L.) was screened and demonstrated repellent effects against imported fire ants. GC-QToF-MS analysis of the oil identified carvacrol (63.9%) as the major constituent, followed by linalool (8.4%), p-cymene (7.6%), γ-terpinene (5.9%), and thymol (2.6%). In the digging bioassay, these phytochemicals demonstrated a moderate to potent fire ant repellency. A structural analysis of carvacrol, thymol, and p-cymene indicated that the presence and position of a hydroxy group on the substituted aromatic ring significantly influenced repellency. Inspired by these results, a set of phenolic compounds were further procured/synthesized, in which the hydroxy groups were intact or further converted into esters and carbamates. Several of these molecules demonstrated potent repellency in the digging bioassay. A preliminary structure-activity relationship of these compounds was thus deduced, suggesting the potential role of phenolic functional group and their derived functionalities in fire ant repellency. The observed repellency activity was further corroborated with molecular docking of the

compounds with the hybrid imported fire ant pheromone-binding protein GP-9 (Source: *S. invicta* x *S. richteri*). The screened compounds gave comparable docking scores. Overall, these findings highlight the repellent effects of oregano essential oil phytochemicals and their derivatives in fire ant management, and further structural optimization might be helpful for the development of fire ant control products.

PE-11

Development of an efficient HS-SPME GC/Q-ToF method for analyzing Lavender oil components in cosmetic and personal care products

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Cosmetic and personal care products often contain complex mixtures of ingredients, making analysis challenging. Traditional extraction methods are time-consuming and susceptible to errors. Headspace solid-phase microextraction (HS-SPME) provides a faster, solvent-free, and cost-effective alternative, offering efficient extraction of volatile analytes with reduced error potential through automation.

This project aimed to develop an HS-SPME method coupled with GC/Q-ToF to analyze three commercial personal care products (2510PR-2512PR) for components found in lavender essential oil. The analysis focused on qualitative assessment and the quantification of linalool, linalyl acetate, and lavandulyl acetate. Separation was achieved using an HP-5MS column, and a 100 µm PDMS SPME fiber was utilized, with dodecane as the internal standard. Calibration curves showed excellent linearity (R^2 > 0.99) across concentrations ranging from 0.5 µg/mL to 60 µg/mL for all compounds. Linalool was detected in the products at concentrations of 12.20 ± 0.01 mg/g, 0.43 ± 0.01 mg/g, and 10.64 ± 0.90 mg/g, respectively. While linalyl acetate and lavandulyl acetate were not detected in 2511PR, they were present in 2510PR at 0.77 ± 0.03 mg/g, 0.18 ± 0.02 mg/g, and at 0.20 ± 0.00 mg/g, 0.32 ± 0.00 mg/g for 2512PR, respectively. These results demonstrate that HS-SPME is a fast, cost-effective, and efficient method for analyzing cosmetic and personal care products.

PE-12

Quality assessment of Cinnamon essential oil using GC-MS and chemometrics

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Cinnamon essential oil (EO), derived from species within the Cinnamomum genus, has been highly valued throughout history for its health benefits and use as a flavoring agent. Commercially produced EO is typically extracted from either the leaves or bark of two primary Cinnamomum species: Cinnamomum zeylanicum (CZ) (also known as Cinnamomum verum) and Cinnamomum cassia (CC) (also known as Cinnamomum aromaticum). EO from CZ is regarded as higher quality and more expensive compared to that from CC. As a result, CZ EO is often adulterated with the more affordable CC EO.

Given this issue, the objective of our project was to develop a GC-MS method for analyzing both authentic and commercial EO samples. Authentic EO samples analyzed included 25 CZ bark, 35 CZ leaves, and 12 CC. Principal component analysis was

performed using the data. Good separation of the sample classes was achieved with PC1 explaining 52% of the variability. A class prediction model was constructed which demonstrated 99% accuracy when cross-validated. Thirty commercial samples were analyzed using the model which indicated that approximately 40% of the samples were outliers. The GC-MS analysis of these outliers indicated either poor quality or adulteration with a variety of compounds including triethyl citrate, isopropyl myristate, and diethyl phthalate.

PE-13

Eucalyptus essential oil, an update on the safety and causality evaluation of published case reports

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Eucalyptus (Eucalyptus spp., Myrtaceae) essential oil (EO) and preparations containing eucalyptus have been used in traditional medicine for centuries. However, their use has been associated with cases of central nervous system (CNS) depression and seizures in such a proportion that a better understanding of the cause is deemed necessary. 91 case reports reviewed showed evidence of CNS depression and/or seizure in patients from ages <1 to 74 years old with different doses of eucalyptus EO (3 drops to 1000 mL). Most of the cases were in children; some reacted to small doses (3 drops of EO), mostly from ingestion but also inhalation or after topical application. Most children who developed seizures did not have a history of seizures before the event. The main constituent of eucalyptus EO is 1,8-cineole. Camphor, a constituent previously linked to seizures, is also present in some preparations used to alleviate respiratory symptoms. A literature review was done to better understand the CNS effects of eucalyptus EO, its major constituents and associated ingredients such as camphor. The data available was limited and sometimes contradictory on the potential CNS effects of these materials. In addition to this research, one cannot exclude other factors such as potential adulteration or contamination of the essential oils. However, the composition or even the product's name is rarely documented in case reports. As part of the investigation and review of the data, it was noted that a large proportion of the reports of seizures or CNS depression came from India with patients aged 17 months to 60 years exposed to a few drops by inhalation to 15 mL ingested EO. A small collection of eucalyptus essential oil samples commercially available in India were collected, analyzed by GC-MS, and compared to authentic standard eucalyptus EOs. Although the GC-MS profiles of commercial samples included the typical constituents of eucalyptus EO, such as 1,8-cineole, alpha-pinene, limonene, or para-cymene, the concentration of some compounds did not align with recognized standards and some materials contained synthetic markers suggesting possible adulteration. Most samples contained some levels of camphor, with concentrations ranging from 0.01% to about 12%.