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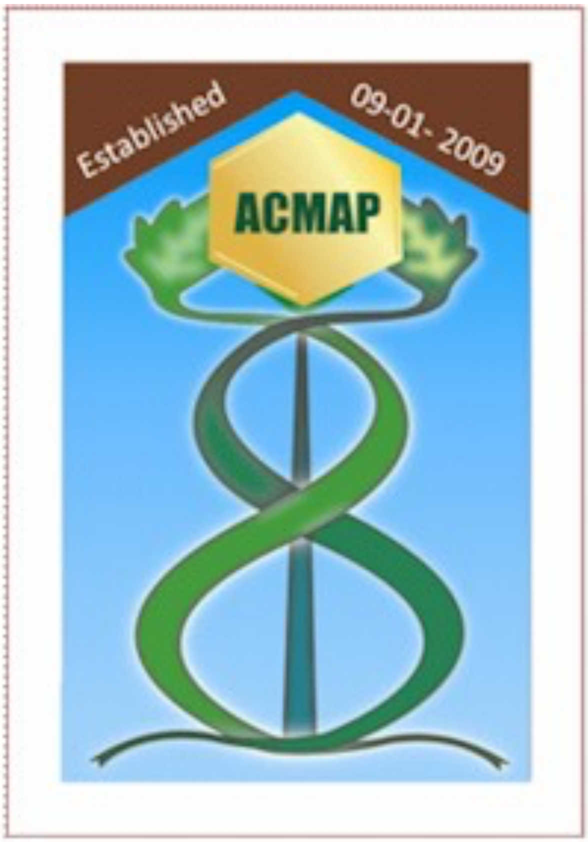

Abstract Book

PLENARY SPEAKER

“Ethnobotany as a tool for targeted discovery of bioactive natural products”

Cassandra Quave, Ph.D.

Emory University

Dr. Cassandra L. Quave is Curator of the Herbarium and Associate Professor of Dermatology and Human Health at Emory University. She was appointed as Thomas J. Lawley, MD Professor of Dermatology and Assistant Dean of Research Cores at Emory University School of Medicine in 2023. Dr. Quave earned degrees in biology and anthropology (B.S.) from Emory University in 2000 and a Ph.D. in biology in 2008 from Florida International University under the direction of Dr. Bradley Bennett. She completed postdoctoral fellowships in microbial pathogenesis at the University of Arkansas for Medical Sciences under the direction of Dr. Mark Smeltzer (2009-2011) and in human health at Emory University under the direction of Dr. Michelle Lampl (2011-2012). As a medical ethnobotanist, her work focuses on the documentation and pharmacological evaluation of plants used in traditional medicine. The National Institutes of Health, industry contracts, and philanthropy support Dr. Quave’s research. She is a Fellow of the Explorers Club, a past President of the Society for Economic Botany, a recipient of the Emory Williams Teaching Award, Charles Heiser, Jr. Mentor Award, American Botanical Council James. A. Duke Excellence in Botanical Literature Award, and American Herbal Products Association Herbal Insight Award. In 2022, she received The National Academies Eric and Wendy Schmidt Awards for Excellence in Science Communication. Dr. Quave serves on the Board of Directors for the Society for Investigative Dermatology and the editorial boards for *Natural Product Reports, Scientific Reports,* and *Journal of Ethnobiology and Ethnomedicine* and is an associate editor for *Frontiers in Pharmacology*.

Beyond her academic research and teaching activities, Dr. Quave is the co-creator and host of Foodie Pharmacology, a podcast dedicated to exploring the links between food and medicine, now in its fifth season. She is the creator and host of the Teach Ethnobotany channel on YouTube, through which she creates and shares educational videos about botanicals, pharmacology, and natural products. She writes a weekly newsletter, Nature’s Pharmacy, to reveal the science behind natural remedies and address misinformation on herbs. Dr. Quave has authored more than 100 scientific publications, two edited books, twenty book chapters, and seven patents; her work has been cited in the scientific literature more than 6,600 times. Dr. Quave is co-founder and CSO of Verdant Scientific—a biotech company focused on developing new therapies for inflammatory skin disease. Her research has been featured in the *New York Times Magazine*, *BBC Science Focus*, *National Geographic Magazine*, NPR, PBS, and the National Geographic Channel. She has written opinion essays for *The Wall Street Journal* and *The Conversation.* Quave is the author of an award-winning science memoir *The Plant Hunter: A Scientist’s Quest for Nature’s Next Medicines*, acclaimed as one of the best books of 2021 by Kirkus Reviews. Learn more about her research by visiting her website or following her on Facebook or Twitter.

Plenary Speaker's Abstract

O1. Ethnobotany as a tool for targeted discovery of bioactive natural products

Cassandra Quave. Emory University, Atlanta, GA. E-mail: cassandra.leah.quave@emory.edu

Out of the estimated 374,000 species of plants on Earth, 9% have been documented as being used as a source of medicine. Billions of people across the globe currently rely solely on plants to meet their primary healthcare needs. Many of our life-saving therapies for the treatment of cancer, pain, heart disease, and infection were developed based on chemical scaffolds first discovered in plants. Yet, most medicinal plants have never been examined through the lens of modern science. In this lecture, I review the historical importance of plants in the evolution of current therapeutics and explore the emerging technologies enabling scientists to look deeper into the pharmacological properties of plants. I will illustrate the utility of the highly targeted ethnobotanical approach to drug discovery with examples of antifungals, antibiotics, and anti-virulence agents discovered in plants that are used in the traditional treatment of infectious and inflammatory skin disease. Lastly, I will review critical issues in modern bioprospecting, from the importance of ethical engagement with communities and plant ecosystems to the long-reaching impacts that we can all make in supporting research capacity-building initiatives in countries rich in biodiversity that lack sufficient support for their scientific infrastructure.

Session I Ethnobotany, Bioprospecting, and Production

Chair: Rebecca Linger, University of Charleston, USA
Co-chair: Emily Merchant, Rutgers University, USA

Keynote

O2. Developing turmeric as a high value medicinal crop in Alabama

Srinivasa Rao Mentreddy[1], Lam Duong[2], Trang Pham[1], Cuong Nguyen[1], Sravan Kumar Sanathanam[1], Suresh Kumar[1], Charles L. Cantrell[3], and Mei Wang[3].
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Turmeric (*Curcuma* spp.), a traditional medicinal crop for >4000 years in India, is gaining popularity in the US as a health supplement to combat inflammatory diseases, memory loss, and other benefits. Field trials were conducted in three phases at university research stations and farms across Alabama to determine turmeric varieties with yield and curcumin content desirable for commercial production in Alabama. Fifteen genotypes in Phase I (2007 – 2018) and fifty-two genotypes in Phase II were evaluated for adaptation, yield, and curcumin content; and in Phase III (2020 – 2022), agronomic practices (three organic manures and plant population density) were evaluated to promote commercial production of turmeric in Alabama. In all trials, five genotypes were planted in single-row plots arranged in a randomized block design with four replications. The middle three plants/plot were harvested to determine fresh & dry rhizome yields and their curcumin (HPLC) content. The genotypic variation for yield and curcumin content was significant in all trials. In phase 1 trials, the fresh rhizome yield ranged from 10 for CL9 to 28 MT/ha for CL7; and the curcumin content varied from 0% in *C. zedoaria* genotypes to 2.5% in *C. longa* genotype (CL6). In Phase II trials, the yield of Vietnamese genotypes ranged from 0 MT/ha (unadapted genotypes, VN 21,23,24) to 18.1 MT/ha (VN 27). The total curcumin content varied between 0 for all black and white turmeric and 6.8% for red turmeric genotypes. Organic manures increased yield by 2 to 5 MT/ha over the control. Two t/ha chicken manure or 3 t/ha vermicompost increased the yield by 3.7 MT/ha over the Control. The fresh yield was 7, 14, and 18 MT/ha at 3, 5, and 7 plants/m^2, respectively. Five turmeric genotypes that combine high rhizome yield and curcumin content were selected for commercial production in Alabama.

Invited I

O3. The pharmacognostical history of the Appalachian may apple

Rebecca S. Linger, Ph.D., Professor of Medicinal Chemistry, University of Charleston School of Pharmacy, 2300 MacCorkle Avenue, SE, Charleston, WV 25312. Email: rebeccalinger@ucwv.edu

Etoposide and teniposide are integral components of cancer chemotherapy. These agents inhibit the action of topoisomerase II and are used to treat testicular, bladder, prostate, stomach, and lung cancers (etoposide) and small cell lung cancer (teniposide). These medications were developed from the natural product, podophyllotoxin, which is extracted from the May Apple, a small, low-growing plant of the Appalachian Forest. Historically, the fruit of the plant has been gathered to make jams, jellies, and pies, however the rest of the plant is known to be highly toxic. This lecture will delve into the historical use of the plant as medicine and how this led to the formulation of official medicines previously described.

Invited II

O4. Efforts in bioprospecting *Scutellaria*: A little-known genus of about 400 species

Nirmal Joshee. College of Agriculture, Family Sciences, and Technology, Fort Valley State University, Fort Valley, GA 31030, USA, Email: josheen@fvsu.edu

Scutellaria (skullcap, family Lamiaceae) species have been traditionally used as herbal medicine for their anti-bacterial, antitumor, anti-angiogenesis, hepatoprotective, antioxidant, anticonvulsant, antibacterial, and antiviral properties in China since ancient times. Therapeutic herbs have been a source of medication for the mitigation of human diseases. The genus *Scutellaria* is comprised of about four hundred species, cosmopolitan by habitat, most of the species found in the northern hemisphere. According to the survey report by the World health organization (WHO), approximately 80% of the population of the world is dependent on herbal-based conventional remedies for primary health care. Herbal medicines have grown in popularity and acceptance in the Western world annually. Many people are adopting natural remedy and lifestyle, placing their trust in alternate therapies as natural, reliable, and efficient. Americans spent more than USD 12 billion on natural additions and more than USD 27 billion on natural products in 1998. For instance, 125 million of 500 million prescriptions by physicians each year involve preparation derived from natural sources. Anticancer, antifeedant, phytotoxic, antimicrobial, and nitric oxide production inhibitory activities are the most reported properties for the diterpenoids from the *Scutellaria* genus. Diterpenes of *Scutellaria* spp. have therapeutic potentials to be used for the treatment of different diseases. Further phytochemical, pharmacological, and clinical studies are required in this regard as a global research effort. Four *Scutellaria* spp. have been researched well and are widely available commercially (*S. baicalensis, S. lateriflora, and S. barbata, and S. racemosa*). We have a collection of *Scutellaria* species at Fort Valley State University and progress in research in the past 20 years will be discussed.

Oral I

O5. Molecular physiological analyses of turmeric (*Curcuma longa*) under various environmental stresses

Chhandak Basu[1], Kirill Musaev[1], Sanjeevi Nagalingam[2], Alex Guenther[2], Bhiolina Bharadwaj[1], Lipi Patel[1], Avetis Mishegyan[1], Lam Duong[3], Trang Pham[4], S. K. Sanathanam[4], Mei Wang[5], and S.R. Mentreddy[4,1] Department of Biology, California State University, Northridge, [2]Department of Earth Systems Science, University of California, Irvine,[3]US Citrus, LLC Hargill, TX.[4]Department of Biological and Environmental Sciences, Alabama A & M University, Huntsville, AL, [5]USDA-ARS-Natural Products Utilization Research Unit, Oxford, MS

Turmeric (*Curcuma longa*) has immense medical properties, including anti-inflammatory and antioxidant effects. The active ingredient of turmeric is curcumin, which contributes to most of the plant's medicinal properties. However, climate change and environmental stress can affect curcumin production and negatively impact turmeric plant growth. We have conducted observations on turmeric plants' responses to four types of abiotic stresses, namely, heat stress, drought stress, salt stress, and flood stress. All environmental stresses were mimicked in a laboratory setting. We observed reduced catalase activities for drought stress, probably due to leaf senescence. Drought stress also led to a reduction in photosynthesis and chlorophyll content. Regarding salt stress, we expected to see more catalase activity in the salt-stressed plants compared to the control plants. However, contrary to our expectations, we did not see a higher catalase activity in the salt-stressed plants. We speculate that cell deaths due to salt injury may be responsible for less catalase activity in salt-stressed plants. Interestingly, we did see an increase in sugar production in salt-stressed plants compared to control plants. We hypothesize that the accumulation of soluble sugar helped the plants to maintain osmotic potential during salt stress. In the context of flood-stressed plants, we observed increased sugar content, but we did not observe a corresponding higher catalase activity. We also saw no difference in chlorophyll content in control vs. flood-stressed plants. We analyzed the biogenic volatile emissions from heat-stressed turmeric plants using a gas chromatograph-mass spectrometer. We found that the emissions of monoterpenes were strongly induced in the heat-stressed turmeric plants compared to the control plants. Our analysis also revealed differential emissions of a-phellandrene and terpinolene from heat-stressed plants. Our work on understanding turmeric plants' molecular and physiological responses to environmental stress will give the researchers genetic and molecular insights into developing and screening stress-tolerant turmeric varieties.

Oral II

O6. Phytochemical and ethnobotanical investigations of ghost pipe (*Monotropa uniflora*), a North American medicinal plant

Savannah G Anez[1], Joshua J. Kellogg[1,2], Eric Burkhart[3,4]. [1] Intercollege Graduate Degree Program in Plant Biology, [2]Department of Veterinary and Biomedical Sciences, [3]Department of Ecosystem Science and Management, [4]Shaver's Creek Environmental Center, Pennsylvania State University, University Park, PA 16802, USA. E-mail: sga5169@psu.edu; jjk6146@psu.edu, epb6@psu.edu

Ghost-pipe (*Monotropa uniflora*, Ericaceae) is a widely distributed North American plant that is used in contemporary folk herbalism in the United States. The species also has a rich ethnobotanical history, especially in the Appalachian region of the United States, and has been used as an analgesic, to treat inflammation, and to allay symptoms of emotional distress. Despite this history of use, little is known about phytochemistry, and it is unclear whether it possesses compounds of medicinal or toxicological activity. Additionally, ghost-pipe is myco-heterotrophic, or parasitic to mycorrhizal fungi. Thus, its secondary metabolite profile, and therefore medicinal properties, may be more affected by changes in local environment and/or host compared to autotrophic plants. To address these knowledge gaps, I am pairing untargeted metabolomics approaches with a survey and key informant interviews to characterize this plant's phytochemistry to in relation to its documented uses. These results will be used to guide bioassay selection to more precisely determine ghost-pipe's bioactivity. Results will collectively be used to better understand therapeutic potential of this plant using ethnobotanical knowledge as a guide.

Session II Chemistry of Medicinal Plants

Chair: Michael Fultz, West Virginia State University, USA
Co-chair: Joshua Kellogg, Pennsylvania State University, USA

Keynote

O7. Exploring Californian lichens and their mycobionts for bioactive compounds

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Lichens are symbiotic organisms comprised by mycobionts and photobionts. Bioactive marine organisms such as algae, cyanobacteria and fungi, including Ascomycetes and Basidiomycetes, can form endemic lichens in coastal regions. These newly formed, restricted, and highly stressed marine lichen *bionts* survive symbiotically under unique conditions and can be sources of new bioactive secondary metabolites with chemotypes and pharmacophores different from those already discovered. Due to many successful identifications of bioactive chemotypes and pharmacophores that are present in each family of natural products, investigation of new and unexplored natural product sources is needed to discover novel and unique bioactive chemotypes and pharmacophores for new generations of drug leads. The laboratory of Dr. Rakotondraibe focuses on the secondary metabolites of liverworts and their endophytes, microbial associates of U.S. endemic lichens, and endemic Malagasy plants to discover compounds that (1) can be used to control insect vectors and (2) are active against human diseases, including cancer. Results obtained in the search for bioactive metabolites from fungal mycobionts of U.S. endemic lichens will be presented. Moreover, a recently developed in-house one-dimensional NMR-based dereplication method utilizing Total Spectroscopy Scanning to assist mass spectrometry, can detect minute amounts of known compounds, and prioritize new and active compounds, and will be also discussed.

Invited I

O8. New strategies for natural product discovery with interaction metabolomics

Warren S. Vidar[1], Tim U. H. Baumeister[2], Lindsay K. Caesar[3], Joshua J. Kellogg[4], Daniel A. Todd[1], Roger G. Linington[2], Olav M. Kvalheim[5], and Nadja B. Cech[1]. [1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC 27402, [2]Department of Chemistry, Simon Fraser University, Burnaby V5A 156, BC, Canada, [3]Department of Chemistry and Biochemistry, James Madison University, Harrisonburg, VA 22807, [4]Department of Veterinary and Biomedical Sciences, Pennsylvania State University, University Park, PA 16802, [5]Department of Chemistry, University of Bergen, Bergen, 5020, Norway. E-mail: wsvidar@umd.edu; tim.baumeister@gmx.de; caesarlk@jmu.edu; jjk6146@psu.edu; todd.da@pg.com; rliningt@sfu.ca; olav.kvalheim@uib.no; nbcech@uncg.edu

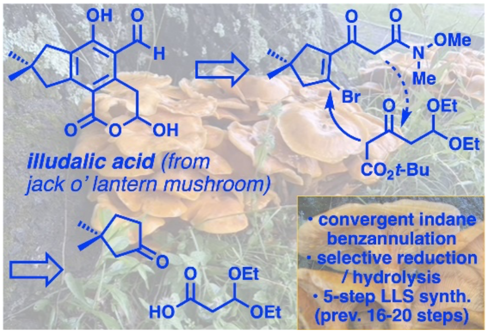
Interaction metabolomics, a novel approach developed in our study, addresses the shortfall in traditional mass spectrometry metabolomics which fails to consider potential synergistic relationships between components of natural mixtures. This innovative method incorporates compound interaction terms (CITs), representing the product of paired feature intensities in the data matrix. Validated through spiking inactive matrices with known quantities of antimicrobial compound berberine and synergist piperine, and testing the mixtures' antimicrobial activity against *Staphylococcus aureus*, we demonstrated that classical metabolomics led to false positives, while interaction metabolomics accurately identified the synergistic compounds. Further validation, using mixtures of goldenseal (*Hydrastis canadensis*) and habanero pepper (*Capsicum chinense*) extracts, confirmed the ability of interaction metabolomics to correctly link the enhanced activity of these mixtures to the combined action of berberine and capsaicinoids, reinforcing its potential utility in comprehensive mixture analysis across various research areas, including natural products research.

Invited II

O9. Synthetic analogues of illudalic acid from the Eastern Jack O' Lantern mushroom

Gregory Dudley, West Virginia University, Morgantown, West Virginia 26506, USA. Email: Gregory.dudley@mail.wvu.edu

The fungal natural product illudalic acid, from the toxic jack o'lantern mushroom *Omphalotus illudens*, has been a long-standing challenge for chemical synthesis. The significance of this challenge rose in recent years due to the discovery of its biological activity as a potent and selective inhibitor of the LAR family of protein tyrosine phosphatases (PTPs). We will describe benzannulation methodologies and convergent syntheses of illudalic acid and analogues for pharmacological characterization aimed at advancing synthetic and medicinal chemistry.



Illudalic acid structure, summary retrosynthesis, and highlights

Invited III

O10. Everything about methyl salicylate

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Methyl salicylate possesses a rich history of therapeutic advantages, primarily sourced from birch and wintergreen essential oils. Our research encompassed a market survey of these oils. None of the 27 birch samples examined were authentic: most comprised wintergreen, synthetic methyl salicylate, or mixed synthetic methyl salicylate with wintergreen EO. Despite birch and wintergreen containing 99% methyl salicylate, they have some differences in minor constituents. The precursor to methyl salicylate is gaultherin (glycosides), which degrades into methyl salicylate when exposed to heat and water. Our research delved into birch and wintergreen's antimicrobial and antileishmanial properties, revealing promising attributes. Notably, Gaultherin exhibited superior effects compared to aspirin regarding efficacy and side effects. Consequently, Gaultherin extraction emerges as a potential future avenue for regulatory compliance and solutions of methyl salicylate.

Oral

O11. Unlocking novel ligand-receptor associations from medicinal plants and fungi using untargeted metabolomics, molecular networking, docking and data mining

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Nuclear receptors (NRs) constitute an essential family of transcription factors with significant roles in both physiological and pharmacological processes. Among them, the aryl hydrocarbon receptor (AHR) has been shown to have numerous biological functions including cell cycle regulation, liver development, circadian rhythm regulation, as well as gut homeostasis regulation. Importantly, AHR is abundant in the gastrointestinal tract, and diet-derived AHR ligands have potential to maintain homeostasis in the gut. A significant source of AHR modulating compounds are derived from phytochemicals and mycochemicals from foods. However, among the myriad bioactive compounds from plants and fungi, identifying novel functional ligands which would significantly impact gastrointestinal health is a challenge. To overcome this, a combination of untargeted metabolomics, molecular networking, molecular docking, and data mining could be used to predict biologically functional compounds accurately and efficiently. This study predicted, identified, and characterized a novel AHR modulator in the white button mushroom (*Agaricus bisporus)*, and introduced a protocol to identify more novel AHR modulators in other fungi with nutritional and medicinal significance. The metabolome of *Agaricus bisporus* was analyzed in combination with known AHR ligands to find structural associations between the compounds constituting the two groups. Molecular networking of these compounds revealed that a methylated analog to benzothiazole was indicated in *Agaricus bisporus*, which was subsequently isolated and identified as 2-amino-4-methyl-benzothiazole(2A4). Cell-based AHR transcriptional assays revealed that 2A4 possesses agonistic activity and upregulated *CYP1A1* expression. Additional cell based assays on medicinal and culinary fungi has shown that *Ganoderma lucidum*, *Pleurotus ostreatus*, *Lentinula edodes*, and *Hypsizygus tessellatus* all have significant AHR modulating activity and are prime candidates for sources of novel AHR modulating compounds using this method. Future results have potential to guide dietary advice in maintaining gut health and systemic health through the multitude of biological functions AHR regulates.

Session III Production strategies for medicinal biomass and bioactive compounds

Chair: Nirmal Joshee, Fort Valley State University, USA
Co-chair: Barbara Liedl, West Virginia State University, USA

Keynote

O12. Plants in space: Enabling health on earth

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The quest to explore and colonize space has advanced the technology for sustained production of crops to regenerate the atmosphere, purify water and produce food. Plants are also emerging as pivotal tools to mitigate the impact of high dose radiation and extended exposure to microgravity on crew health. The impacts of spaceflight on crew health can be reduced by selecting species with phytochemicals that scavenge free radicals associated with high dose radiation, stimulate calcium uptake and metabolism to offset bone loss, increase resistance to common infections, and protect sensory perception. The techniques for optimizing the growth, and increasing the concentration of phytochemicals, of plants in space have broad application on Earth. The application of controlled environment agriculture (CEA) techniques developed for growing plants in space has the potential to increase phytochemical content, ensure uniform quality, and enable year-round production of medicinal plants.

Invited I

O13. Hairy root culture: A sustainable approach for high-level production of bioactive compounds

Fabricio Medina-Bolivar. Arkansas Biosciences Institute and Department of Biological Sciences, Arkansas State University, Jonesboro, AR 72401. E-mail: fmedinabolivar@astate.edu

Plant natural products have attracted significant attention because of their wide-ranging therapeutic capabilities and their use in pharmaceuticals, nutraceuticals, and cosmeceuticals. Nevertheless, conventional approaches to obtaining these compounds from their original organisms frequently encounter issues pertaining to sustainability, scalability, and intensive cultivation. In recent years, hairy root cultures produced via *Agrobacterium rhizogenes*-mediated genetic transformation have arisen as a highly promising alternative for the sustainable production of bioactive natural products. These cultures effectively replicate the biosynthetic pathways responsible for bioactive compounds found in their parent plants, while offering advantageous features. Hairy root cultures demonstrate continuous growth, straightforward genetic manipulation, and controlled production facilitated by chemical elicitors. As such, they are an ideal system for achieving high yields and consistent production of bioactive molecules. This presentation will provide an in-depth exploration of the pivotal strategies and recent breakthroughs in harnessing hairy root cultures for the synthesis of bioactive natural products and elucidating their biosynthetic pathways. The focus will be on bioactive phenolic compounds, including non-prenylated and prenylated stilbenes, flavonoids, and benzofurans found in peanut, pigeon pea, and mulberry. Growth conditions and elicitation techniques to maximize productivity, as well as efficient downstream processing methods for product extraction and purification, will be presented.

Invited II

O14. Enhancing health benefits: Investigating Brix% of specialty crops

Bipul Kumar Biswas, Aka Saha, and Arthur Robeson, Specialty Plants Research Lab., College of Agriculture Family Sciences and Technology, Fort Valley State University, Fort Valley, GA 31030, E-mail: biswasb@fvsu.edu

Research conducted at Fort Valley State University (FVSU) has shed light on the health benefits of high Brix% in various specialty crops, such as lettuce, stevia, tomatoes, and turmeric, grown through both conventional and hydroponic farming methods. Turmeric, renowned for its curcumin content, exhibited increased curcuminoid levels in crops with higher Brix%. These compounds possess potent anti-inflammatory and antioxidant properties, potentially offering enhanced therapeutic benefits. Similarly, stevia, a natural sweetener, demonstrated elevated levels of its sweetening compounds in high Brix% plants, which could contribute to its utility for calorie-conscious individuals. Lettuce varieties, when cultivated hydroponically with elevated Brix%, exhibited improved nutrient density, particularly in vitamins and minerals. This suggests that hydroponic systems, coupled with high Brix%, could offer nutrient-rich lettuce for health-conscious consumers. Tomatoes, a popular dietary source of antioxidants like lycopene, displayed increased lycopene concentrations with higher Brix% across both farming methods. Lycopene's potential role in reducing the risk of chronic diseases, including certain cancers and cardiovascular conditions, underscores the importance of Brix% optimization. Conventional farming showed promising results too. High Brix% levels in conventionally grown crops positively influenced phytochemical content, contributing to their potential health-promoting properties. These crops could provide accessible options for individuals without access to specialized farming techniques. FVSU's research indicates that both conventional and hydroponic methods, when combined with high Brix%, can enhance the health benefits of specialty crops. The increased levels of bioactive compounds such as curcuminoids, sweetening agents, and antioxidants in turmeric, stevia, lettuce, and tomatoes suggest a potential for improved therapeutic and nutritional value. These findings highlight the significance of agricultural practices that prioritize Brix% to produce crops with enhanced health benefits, catering to a spectrum of consumer preferences and dietary needs.

Oral I

O15. Silicon priming and *in vitro* morphology improvement of three medicinal *Scutellaria* spp.

Samantha H. Sherman, Poonam Khatri, Birat Sapkota, and Nirmal Joshee, Department of Agriculture, Fort Valley State University, Fort Valley, GA 31030. josheen@fvsu.edu

Three *Scutellaria* species (*S. baicalensis*, *S. barbata*, and *S. lateriflora*) are used in the herbal industry. The large-scale biomass production can be made possible through micropropagation. However, *Scutellaria* spp. are prone to a physiological disorder hyperhydricity leading to high mortality of micropropagated plants during acclimatization and to the field or greenhouse transfer. Literature suggests that cytokinins and sugar in the medium play a role in the induction of hyperhydricity. In this study Driver and Kuniyuki medium was supplemented with $CaSiO_3$ at 0, 1, 5, and 10 mM. concentrations. Nodal explants were exposed to silicon treatments for four weeks and then subcultured onto shoot induction medium incorporated with 0, 1, 5, and 10 µM of meta-Topolin for four weeks to see if there is an onset of hyperhydricity. After two passages of four weeks each on elongation medium, destructive count was performed. Plants were assessed for severity of hyperhydricity and browning. Chlorophyll analysis was conducted to determine whether silicon supplementation had any effect on chlorophyll content. *Scutellaria baicalensis* responded best to DKW+1 mM $CaSiO_3$ and DKW+10 µM mT and produced high quality explants with minimal browning and no hyperhydricity. High frequency of *in vitro* flowering was reported in *S. barbata* exposed to 1-, 5-, and 10-mM silicon without plant growth regulators. The best treatment for *Scutellaria lateriflora* for reduction in browning and hyperhydricity was DKW+5 mM $CaSiO_3$ and DKW with no plant growth regulators. Microscopy revealed the presence of silicon bodies in treated samples and differences in surface structure features such as stomata structure were observed.

Oral II

O16. Sterilization, explant selection, and media optimization for the micropropagation of highly nutraceutical *Aronia* species

Sramika Rijal, Samantha Sherman, and Nirmal Joshee, Department of Agriculture, Fort Valley State University, Fort Valley, GA 31030. Presenter Email: srijalti@wildcat.fvsu.edu; Corresponding author Email: josheen@fvsu.edu

Aronia species (chokeberries, Rosaceae) are of north American origin and are known for their nutrient-rich underutilized berries. *Aronia* berries have been reported to have higher antioxidant capacity than blueberries and are used in food, bakery, herbal, and green cosmetic industries. The demand for these products is increasing and so is for desirable planting stock. Earlier studies have shown heavy pathogen load in greenhouse raised and field plants and slow growth of nodal and shoot tip cultures. For direct organogenesis, plants from greenhouse were selected and stock cultures were established with series of sterilization using Clorox and mercuric chloride at various concentrations and for different time length. In explants sterilized with bleach, the contamination rate was 80% whereas in mercuric chloride contamination was not seen but explant survival was only 10%. Three media; Murashige and Skoog, Driver and Kuniyuki and Quiorine and Lepoivre were used for *in vitro* propagation of *A. melanocarpa* cv Viking and *A. arbutifolia* 'red' and 'black' cultivars. For indirect organogenesis, *in vitro* leaves were harvested to prepare transversely cut explants and placed in MS media modified into 25 treatments of Indole Butyric Acid (IBA), Indole Acetic Acid (IAA), 1-Naphthaleneacetic Acid (NAA), 6-Benzylaminopurine (BAP) and Thidiazuron (TDZ), in the form of auxin-cytokinin combination. After two weeks of incubation, regenerating callus was found in *A. arbutifolia* 'black' in response to IBA and TDZ, whereas a minimum callus was observed in the *A. arbutifolia* 'red' cultivar. Upon subculturing in the fresh media, new leaves, and shoot buds were noticed in the media supplemented with TDZ compared to BAP and meta-Topolin. Our studies indicate that leaves could be a better and rapid way to multiply *Aronia* spp. Acclimatized plants were successfully transferred to the greenhouse and antioxidant level of berries will be determined in the field studies.

Session IV Hemp and Medicinal Cannabis

Chair: Diana Roopchand, Rutgers University, USA
Co-chair: Aruna Weerasooriya, Prairie View A&M University, USA

Keynote

O17. Antinociceptive effects of cannabichromene in animal pain models

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For centuries, cannabis has been utilized to address various medical conditions, particularly those that are associated with perceived pain. More recently, individual cannabinoid compounds have also been examined, including THC and CBD. However, the euphoric effects associated with some of these compounds have raised concerns about their therapeutic value, especially in light of the opioid epidemic. To address the need for non-euphoric pain treatments, we have focused our investigation on identifying non-psychoactive cannabinoids with anti-nociceptive properties. Cannabichromene (CBC) is a minor, non-psychoactive cannabinoid whose pharmacological profile suggests that it has the potential to reduce pain. Therefore, we assessed the analgesic potential of CBC in: 1) a model of chemotherapeutic-induced peripheral neuropathy (CIPN); 2) hot and cold thermal assays; and 3) the formalin inflammatory pain assay. The results showed that CBC (at concentrations of 20 mg/kg, i.p.) reduced mechanical allodynia in a mouse model of CIPN. Additionally, we found that CBC increased the tail-flick latency in the hot thermal assay, while reducing the pain score in both phases of the formalin test (initial acute pain followed by delayed inflammatory pain). We found no CBC effect in the acetone cold thermal assay. Lastly, we did not observe any sex differences in CBC's analgesic properties, which is in contrast to the analgesic properties of other cannabinoids that have been previously examined. Overall, our results suggest that CBC has antinociceptive properties that may be pain-type specific. CBC is reported to be a potent agonist of TRPA1 and also have activity at the CB2 receptor, activation of either or both of these receptors may be responsible for the antinociceptive activity of CBC and is currently under investigation.

Invited I

O18. Microbiome Diversity of Industrial CBD and Fiber Hemp Genotypes

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Microbiomes (bacterial and fungal species) are crucial for host medicinal plant growth and development. The microbial communities associated with host plants (either in endophytic or epiphytic modes) can perform functions, e.g., producing specialized metabolites and nutrient mobilization from the soil. However, environmental factors and host genotypes can influence microbiome composition and diversity, impacting the plant's ability to cope with climatic stresses and pathogenic attacks. To understand the microbiome diversity and composition of industrially important hemp (*Cannabis sativa* L.), we evaluated the endophytic and rhizosphere microbial communities in different plant organs. For this purpose, we performed next-generation sequencing and bioinformatics analysis of the DNA extracted from soil, root, stem, and leaf parts of two fiber-rich (American Victory-1 and Unknown) and cannabidiol (CBD; Sweet Sensi and Cherry Wine) genotypes. The results showed significantly higher microbiome diversity across CBD vs. fiber genotypes. This was highly pronounced in the root's fungal and bacterial endosphere, whereas the stem part had a significant richness in fungal diversity compared to bacteria between CBD and fiber genotypes. Interestingly, the soil system showed no significant diversity variation across CBD vs. fiber genotypes – suggesting that the endophytic microbiome is significantly variable in root>stem>leaf>soil parts. In fungal diversity, Ascomycota, Basidiomycota, and Mucuromycota were highly abundant root and stem than leaves in CBD than fiber genotypes, whereas *Mortierella, Cladosporium*, and *Neocosmospora* were the core-microbiome species. The results suggest that microbiome species, specifically endophytes, can be selective breeding approaches for hemp cultivars as these can drastically influence industrially essential traits. This work can help build a culturome of genotypes and specialized microbiome that is helpful to improve plant growth and synthesize metabolites or fibers.

Invited II

O19. Regulation of the cannabinoid and terpene metabolic network in industrial hemp (*Cannabis sativa*) under biotic stress

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Since its legalization in the 2018 farm bill the cultivation of industrial hemp (*Cannabis sativa*) has gained significant attention. However, the renewed interest in this crop and medicinal plant has demonstrated that our knowledge about its cannabinoid and terpene metabolic network is still limited. Such detailed understanding is highly relevant considering the potential medicinal applications as well as legal regulations of certain cannabinoids. We have taken a chemical ecology approach to study the regulation of cannabinoid and terpene metabolism in hemp. In general plant species respond with an increase in specific specialized metabolites when exposed to biotic stresses like pests and pathogens. Thus, insect or mammalian herbivory could trigger biotic stress, by activating the jasmonic acid signaling pathway, and lead to an increase in cannabinoid and terpene levels, the two prominent specialized metabolites produced in glandular trichomes of hemp. To test this hypothesis, we used hemp varieties for feeding assays with beet armyworm (*Spodoptera exigua*) and treatments with methyl jasmonate and oral saliva. Flower samples were extracted, and cannabinoid and terpene contents quantified by LC/MS and GC/MS analysis. Our studies demonstrated that these biotic stress treatments resulted in altered terpene profiles and increased levels of major and minor cannabinoid compounds. Subsequent qRT-PCR analysis revealed respective changes in the expression of known biosynthetic genes in the terpene-cannabinoid metabolic network.

Invited III

O20. Early sex-determination in hemp using transcriptomic analysis

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Hemp (*Cannabis sativa*) produces plethora of medicinally important chemicals, including the well-known CBD (cannabidiol). These chemicals are primarily produced in the bracts of female hemp inflorescences. Thus, early detection of female hemp plants will be highly advantageous from agronomic, economic, and environmental standpoints. The current project hypothesized that genetic regulation of early vegetative development in two sexes of hemp plants differs and thus there will be differentially expressed genes between the two sexes of hemp plants even at the early vegetative stage. Our research employs next-generation sequencing based transcriptome analysis using a 3′-end-directed approach to identify differentially expressed genes between two sexes of hemp plants at early vegetative stage. Our study identified several candidate genes and validated their sex-specific expressions using RT-PCR analysis. Identified sex-specific genes can be utilized to develop biomarkers to facilitate accurate determination of sex of hemp plants at an early developmental stage, leading to potential resource savings. Additionally, identification of such genes will lay the foundation of development of female-only hemp lines.

Oral I

O21. Untargeted Lipidomics of Liver Tissues from CBD-treated Estrogen Deficient and Sufficient Mice

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Low levels of 17β-estradiol (E2) in postmenopause can increase the risk of metabolic disease. Hormone replacement therapy (HRT) is not recommended for chronic disease prevention as use beyond five years can increase risk of cancer, heart disease, and stroke. Our previous work indicated that cannabidiol (CBD), a non-psychotropic phytocannabinoid component of *Cannabis sativa*, may be useful in postmenopause. Markers of inflammation and symptoms of metabolic disease were reduced in CBD-treated ovariectomized (OVX) mice compared to vehicle (VEH)-treated OVX. Other studies reported that lipid metabolism is dysregulated in E2 deficiency. In this study, lipid extraction of liver tissues from VEH or CBD-treated OVX and sham surgery (SS) mice was performed. Vanquish Horizon UHPLC system (Thermo Fisher Scientific, Waltham, MA) was employed for lipidome detection. MS/MS raw data were analyzed using MS-DIAL, and lipid annotations were generated by matching identified lipids with the built-in MS/MS database. To enhance accuracy, the quantification of annotated lipid species was performed from Mass Spec1 (MS1) runs using El-MAVEN, followed by normalization using SPLASH® Lipidomix® MS Internal Standard. Chemometrics analysis was performed using Metaboanalyst 5.0. We found alterations in several lipid classes including phosphatidylethanolamine (PE), lysophosphatidylethanolamine (LPE), phosphatidylglycerol (PG), cardiolipin (CL), and fatty acids (FA). For example, loss of E2 (VEH-treated SS vs. VEH-treated OVX) resulted in increased hepatic levels of: 1) PE, which is associated with liver diseases and 2) CL, which is associated with non-alcoholic fatty liver diseases; however, PE and CL were found to be decreased in livers of CBD-treated OVX mice. Interestingly, PE and CL levels were increased in CBD-treated SS mice relative to CBD-treated OVX mice, suggesting CBD can have differential effects depending on E2 levels. These findings provide insight for continued investigation of the role of CBD in modulating lipid profiles and regulating cardiometabolic health in E2 deficient and sufficient females.

Session V Bioactive compounds of plants and their roles in human health I – Cancer

Chair: Gerald Hankins, West VA State University, USA
Co-chair: Anait S. Levenson, Long Island University, USA

Keynote

O22. Searching Nature for the Cure

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Cancer remains a serious health concern with over 8 million deaths each year throughout the world. Treatment of cancer patients is often complicated by the many forms of the disease (over 270 different cancer types), side effects of current chemotherapeutic agents and the development of chemoresistance to many drugs. As a result, there is a need to find new therapies that are effective and safer than current chemotherapeutic agents or can be used with current therapies to increase the therapeutic efficacy of treatment regimens. Natural products have been used in treating various cancers for decades, and these agents have come from a wide variety of life forms, including microorganisms, plants, and amphibians. Plant-derived anticancer agents include drugs such as paclitaxel, etoposide, the vinca alkaloids and irinotecan, with many other plant-based chemicals (e.g. resveratrol, curcumin, lycopene, flavonoids, gallacatechins) showing potential for use as adjunctive cancer chemotherapeutic agents. While over 390,000 plant species have been identified worldwide, only limited studies have characterized the potential of many of these species to contain secondary metabolites with the ability to selectively kill cancer cells or act as chemopreventive agents. Thus, it is quite likely that these plants contain a potential wealth of compounds that could lead to newer and safer agents for treating one or more of these deadly diseases. As a result, the search in nature for the cure for cancers continues, finding new compounds with unique chemical structures and novel mechanisms of action, which is the focus of this presentation.

Invited I

O23. Soy isoflavone genistein reboosts targeted therapy sensitivity in triple negative breast cancer

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Triple-negative breast cancer (TNBC) is a very aggressive type of breast cancer that does not expression important proteins called estrogen, progesterone and HER2. This makes TNBC hard to treat because it does not respond to common targeted treatments. However, recent treatments using the immune system to target key immune proteins such as PD-1 and PD-L1 have been successful in many types of cancer. This form of immunotherapy works by blocking PD-1/PD-L1 pathway and leads to stimulating the body's own immune system to recognize and attack cancer cells, thus providing a potential new treatment option for TNBC patients. Nevertheless, only approximately 30% of TNBC patients may respond to these drugs, suggesting the majority of TNBC patients would not benefit from these innovative immunotherapies. It is believed that low levels of immune cells and PD-L1 expression in tumors are the primary reasons for the lack of response or resistance to anti-PD-1/PD-L1 treatments. The use of nontoxic and healthy food and nutrition to increase treatment response is an emerging field of cancer research. Our studies showed that adding genistein (GE), a bioactive dietary component found in soybean products such as soy milk, soy protein and tofu, could significantly enhance the efficacy of anti-PD-1 immunotherapy in a TNBC mouse model that was previously not responsive to the treatment. Our research also found that soybean GE can increase tumor immune response by significantly drawing immune cells into the tumor and increasing PD-L1 expression. Our studies will help to establish a novel nutrition-based intervention targeting TNBC patients who might not qualify for this treatment initially. Eventually, we will develop a novel strategy of administering a tailored dietary plan to resensitize these patients to emerging anti-PD-1/PD-L1 immunotherapies.

Invited II

O24. Current advances of dietary stilbenes as targeted anticancer agents for cancer prevention and therapy

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Cancer is among the leading causes of death worldwide. Developing novel chemopreventive, interceptive and therapeutic strategies for cancer management is a major goal in clinical oncology. Polyphenols found in plants and food have gained increased importance in cancer prevention/therapy due to their potent biological properties including anti-inflammatory, anti-oxidative, antiproliferative and pro-apoptotic effects. Numerous studies have shown that stilbene polyphenols can affect multiple signaling pathways involved in inflammation, cell proliferation and survival, EMT, invasion, angiogenesis, and metastasis by modulating transcription and growth factors, enzymes, and non-coding RNAs. I will discuss the importance of targeted strategies and, particularly, MTA1-mediated anticancer effects of stilbenes including resveratrol, pterostilbene, and Gnetin C in prostate cancer *in vitro* and *in vivo*. Moreover, challenges in combinatorial approaches using natural compounds with approved drugs will be discussed. In addition, an overview on human clinical trials with resveratrol, pterostilbene and Gnetin C will be presented.

Oral I

O25. The bioactive natural compound, theaflavin and cancer

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Consumption of black tea, rich in polyphenols, has been found to reduce ovarian cancer risk. Theaflavin (TF1), theaflavin-3-gallate (TF2a), theaflavin-3'-gallate (TF2b) and theaflavin-3, 3'-digallate (TF3) are four main theaflavin derivatives found in black tea. All four theaflavin derivatives inhibited ovarian cancer cells. Theaflavin-3, 3'-digallate (TF3), the major theaflavin monomer in black tea, exhibited a potent growth inhibitory effect on the cisplatin-resistant ovarian cancer A2780/CP70 cells (IC50, 23.81 μM), and was less cytotoxic to normal ovarian IOSE-364 cells (IC50, 59.58 μM) than to the cancer cells. Flow cytometry analysis indicated that TF3 induced preferential apoptosis and G2 cell cycle arrest in A2780/CP70 cells with respect to IOSE-364 cells. TF3 induced apoptosis through both the intrinsic and extrinsic apoptotic pathways and caused G2 cell cycle arrest via cyclin B1 in A2780/CP70 cells. The p53 protein played an important role in TF3-induced apoptosis and G2 cell cycle arrest. TF3 upregulated the p53 expression via the Akt/MDM2 pathway. Our findings indicated that TF3 plays an important role in the prevention and treatment of platinum-resistant ovarian cancer.

Oral II

O26. TET enzymes are novel epigenetic target for curcuminoids in leukemia cells

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Curcuminoids are natural polyphenolic compounds derived from turmeric and include curcumin, demethoxycurcumin and bisdemethoxycurcumin. The development of curcumin as a small molecule drug was not successful because of its low solubility and metabolic instability. Accordingly, the development of metabolically stable and soluble curcumin analogs is necessary. Dimethoxycurcumin (DMC) is a metabolically stable analog of curcumin that demonstrated antitumor activity in leukemia cells by an unclear mechanism. In this study, we are investigating the effect of both curcumin and DMC on DNA hydroxymethylation (5hmC), an epigenetic mark that is known to affect gene expression, cellular differentiation and is catalyzed by a family of enzymes known as Ten Eleven Translocation (TET).

HL60 and U937 leukemia cell lines were treated with different concentrations of curcumin and DMC for 48 hours. Nuclear extracts were used to measure the activity of TET isoforms using a fluorometric ELISA-based assay and an antibody against 5hmC. Additionally, DNA extracted from treated cells were used to quantitate the epigenetic mark 5hmC using a fluorometric assay that uses a specific antibody against 5hmC. Global 5hmC epigenetic mark was significantly increased upon treatment with either curcumin or DMC in both cell lines cells. Concordantly, both drugs increased the activity of the TET enzymes in both HL60 and U937 cells. In conclusion, we identified TET enzymes as a novel epigenetic target for curcumin and its analog DMC that may affect gene expression, differentiation of leukemia cells and the antitumor activity of these compounds.

Session VI Bioactive compounds of plants and their roles in human health II – Inflammatory diseases and beyond (IV)

Chair: Andera Doseff, Michigan State University, USA
Co-chair: Jeremy Johnson, University of Illinois at Chicago, USA

Keynote

O27. Gut microbial metabolism of dietary polyphenols in health and disease

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The molecular mechanisms by which dietary fruits and vegetables confer health benefits remain poorly understood. Historically, beneficial properties in the amelioration of conditions like cardiometabolic disease or intestinal cancer have been attributed to the antioxidant activity of flavonoids. However, flavonoids are consumed in a largely glycosylated form, rendering them poorly available for small intestinal absorption and subsequent systemic distribution. Upon reaching the colon, select commensal gut bacteria can catabolize these flavonoid substrates into smaller monophenolic acids.

Our lab uses several mouse disease models to assess the contribution of microbial catabolism to the beneficial effects exerted by dietary flavonoids. In a diet-induced obesity model, we identified gut microbial flavonoid catabolites, which on their own are sufficient to reduce cardiometabolic disease burden in mice. Dietary supplementation with flavonoid composites attenuated obesity and continuous delivery of the catabolite 4-hydroxyphenylacetic acid was sufficient to reverse hepatic steatosis. We showed that this anti-steatotic effect is associated with the activation of AMP-activated protein kinase a. Analysis of healthy human gut metagenomes revealed that under one percent of individuals contains a complete flavonol catabolic pathway, underscoring the rarity of this process in people. Our study will impact the design of dietary and probiotic interventions to complement traditional cardiometabolic treatment strategies.

Invited I

O28. Dietary intervention for castration-resistant prostate cancer

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Androgen ablation therapy (AAT) and bilateral orchidectomy have been the primary therapeutic options for locally advanced and/or metastatic Castration-Resistant Prostate Cancer (CRPC). While most patients initially respond to AAT, a significantly high number of cases ultimately transition to CRPC. Currently, available therapeutic options against CRPC are not effective. Therefore, the identification of novel and safe targeted therapies for prostate cancer is of high clinical relevance. Urolithins are naturally occurring dietary precursors produced as metabolites of ellagitannins (ET) and ellagic acid (EA) by gut microbiota. Urolithin A (UroA) is the most abundant isoform of urolithins found in plasma and urine after ET and EA intake. UroA exerts anti-proliferative activity against many cancer types in vitro. Through structure-activity relationship (SAR) studies based on naturally occurring Urolithin A (UroA) structure, we developed a novel molecule UroBoc that directly binds to the NTD and inhibits CRPC growth. UroBoc inhibits the growth of Androgen receptor (AR)+ CRPC, including enzalutamide-resistant cells, at 800nM concentration, 40 times lower than that of the parent compound, UroA. UroBoc is non-toxic to normal prostate epithelial cells or other cancer cells at this concentration. UroBoc inhibited AR, AR-SV, and PSA expressions in AR-FL (LNCaP, C42B) and AR-V7 (22-RV1) CRPC cell lines. Molecular studies suggested that UroBoc selectively promotes ubiquitination and subsequent degradation of AR and AR-V7 by proteasome activation. Further, oral administration of UroBoc (20mg/kg) for six weeks significantly inhibited tumor growth in CRPC in both castrated and non-castrated mice models as compared to the parent-UroA (50mg/kg) treatment—immunohistochemistry analysis confirmed the in vitro findings, such as the downregulation of AR signaling in UroBoc-treated tumor tissues. UroBoc, a safe dietary supplement, is highly effective in controlling AR+ CRPC. In addition, UroBoc treatment exerts the anti-cancer effects outlined in our studies. Therefore, it may improve survival in prostate cancer patients.

Invited II

O29. Flavone apigenin triggers transcriptome reprograming reducing inflammation through Its association with novel protein target

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Chronic inflammatory diseases, including cancer and obesity, are reaching epidemic levels worldwide, inflicting life-threatening conditions and significant socioeconomic burdens. Flavonoids, including flavones, are the largest class of bioactive dietary phytochemicals attracting great interest for their beneficial effects in treating and preventing chronic inflammatory diseases. We showed that the dietary flavone apigenin has anti-inflammatory activity in macrophages. Apigenin reduces macrophage infiltration at inflammatory sites resulting in decreased tumor growth. Additionally, apigenin reduces obesity-induced inflammation, reducing macrophage infiltration and modulating microbiome dysbiosis. Apigenin associates with the RNA-binding protein hnRNPA2. RNAseq analyses revealed the gene regulatory networks modulated by the hnRNPA2/apigenin axis. HnRNPA2 associates with a critical transcription factor in inflammation. Through this interaction, apigenin via hnRNPA2 reduces inflammation. These findings provide novel insights into how medicinally active phytochemicals exert anti-inflammatory and anti-cancer activities, providing critical opportunities for preventing and treating inflammatory diseases, including cancer and obesity.

Oral I

O30. Dietary curcumin intake and its effects on the transcriptome and metabolome of *Drosophila melanogaster*

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Curcumin (*Curcuma longa*) is a yellow pigment found primarily in turmeric, it is from the ginger family and is best known as a spice from culinary dishes and its medicinal properties. Curcuminoids represent an economically important horticultural crop worldwide due to the beneficial properties of their phytochemicals including turmeric, curcumin, and its polyphenolic compounds. Diets supplemented with these phytochemicals may cause a shift in the organism's gene expression and metabolism. Understanding how these interactions can occur, can reveal the potential health effects associated with these changes. To understand, we performed transcriptomic and metabolomic analyses of adult *Drosophila melanogaster* flies reared on curcumin supplemented diets. When comparing physiological changes between flies reared on control and curcumin diet, there was a substantial decrease noted in weight between curcumin as compared to the control. Metabolome analysis revealed that Erythritol, Butanoic acid (3-mehtyl-2-hydroxy), and Ornithine were downregulated and Sucrose, Gluconic acid, and Maltose were highly upregulated in response to the curcumin diet. Further investigation using integration analysis between the transcriptome and metabolome data showed Signal Transduction, G Protein Coupled Receptors (GPCR) Signaling, G Protein Coupled Receptors (GPCR) downstream signaling, and Purine nucleotides nucleosides metabolism, displayed the highest number of overlapping metabolites with varying overlapping genes. Using a Metabolite-Disease Interaction Network analysis showed with upregulated metabolites such as Dopamine; Succinic, Fumaric, L-Aspartic Acids being connected to Alzheimer's Disease. For down-regulated metabolites Ornithine and Citric Acid are connected to Schizophrenia.

Oral II

O31. Human-derived *Lactobacillus* strains may deconjugate estrogens

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Estrogens, which are C18 steroid hormones biosynthesized from low-density lipoprotein (LDL) cholesterol, are important for both male and female reproductive, metabolic, bone, and gastrointestinal function. Estrone (E1) and 17β-estradiol (E2) constitute the primary bioactive forms of estrogen in non-pregnant females. The estrobolome is the collection of gut bacteria that can metabolize estrogens. Many gut bacteria harbor a *gus* gene, which encodes β-glucuronidase (GUS), a group of substrate specific enzymes responsible for removing the glucuronide moieties that make compounds more water soluble for subsequent excretion via bile into urine/stool. GUS-mediated deconjugation of glucuronidated E1 (G-E1) and E2 (G-E2) allows reabsorption of active E1 and E2 into enterohepatic circulation. Basic knowledge of the bacterial strains with estrogen-specific GUS activity is limited but required to understand the impact of gut bacteria on disorders associated with hypo- and hyper-estrogenic conditions. Additionally, dietary compounds, medications, or supplements may influence the composition of the estrobolome and, subsequently, the levels of circulating estrogens. To explore this, we first sought to identify strains of *Lactobacillus* that could deconjugate glucuronidated estrogens. Eight human derived *Lactobacillus* strains with and without putative GUS activity were anaerobically cultured in de Man, Rogosa & Sharpe broth in the absence or presence of G-E1 and G-E2. Over a 24 h time course, aliquots of culture were collected for LC-MS analysis of free and conjugated estrogens. Among the tested strains, *L. jensenii* 269-3 was able to deconjugate G-E1 and G-E2 while *L. rhamnosus* LMS2-1 efficiently deconjugated only G-E1. Conversely, the remaining six *Lactobacillus* strains were unable to deconjugate G-E1 or G-E2. Ongoing work will determine if GUS is active when bacteria are grown in other carbon sources. Future work will evaluate GUS activity towards G-E1 and G-E2 in the absence/presence of various natural product compounds.

Session VII Plant and algae derived cosmeceuticals and nutraceuticals

Chair: Alok Arun, Interamerican University of Puerto Rico – Barranquitas, Puerto Rico
Co-chair: Eyas Mahmoud, West Virginia State University

Keynote

O32. Take a deep breath and thank the algae

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The algae have been used medicinally for over 5000 years in countries spanning the globe including China, Japan, Pan Pacific Islands, and the northern Atlantic Ocean countries. Uses include: antiparasitic, antitumor, antibiotic, antiviral, and antioxidant. Nutraceutical and supplement usages to supply and/or affect: constipation, goiter, juvenile herpes, omega 3's, iodine, Beta Carotene, trace minerals, Alzheimer's, aphrodisiac, anxiety, and miscarriages. Cosmeceutical developments include facial and skin applications for the minimization of wrinkles, dry skin, color and crème-based compounds, hair luster, vitality and natural sunscreen. Algae products include: the development of biodegradable plastics, commercial pigments, concrete, and ecosystem services.

Invited I

O33. Exploration into natural variation associated with nutraceuticals and cosmeceuticals in *Capsicum chinense*

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Capsicum chinense is an important cultivated crop with color diversity, abundant biochemical and mineral constituents of nutritional and cosmetic value. These horticultural crops are good sources of bioactive compounds contributing to the fruit color diversity. However, some alterations in the genome often impact the metabolism, whether positive or negative, control of the phenotype. Therefore, we used a genome-wide association study (GWAS) employing the multi-locus mixed model (MLMM) in pepper collections to elucidate causal SNPs and the genetic factors that affect pepper color formation, which is crucial while ripening. We identified 43,357 single nucleotide polymorphisms (SNPs) from the genotyping by sequencing of 244 collections. Furthermore, we used the contents of capsaicinoids, carotenoids, and flavor compounds to associate the genetic variants involved in producing these metabolites and manifesting into diverse fruit colors. The GWAS revealed common candidate genes related to multiple metabolites, hence regulating diverse metabolite biosynthesis pathways. Overall, it was noted that the several genes controlling more than one trait indicated crosstalk between pathways or finding the pentatricopeptide repeat-containing protein, transcription factor BIM2, kinesin-like protein FRA1, protein NRT1/ PTR FAMILY 7.3-like, protein FAR1-related sequence 5-like, zinc finger A20, among others. Allelic effects resolved for various candidate genes indicated variable levels of secondary metabolite accumulation. Therefore, the genes identified in the studied accessions have essential roles in fruit color development and metabolite accumulation.

Invited II

O34. Research on nutraceutical properties on Sericea Lespedeza by the American Consortium for Small Ruminant Parasite Control

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Sericea lespedeza (SL; *Lespedeza cuneata*) is a widely adapted, tannin-rich warm season perennial legume that can be used for grazing, hay, or as a conservation plant. It has been used for soil conservation and as forage for livestock for over 100 years in the United States and can be grown on a wide range of soil types, including acidic, infertile soils that will not support growth of other forage legumes. Research over the last 15 years by the American Consortium for Small Ruminant Parasite Control (ACSRPC.org) has focused on the nutraceutical (nutritional + pharmaceutical) properties of this forage, with a particular focus on its health benefits as an anti-bloat, anti-bacterial, and anti-parasitic feed for livestock. The ACSRPC has evaluated the anti-parasitic efficacy of SL in fresh (grazed), dried (hay, pellets), and preserved (ensiled) forms for both sheep and goats under differing production conditions (various U.S. states, South Africa), and found similar positive results. The bioactivity of SL is thought to be related to the high concentration and unique molecular structure of its condensed tannins (CT), which have up to 98% ‘prodelphinidin-type’ subunits and are very reactive with protein. Other benefits of these bioactive compounds in SL include reduced ruminal production of methane and reduction in viability of fly larvae in manure. Achieving an optimal dietary inclusion rate of SL for livestock to minimize potential negative effects of CT on digestibility while maintaining their health benefits for livestock will be a focus of future research of the ACSRPC with this forage.

Oral

O35. Production of a prenylated stilbene-rich extract from elicited hairy root cultures of peanut and assessment of its bioactivities for human health applications

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Plant-based natural products have garnered considerable attention due to their wide range of biological activities, including antioxidant, cardioprotective, and anti-inflammatory properties. One group of these natural products, known as prenylated stilbenes, has demonstrated numerous health benefits, and it is particularly produced in peanuts as phytoalexins. However, obtaining these specialized metabolites from natural sources can be a time-consuming and challenging process. Therefore, the objective of this project was to utilize a peanut hairy root system to produce a prenylated stilbene extract rich in prenylated stilbenes and evaluate its potential health benefits. To accomplish this objective, previously established hairy root cultures of peanut cultivar Hull were co-elicited with methyl jasmonate, methyl-β-cyclodextrin, hydrogen peroxide, and magnesium chloride for 168 hours. The levels of prenylated stilbenes in ethyl acetate extracts from the culture medium were then determined by high-performance liquid chromatography analysis. The resulting extract was found to be rich in the prenylated stilbenes arachidin-1 and arachidin-3, with concentrations of 162.37 ± 1.33, and 72.24 ± 2.05 mg/g of extract, respectively. Additionally, the extract exhibited considerable antioxidant activity with an IC_{50} value of 8.147 µg/mL in the DPPH antioxidant assay. Furthermore, the extract was evaluated for its potential anti-inflammatory and cardioprotective effects in cell-based assays. Based on these findings, it is evident that peanut hairy root extracts rich in prenylated stilbenes hold great promise as potential ingredients for the development of nutraceutical formulations aimed at enhancing human health.

Session VIII Plant based antimicrobials

Chair: Rao Mentreddy, Alabama A&M University, USA
Co-chair: Padma Nimmakayala, West Virginia State University

Keynote

O36. Bioactive components of medicinal plants and their effects on antibiotic-resistant human pathogenic bacteria.

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Antibiotic resistance is a significant public health issue of the 21st century and represents an important threat to the global economy. Healthcare-associated infections are one of the major complications in medicine, mainly caused by drug-resistant bacteria such as *Clostridioides difficile*. The spread of drug-resistant pathogens limits the efficacy of available drugs and complicates the treatment of bacterial diseases. Therefore, there is an urgent need for new compounds with mechanisms of action capable of limiting bacterial resistance. Regulating antibiotic prescription, instituting antimicrobial stewardship programs, and developing new drugs with improved potency against multi-drug-resistant pathogens are approaches with improved potency against multi-drug-resistant pathogens are some of the approaches that can be instituted to help solve this major public health problem. Plants synthesize and utilize several aromatic metabolic compounds to fight against pathogens and predators. These metabolites could be a source of potent antimicrobial drugs and a promising strategy for discovering bioactive products against multidrug-resistant bacteria. In this study, the antimicrobial effects of medicinal plants were evaluated on multidrug-resistant strains of *C. difficile*. Results showed that extracts from these plants have potent antimicrobial activities against this multidrug-resistant pathogen. Furthermore, sub-inhibitory amounts of the active compounds of the select plant extract affected virulence factors of *C. difficile* by targeting cell-wall synthesis, toxin production, and/or spore formation. This study also demonstrated that a significant number of *C. difficile*-challenged mice treated with a partially purified extract from a select medicinal plant survived the acute phase of the infection as compared (60% versus 40%) to vancomycin, the treatment of choice of *C. difficile* infections. A deeper understanding of the mechanism of action of these compounds is still needed. Further investigation of medicinal plants would be a cost-effective and innovative strategy to develop next-generation novel antimicrobials to combat the emerging threat of antibiotic resistance.

Invited I

O37. The biosynthesis and regulation of the anti-malarial artemisinin in *Artemisia annua* (Asteraceae)

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Artemisinin is a sesquiterpene lactone with potent anti-malarial properties, primarily synthesized in the glands of *Artemisia annua*. Recognized by the WHO as a critical component in malaria treatment, the unique structural feature of artemisinin – its endoperoxide bridge – renders in vitro synthesis economically inviable. Our study explores the evolution of artemisinin biosynthesis within the large *Artemisia* genus, which consists of approximately 400 species but only a select few have evolved to produce artemisinin. Central to our research is the examination of genome evolution in the context of transposon activity and its influence on regulating gene expression. We place particular emphasis on genes encoding for terpene synthases and P450 monooxygenases that are central to artemisinin biosynthesis. Furthermore, we also aim to understand the genetic and metabolic control directing carbon flux towards the general terpene pathway and the committed artemisinin biosynthesis in different accessions of *Artemisia annua* of our germplasm collection.

Invited II

O38. Antimicrobial activities of the crude extract of leaf, flower, and twig of *Artemisia tridentata*

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With the rise of multidrug resistance and declining in the discovery of new antibiotics, medicinal plants are gaining attention for the discovery of new natural antibiotics. These plants contain secondary metabolites with antimicrobial activities. *Artemisia tridentata, or* Big Sagebrush, has been used as a medicinal herb in Native American cultural medicine. The essential oil of Big Sagebrush has antimicrobial activities. A close relative, *Artemisia annua*, a Chinese herb, was used to derive Artemisinin, a common antimalarial drug, and potential anticancer treatment. We report here the antimicrobial activity in the crude extract of Big Sagebrush's leaves, twigs, and flowers. A crude extract from the leaves, twigs, and flowers was prepared in methanol and tested for antimicrobial activity by well and disk diffusion agar method against *Bacillus cereus, B. subtilis, Agrobacterium tumefaciens; E. coli* Dh5α, and two plant pathogens *Pseudomonas syringae* pv DC3000, *P. syringae* pv tabaci. The crude extract for all three tissues showed antimicrobial activity against all tested microbes except *E. coli* and *P. syringae* pv DC3000. *B. subtilis* showed maximum growth inhibition to flower extract with a zone of inhibition of 18mm followed by leaf 12mm. We also tested *crude e*xtracts from leaves and flowers against four human pathogens *Staphylococcus aureus, S. epidermidis, Proteus vulgaris, and Micrococcus luteus*. The crude extract of the flowers showed a zone of inhibition against all the human pathogens. In contrast, crude leaf extract inhibits all growth except *S. aureus.* Leaf and flower extract showed maximum inhibition to *Micrococcus luteus* with 18, 22 mm of inhibition diameters close to the inhibition zone created by Vancomycin 30µg, an antibiotic used to treat *Micrococcus luteus* infection. We separated the metabolites in the leaf crude extract by High-Performance Liquid Chromatography (HPLC) and found that fraction four out of thirty fractions inhibited bacterial growth against *B. subtilis*.

O39. Variation among *Ocimum* species for antimicrobial activity against *Escherichia coli* and *Streptococcus pyogenes*

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Plant-derived antimicrobials are extensively used in alternative therapeutic strategies to combat microbial infections. The genus *Ocimum* (Lamiaceae family), with over 150 species, has proven antimicrobial properties. In this study, the variation among *Ocimum* species for antimicrobial activity against two human pathogens, *Escherichia coli, and Staphylococcus pyogenes,* was evaluated using leaf methanolic extracts of nineteen accessions belonging to seven *Ocimum* species. The cultures were grown overnight on BHI broth at 37ºC and diluted to a final concentration of ~10^5 CFU/ml. Pure cultures served as the Control. Bioscreen C at a wideband filter between 420 and 580 nm determined bacterial growth rate. The percentage reduction in pathogen colonies was calculated based on the difference in OD absorption units between treatments and Control. There were significant differences among Ocimum species in their activity against the two pathogens. The growth reduction of *E. coli* ranged from 20% (*O. americanum*) to 91% (*O. basilicum*). *O. basilicum* and *O. tenuiflorum* reduced the growth of *E. coli* by 91 and 88%, respectively, and were the most effective species. The growth reduction in *S. pyogenes* ranged from 10% by *O. campechianum* to 76% by *O. tenuiflorum* (MSR 1). *O. basilicum* (PI 652071), *O. selloi* (PI 511865), *O.* × *africanum* (PI 500942), and *O. gratissimum* (PI 652067) reduced the growth of *S. pyogenes* by more than 50% of that of Control. The *Ocimum* species showed greater efficacy in controlling the growth of *E. coli* than *S. pyogenes*. Some accessions of *O. basilicum*, *O. gratissimum, O.selloi*, and *O. tenuiflorum* inhibited the growth of *E. coli* and *S. pyogenes* more than the antibiotic. The study validated the antimicrobial activity of *Ocimum* species against *E. coli* and *S. pyogenes*. The variation between and within species indicates the need for further research to increase the potency of *Ocimum* species as antimicrobial agents

Poster Presentations

Session I Ethnobotany, bioprospecting, and Production

P1. The effects of direct low-temperature plasma treatment vs plasma activated water on seed germination, seedling growth and yield of microgreens

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Microgreens, characterized by early harvest within 7-21 days, are nutritionally dense and rich in health-beneficial phytochemicals. Due to their antioxidant, polyphenol, indole, and isothiocyanate content, microgreen consumption has been associated with reduced inflammation, oxidative stress, and chronic disease risk. Furthermore, microgreens exhibit medicinal potential through their impact on cellular pathways, including miRNA and DNA methylation. However, poor germination, leading to poor stands, seed-borne diseases, and high-water consumption often limit microgreens production. Cold/low-temperature plasma (LTP), featuring unbound electrons, ions, and neutral particles, presents an innovative non-chemical solution. LTP can generate reactive nitrogen species, reactive oxygen species, and ultraviolet light, making them a valuable tool for seed priming, effectively breaking seed dormancy, expediting germination, and disinfecting seeds, thereby addressing key limitations in microgreens cultivation. In this experiment, 'Amara' mustard greens seeds were directly exposed to LTP or Plasma Activated Water (PAW) for T0=0s, T1=30s, T2=60s, and T3=90s and planted in seed germination trays (6 seeds/tray) after appropriate treatment. The days to first seed germination, germination rate, and seedling height were recorded. Seeds directly exposed to either LTP or PAW had no significant impact on germination percentage except for PAW-Ar90s, which increased by 36% compared to the Control group. Plant height significantly increased with direct Ar and He plasma treatments by approx. 98% in Ar30s and 50% (all other treatments) compared to Control. Exposure of seeds directly to He and Ar plasma also produced more seedling biomass. He30s, He 60s, He 90s, and Ar 90s significantly enhanced biomass by 250%, 200%, 215%, and 120%, respectively compared to Control. In contrast, PAW treatments had no significant impact on these parameters. Thus, this study demonstrated that direct seed treatment of LTP using Argon or Helium gas improved plant stand and yield and could potentially reduce water consumption due to quicker seedling growth rates.

Session II Chemistry of Medicinal Plants

P2. Natural variation in watermelon lines for Selenium (Se) tolerance to identify the candidate genes by genome-wide association study

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Selenium (Se) is a naturally occurring element with a narrow range of necessity and toxicity effects on living organisms. Rapid industrialization, modern agricultural practices, and other anthropogenic activities add a significant quantity of Se into the environment, which induces severe toxic effects on all life forms, altering the soil properties and biological activities at elevated concentrations. Agriculture sites and water bodies of West Virginia have been reported with high concentrations of Se due to coal mining and agriculture activities and erosion of natural deposits, which could cause various health hazards through biomagnification in the food chain. Therefore, it's important to ensure the food safety of the local population by minimizing the entry of Se into the food chain. Hence, the present study was designed to screen the low Se accumulating watermelon lines through a pollution-safe cultivar (PSC) screening approach. In this study, a total of 180 watermelon lines are screened for their Se tolerance. Among the tested lines, PI525084, PI482248, and PI500318 showed better tolerance to the Se at the seedling stage. However, watermelon lines PI490379 and PI494821 were susceptible to Se exposure. Once all the genotypes are screened, the potential tolerant lines can be identified. Metabolic profiling and Genome-wide association analysis (GWAS) is in progress. Our results will provide critical insight into how Se affects plant development, metabolism, and genes involved in transport, which can help to develop pollution-free cultivars (PSC) and ensure food safety.

Session III Production Strategies for medicinal biomass and bioactive compounds

P3. Column chromatography and semi-preparative HPLC purification of arachidin-1 and arachidin-3 from elicited hairy root cultures of peanut (*Arachis hypogaea* L.)

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Stilbenoids are phenolic compounds that have gained significant attention due to their diverse biological activities and potential benefits to human health. Peanut hairy root culture is an ideal platform to produce stilbenoids, particularly the prenylated stilbenoids arachidin-1, arachidin-2, arachidin-3, and arachidin-5. Among these stilbenoids, arachidin-1 and arachidin-3 have demonstrated anti-inflammatory, anticancer, antiviral, and antioxidant activities *in vitro*. However, the research on arachidin-1 and arachidin-3 has been challenging due to their limited availability. To overcome this limitation, the objective of this study was to establish a highly efficient bioproduction and purification platform for arachidin-1 and arachidin-3. To achieve this goal, peanut hairy root cultures were co-elicited for 192 hours with 18 g/L cyclodextrin and 125 μM methyl jasmonate to selectively enhance the production of arachidin-3 (yield: 178.2 ± 6.8 mg/L), whereas co-elicitation with 18 g/L cyclodextrin and 3 mM hydrogen peroxide was used to selectively enhance the production of arachidin-1 (yield: 132.6 ± 20.4 mg/L). These specific elicitation conditions significantly increased the yield of the targeted stilbenoids, making their purification more feasible. The ethyl acetate extract derived from the elicited culture medium was fractionated sequentially using normal-phase and reversed-phase column chromatography techniques. Subsequently, semi-preparative high-performance liquid chromatography purification was performed on a C18 column to obtain arachidin-1 and arachidin-3. The recovery rates for arachidin-1 and arachidin-3 were 32% and 39%, respectively, and both compounds were obtained with >95% purity. This study presented a sustainable method to produce arachidin-1 and arachidin-3 with a high degree of purity by utilizing hairy root cultures of peanuts in combination with column chromatography and semi-preparative HPLC. This strategy successfully obtained sufficient amounts of arachidin-1 and arachidin-3 for conducting *in vivo* experiments.

Session IV Hemp and medical cannabis

P4. Cannabidiol may alter intestinal inflammasome activity in an E2-dependent manner

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The reduction of 17β-estradiol (E2) in postmenopause is associated with a range of metabolic disorders. Hormone replacement therapy (HRT) is not appropriate for everyone therefore other options are needed. Cannabidiol (CBD) is a non-psychotropic phytocannabinoid derived from the industrial hemp plant (*Cannabis sativa* L.). CBD supplementation may be beneficial for post-menopausal women as it has been shown to reduce inflammation, improve gut barrier integrity, limit bone loss, and improve cognitive function. Prior work showed that ovariectomized (OVX) mice supplemented with CBD (25 mg/kg delivered perorally in a sesame oil and peanut powder vehicle) had improved metabolic phenotypes and an increase in relative abundance of fecal *Lactobacillus* species. RNA sequencing was performed on colon tissue collected from sham surgery (SS) or OVX mice treated with CBD or vehicle (VEH) (n = 4 /group) and differentially expressed genes (DEGs) were sorted using ShinyGo to identify pathways altered by E2 deficiency and/or CBD treatment. Compared to VEH-treated OVX mice, CBD-treated OVX mice showed decreased expression of several inflammatory response pathway genes. In particular, the expression of 13 inflammasome signaling pathway genes were analyzed and revealed differential gene expression related to five different inflammasomes – NLRP1b, NLRP9b, NLRP10, NLRC4, and AIM2. The present work aims to investigate how CBD may alter the expression of inflammasome-related proteins.

Session V Bioactive compounds of plants and their roles in human health I – Cancer

P6. Investigating the impact of cyanide ions (CN-) derived from cassava (*Manihot esculenta* Crantz) on A549 (alveolar epithelial) cells

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Cassava (*Manihot esculenta* Crantz) holds a pivotal position as one of the most vital tropical root crops. It serves as a prominent source of cyanogenic glycosides, namely linamarin and lotaustralin, those, upon decomposition release HCN and ketone. Utilizing cassava cyanide extract (CCE) derived from cassava leaves and tuber rinds, a biopesticide was formulated to combat specific borer insect pests infesting horticultural crops. In the study, Adenocarcinomic human alveolar basal epithelial cells (A549) were subjected to three distinct concentrations (100, 200, 400 ppm) of CCE. The MTT and NRU assays revealed a striking dose-dependent cytotoxicity, emphasizing the impact of concentration; surprisingly, the DCFH-DA assay failed to exhibit any notable free radical scavenging activity. However, the NRR assay showcased a remarkable decline in nitrile radicals as the concentration of the bioactive compound escalated. Interestingly, a negative correlation emerged between the concentration of the bioactive principles and the functionality of both mitochondria and lysosomes, underscoring their interplay in cellular processes. Through a diverse array of cellular assays, the profound cellular response to CCE was elucidated, revealing a significant shift towards necrotic cell death rather than apoptosis at higher concentrations (400 ppm). These findings underscore the remarkable anti-proliferative capability exhibited by CCE, emphasizing its potential as a powerful tool in combating cellular proliferation.

P7. Flavone apigenin reprograms transcriptome-wide alternative splicing through hnRNPA2 to inhibit therapeutic resistance in triple-negative breast cancer

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Triple-negative breast cancer (TNBC) is characterized by cancer-specific transcriptome alterations owing to alternative splicing (AS) thereby promoting tumor growth and resistance to anti-cancer therapy. The lack of targeted treatments and the refractory nature of TNBC underscores the need to identify approaches that reprogram AS circuitry toward transcriptomes favoring tumor suppression and therapeutic responsiveness. Medicinally active plant compounds provide untapped opportunities to fight cancer. Among them, the flavone apigenin showed anti-cancer activity against TNBC. To understand the mechanisms responsible for its anti-tumorigenic effects, we identified direct targets of apigenin by screening a human BC-derived phage-display peptide library coupled with next-generation sequencing (PD-Seq). Among the high-affinity targets, we found hnRNPA2, an RNA-binding protein and a critical regulator of AS. Thus, the goal of this study was to elucidate the mechanisms by which apigenin through hnRNPA2 regulates TNBC cell death and therapeutic resistance. We found that apigenin inhibited the growth of TNBC MDA-MB-231 three-dimensional cultures (spheroids), which mimic the complexity of clinical tumors, more effectively than the other flavonoids studied. Apigenin inhibited the growth of TNBC patient-derived organoids at an *in vivo* achievable concentration. Furthermore, apigenin-sensitized TNBC spheroids to first-line chemotherapeutic drug doxorubicin-induced apoptosis in an hnRNPA2-dependent manner by modulating the expression of drug transporters *ABCC4* and *ABCG2*. To elucidate the network by which apigenin reprograms cancer-associated AS in TNBC, we performed RNA-seq analyses of MDA-MB-231 scramble and hnRNPA2 knockout spheroids treated with vehicle or apigenin. Analyses of the transcriptome-wide AS changes along with spliced isoform-specific RT-PCR will reveal the aberrant splicing events impacted by apigenin in an hnRNPA2-dependent and independent fashion to induce cell death and inhibit therapeutic resistance in TNBC. Together, these findings provide novel insights into the role of flavones in altering cancer transcriptomes to sensitize tumors to therapeutic agents, highlighting the relevance of using natural compounds to treat TNBC.

P8. Evaluation of the anticancer potential of cassava-cyanide extract (CCE) against Human Glioblastoma (LN229) Cell Line

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Numerous natural compounds have shown promise in inhibiting tumor cell growth and metastasis by inducing programmed cell death. Cassava (Manihot esculenta Crantz) is known to contain cyanogenic glycosides with anticancer properties. In this study, we developed a technology to isolate bio-active principles from cassava leaves and investigated the cytotoxic effect of cassava cyanide extract (CCE) on human glioblastoma cells (LN229). Our results demonstrated concentration-dependent cytotoxicity of CCE against LN229 cells, as confirmed by cell viability assays (MTT, neutral red, and LDH) and free radical generation studies (DCFH-DA and Griess reagent assay). Microscopic imaging using fluorescent dyes revealed impairment in the integrity of lysosomes, cytoskeletal network, nucleus, and mitochondria in CCE-treated cells. Coomassie brilliant blue staining further confirmed morphological aberrations in LN229 cells after CCE treatment. Additionally, flow cytometry analysis showed that CCE induced programmed cell death, as evidenced by calcein-AM/PI and annexin V/PI staining. The findings highlight the potential of CCE as an antineoplastic agent and provide insights into its cytotoxic mechanisms against human glioblastoma cells. Further investigations are warranted to elucidate the underlying molecular pathways and evaluate the efficacy of CCE in preclinical and clinical setting.

P9. Anticancer potential of cyanide ion (CN-) extract derived from cassava (*Manihot esculenta* Crantz) on human glioblastoma (LN229) and alveolar epithelial cells (A549 Cells)

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Cassava (*Manihot esculenta* Crantz), a staple food for over 800 million people, is extensively cultivated in the tropical regions. Cyanogenic glycosides like lotaustralin and linamarin are rich in cassava which are enzymatically hydrolyzed into hydrogen cyanide (HCN) and ketones. The remarkable ability of cyanogenic glycosides to freely traverse the plasma membrane and elicit a potent bystander effect confers therapeutic significance in diverse conditions, including hypertension, asthma, and cancer. The current study aims to elucidate the cytotoxic effects of cassava cyanide extract (CCE) on the cell lines of A549 and LN229. The antineoplastic effect CCE on these two cell lines are mediated by programmed cell death. Exposure to CCE resulted in notable morphological changes, compromised stability of mitochondria and lysosomes, release of lactate dehydrogenase (LDH) enzymes, and generation of reactive oxygen species (ROS) and also the bioactive compound displayed modulatory effects on the structural and functional properties of cell organelles, including lysosomes, mitochondria, cytoskeleton, and nucleus. Multiple cellular assays were conducted to evaluate the cellular response to CCE, revealing a notable concentration-dependent effect. Particularly, at a higher concentration of 400 ppm, CCE induced a substantial incidence of necrotic cell death rather than apoptosis. These findings highlight the pronounced anti-proliferative potential of CCE, suggesting its efficacy in inhibiting cell growth and proliferation.

P10. Evaluating the antiproliferative capacity of extracts from *Syringodium filiforme* in colorectal cancer cell line.

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Colorectal cancer is one of the most frequent cancers affecting males and females. Although several treatment options are available, not all are effective. There is need to find novel sources of treatment against aggressive cancers, including those from the colon. Algae-derived products are a promising alternative found to be effective against several cancers. *Syringodium filiforme* is a type of seagrass commonly found in tropical areas that contain active metabolites typically described as having antiproliferative capabilities. However, there are not many studies directly focused in evaluating the potential of this alga against colorectal cancer. The aim of this study was to evaluate the antiproliferative capacity of extracts obtained from *Syringodium filiforme* and to verify possible routes by which this activity might be promoted. Water-based and polar-based extracts from the alga were obtained and their antiproliferative potential was evaluated by cytotoxic assay. More studies will be required to better understand the mechanisms involved. There is a potential to use molecules obtained from *Syringodium filiforme* to reduce the proliferative capacity of colorectal cancer and other similar aggressive cancers.

P11. Gene expression changes elicited by *Nigella sativa* compounds in glioblastoma cells.

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Approximately 1.9 million new cases of cancer will be diagnosed in the United States in 2023, and around 600,900 will die of the disease. While cancer survival rates have improved over time, the survival rates are still low for many cancers. Examples include the deadly brain cancer glioblastoma, for which there is a five-year post-diagnosis survival rate of 6.9%. Thymoquinone, the most abundant and well-studied bioactive compound of *Nigella sativa* seed oil, has shown anticancer effects in several human cancers. Although some mechanisms for its anti-cancer activity have been identified, knowledge of the exact anti-cancer mechanisms and pathways affected in glioblastoma is still in its infancy. So, we present the first RNA sequencing study directed towards identifying differentially expressed genes (DEGs) and comprehensively analyzing the pathways affected by thymoquinone in glioblastoma. RNA-seq using control, 2.5 μM, and 5 μM TQ didn't yield any statistically significant changes in gene expression. Second RNA sequencing was performed using TQ concentrations (25 μM and 50 μM), at which significant death of glioblastoma cells was observed, including 0.1% DMSO as control. This generated 1548 DEGs in 25 μM TQ and 2797 DEGs in 50 μM TQ. In total, we found 1202 common DEGs between 25 and 50 μM TQ treatment. KEGG pathway analysis revealed that TQ significantly inhibited the PI3K-Akt signaling, calcium signaling, focal adhesion, and ECM-receptor interaction pathway in A172 glioblastoma cells. It upregulated the P53 signaling pathway and tumor suppressor genes. TQ downregulated several potential oncogenes, prognostic markers, and some highly expressed genes in GBM. It regulated the expression of core components and the regulator of Wnt signaling in GBM cells. Finally, we validated the expression of some DEGs using qRT-PCR. Hence, we identified some anti-cancer mechanisms of TQ on A172 GBM cells by revealing effects on several signaling pathways.

Session VI Bioactive compounds of plants and their roles in human health II – Inflammatory diseases and beyond (IV)

P12. Effects of capsaicin supplemented diet in the neuronal response of *Drosophila melanogaster*

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Capsaicin is a bioactive component found in *Capsicum spp.* fruits that provoke heat and pain sensation due the activation of primary sensory neurons. Capsaicin has been related to antioxidant and fat mobilization properties, deactivation of neurodegenerative-related genes and is employed in neuropathies and fibromyalgia therapies. *Drosophila melanogaster* is a genetic biomodel that can be fast and easily reproduced as well as tissue-dissected with low culture and growth expenses for molecular projects due its >70% sharing with human genes and their regulation. Until this study, Bulk transcriptomics and microbial community changes have been screened in *D. melanogaster* through RNA-seq and 16sRNA-based microbiome methods, however, there is a lack of knowledge of how brain genes and pathways could be modulated during the feeding process with Capsaicin, important to understand the benefits of consuming spicy food for our central neuronal system. Thus, we aim to reveal the molecular mechanisms that are specifically activated and deactivate in *D. melanogaster* dissected brain under a Capsaicin supplemented diet employing RNA-seq transcriptomics based on previous studies where neurons and nervous system participating genes such as olfaction, taste, hearing, vision, locomotion, memory and learning related responses have been reported that are complex to understand with non-tissue specificity.

P13. Unlocking novel ligand-receptor associations from medicinal plants and fungi using untargeted metabolomics, molecular networking, docking and data mining

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Nuclear receptors (NRs) constitute an essential family of transcription factors with significant roles in both physiological and pharmacological processes. Among them, the aryl hydrocarbon receptor (AHR) has been shown to have numerous biological functions including cell cycle regulation, liver development, circadian rhythm regulation, as well as gut homeostasis regulation. Importantly, AHR is abundant in the gastrointestinal tract, and diet-derived AHR ligands have potential to maintain homeostasis in the gut. A significant source of AHR modulating compounds are derived from phytochemicals and mycochemicals from foods. However, among the myriad bioactive compounds from plants and fungi, identifying novel functional ligands which would significantly impact gastrointestinal health is a challenge. To overcome this, a combination of untargeted metabolomics, molecular networking, molecular docking, and data mining could be used to predict biologically functional compounds accurately and efficiently. This study predicted, identified, and characterized a novel AHR modulator in the white button mushroom (*Agaricus bisporus)*, and introduced a protocol to identify more novel AHR modulators in other fungi with nutritional and medicinal significance. The metabolome of *Agaricus bisporus* was analyzed in combination with known AHR ligands to find structural associations between the compounds constituting the two groups. Molecular networking of these compounds revealed that a methylated analog to benzothiazole was indicated in *Agaricus bisporus*, which was subsequently isolated and identified as 2-amino-4-methyl-benzothiazole(2A4). Cell-based AHR transcriptional assays revealed that 2A4 possesses agonistic activity and upregulated *CYP1A1* expression. Additional cell based assays on medicinal and culinary fungi has shown that *Ganoderma lucidum*, *Pleurotus ostreatus*, *Lentinula edodes*, and *Hypsizygus tessellatus* all have significant AHR modulating activity and are prime candidates for sources of novel AHR modulating compounds using this method. Future results have potential to guide dietary advice in maintaining gut health and systemic health through the multitude of biological functions AHR regulates.

P14. Effect of bacterial-derived polyphenol metabolites on mammalian cell inflammation

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B-type proanthocyanidin (PAC) are major polyphenols constituents found in grape berries and seeds, which have been associated with protection from chronic metabolic disease. Although parent PAC compounds are not well absorbed, they are metabolized by the gut microbiota into more bioavailable metabolites, which may contribute to metabolic health benefits. Mice supplemented with PAC-rich grape polyphenol (GP) showed improved glucose metabolism. Here we compared bacterial-derived GP metabolites individually and in mixture for anti-inflammatory activity. RAW 264.7 macrophages were treated with 25, 50, 75, 100μM of bacterial-derived GP metabolites (HCA, 3HPAA, VA, DAT, 4HBA, 3-4CA, PCA) in the absence or presence of pro-inflammatory TNFα and lipopolysaccharide (LPS). Results showing qPCR quantified changes in inflammatory marker expression will be presented.

P15. Investigating inhibitors of autoinducing peptides in methicillin-resistant *Staphylococcus aureus (*MRSA*)* from Pennsylvania native plants and fungi

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Due to the widespread use of antibiotics to treat bacterial infections, pathogenic bacteria like methicillin-resistant *Staphylococcus aureus* (MRSA) are well known to develop resistance to current antibiotic therapies, which poses a serious threat to public health. The peptide signaling system of MRSA, *Staphylococcus aureus* accessory gene regulator (agr), secretes many destructive virulent agents and is activated by cyclic peptides known as autoinducing peptides or AIPs. An anti-virulence approach to bacterial treatment has been proposed that limits toxin production through inhibition of AIP with the goal of lowering the severity of bacterial infections while allowing the body to rid itself of infection without the development of resistance. Natural resources, especially plants, hold possible small molecules capable of combating antibiotic resistance and thus contribute to the development of new classes of antibiotics. In this study we tested 28 endemic Appalachian plants and fungi commonly used in traditional medicinal systems for their antibacterial and AIP inhibitory activity via a rapid, quantitative liquid chromatography-mass spectrometry (LC-MS) method. Several of the plants surveyed have shown reduction in quorum sensing peptides; two of the most noteworthy being *Ganoderma lucidum* at 56% AIP inhibition and *Inonotus obliquus* at 62%.

P16. Evaluation of anti-tuberculosis phytochemicals from *Artemisia* spp.

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Tuberculosis (*Mycobacterium tuberculosis*, TB) kills 1.6 million people each year, and a major obstacle to lowering this number is the suboptimal nature of current TB therapies. *Artemisia* species are a rich source of phytochemicals, including artemisinin. Our preliminary studies indicate that *Artemisia* spp. extracts are antimycobacterial, yet the activity cannot be attributed to artemisinin alone. Using a biochemometric approach, we discovered compounds from *Artemisia afra* with potential anti-TB activity. Successive rounds of fractionation were profiled via untargeted LC-MS metabolomics and correlated with *in vitro* antimycobacterial assays using supervised machine learning methods. Tentative identification of bioactive metabolites highlighted phytochemicals from various classes possessing anti-TB activity; next we will analyze combination effects to better understand their mechanism of action.

P17. Screening North American Native Plants for α-glucosidase Inhibitory Activity

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Diabetes is a leading chronic disease in the Unites States with over 40% of the population being diabetic or prediabetic. Natural alternatives to pharmaceuticals for a variety of chronic conditions have received increased public interest in recent years. A leading method for decreasing blood sugar levels is to inhibit carbohydrate digestion. This is accomplished by blocking the activity of a main carbolytic enzyme: α-glucosidase. For this project, North American native plants were harvested, extracted, and screened using a rapid in vitro assay to demonstrate their inhibitory activity against alpha-glucosidase. High inhibitory activity was seen in the assay for *Inonotus obliquus, Asimina triloba, Pilea pumila,* and *Rhus glabra.* Average inhibitory activity ranged from 10% to 40%, revealing promising results for understanding new natural sources of anti-diabetic medicine.

Session VIII Plant based antimicrobials

P18. Plant and fungi inhibitors of autoinducing peptides in methicillin-resistant *Staphylococcus aureus (*MRSA*)*

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Due to the widespread use of antibiotics to treat bacterial infections, pathogenic bacteria like methicillin-resistant *Staphylococcus aureus* (MRSA) are well known to develop resistance to current antibiotic therapies, which poses a serious threat to public health. The peptide signaling system of MRSA, *Staphylococcus aureus* accessory gene regulator (agr), secretes many destructive virulent agents and is activated by cyclic peptides known as autoinducing peptides or AIPs. An anti-virulence approach to bacterial treatment has been proposed that limits toxin production through inhibition of AIP with the goal of lowering the severity of bacterial infections while allowing the body to rid itself of infection without the development of resistance. Natural resources, especially plants, hold possible small molecules capable of combating antibiotic resistance and thus contribute to the development of new classes of antibiotics. In this study we tested 39 plants and fungi, some North American native plants and some widely used plants and fungi of commerce, for their antibacterial and AIP inhibitory activity via a rapid, quantitative liquid chromatography-mass spectrometry (LC-MS) method. Several of the plants surveyed have shown reduction in quorum sensing peptides; two of the most noteworthy being *Inonotus obliquus* at 62.41% AIP inhibition and *Typha species* at 63.19%.

P19. Bioprospecting of early-evolving land plants for antimicrobial activities.

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Plants are known for their unique biochemical properties. Recently, early evolving land plants are emerging model systems to extract plant metabolites. Bryophytes and pteridophytes are early evolving land plants that have been utilized in primitive eras for folklore medicines due to their medicinal properties. Research suggests that bryophytes and pteridophytes have been able to lower proliferation of cancer cells in addition to their antibiotic properties. Early evolving land plants from Puerto Rico remains to be explored. Our research aims to evaluate the preliminary medicinal properties of three bryophyte and two pteridophyte species of Puerto Rico, by estimating the amount of flavonoid, total polyphenol, antioxidant capacity and antimicrobial properties. Our preliminary results show that the tissues from pteridophytes have more amount of total flavonoids and total polyphenol content. Further, we determined the antimicrobial effect of these plants on four different gram-positive and gram-negative bacterial strains, using the methanolic extracts of each plant obtained by Soxhlet extraction method. This assay demonstrated once again that pteridophytes have effect inhibiting the growth of some of these bacteria compared to the bryophytes in study. Our study provides a preliminary data to carry out an in-depth bioprospecting of early evolving land plants from Puerto Rico.

P20. Antibacterial effects of some common medicinal plants extracts on food-borne bacteria

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Medicinal plant extracts exhibited antibacterial effects against various bacteria. We aim to examine the antibacterial effects of extracts from *Quercus alba* and *Liriodendron tulipifera* against foodborne bacteria and explore the potential of these plants as food preservatives. In this study, methanolic extracts of leaves of *Quercus alba* and *Liriodendron tulipifera* were partitioned successively through hexane, chloroform, ethyl acetate, and the antibacterial activity of each fraction was tested against *Listeria monocytogenes*. Our preliminary data suggested that the butanol fraction of Quercus alba displayed an inhibition on the growth of *L. monocytogenes* by 10^3 times at a concentration of 3mg/mL, while the chloroform fraction of *Liriodendron tulipifera* showed about a 10^4 times inhibition on the growth of the *L. monocytogenes*. In addition, essential oils from *Liriodendron tulipifera* resulted in a 50% reduction in the growth of L. monocytogenes. Our results indicated that the plant extracts, in particular, the chloroform fraction of *Liriodendron tulipifera* and the butanol fraction of *Quercus alba,* may have the potential to serve as food preservatives to extend food shelf-life and increase food safety. We will further examine the antibacterial effects of these plant extracts against other foodborne bacteria and analyze the chemical components of the extracts using GC/MS.

P21. Identification and analysis of antibacterial compounds from model mosses *Physcomitrium patens* and *Ceratodon purpureus*

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Plants synthesize a large spectrum of secondary metabolites with substantial structural and functional diversity, making them a rich reservoir of new biologically active compounds. Here, we aim to identify and characterize novel antimicrobial natural products from model mosses *Physcomitrium patens* and *Ceratodon purpureus*. We collected moss exudates (soluble secreted fractions) from one *P. patens* strain, Gransden (Gd) and two *C. purpureus* strains, male R40 and female GG1. Exudates from the male *C. purpureus* strain R40 and the *P. patens* strain Gd exhibited strong inhibitory properties against several species of Gram-positive bacteria, including *Staphylococcus aureus* and *Enterococcus faecium*, though they did not inhibit the growth of Gram-negative bacteria. Interestingly, exudates from the female *C. purpureus* strain GG1 did not exhibit strong inhibitory activity against any tested bacteria, providing an interesting case of sexual dimorphism. Size fractionation experiments indicated that the secreted bioactive compounds from *C. purpureus* R40 have a relatively low molecular weight of less than 1 kDa, while secreted bioactive compounds from *P. patens* Gd have much larger molecular weights. Additionally, the R40 exudate compounds are thermostable and not sensitive to proteinase K treatment, whereas Gd exudate compounds are sensitive to heat and proteinase K treatments, suggesting different chemical structures of analyzed bioactive metabolites. Current efforts are underway to determine the chemical structures of identified bioactive plant metabolites. Overall, our results suggest that the antimicrobial compounds present in *C. purpureus* R40 and *P. patens* Gd exudates can potentially add new options for treating infections caused by antibiotic-resistant Gram-positive bacteria. Further analysis and molecular identification of antibacterial exudate components, combined with bioinformatic analysis of genomes of these two model mosses, will be instrumental in the identification of specific genes and biochemical pathways involved in the bioactive metabolite biosynthesis. Supported by USDA grant 58-6060-2-006 and NIGMS grant P20GM103434.

Other

P22. Microstructural variability and histochemical differences in the nutritional peanut cultivars grown in Georgia

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Georgia is the largest producer of peanut crop in the US. It is considered one of the major plant protein sources in the US. Aflatoxin contamination triggered by *Aspergillus* spp. results in a loss of approximately $126 million each year in the US alone. The hull, seed coat, and cotyledon of the peanut fruit have differences in the cellular structure and presence of chemical compounds, among cultivars. The study of microstructures and chemical localization of various compounds and their possible role in *Aspergillus* infection is limited. Thus, the microstructures (sclerenchyma fiber, cuticle, stomata) and the presence of lignin, pectin, terpenes, starch, total proteins, calcium, and silicon in different parts of the fruit were studied. Three cultivars of peanuts (Georgia-06 G, Georgia-12Y, and Georgia-18RU) were selected for comparative studies at R3-R8 reproductive developmental stages. The differences in the cellular structure of the hull, seed coat, and cotyledons at different developmental stages were observed in paraffin sections. Also, stomata were observed in the longitudinal sections of the cotyledon which could be a potential route for fungal invasion in cotyledons. In addition to that, the difference in the starch localization was found among cultivars at the R8 stage where the Georgia-06 G had the highest starch [area (%)], using ImageJ software. The use of specific stains to investigate the localization of chemical compounds possibly involved in antifungal and antifeedant activities will hopefully provide some direction in the host-pathogen relationship. The variations in the metabolite localization are expected to aid in the identification of peanut cultivar tolerant/susceptible to *Aspergillus* spp. If a relationship based on histochemical differences and incidence of fungal infection is established, the information may help future peanut breeding programs for mitigating biotic or abiotic stress.

P23. Micromorphological structures in medicinally important *Scutellaria* species

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Scutellaria (skullcap) species have been traditionally used as herbal medicine for their anti-bacterial, antitumor, anti-angiogenesis, hepatoprotective, antioxidant, anticonvulsant, antibacterial, and antiviral properties in China since ancient times. Medicinal biomass that is wildcrafted and used for the herbal supplement industry usually gets adulterated with other plants due to the lack of plant identification expertise available or intentionally. Therefore, it is important to develop methods to taxonomically classify and identify the correct species to minimize the negative effects of adulteration. Micromorphological structures are an inexpensive way to differentiate adulterants. Trichomes, stomata, and other minute morphological structures play a key role in taxonomical classification and can also be useful in differentiating adulterants. This study focused on cataloging morphological variation among medicinally important S*cutellaria* species present in our germplasm collection. Cataloging of microstructures such as stomata type, arrangement and density, stomatal index, trichome (glandular and non-glandular) diversity and morphology, and palisade cell ratio provide reliable taxonomic markers. *Scutellaria havanensis, Scutellaria* sp. (Puerto Rico)*, S. costaricana, S. suffrutescens, and S. barbata* were used in this study and a clearing method coupled with safranin staining was optimized by calibrating concentration and exposure of the tissue to 70% ethanol, 5% NaOH, incubation at 80ºC, and 20% bleach. Leaf, flower, stem, and petiole tissues were observed under microscope and scored for microstructures. Preliminary studies indicate differences in the number of vein islets, vein endings and trichomes among *Scutellaria* species. In *S. costaricana* and *Scutellaria* sp. (Puerto Rico) there were average of 301.6 and 202.5 vein endings and 285.8 and 265.8 vein islets. In *S. costaricana* there were 73.3 glandular and 53.3 non-glandular trichomes were present on the leaf margin and lamina, whereas in *Scutellaria* sp. (Puerto Rico) only 37.5 non-glandular trichomes were recorded with no glandular trichomes on the leaf lamina, stressing the importance of microstructures.

P24. A comprehensive evaluation of heavy metal contaminations in agricultural soil and vegetables and its implication in human health risk assessment

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Contamination of agricultural soil and agri-products with heavy metals poses a significant environmental challenge in the United States, while the status of metal contamination and associated health risks have been merely studied. Hence, this study aimed to assess metal contamination levels in soils from major agricultural sites in West Virginia and Ohio, and evaluate the potential health risks associated with consuming metal-contaminated vegetables. In this study, Fe was the most predominant contaminant in agricultural sites, ranging from 28.41 to 67.36 g/kg soil, exceeded the permissible limit of WHO, while Cd was the least contaminant, ranging from 0.13 to 2.91 µm/g of soil. In pollution indicators analysis, soil samples indicated minimal to moderately contaminated with Cu, Fe, Mn, Ni and Zn. Among the 13 sampling sites, RS3 and RS6, exhibited a moderate degree of pollution load index (<1 PLI<2). Concentrations of metals in vegetable samples were within the permissible limits WHO. Principal component and hierarchical cluster analysis indicated the different natural and anthropogenic sources of metals in soil. In noncarcinogenic health risk assessment, the values of EDI, THQ and HI levels for all the metals were lower than their daily uptake. However, cancer risk assessment revealed that Ni pose significant risks to the adult and children population associated with these agricultural sites. Therefore, regular monitoring of metal concentrations in soil and vegetables, as well as associated health risks, is strongly recommended to prevent potential health hazards in the future.

P25. Identifying genes involved in metabolite biosynthesis in *Capsicum* spp.: integrating metabolome and genome approaches to improve organoleptic and nutritional features of pepper.

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Chili pepper is an important cultivated crop which have abundant biochemical and mineral constituents of nutritional value. These horticultural crops are good sources of bioactive compounds such as carotenoids, capsaicinoids, volatile and non-volatile compounds, and vitamins. However, the plant domestication has altered the different pepper species causing crop diversification, affecting principally their organoleptic characteristics which are important for consumers. These alterations in the genome often impact the metabolism, whether positive or negative influence their phenotype. Therefore, for this study we used genome-wide association study (GWAS) employing the multi-locus mixed model (MLMM) in peppers collections to elucidate causal mutations and the genetic factors that affect pepper color formation which is crucial for fruit growth. We identified 43,357 single nucleotide polymorphisms (SNPs) from the genotyping by sequencing. Furthermore, we used the contents of carotenoid metabolites namely α-carotene (26-63 µg/g), β-carotene (38-317 µg/g), capsanthin (38-58 µg/g), β-cryptoxanthin (11-22 µg/g), lutein (21-266 µg/g), and zeaxanthin (23-58 µg/g), to screen the collection of three different pepper species *C. baccatum, C. chinense, C. frutescens* (117, 136, 183 accessions respectively) and associate them for gene identification analysis. The GWAS revealed candidate genes that were observed on each metabolite, hence, the candidate genes might be affecting completely the carotenoid biosynthesis pathway. Overall, genes identified are involved primarily in transcriptional regulation, stress response related proteins, carotenoid biosynthesis, and fruit development. Similarly, it was analyzed the genes that were also shared among the three species, finding the pentatricopeptide repeat-containing protein, F-box/LRR-repeat protein, ABC transporter B family, geraniol 8-hydroxylase-like, U-box domain-containing protein, as common genes. Further studies are going to validate the candidate genes to color development to elucidate expression of genes selected via RNA-sequencing of fruits showing different colors in the harvested samples on the ripening stage (7-10 days after pollination, DAP), breaker (15-20 DAP), and mature stage (35-40 DAP).

P26. Natural variation in watermelon lines for Selenium (Se) tolerance to identify the candidate genes by Genome-wide association study.

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Selenium (Se) is a naturally occurring element with a narrow range of necessity and toxicity effects on living organisms. Rapid industrialization, modern agricultural practices, and other anthropogenic activities add a significant quantity of Se into the environment, which induces severe toxic effects on all life forms, altering the soil properties and biological activities at elevated concentrations. Agriculture sites and water bodies of West Virginia have been reported with high concentrations of Se due to coal mining and agriculture activities and erosion of natural deposits, which could cause various health hazards through biomagnification in the food chain. Therefore, it’s important to ensure the food safety of the local population by minimizing the entry of Se into the food chain. Hence, the present study was designed to screen the low Se accumulating watermelon lines through a pollution-safe cultivar (PSC) screening approach. In this study, a total of 180 watermelon lines are screened for their Se tolerance. Among the tested lines, PI525084, PI482248, and PI500318 showed better tolerance to the Se at the seedling stage. However, watermelon lines PI490379 and PI494821 were susceptible to Se exposure. Once all the genotypes are screened, the potential tolerant lines can be identified. Metabolic profiling and Genome-wide association analysis (GWAS) is in progress. Our results will provide critical insight into how Se affects plant development, metabolism, and genes involved in transport, which can help to develop pollution-free cultivars (PSC) and ensure food safety.

P27. Effect of pepper different rootstocks as a sustainable alternative to improve yield and nutritional parameters of watermelon

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Grafting is a widely used practice in fruit-bearing vegetables. However, it remains unclear why grafting influence plant growth, fruit yield, and quality, particularly from the prospective of mineral nutrition. Therefore, the present study aimed to investigate the positive influence of grafting on fruit yield and nutritional parameters of watermelon under field conditions. In this study, watermelon was grafted onto CSB (Carolina Strongback), LG3 (Lagenaria), and MM30 (Pumpkin) root stocks. Non-grafted plants were used as the control. The results show that rootstock grafting significantly increases the fruit yield and nutritional properties of watermelon. Compared to the parental plants, grafted plants showed a significant increase in fruit weight (21.11%), fruit firmness (16.39%), fruit length (21.07%), fruit width (4%), rind thickness (4%), flesh firmness (6.45%), and soluble sugar content (3.54%). Among the three different rootstock combinations, the combination of watermelon and pumpkin rootstock (MM30) showed exceptional results with a surprising 10% increase in various traits. These results confirm that, when using the appropriate rootstock/scion combination, grafting can improve the marketable yield of watermelon fruit without reducing the sensory quality of the fruit or its biochemical parameters.

P28. Exploring the medicinal properties of underutilized crop *Arracacia xanthorrhiza*.

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Secondary metabolites are compound of the metabolic pathway in plants. It is known that plants use these compounds to protect themselves and ensure their propagation. Flavonoids are one of the most common and widely studied secondary metabolites in plants. *Arracacia xanthorrhiza*, locally known as *apio* in Puerto Rico, has very high nutritional and folklore-associated medicinal values. *Arracacia xanthorrhiza* belongs to the plant family Apiaceae, members of which are supposed to have very high medicinal value due to the presence of secondary metabolites. In this study we wanted to verify the flavonoid content and explore medicinal properties of the *Arracacia xanthorrhiza*. We wanted to verify the phenolic and flavonoid content in *Arracacia xanthorrhiza* samples. In addition, we identified the putative genes involved in flavonoid biosynthesis, the presence of trichomes and the antiproliferative capacity of apio extracted total flavonoids against a metastatic colorectal cancer cell line. Our results showed that *Arracacia xanthorrhiza* had significant flavonoid content, especially in the leaves. Also, we identified potential homologs of secondary metabolite genes, including *Flavonol synthase*, that could be potential gene involved in secondary metabolite synthesis. We also identified trichomes from the leaf tissues using Scanning Electron Microscopy. Additionally, we observed antiproliferative action of the *Arracacia xanthorrhiza flavonoid* leaf extract in colorectal cancer cells. Although more specific characterization methods could be used to better understand the flavonoids from *A. xanthorrhiza*, this study highlights their abundance and potential benefits. *A. xanthorrhiza* is understudied, but as shown in this study, it has potential benefits that prompts further evaluation.

P29. Effect of watermelon rind extract on cell proliferation, apoptosis, senescence, and universal transcriptomic profile in human renal adenocarcinoma cells

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Cancer researchers are fascinated by the diverse chemistry of natural products, which show exciting potential as anticancer agents. In this study, we aimed to investigate the anticancer properties of watermelon rind extract (WRE) by examining its effects on cell proliferation, apoptosis, senescence, and global gene expression in human renal cell adenocarcinoma cells in vitro. Human renal adenocarcinoma cells were cultured in RPMI-1640 media and treated with 25, 45, 65, 85, 105, 125, and 150 mg mL-1 of WRE for 24, 48, and 72 hours. At 24 hours after treatment, (45 mg mL-1 of WRE) cell proliferation significantly reduced, more than 34% compared to the control. Cell viability decreased at 48 and 72 hours after treatment to 45% and 37%, respectively. We also examined polycaspase, beta-galactosidase (SA-beta-gal), and wound healing activities using WRE. All treatments induced an early polycaspase response and a significant reduction in cell migration. Further, we analyzed the global gene expression profile of the cells grown at 45 mg mL-1 of WRE after 24 hours using RNA sequencing (RNAseq) analysis. We identified 186 differentially expressed genes, including 149 upregulated genes and 37 downregulated genes, in cells treated with WRE compared to the control. The differentially expressed genes were associated with intrinsic and extrinsic apoptotic pathways, such as the NOD-like receptor signaling pathway and tumor necrosis factor signaling pathway. These findings indicate that WRE regulates various transcripts involved in apoptotic pathways. The study suggests the potential anticancer properties of WRE and provides insights into its effects on cellular processes and gene expression in human renal cell adenocarcinoma cells.

P30. Assessing the effects of *Thalassia sp.* extracts in metastatic colorectal cancer cell line

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Autotrophic marine organisms such as plants and algae represent a relatively unexplored source of potential antitumoral molecules. Several metabolites obtained from these organisms are well known to reduce the proliferation of cancer cells. However, there is not enough information related to others, as it is the case with the marine seagrass *Thalassia*. The aim of this study was to evaluate the effects of solvent-based and aqueous extracts prepared from *Thalassia* obtained from the southwestern region of Puerto Rico in the proliferation of the aggressive colorectal cancer cell line HCT-116. *Thalassia* extracts reduced the cell viability of HCT-116 cells. This finding prompts more studies to better elucidate the mechanisms related to this impairment of cellular viability. Obtaining new options that potentially improve therapeutic approaches to aggressive cancers such as colorectal cancer is extremely necessary.

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