



American Council for Medicinally Active Plants  
**7<sup>th</sup> ANNUAL  
CONFERENCE**



Lima, Peru  
June 29 - July 1, 2016

**ACMAP**

# THANK YOU TO OUR SPONSORS



Welcome to the 7<sup>th</sup> Annual Conference of the American Council for Medicinally Active Plants  
June 29 - July 1, 2016 - Lima, Peru

On behalf of American Council for Medicinally Active Plants (ACMAP), I am honored to Chair our 7th Annual Conference here, in the beautiful country of Peru. When I had the opportunity to become a co-founder of ACPMAP in 2009, I would never have imagined that I would be representing ACPMAP as Conference Chair in my home country of Peru. Hosting the 2012 ACPMAP conference at Arkansas State University became a highlight of my career and now, it has been a dream come true to bring ACPMAP to Peru, a country that is so diverse in a culture that is built around the healing properties of plants.

This conference provides a forum to bring scientists, students, educators, and industry professionals from all over the world to share their common interest in plant natural products. It is our hope that these individuals will exchange ideas and research findings that will stimulate the development of innovative means for production of medicinally active plants and plant-derived medicinal products.

ACMAP's goal is to discover new ways to link agriculture to biotechnology, health, nutrition, and medicine and to increase awareness of the need of plant-based medicinal research.

I would like to invite you to take advantage of this opportunity to participate, share, and interact with one another as we attempt to understand the science behind traditional medicine and push the frontiers of cutting-edge technology.

It is my hope that you will appreciate the beauty, culture and history of my country and you will be captivated by the people and the traditions found here. It is my wish that you will be able to take a small part of Peru with you as you return to your home.

I would like to extend a thank you to REDBIO for allowing us this opportunity to bring scientists together at this joint forum.

Thank you for your attendance and we welcome any feedback that may help ACPMAP increase awareness of the need for research on medicinally active plants.

I look forward to meeting each of you and exchanging ideas.

Sincerely,

*Fabricio Medina-Bolivar*

Fabricio Medina-Bolivar, Ph.D.  
ACMAP Conference Chair  
2014-2015, ACPMAP President

Dear Colleagues:

It is indeed my pleasure and privilege to welcome you to the 7<sup>th</sup> Annual Conference of the American Council for Medicinally Active Plants (ACMAP). This being the first international ACMAP conference it has a special place in the timeline of this young organization, which within a short 8 years since its inception in 2009 in the US has grown to host its Annual conference in a foreign country, Lima, Peru - in a different continent!

Another first step for ACMAP is the fact that this is its first joint annual conference with another international society, REDBIO which draws its membership from South American countries and the Caribbean. Thus, it is a giant step forward for both REDBIO and ACMAP in the age of collaborations, partnerships and bridges between institutions, disciplines and cultures.

Much of the credit goes to the Program Chair, Dr. Fabricio Medina-Bolivar who conceptualized the idea, collaborated with REDBIO and brought the idea to fruition with due diligence and extraordinary perseverance. Kudos to the members of the ACMAP Board of Directors who encouraged and worked with Fabricio at every step of the conference planning and execution.

Through this joint international conference, ACMAP provides its attendees diverse yet related topics of profound scientific content, cutting edge research presentations by world-reknown scientists carefully chosen by the two societies!

I extend our sincere thanks to all presenters and participants for making this conference a reality. I welcome each one of you to participate, share, and interact with one another as we strive to understand the science behind folkloric/anecdotal medicine and push the frontiers of science of plant-based medicine further. On behalf of the ACMAP Board of Directors, we hope that you'll find this conference and this venue most productive and enjoyable. Please feel comfortable to contact any of our organizers if we can help make your ACMAP-REDBIO participation more exciting and rewarding in many ways. Again, thank you all and welcome to the 7<sup>th</sup> Annual Joint Conference of ACMAP and REDBIO.

Special thanks to the Program Chair, Dr. Fabricio Medina-Bolivar, organizers of REDBIO and each member of the ACMAP Board of Directors without whose diligence and dedication, this joint conference could not have been possible.

Humbly,

*M.S. Srinivasa Rao*

Srinivasa Rao Mentreddy, Ph.D.  
2016 ACMAP President

**7th Annual Conference  
American Council for Medicinally Active Plants  
(ACMAP)  
June 29 – July 1, 2016**

**Jointly held with the IX Conference of the Latin American and Caribbean  
Agricultural and Forestry Biotechnology Network (REDBIO 2016-PERU)  
June 27 – July 1, 2016**

**Conference Venues:**

**Wednesday June 29:**

Casa Andina Private Collection Hotel  
Av. La Paz 463, Miraflores, Lima, Peru

**Thursday June 30 and Friday July 1:**

Centro de Convenciones del Colegio Medico del Peru “Daniel Alcides Carrion” (Peruvian Medical Association Convention Center “Daniel Alcides Carrión”)  
Av. 28 de Julio 776, Miraflores, Lima, Peru

**WEDNESDAY – June 29**

**ACMAP FIELD TRIP**

**8:30 am – 2:00 pm**

**International Potato Center (CIP)**

Advanced registration required

Bus will depart from Casa Andina Private Collection Hotel

(Departure time will be confirmed to registrants)

Peruvian lunch buffet at Casa Andina Private Collection Hotel

**ACMAP BOARD MEETING**

**2:00 pm - 2:30 pm**

**Casa Andina Private Collection Hotel**

*(ACMAP Board Members and Conference Chair)*

**ACMAP REGISTRATION**

**2:00 pm - 3:45 pm**

**Casa Andina Private Collection Hotel**

**(Conference Room – Level 2)**

*(Travel vouchers to field trip to Cusco/Machu Picchu will be provided at this time – advanced purchased required)*

**ACMAP SESSION**

**Casa Andina Private Collection Hotel  
(Conference Room – Level 2)**

**3:45 pm - 4:00 pm**

**Welcome Remarks**

**Fabricio Medina-Bolivar**, Ph.D. (ACMAP Program Chair, 2014-2015 ACPMAP President, Arkansas State University, USA)

**4:00 pm - 4:30 pm**

**O1. Research on *Lepidium meyenii* (“Maca”): A Hypocotyl from the Peruvian Highlands**

**Dulce Alarcón-Yaquette**, B.S. and **Gustavo Gonzales**, M.D., Ph.D. (Universidad Peruana Cayetano Heredia, PERU)

**4:30 pm - 6:00 pm**

**Session: Plant Constituents for Obesity and Related Metabolic Disorders**

Chair: **Agnes Rimando**, Ph.D. (U.S. Department of Agriculture, USA)

Co-Chair: **Masuko Kobori**, Ph.D. (National Food Research Institute, JAPAN)

*4:30 pm - 4:55 pm*

**O2. “Dietary Quercetin Suppresses Inflammation of Visceral Adipose Tissue and Improves Metabolic Syndrome in Diet-Induced Obese Mice”**

**Masuko Kobori**, Ph.D. (National Food Research Institute, JAPAN)

*4:55 pm - 5:20 pm*

**O3. “Grape Seed Flour and Polyphenols Reduces Metabolic Dysfunction and Numbers of Gut Bacteria in Hamsters and Mice on High Fat Diets”**

**Wallace Yokoyama**, Ph.D. (U.S. Department of Agriculture, USA)

*5:20 pm - 5:45 pm*

**O4. “Hypolipidemic Effect of Soy Foods and their Components”**

**Yoko Takahashi**, Ph.D. (National Food Research Institute, JAPAN)

**5:45 pm - 6:00 pm**

**BREAK**

**6:00 pm - 6:50 pm**

*Selected Oral Presentations:*

*6:00 pm - 6:25 pm*

**O5. “Cardioprotection with Polyphenols”**

**Thomas Netticadan**, Ph.D. (Canadian Centre for Agri-Food Research in Health and Medicine, CANADA)

6:25 pm - 6:50 pm

**O6. “Neuroprotective Role of Antioxidants in Methamphetamine-Induced Dopamine Neurotoxicity”**  
Syed Ali, Ph.D. (Food and Drug Administration, USA)

**ACMAP RECEPTION**

7:00 pm - 9:00 pm

**Cocktail reception at Casa Andina Private Collection Hotel**  
*(Light hors d’oeuvres will be provided)*

**ACMAP Presentation**

**Rao Mentreddy**, Ph.D. (2016 ACPMAP President; Alabama A&M University, USA)

**Jeffrey Adelberg**, Ph.D. (ACMAP Secretary; Clemson University, USA)

**Adolfina Koroch**, Ph.D. (Journal of Medicinally Active Plants Executive Editor; City University of New York, USA)

**Fabricio Medina-Bolivar**, Ph.D. (ACMAP Program Chair; 2014-2015 ACPMAP President; Arkansas State University, USA)

**THURSDAY – June 30**

**Room A – Convention Center Auditorium**

8:30 am - 8:40 am

**ACMAP Welcome**

**Fabricio Medina-Bolivar**, Ph.D. (ACMAP Conference Chair, 2014-2015 ACPMAP President, Arkansas State University)

8:40 am - 3:30 pm

*Session:* **Biodiversity and Biotechnology for Human Health (ACMAP and REDBIO joint Plenary Session)**

8:40 am - 9:00 am

**Opening Remarks**

Chair: Carlos Malpica, Ph.D. (Metabolon Inc., SPAIN)

9:00 am - 9:30 am	<p><b>O7. “Bioactive Compounds from the Andean and Amazonian Plant Biodiversity”</b>  <b>David Campos</b>, Ph.D. (Universidad Nacional Agraria La Molina, PERU)  <i>(Talk in Spanish – simultaneous English translation provided)</i></p>
9:30 am - 10:00 am	<p><b>O8. “Metabolomics Applied to Personalized Medicine”</b>  <b>John Ryals</b>, Ph.D. (Metabolon Inc., USA)</p>
10:00 am - 10:30 am	<p><b>O9. “Valeriana officinalis as a Novel Platform for Plant Natural Product Drug Discovery”</b>  <b>Joe Chappell</b>, Ph.D. (University of Kentucky, USA)</p>
10:30 am - 11:00 am	<p><b>COFFEE BREAK</b></p>
11:00 am - 11:30 am	<p><b>O10. “Next Generation Plant-Made Therapeutic Proteins – Exploiting Plant Lectins to Deliver Human Enzymes”</b>  <b>Carole L. Cramer</b>, Ph.D. (Arkansas State University &amp; BioStrategies LC, USA)</p>
11:30 am - 12:00 pm	<p><b>O11. “Peanut Hairy Roots: A Sustainable System for the Production and Discovery of Bioactive Compounds for Human Health”</b>  <b>Fabricio Medina-Bolivar</b>, Ph.D. (Arkansas State University, USA)</p>
12:00 pm - 12:30 pm	<p><b>O12. “Making the Best Match: Designing Transcriptomics to Understand Plant Metabolic Specialization and Reveal Gene Functions”</b>  <b>Keithanne Mockaitis</b>, Ph.D. (Indiana University &amp; Dow AgroSciences, USA)</p>
12:30 pm - 1:00 pm	<p><b>Discussion</b></p>
1:00 pm - 2:30 pm	<p><b>LUNCH BREAK</b></p>
2:30 pm - 3:00 pm	<p><b>O13. “Protein-Polyphenol Aggregate Particles: Hypoallergenicity and Food Functionality”</b>  <b>Mary Ann Lila</b>, Ph.D. (Plants for Human Health Institute, North Carolina State University, USA)</p>



- 3:00 pm - 3:30 pm                    **O14. “Metabolic Engineering of Bioactive Compounds in Tomato”**  
Eugenio Butelli, Ph.D. (John Innes Centre, UNITED KINGDOM)
- 3:30 pm - 4:00 pm                    **O15. “Differences in Human Diversity from the Andes: Implications for Human Health**  
Ricardo Fujita, Ph.D. (Universidad San Martin de Porres, PERU)
- 4:00 pm - 8:30 pm                    **REDBIO Session: Advances on Genome Editing**
- 4:30 pm - 5:00 pm                    **COFFEE BREAK**

**POSTER SESSION**

- 6:30 pm - 9:00 pm                    **REDBIO & ACMAP POSTER SESSION**

**FRIDAY – July 1 (concurrent sessions: rooms D and E)**

**Room D – Convention Center Level 4 (ACMAP session)**

- 8:30 am - 10:10 am                    Session. Micropropagation, Conservation and Sustainable Use of Medicinal Plants  
Chair: Jeffrey Adelberg, Ph.D. (Clemson University, USA)
- 8:30 am - 8:55 am                    *Keynote Talk:*
- O16. “Look Under Your Feet! The Wondrous Roots and Tubers of the Andes”**  
David Ellis, Ph.D. (International Potato Center, PERU)
- 8:55 am - 9:20 am                    **O17. “Multifactor Designs to Enable Medicinal Plant Research”**  
Jeffrey Adelberg, Ph.D. (Clemson University, USA)
- 9:20 am - 9:45 am                    **O18. “Advanced Roots System: Unlocking Plant Biodiversity through a High Volume and Low Cost Bioreactor”**  
Pierre-Antoine Mariage, Ph.D. (Green2Chem, BELGIUM)

<b>10:10 am - 10:30 am</b>	<b>COFFEE BREAK (Level 4)</b>
	<u><b>Session: Strategies to Increase Levels of Bioactives in Plants</b></u> <b>Chair: Lena Galvez, Ph.D.</b> (Universidad Nacional Agraria La Molina, PERU & Pontificia Universidad Catolica de Valparaiso, CHILE)
<i>10:30 am - 10:55 am</i>	<i>Keynote Talk:</i>
	<b>O20. “Metabolic Innovations and Fermentation Biology for Medicinally Active Functional Foods”</b> <b>Kalidas Shetty, Ph.D.</b> (North Dakota State University, USA)
<i>10:55 am - 11:20 am</i>	<i>Keynote Talk:</i>
	<b>O21. Bioactivity and Metabolic Engineering of a Nutraceutical”</b> <b>Agnes Rimando, Ph.D.</b> (U.S. Department of Agriculture, USA)
<i>11:20 am - 11:45 am</i>	<b>O22. Space-Age Technology to Increase Yield and Improve Quality of Traditional Medicinal Plants”</b> <b>Gary Stutte, Ph.D.</b> (NASA, USA)
<i>11:45 am -12:10 pm</i>	<b>O23. “Effects of Agronomic Treatments on Growth and Essential Oil Composition of Basil”</b> <b>Rao Mentreddy, Ph.D.</b> (Alabama A&M University, USA)
<i>12:10 pm - 12:35 pm</i>	<b>O24. “Passion Fruit Peel and Sorghum Flour with High Tannin Content as Alternative Fiber- and Antioxidant-Rich Food”</b> <b>José Luis Ramírez Ascheri, Ph.D.</b> (Embrapa Food Technology, BRAZIL)
<b>12:35 pm - 2:00 pm</b>	<b>LUNCH BREAK</b>
<b>2:00 pm - 3:15 pm</b>	<u><b>Session: Traditional Knowledge and Use of Medicinal Plants</b></u> <b>Chair: Luis Nopo-Olazabal, Ph.D.</b> (Arkansas State University, USA)
<i>2:00 pm - 2:25 pm</i>	<b>O25. “Pharmacist’s View on Medicinal Plants Legal Access”</b> <b>Michel Sauvain, Ph.D.</b> (Institut de Recherche pour le Développement, FRANCE)

2:50 pm - 3:15 pm                    **O27. “Characterization of Phenolic Bioactives from Peruvian Corn (*Zea mays* L.) Diversity”**  
Lena Galvez Ranilla, Ph.D. (Universidad Nacional Agraria La Molina, PERU & Pontificia Universidad Católica de Valparaíso, CHILE)

3:25 pm - 3:45 pm                    **COFFEE BREAK (Level 4)**

**Room E – Convention Center Level 5 (ACMAP session)**

8:30 am - 10:10 am                    *Session: Plant Bioactives for Cancer Prevention and Therapy*  
**Chair: Anait S. Levenson, M.D., Ph.D.** (Long Island University - Brooklyn, USA)

8:55 am - 9:20 am                    **O29. “Bitter Melon and Pancreatic Cancer”**  
Rajesh Agarwal, Ph.D. (University of Colorado Skaggs School of Pharmacy and Pharmaceutical Sciences, USA)

9:20 am - 9:45 am                    **O30. “Epigenetic Chemopreventive Nature of Dietary Phytochemicals”**  
Anait S. Levenson, M.D., Ph.D. (Long Island University, USA)

9:45 am - 10:10 am                    **O31. “Chinese Medicinal Plants Inhibit Arthritis by Altering Pathogenic Immune Events”**  
Kamal Moudgil, Ph.D. (University of Maryland School of Medicine, USA)

10:10 am - 10:30 am                    **COFFEE BREAK (Level 4)**

10:30 am - 12:35 pm                    **Session: Preclinical and Clinical Evaluation of Medicinally Active Plants**  
**Chair: Rebecca Parr, Ph.D.** (Stephen F. Austin State University, USA)

10:30 am - 10:55 am                    **O32. “Antiplasmodial and Antileishmanial Activities of Herbal *Pseudelephantopus spiralis* (Less.) Cronquist and Isolated Hirsutinolide-Type Sesquiterpenoids”**  
Valerie Jullian, Ph.D. (Institut de Recherche pour le Développement, FRANCE)

10:55 am - 11:20 am                    **O33. “Oregano (*Origanum vulgare*) Extract Improves Intestinal Mucosa Development in Young Individuals”**

	<b>Diego Martinez</b> , Ph.D. (LIAN Development and Service, PERU)
11:20 am - 11:45 am	<b>O34. "Seabuckthorn (<i>Hippophae</i> spp) for Medicine and Health Food"</b> <b>Ashish Yadav</b> , Ph.D. (Indian Council for Agricultural Research, INDIA)
11:45 am - 12:10 pm	<b>O35. "Discovering the Mechanism of Action of Stilbenoids on Rotavirus Infected Cells"</b> <b>Rebecca Parr</b> , Ph.D. (Stephen F. Austin State University, USA)
12:10 pm - 12:35 pm	<b>O36. "Pharmacokinetics of Phytochemicals: Implications for Clinical Trial Design"</b> <b>Jeremy Johnson</b> , Ph.D. (University of Illinois at Chicago, USA)
<b>12:35 pm - 2:00 pm</b>	<b>LUNCH BREAK</b>
2:00 pm - 2:20 pm	<b>O37. "Effect of D-004, a Lipid Extract from Cuban Royal Palm (<i>Roystonea regia</i>) Fruits on Prostate Hyperplasia and Urodynamic Changes in Experimentally Induced Rats"</b> <b>Vivian Molina</b> , Ph.D. (National Centre for Scientific Research, CUBA)
2:20 pm - 2:40 pm	<b>O38. "Characterization of the Mechanism of Action of D-004, a Lipid Extract of <i>Roystonea regia</i> fruits in Benign Prostatic Hyperplasia"</b> <b>Yohani Perez</b> , Ph.D. (National Centre for Scientific Research, CUBA)
<b>2:40 pm - 3:25 pm</b>	<u><b>Session: Selected Student Presentations</b></u> <b>Chair: Adolfin Korocho</b> , Ph.D. (City University of New York, USA)
2:40 pm - 2:55 pm	<b>O39. "Bioproduction and Anti-inflammatory Activity of Delta-Tocotrienol-Enriched Extracts from Hairy Roots of Achiote (<i>Bixa orellana</i>)"</b> <b>Jarrold Creameans</b> (Arkansas State University, USA)
2:55 am - 3:10 pm	<b>O40. "Sacha Inchi (<i>Plukenetia volubilis</i> Linneo) Oil and its Effects on Growth and Lipid Profile in Rats"</b> <b>Marlene Julca</b> (Universidad Nacional Mayor de San Marcos, PERU)
3:10 pm - 3:25 pm	<b>O41. "Extraction of Natural Analgesic Compounds from Plants used Traditionally in Mali: Study of <i>Pericopsis laxiflora</i>"</b> <b>Ombeline Dalton</b> (Universite Cleremont-Auvergne, FRANCE)

3:25 pm - 3:45 pm

**COFFEE BREAK (Level 4)**

Session: Phytochemistry of Medicinally Active Plants

**Chair: Rodolfo Juliani, Ph.D. (Rutgers University, USA)**

3:45 pm - 4:10 pm

**O42. “An Ancient Seed Revisited: Composition of Pine Nuts (*Pinus pinea* L.) Grown in Chile, Argentina, Turkey, Israel, Italy and Spain”**

**Mariane Lutz, Ph.D. (Universidad de Valparaíso, CHILE)**

4:10 pm - 4:25 pm

**O43. “Using Headspace Gas Chromatography for the Determination of Volatile Components and Sensory Profiles in Medicinally Active Plants”**

**Rodolfo Juliani, Ph.D. (Rutgers University, USA)**

4.40 pm

**ACMAP PICTURE**

*(Location to be announced)*

5:00 pm - 5:30 pm

**CLOSING CEREMONY AND AWARDS (Room E, Level 5)**

**ACMAP CLOSING DINNER AND PERUVIAN SHOW**

7:30 pm - 10:30 pm

**Bus will depart from Casa Andina Private Collection Hotel  
(Advanced registration required)**

**Saturday – July 2**

ACMAP Optional Field Trip – Advanced Reservation Required  
(Day 1- Depart from Lima to Cusco)

**SUNDAY – July 3**

ACMAP Optional Field Trip  
(Day 2- Tour of Cusco)

## **MONDAY – July 4**

Optional Field Trip  
(Day 3 - Machu Picchu)

## **TUESDAY – July 5**

Optional Field Trip  
(Day 4 – Depart from Cusco to Lima)

# ORAL PRESENTATIONS

## **O1. Research on *Lepidium meyenii* (“Maca”): A Hypocotyl from the Peruvian Highlands**

Dulce E. Alarcón-Yaquette<sup>1,2</sup>, Gustavo F. Gonzales<sup>1,2</sup>

<sup>1</sup>Research Circle of Plants with Effect on Health; <sup>2</sup>Endocrinology and Reproduction Unit, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru

E-mail: dulce.alarcon@upch.pe

People from the Peruvian Central highlands have been using a hypocotyl, maca (*Lepidium meyenii*) as a nutraceutical for centuries. This Andean crop grows exclusively over 4,000 meters above sea level and scientific research has prompted a wave of interest on its medicinal properties.

At the beginning, maca was marketed as an aphrodisiac but several studies showed that its effects on libido are not immediate. Other properties were demonstrated for several parameters such as enhancing fertility, reducing inflammatory cytokines, reversing prostatic benign hyperplasia and improving cognitive parameters including learning and memory in experimental animal models and as a neuroprotector and glycemic reducer on *in vitro* studies. Nevertheless, the mechanisms behind these functions are not fully understood.

This update aims to bring the latest findings on research of this Peruvian crop, including the differential properties among three phenotypes, toxicity studies and proposed mechanism of action involving the modulation of the novel signaling pathway: the endocannabinoid system.

## **O2. Dietary Quercetin Suppresses Inflammation of Visceral Adipose Tissue and Improve Metabolic Syndrome in Diet-Induced Obese Mice**

Masuko Kobori, Yumiko Takahashi, Yukari Akimoto, Hideaki Oike, Katsunari Ippoushi

National Food Research Institute, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, 305-8642 Japan

E-mail: kobori@affrc.go.jp

Quercetin is a common flavonoid contained in vegetables, fruits and tea as the glycosides. Epidemiological studies have suggested that quercetin reduces the risk of cardiovascular diseases and some other lifestyle-related diseases. Recently we determined the quercetin content of the food and estimated the daily quercetin intake by residents in a town of Japan. The daily intake of quercetin was mainly provided by onions and green tea. Our result suggested that quercetin intake was negatively correlated with diastolic blood pressure. To evaluate the mechanism of the suppressive effect of quercetin on metabolic syndrome, we examined the effect of quercetin on diet-induced obese mice. We fed C57BL/6J mice with a high-fat, high-sucrose and high-cholesterol Western diet or a Western diet containing quercetin for 18-20 weeks. Chronic dietary intake of quercetin alleviates obesity and metabolic syndrome in the diet-induced obese mice. The consumption of quercetin significantly reduced fat accumulation of the liver and the visceral adipose tissues in mice fed a Western diet. Obesity induces the chronic inflammation in visceral adipose tissue and leads to systemic insulin resistance related to metabolic syndrome. Comprehensive gene expression analysis showed that quercetin decreased the genes expression associated with the accumulation and activation of immune cells, such as macrophages, T and B lymphocytes, dendritic cells, and mast cells, in the epididymal adipose tissues of diet-induced obese mice. Quercetin suppressed the plasma levels of proinflammatory cytokine TNF $\alpha$ , which is shown to induce the insulin resistance. Suppression of oxidative stress and NF $\kappa$ B expression likely contribute to the prevention of the accumulation and activation of immune cells and resulting chronic inflammation. Significant amount of quercetin metabolites were contained in the plasma and the epididymal adipose tissues.

### **O3. Grape Seed Flour and Polyphenols Reduces Metabolic Dysfunction and Numbers of Gut Bacteria in Hamsters and Mice on High Fat Diets**

Wallace Yokoyama<sup>1</sup>, Hyunsook Kim<sup>2</sup> and Torey Arvik<sup>1,2</sup>

<sup>1</sup>USDA, ARS, Western Regional Research Center, Albany, CA and <sup>2</sup>Hanyang University, Seoul, S Korea, <sup>3</sup>Sonomaceuticals, LLC, Santa Rosa, CA, USA

E-mail: wally.yokoyama@ars.usda.gov

Grape seeds contain high levels of flavonoids including catechin, epicatechin, and their dimers, trimers, higher oligomers and polymers. While the monomers, catechin and epicatechin, are absorbed into the blood stream, dimers and higher oligomers pass into the cecum and colon. In normal weight or obese mice and hamsters the feeding of high fat diets supplemented with grape seed flour prevents or reduces, respectively, characteristics of metabolic dysfunction including high levels of plasma and liver lipids, body weight gain, fatty liver, and higher adipose weight. Expression of liver and adipose genes show differences in fat, carbohydrate, cholesterol and bile acid metabolism between control and grape seed supplemented animals. The numbers of gut microbes were reduced by 1 log in hamsters fed grape seed flour and changes in plasma lipoproteins and weight gain were correlated with some species of bacteria. These results suggest that metabolic effects of grape seed consumption may be due to modulation of gut bacteria numbers and/or species.

### **O4. Hypolipidemic Effect of Soy Foods and their Components**

Yoko Takahash

National Food Research Institute, NARO, Tsukuba, Ibaraki 305-8642 Japan

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Soybean and soyfoods are known to reduce the risk of developing cardiovascular diseases through regulating circulating lipid levels. Soyfoods contain various compounds with hypolipidemic properties, such as soy protein, polyunsaturated fatty acids, and possibly isoflavones. Although several clinical studies failed to conclude that soy protein and isoflavones significantly decreased blood cholesterol levels, we suppose that the ingestion of whole soyfoods, rather than single soy compound, may be beneficial to the improvement of the blood lipid profiles. In order to clarify the contribution of the soy compounds in regulating lipid metabolism, we compared the effect of soyfoods with their hypolipidemic compounds in rats. Tofu (soybean curd) and soy protein significantly lowered serum triacylglycerol and cholesterol levels compared to casein. The reduction of serum lipid levels was accompanied with exclusive alteration of mRNA levels involved in fatty acid and/or steroid synthesis in the liver. By contrast, isoflavone supplementation had little effect on serum lipid levels or hepatic mRNA expressions. Also, isoflavones exerted no synergistic effects with soy protein.

### **O5. Cardioprotection with Polyphenols**

Thomas Netticadan

Canadian Centre for Agri-Food Research in Health and Medicine, Winnipeg, Canada

E-mail: TNetticadan@sbrca.ca

Despite the success of existing therapies, cardiovascular disease (CVD) remains to be one of the major causes of death worldwide. Exploring new strategies to improve survival of patients with CVD is therefore of great importance; examining the potential of phytochemicals may be one such avenue. Polyphenols are



a class of compounds that have received increased attention for their potential health benefits. These compounds are synthesized by plants to serve a wide variety of functions, including defense.

Over the past decade, we studied the cardioprotective properties of resveratrol, a polyphenol found in grapes, berries and peanuts, as well as in Japanese knotweed. We examined *in vivo* the ability of resveratrol to prevent or reverse the development of abnormalities in cardiac structure and function in animal models of CVD. We also examined *in vitro* the effects of polyphenols in protecting diseased adult rat cardiomyocytes. The molecular mechanisms underlying the effects of resveratrol was studied in both diseased adult rat cardiomyocytes and heart tissues from the animal models of CVD. Our results showed that administration of 2.5 mg/kg/day of resveratrol was able to prevent/reverse abnormalities in cardiac structure and function in animal models of hypertension (the spontaneously hypertensive rat) and obesity/type II diabetes (high fat fed rat) and myocardial infarction (coronary artery ligated rat). We also demonstrated that 30 micromolar resveratrol protected cardiomyocytes against exposure to norepinephrine (NE), a potent hypertrophic and cell-death trigger. The beneficial effects of resveratrol observed *in vivo* and *in vitro* were associated with its antioxidant properties.

The strong cardioprotective effects observed with resveratrol, led us to examine the potential of a polyphenol rich source – blueberry. Our results showed cardiomyocyte protection with total blueberry polyphenol enriched fraction in NE-exposed cardiomyocytes, similar to that observed with resveratrol. On the basis of the results from our studies, we conclude that polyphenols have strong cardioprotective properties, and may therefore have a potential in the prevention and treatment of CVD.

## **O6. Neuroprotective Role of Antioxidants in Methamphetamine-Induced Dopaminergic Neurotoxicity**

Syed Ali<sup>1</sup>, Syed Imam<sup>1</sup> and Yosef Itzhak<sup>2</sup>

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Oxidative Stress produces free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) and these free radicals have been known to be involved in a multitude of neurodegeneration. Recently, we have developed an HPLC/EC method to identify 3-nitrotyrosine (3-NT), an *in vivo* biomarker of peroxynitrite production, in brain to evaluate if an agent-driven neurotoxicity is produced by the generation of peroxynitrite. A single or multiple injections of methamphetamine (METH) produced a significant increase in the formation of 3-NT in the striatum. This formation of 3-NT correlated with striatal dopamine (DA) depletion caused by METH administration. Pretreatment with antioxidants such as selenium and melatonin can completely protect against the formation of 3-NT and depletion of striatal DA. We also used two different approaches, pharmacological manipulation and transgenic animal, in order to further investigate the role of peroxynitrite. We have shown that a selective nNOS inhibitor, 7-nitroindazole, significantly protected against the formation of 3-NT as well as striatal DA depletion. Similar results were observed with nNOS knockout mouse. In addition, we have shown that peroxynitrite also played an important role in apoptotic gene expression. METH administration up-regulated the protein expression of p53 and down-regulated *bcl-2* protein expression in the striatum of wild type mice but not in the nNOS knockout mice. Together, these data clearly support the hypothesis that free radicals and oxidative stress plays a major role in METH-induced dopaminergic neurotoxicity and selective NOS inhibitors and antioxidants can protect against METH-induced neurotoxicity.

## **09. *Valeriana officinalis* as a Novel Platform for Plant Natural Product Drug Discovery**

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Valerian is a nutraceutical preparation from the roots of *Valeriana officinalis* that is commonly recommended for relief of tension, anxiety and insomnia. The greatest biological efficacy of valerian has been correlated with freshly harvested and carefully dried root preparations, and with the iridoid alkaloid and sesquiterpene content of these preparations. The valepotriates are epoxyiridoid esters with the dominant species being valtrate. Because of putative instability and water insolubility of the valepotriates, some investigations have suggested that the sesquiterpene compounds are more important for the biological activity of valerian. To determine which chemical components of *V. officinalis* are important for its biological activities, we have developed several important capabilities and tools to support this effort. First, we have developed and relied upon transcriptomic and metabolomic resources to identify the genes encoding for these unique biosynthetic capabilities. Second, the methodology for genetic engineering hairy root cultures of *V. officinalis* having diverse chemical profiles has been developed. Third, we have been working on the development of a novel test platform for assessing the anxiolytic activity of the various engineered hairy root culture lines. Progress in all of these areas will be presented.

## **010. Next Generation Plant-Made Therapeutic Proteins – Exploiting Plant Lectins to Deliver Human Enzymes**

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The use of plants as 'factories' for complex pharmaceutical proteins for vaccine and therapeutic applications has been the focus of active research and development for more than two decades. Key advantages of plants compared to mammalian cell-based bioproduction include safety (plants do not support human/animal viruses), costs (especially reduced up-front capitalization), and tremendous flexibility in scale - from personalized medicine approaches to rapid-response large-scale bioproduction of vaccine antigens. However, the number of products reaching clinical testing and regulatory approval remains low. Our strategy has been to use plant components to selectively enhance the efficacy of plant-made pharmaceuticals. Plant lectins, such as the RTB carbohydrate binding subunit of ricin, are known to bind to the surface of mammalian cells and to direct endocytosis, transcytosis, and lysosomal delivery. RTB fusions with human lysosomal enzymes were produced in the leaves of *Nicotiana benthamiana*. The plant-made fusion proteins retained both lectin-binding and lysosomal enzyme activities, were efficiently taken up into human cells, and facilitated disease correction of fibroblasts from patient having a lysosomal storage disease based on genetic deficiency of that enzyme. Results indicated that RTB-mediated delivery provides key advantages in uptake kinetics/capacities at the cellular level compared to mammalian cell-derived enzyme. In lysosomal disease mouse models, the RTB fusion products showed broad biodistribution and reduced the lysosomal disease burden including reaching "hard-to-treat" tissues that are not addressed by current mammalian cell-derived enzyme replacement therapies. Our result highlight novel approaches to exploit plant biology in support of human health and treatment of rare genetic diseases.

## **O11. Peanut Hairy Roots: Sustainable Systems for Production and Discovery of Bioactive Compounds for Human Health**

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Hairy root cultures produced via genetic transformation with *Agrobacterium rhizogenes* provide a sustainable platform for the production and discovery of bioactive compounds made by plants. As example, we developed hairy roots of peanut and maintained them as clonal cultures over several years. Peanut hairy roots have the capacity to produce and secrete prenylated stilbenoids, such as arachidin-1 and arachidin-3, into the culture medium upon treatment with elicitors. Several compounds were investigated as elicitors and the co-treatment of the hairy root cultures with methyl jasmonate and cyclodextrin led to very high yields of prenylated stilbenoids. In particular, the yield of arachidin-1 was 1,000 times higher when compared to methyl jasmonate treatment alone. High performance countercurrent chromatography methods were developed to purify the prenylated stilbenoids from the culture medium and subsequently study them in different bioassays. In addition to its antimicrobial role in the peanut plant, arachidin-1 showed antioxidant and anti-adipocytic activity as well cytotoxicity in different cancer cell lines. Due to these important bioactivities for plant and human health, transcriptomic approaches are being conducted to identify the enzymes involved in the biosynthesis of prenylated stilbenoids and elucidate the mechanisms that regulate the accumulation of these compounds in peanut. We demonstrated that hairy roots are a valuable tool for production of bioactive compounds which may lead to the development of drugs for the prevention and treatment of human diseases.

## **O12. “Making the Best Match: Designing Transcriptomics to Understand Plant Metabolic Specialization and Reveal Gene Functions”**

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In collaboration with researchers in legumes, gymnosperm and angiosperm trees and other woody perennials, we have undertaken experiments to generate high-quality whole transcript sequence references and to associate these with secondary metabolite profiles. We have mined transcriptomic and genomic references to piece apart enzyme pathways responsible for the production of valuable natural products, including lineage-specific oils associated with valued wood properties or insect repellent qualities, and phytoalexin defense compounds that offer human health benefits. In many cases, intraspecific variation in genomic capacity to produce such compounds is known to correlate well with a plant's ability to adapt to environmental stresses, both biotic and abiotic. The recent completions of whole genome references for a vast array of diverse plants has allowed us to observe not only generalities of secondary metabolic diversity among the plant kingdom, but to uncover gene functional uniqueness among well- versus poorly-adapted plants. A major source of this uniqueness derives from gene copy number differences and other forms of genomic structural variation that occur during the course of plant adaptation. Emerging technologies and experimental approaches to genomics studies are only now beginning to enable plant breeding programs to recognize and directly utilize these types of genomic variation. Here I will summarize some of the challenges currently faced in genomics approaches. I will present examples of how well-designed transcriptomics can elevate the value of genome sequence references, and can importantly, with or without a genome sequence reference, greatly accelerate gene functional discovery in targeted experiments.

### **O13. Protein-Polyphenol Aggregate Particles: Hypoallergenicity and Food Functionality**

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Dietary berry fruits are a particularly rich and abundant resource for a wealth of phytoactive polyphenolic compounds, and their efficacy has been well demonstrated *in vivo* and in clinical trials for antidiabetic, neuroprotective, anticancer, cardioprotective and other health-relevant benefits. Despite well-documented positive associations between berry consumption and health maintenance, the general public still fails to incorporate adequate fruit intake in the daily diet, largely due to issues of perishability, inconvenience, lack of stability in processed food products, and perceived higher costs. In addition, it is a challenge to deliver a credible placebo in clinical trials with berry fruit, and therefore most researchers resort to using relatively unstable (and less efficacious) powdered berry and placebo formulations. By taking advantage of the natural affinity of polyphenols for edible proteins, it has been possible to form stable, chimeric micrometer scale dry protein-polyphenol aggregate particles with multiple food product, clinical and therapeutic applications. In the protein-polyphenol aggregate particles, proteins are rendered less reactive, and phytoactive constituents are stabilized and concentrated. Polyphenols strategically bound to allergenic epitopes on certain proteins (e.g. peanut, egg, soy, whey) result in protein-dense food ingredients with significantly attenuated allergenicity (triggering reduced mast cell degranulation on oral challenge to allergic mice *in vivo*). In addition, the protein-polyphenol aggregate particles modulate gastric digestion of allergenic proteins, reducing the immunoreactivity of digestive peptides. While protein aggregation, sugar crystallization and/or phase separation typically cause an adverse reaction in high-protein power bar formulations called bar hardening, incorporation of the protein-polyphenol aggregate particles (as at least a percentage of the total protein ingredient) mitigated this destabilizing event, and supplemented overall nutritional and immunoprotective value of food products. Percent polyphenol content in particles was inversely correlated with percent creep recovery (lower creep recovery is characteristic of more viscous, fluid-like textures).

### **O14. Metabolic Engineering of Bioactive Compounds in Tomato**

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There is growing evidence that bioactives in the diet play an important role in promoting health. Flavonoids and related phenolics are examples of bioactives from plants that have beneficial influences on a number of important risk factors associated with cardiovascular disease, cancer, obesity and age-related degenerative diseases. Despite increasing knowledge about the link between food and health, only few people are meeting the recommended dietary intakes of flavonoid-containing fruit and vegetables and chronic diseases are reaching epidemic proportions in many countries. Consequently, exploring methods to improve the levels of flavonoids and related phenolics in the fruit and vegetables that people eat is an important strategy to promote health through the production of protective foods.

In tomato, the combination of different types of metabolic strategies (including manipulation of transcription factors) with the availability of natural mutant varieties results in the generation of genetically modified plants with very high levels of different specific phenolic compounds. These new genetically improved tomatoes provide a unique opportunity to assess, within a whole-food context, the impact on disease of individual phytonutrients including those (such as resveratrol and isoflavonoids) found at low concentrations in only a limited number of plant species.

Our approach focuses on testing the potential health benefits of functional foods using *in vitro* and animal model systems for different diseases and, eventually, human intervention studies. Our work will be of interest to different research areas including fundamental research on plants and human nutrition and can provide a unique platform for large-scale producing of valuable compounds derived from aromatic amino

acids, including active compounds from medicinal plants that are often poorly productive or difficult to grow and manage.

### **O16. "Look Under Your Feet! The Wondrous Roots and Tubers of the Andes"**

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The International Potato Center (CIP) holds *in trust* the global collections of eleven different root and tuber crops (potato, sweetpotato, oca, mashua, ulluco, yacon, arracacha, maca, ahipa, mauka and achira), belonging to eleven different botanical families. It is often said that indigenous potato farmers in the Andes annually harvest 10 or more different potato landraces as an insurance policy against the harsh conditions, if one variety does not make it, another will. Yet it has also been said that by planting this diversity, they are also providing a nutrient and vitamin varied diet to their families. The interrelationship of these Andean root and tuber crops is evident with mashua (*Tropaeolum tuberosum*) often ringing potato fields to help keep weevils out of the potatoes. Oca (*Oxalis tuberosa*) which contains a high level of ocatin, a compound with antimicrobial and antibacterial properties is also interplanted in the potato fields yet protective properties to potatoes has yet to be documented. Most of the Andean root and tuber crops come in a variety of colors with deep purple varieties providing antioxidant properties, orange and yellow varieties provide carotenoids as a source of vitamin A and all have been used for centuries as folk medicines for Andean communities. Maca (*Lepidium meyenii*) for example has been referred to as the Andean Viagra while mashua is said to have a mellowing effect on men. Both maca and mashua are high in glucosinolates known to have anticancer activity and mashua has also been found to have anti-inflammatory properties. Arracacha (*Arracacia xanthorrhiza*) is an excellent food for babies, the starch is easily digestible and it has a multiple vitamins and minerals. Yacon (*Smallanthus sonchifolus*), a very sweet tasting root is known its high content of fructooligosaccharides (FOS) and prebiotic and antioxidant properties. Potato and sweetpotato are also both sources of several bioactive compounds. In many markets around Lima and other Andean cities one can find extracts or dried powder of all these crops, often segregated by color of the root or tuber and each color purported to provide differing medical effects. In this talk, we will overview properties and uses of these crops as they relate to agriculture, nutrition and health.

### **O17. Multifactor Designs to Enable Medicinal Plant Research**

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Controlling plant growth is an inherently multifactor problem. Cloned plants in an *in vitro* environment eliminates variation due to genetics, temperature, light, moisture, pests and peculiarities in soil. This stringent environmental control allows much more complex experimental designs to be used *in vitro*, compared to what we have traditionally used in field agriculture. DOE is a branch of statistics for solving multifactor problems efficiently. Mineral nutrition is inherently a multi-factor problem with synergisms and antagonism among pairs of nutrients often being as important as a single nutrient factor. In this talk we will see designs for how *in vitro* mineral concentrations, P, Ca, Mg, and KNO<sub>3</sub>, and plant density effected both greenhouse growth and the concentrations of curcuminoids concentrations in plants after 6 months of greenhouse growth. Factors in the greenhouse environment that complicate multi-factor designs will be discussed and strategies to solve for those factors will be shown. This talk will conclude with discussion for making multifactor DOE more robust for use in controlled environment production of high value crops, with secondary metabolites as an important group of responses.

### **O18. Advanced Roots System: Unlocking Plant Biodiversity through a High Volume and Low Cost Bioreactor**

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Green2Chem was founded in 2011 with the objective to unlock biodiversity through a novel technology platform - Advanced Root System (ARS™) - which is a patented new generation bioreactor specially design for adventitious and hairy roots cultivation. ARS technology is a fully controlled disposable bioreactor with a high oxygenation level and low shear stress allowing in-vitro roots cultivation at ton scale and at production cost compatible with plants from the field. With a pipeline of 27 hairy roots species, a strong expertise into elicitation and the ARS technology currently at demo-plant scale on Panax Ginseng, Green2Chem ambition to become a leader in the market of high value plant extract

### **O20. Metabolic Innovations and Fermentation Biology for Medicinally Active Functional Foods**

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It is clear medicinally active food systems are essential to counter diet-linked non-communicable chronic diseases (NCD) that are rapidly increasing globally and solutions have to be integrated as part of solutions to food security challenges. Plant and microbial metabolic innovations through integrated systems-based solutions can be targeted to advance medicinally active functional foods to counter NCDs such as type 2 diabetes and its complications. Targeting diverse and culturally relevant plant food systems coupled to microbiome targeted bioprocessing can be part of metabolic innovations to widen medicinally active functional foods in diverse landscapes and food systems. Using screening technologies for optimizing redox regulated pathways in plant food systems we have enhanced bioactive profiles for early stages of type 2 diabetes such as hyperglycemia and associated complications such as hypertension. These bioactives profiles can be further widened using fermented systems which can be also enriched for beneficial microbiomes. Such systems based strategies are essential to develop and widen health targeted functional foods and to enhance diversity of culture specific food systems.

### **O21. Bioactivity and Metabolic Engineering of a Nutraceutical**

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Pterostilbene exemplifies a compound found in some fruits (such as grapes and blueberries) that has shown multitude of activities that are beneficial to human health, and because it is present only in minute quantities in fruits has attracted studies to engineer its synthesis. This presentation reviews studies demonstrating its hypolipidemic, anxiolytic, anti-obesity, and memory-enhancing effects. Engineering the production of pterostilbene in *Nicotiana tabacum* and *Arabidopsis thaliana* using a two-gene binary vector containing peanut stilbene synthase and sorghum O-methyltransferase, via *Agrobacterium tumefaciens* mediated transformation, will be covered, as well as ramifications of manipulating the biosynthetic pathway towards stilbene synthesis. Observations in the *Nicotiana tabacum* transformants will be presented.

## **O22. Space-Age Technology to Increase Yield and Improve Quality of Traditional Medicinal Plants.**

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Technology to enable the growth of plants on long duration space missions can be used to increase the quality, composition and yield of medicinal plants in controlled environments. Controlled environment agriculture (CEA) enables the production of plants and their products inside structures such as greenhouses, growth chambers, and indoor plant factories. Environmental conditions throughout the growth cycle are managed to optimize the concentration of high value phytochemicals, maximize yields, and minimize microbial and insect contamination on a year round basis. Application of CEA for medicinal plant production removes the geographical constraints to growing by enabling environmental (temperature, photoperiod, light quality, CO<sub>2</sub>) and cultural (rooting media, nutrient composition, irrigation) factors to be managed and replicated anywhere in the world. Enriching the atmosphere with CO<sub>2</sub> has been reported to significantly increase yield, reduce time to harvest, and enhance the concentration of bioactive compounds of a number of medicinal species. Advances in lighting technology enable the optimization of light quality throughout the growth cycle to minimize costs and maximize production of high value bioactive compounds. Management of CEA has potential to increase availability, improve quality, and reduce over-harvesting pressures of medicinal and aromatic plants (MAPs) supplying the commercial market.

## **O23. Effects of Agronomic Treatments on the Growth and Essential Oil Composition of Basil**

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Basil (*Ocimum* spp.) is used for culinary, aromatic and medicinal uses worldwide. Basil has the potential for production as a fresh market herb and as a dry medicinal product in the US, provided agronomic practices and their effects on growth, yield and essential oil composition are known. The objectives of the study were to assess inter- and intra-specific variation for biomass using 50 to 86 genotypes belonging to six *Ocimum* species in a three-year study; and to determine the changes in biomass, leaf area index and essential composition of three *O. tenuiflorum* accessions in response to various agronomic treatments in a five-year study in Alabama. Essential oil composition of stems and leaves were determined using gas chromatography/mass spectrometry. The above ground fresh biomass per plant ranged from 424.0 g for *O. selloi* to 1450.3 g for *O. basilicum* accession. Inter- and intra-specific variation for biomass and dry matter partitioning patterns were observed in the three-year study. Genotypic variation among *O. tenuiflorum* accessions was significant for biomass, leaf area index, and dry matter partitioning in the five year study. The essential oil composition varied with genotype and agronomic treatments. Eugenol and trans- $\beta$ -farnesene were present in variable levels in all agronomic treatments, but Trans- $\beta$ -guaiene,  $\beta$ -caryophyllene, Methyl chavicol and 1,8-cineole, Bicyclogermacrene,  $\beta$ -bisabolene,  $\alpha$ -cadinene, E-methyl cinnamate and linalool were present or absent depending upon the agronomic treatment. The wide genotypic variation among *Ocimum* accessions indicates potential for adaptation to a wide range of environments. The essential oil composition can be varied by changing agronomic treatments.

## **O24. Passion Fruit Peel and Sorghum Flour with High Tannin Content as Alternative Fiber- and Antioxidant-Rich Food**

José Luis Ramirez Ascheri,<sup>1,2</sup> Davy William Hidalgo Chavez<sup>2</sup>; Jhony Willian Vargas Solórzano<sup>2</sup> Elisabete Maria da Graça Costa do Nascimento<sup>2</sup> and Valeria França de Souza<sup>2</sup>

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Brazil is a country known for the cultivation of tropical fruits and has become one of the main producers of passion fruit, the most significant proportion of which commercialized as juice. This generates a considerable amount of residues, due to the peels and seeds, which represent 65-70% of the raw material. For other way, the pericarp of sorghum grains differ in color, thickness, presence of a pigmented testa and secondary plant color, which affect the phenolic composition. The objective this work was to use both raw materials in flour form, processed by extrusion in suitable proportions in order to obtain a pre-cooked product with functional characteristics, palatability and sensory properties. Denaturation of proteins during extrusion leads to open loose structures, which promote polyphenol-protein interaction resulting in the formation of tannin-protein and lignin-protein complexes that could contribute to increase both, insolubility and molecular weight of compounds. Where determined the Phenolic compounds by HPLC; Antioxidant activity by free radical ABTS+, Free Radical DPPH+. The results of blend pre-cooked showed free phenolic ( $\mu\text{g/g}$ ): p-cumaric ac., ferulic ac., sinapic ac., o-cumaric ac., as  $3.0\pm 0.1$ ,  $31.5\pm 2.7$ ,  $2.0\pm 0.2$  and  $0.8\pm 0.1$ , respectively. Antioxidant activity ABTS+ as:  $12.83\pm 0.48$   $\mu\text{Mol trolox/g}$ . Antioxidant activity DPPH+ as  $50.67\pm 1.50$  IC50 DPPH. The results showed the potential of prepared product and the opportunities for its use as food..

## **O25. Pharmacist's View on Medicinal Plants Legal Access**

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Twenty years passed since the international Convention on Biological Diversity of Rio de Janeiro (1992), which declares that the genetic resources are the property of States, which shelter them; and the protocol of Nagoya (2010) that proposes rules for the access to genetic resources and the fair and equitable sharing of benefits arising from their utilization.

Two parallel phenomena make difficult their application: The first is of a legal nature, how to apply the laws which govern the individual intellectual property rights to the collective knowledge of native-born people? The big pharmaceutical industry has little interest in developing medicines based on traditional knowledge because they could be accused of biopiracy.

The second is the poor cultural and environmental state of native people in tropical forests. Currently, there is an urgent need to carry out an exhaustive inventory of what remains as ancestral knowledge.

We will illustrate, through examples of scientific research in the Amazonian forest, how it may be possible to reconcile the remuneration for the traditional knowledge, the actual state of national regulatory laws and the inventory of endangered native pharmacopoeias.



## **O27. Characterization of Phenolic Bioactives from Peruvian Corn (*Zea mays* L.) Diversity**

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Peru has one of the highest corn diversity in the world; however its potential as a source of phenolic bioactives has not been fully studied. The objective of this study was to characterize for the first time 22 native corn accessions corresponding to 5 local races from the region of Arequipa (Peru) through the analysis of their phenolic profiles in both the free and cell wall-bound fractions. The CIELAB color parameters and total anthocyanin contents were also evaluated. Major phenolic levels were found in the bound fraction and phenolic contents measured by the Folin-Ciocalteu method varied from 90 to 207 mg of gallic acid equivalents (GAE) per 100 g of sample in dry weight (DW). Only corn samples with darker kernels (low  $L^*$  values) had higher phenolic contents in their free fraction and this was likely related to their higher total anthocyanin contents. A purple corn sample from the *Kculli* race showed the highest anthocyanin content among evaluated corn accessions (510 ± 23 mg of cyanidin 3-glucoside equivalents per 100 g DW). Major phenolic compounds detected by UPLC-PDA in the bound fraction were phenolic acids such as ferulic acid and *p*-coumaric acid whereas the free fractions were rich in *p*-coumaric acid derivatives. Peruvian corn diversity may be an important natural source of phenolic bioactives.

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## **O29. Bitter Melon and Pancreatic Cancer**

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The median life of pancreatic cancer (PanC) patients post diagnosis is <6 months and overall 5 year survival is 3-5%. The low survival numbers in this patient population is due to the fact that the initial growth and progression phase of this disease is slow and asymptomatic and thus remains unnoticed by the patients; by the time the malignancy is diagnosed, the prognosis is poor as the disease has most likely progressed to advanced stages. For this very reason, there is an urgent need for newer drugs or therapies and alternative strategies to improve the disease outcome in these patients. Recently, considerable research efforts have been directed towards the identification of naturally occurring dietary/non-dietary agents for both prevention/ intervention of PanC. One such dietary agent is 'bitter melon' (*Momordica charantia*, also called bitter gourd, karela, balsam apple, etc.) from *Cucurbitaceae* family which has gained an enormous attention in recent years as an alternative medicine. In folk medicine, bitter melon has been used for its several health benefits including presumed anti-diabetic effects. However, in recent times, bitter melon is now also extensively evaluated for its anti-cancer efficacy against various malignancies including our work in PanC. Our studies have shown bitter melon activity against both gemcitabine (frontline chemotherapeutic in PanC patients) sensitive as well as resistant PanC cell lines. Our presentation will summarize both the efficacy data and molecular mechanisms involved in pre-clinical effects of bitter melon against PanC. This work was supported by R01 CA195708 grant from the National Cancer Institute.

### **O30. Epigenetic Chemopreventive Nature of Dietary Phytochemicals**

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In the last decade, strong evidence has emerged on the link between diet and epigenetic mechanisms of cancer. Dietary bioactive molecules from vegetables and fruits have been shown to exhibit anti-inflammatory, anti-oxidative and anti-cancer activities through multiple mechanisms including epigenetic changes associated with DNA methylation, histone modifications, and modulation of non-coding microRNAs (miRNAs). The epigenetic molecular mechanisms that contribute to the chemopreventive nature of dietary polyphenols have been widely studied in cancer. In addition, immunosensitization and chemosensitization properties of natural agents make them viable candidates for combinatorial strategies leading to additive and/or synergistic beneficial effects. In this presentation, I will briefly summarize current knowledge on the most studied natural products, nutrients and food components and their effects on DNA methylation, histone modifications, miRNAs and other epigenetic regulators in cancer. I will focus on the epigenetically-mediated effects of dietary stilbenes in prostate cancer.

### **O31. Chinese Medicinal Herbs Inhibit Arthritis by Altering Pathogenic Immune Events**

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Rheumatoid arthritis (RA) is a major autoimmune disease affecting people worldwide, and its prevalence in women is 2-3 times higher than that in men. This disease is characterized by inflammation of the inner lining (synovium) of the joints and tissue damage. The currently available mainstream (or allopathic) drugs for arthritis therapy are quite potent, but they also have the disadvantages of severe side effects and high cost. Thus, there is a need for safer and less expensive therapeutic products for RA. Based on the knowledge gained from traditional systems of medicine, plants offer a vast resource of anti-inflammatory compounds. Using the rat adjuvant-induced arthritis model of RA, we examined the anti-arthritis activity of extracts of traditional Chinese medicine (TCM) herbs and their bioactives. We tested *Celastrus aculeatus* Merr. (*Celastrus*) extract, Celastrol, and a multi-herbal formula Huo-luo-xiao-ling dan (HLXL). We elaborated their immunological and biochemical mechanisms of action. These products were administered to arthritic Lewis rats by daily beginning at the onset of arthritis. This treatment resulted in reduced clinical severity of arthritis; a decrease in pro-inflammatory cytokines and chemokines coupled with an increase in anti-inflammatory cytokines; as well as reduction in nitric oxide production and matrix metalloproteinase activity. Using celastrol, we further elaborated an altered balance of pathogenic T helper 17 (Th17) and protective T regulatory (Treg) cells, and changes in the levels of transcription factors associated with these cells. We believe that the above results would help enhance the confidence of the professionals as well as the public in the use of these plant products for arthritis therapy. However, further testing in controlled clinical studies in RA patients would be required to gain approval for their use as an alternative/ adjunct to mainstream drugs.

### **O32. Evaluation of Antiplasmodial and Antileishmanial Herbal Medicine *Pseudelephantopus spiralis* (Less.) Cronquist and Isolated Hirsutinolide-Type Sesquiterpenoids**

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*Pseudelephantopus spiralis* (Less.) Cronquist is distributed in the Caribbean, Mesoamerica and Latin America. Preparations of the plant are traditionally used in Latin America for the treatment of various diseases including fever, malaria, and spleen or liver inflammations. Extracts of the aerial parts of *P. spiralis* were prepared and bio-guided assay led to the isolation and identification of seven hirsutinolide-type sesquiterpenes lactones. The extracts and pure compounds were tested for their *in vitro* antiprotozoal activities against two parasite species: *Plasmodium falciparum* and *Leishmania infantum*. The samples were also assessed for their cytotoxicity on VERO cells and macrophages. Aqueous extracts showed IC<sub>50</sub> on *P. falciparum* of 3.0 µg/mL and on *L. infantum* of 13.4 µg/mL and found to be cytotoxic to VERO cells (CC<sub>50</sub><3 µg/mL). Two sesquiterpene lactones showed activity against both parasites with IC<sub>50</sub> < 10 µM but failed in selectivity.

### **O33. Oregano (*Origanum vulgare*) Extract Improves Intestinal Mucosa Development in Uoung Individuals**

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Traditional use of oregano tea to manage gastrointestinal disorders motivated us to evaluate the effect of a constant low amount oregano essential oil (OEO) intake on digestive function development. In order to work under controlled conditions we applied an animal model with 128 male day-old chicks allotted into 16 experimental cages, 8 each. The chicks received one of the following treatments per cage until day 14: T1, a commercial diet (control group); T2, the same diet plus OEO (25 grams per metric tonne of feed). The OEO was extracted by steam distillation, characterized as having carvacrol as the main secondary metabolite, and adsorbed onto a mineral carrier. On day 14 one chick per cage was taken and humanely sacrificed to take duodenum, jejunum and ileum samples and fixed them on buffered formaldehyde. Samples were processed and stained with hematoxylin eosin and the morphometric characteristics of each intestinal section were determined. All the data were analyzed under a Randomized Complete Block Design with 2 treatments and 8 replications, having intestinal sections as the blocking factor, and the GLM procedure of SAS9.2 software was applied. The results showed a faster growth of intestinal villus (P=0.0066) without changes in crypt length (P=0.9448) and width (P=0.5725), villus width (P=0.8092) nor lamina propria thickness (P=0.4483), indicating absence of any inflammatory process due to the OEO intake. OEO chicks showed a higher goblet cell number per villi (P=0.0009) with no change in cell (P=0.3890). OEO chicks showed a more developed mucosa structure, as seen by the area of mucosa per unit of muscularis area (P=0.0410), and the higher enterocyte area density (P=0.0262). OEO fed chicks exhibited a higher ability to transform feed into body weight (P<0.05). These findings indicate a positive effect on intestinal development due to OEO intake.

### **O34. Seabuckthorn (*Hippophae* spp) for Medicine and Health Food**

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The high altitude regions of the world are characterized by extreme cold and arid climatic conditions with temperatures of -40°C in winter and +35°C in summer, low atmospheric pressure, high wind velocity, long frozen winters, etc. The increase in free radicals production in humans exposed to high altitude environment is a major challenge. Free radicals may be very damaging since these induce oxidative stress, which is considered to play a causative role in deterioration of mental and physical performance of humans. Adequate nutritional support is necessary for humans to maintain high level of physical and mental fitness at high altitudes. Seabuckthorn is a plant species adapted to high altitude cold deserts and is rich in vitamins, omega 3, 6, 7, 9 fatty acids, antioxidants, amino acids, malic acid, tannins, etc. To counteract the oxidative stress and enhance the performance of humans, we have developed a fruit bar using seabuckthorn pulp along with several other ingredients. These bars release energy slowly over a longer period of time, possess thirst quenching properties, and they are easily digestible. Another product, a cloud-stable blended herbal beverage 'seapricot' using seabuckthorn and apricot has also been developed. The 'seapricot' is very rich in antioxidants, unsaturated fatty acids, carbohydrate, proteins, minerals and Vitamins A, B1, B2, B3, B6, B9, B12, 'C' and 'E'. High nutritional profile and substantive medicinal properties have predicated seabuckthorn as a super fruit of high altitude cold desert.

### **O35. Discovering the Mechanism of Action of Stilbenoids on Rotavirus Infected Cells**

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Rotavirus (RV) infections are the leading cause of viral induced severe diarrhea and dehydration resulting in death for infants and young children. Although vaccines are available, their efficacies vary depending on RV strain specificity and the timing of vaccination. Continuing surveillance for emerging RV strains, assessment of vaccine efficacies, and development of cost effective antiviral drugs remain important strategies for the prevention of RV pathology. Our laboratory has shown the stilbenoids, trans-arachidin-1 and -3 (tA-1 and tA-3), significantly decreased RV progeny and RV replication in a human intestinal cell line, HT29.f8. To understand the molecular mechanism(s) of action of the arachidins on RV infections, studies were performed to identify the host genes regulated at eight hours post RV/RV+tA-3 infections using microarray analyses. This provided insight into the regulation of the transcriptome of HT29.f8 cells, and quantitative real-time PCR (qRT-PCR) on RV/RV+tA-1/tA-3 validated the microarray experiments. The data from these studies demonstrated that the intrinsic apoptotic pathway that leads to cell death is activated with an RV infection, and quantitative real time PCR confirmed that the transcripts for caspase 7 and 9 are significantly upregulated. Additionally, transmission electron microscopy (TEM) analyses revealed the gross distribution of the virus particles and the effects on cellular ultrastructures. The results showed a distribution of mature and immature virus particles between 80-115nm. Additionally, the TEM micrographs revealed the structures of the cells treated with RV alone showed signs of apoptosis at 18 hours post infection, whereas the cells treated with RV and the arachidins appeared to have a more normal ultrastructural appearance. This suggests the arachidins have a protective effect on the host cells. Taken

together the data suggests a mechanism of action by the arachidins that interferes with viral replication, and supports the development of tA-1 and -3 as antiviral agents.

### **O36. Pharmacokinetics of Phytochemicals: Implications for Clinical Trial Design**

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Resveratrol is widely promoted as a potential anti-oxidant, anti-inflammatory, and cancer chemopreventive agent, however, a significant shortcoming exists in determining the optimal dose in human clinical trials. The general consensus has been that 'more is better' in regard to all phytochemicals in preclinical and clinical models. As a result doses are selected that can represent 10x to 100x or even more what would be achieved in the human diet. For example, estimates of resveratrol from food sources could range from 1 to 10 mg per day, however, clinical trials have been designed administering 5,000 mg per day. To challenge the assumption that 'more is better' the results of a clinical trial over an 84 day period will be discussed in light of the known research related to the pharmacokinetic studies of resveratrol. This research will be compared and contrasted to previous research performed with resveratrol to begin the conversation about how best to choose a dose for human clinical trials. In light of these findings, it would appear reasonable that a new strategy is needed for the development of diet-derived agents for human disease.

### **O37. Effects of D-004, a Lipid Extract from Cuban Royal Palm (*Roystonea regia*) Fruits, on Prostate Hyperplasia and Urodynamic Changes in Experimentally Induced Rats**

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Benign prostate hyperplasia (BPH) is the non-malignant and uncontrolled growth of the prostate gland that lead to lower urinary tract symptoms (LUTS) in men over 50 years, which affect the quality of life. The conventional treatment for treating BPH includes mainly 5 $\alpha$ -reductase inhibitors and  $\alpha$ 1-adrenoceptors antagonists, but both of them presenting frequent adverse events. Phytotherapy constitutes an alternative to treat BPH, mainly the lipid extracts of the fruits of Saw Palmetto, a palm of the Arecaceae family that contain fatty acids, mainly oleic, lauric, mirystic and palmitic, acids. D-004 is a lipid extract of the fruits of the Cuban palm (*Roystonea regia* - Arecaceae), containing a reproducible mixture of fatty acids, mainly oleic, palmitic, lauric, linoleic, and miristic acids. This study investigated the effects of D-004 on prostate hyperplasia (PH) induced with Testosterona (T) in rats during 15 days and 60 days, respectively, as well as its effects on urodynamic changes (UC) induced by phenylephrine (PHE) in rats. Also, the combined therapies D-004 + finasteride and D-004 + tamsulosin were evaluated on T-induced PH and on PHE-induced UC, respectively, in rats. D-004 (100, 200 and 400 mg/kg) orally administered for 15 days significantly and dose-dependently prevented prostate weigh increase induced by T in rats, while D-004 (200, 400 and 800 mg/kg) orally given for 60 days significantly decreased prostate enlargement in rats with T-induced PH. Combined therapy with D-004 and finasteride, orally administered for 15 days, induced additional benefits in preventing T-induced PH compared with each mono-therapy. Single oral doses of D-004 (400 and 800 mg/kg) significantly prevented the PHE-induced UC in rats inhibiting the micturition volume reduction. The combined therapy with D-004 and tamsulosin significantly inhibited the PHE-induced UC counteracting the micturition volume reduction more effectively than those obtained by the respective mono-therapies.

### **O38. Characterization of the Mechanism of Action of D-004, a Lipid Extract of *Roystonea regia* Fruits, in Benign Prostatic Hyperplasia**

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Benign prostate hyperplasia, a common disease in men over 50 years old, is characterized by the uncontrolled growth of the prostate gland and the occurrence of lower urinary tract symptoms. Oxidative stress has been recently linked with the etiology of benign prostate hyperplasia. D-004, a lipid extract of *Roystonea regia* fruits, has been shown to reduce prostate hyperplasia induced by testosterone in rodents. In order to evaluate mechanism involved in such effects, in vitro studies were conducted. D-004 produced a competitive marked inhibition of 5 $\alpha$ - reductase prostatic enzyme activity without affecting the binding of <sup>3</sup>H- dihydrotestosterone (DHT) to their prostatic receptors. In addition, D-004 antagonized, in a non-competitively mode, the contractile phenylephrine induced response through  $\alpha$ 1 adrenoreceptors (ADR- $\alpha$ 1). On the other hand, D-004 inhibited 5 – lipoxygenase and cyclooxygenase enzymes activities in a non competitive mode and reduced lipid peroxidation and protein oxidation in various tissues, through a OH\* radical scavenger effect. D-004 stimulated endogenous antioxidant system by increasing total antioxidant status of plasma and catalase enzyme activity. Summarizing, D-004 had effects on the static and dynamic components of Benign Prostate Hyperplasia/Lower Urinary Tract Symptoms entity with pleiotropic effects (anti-inflammatory and antioxidant) that can provide additional benefits on prostate tissue.

### **O39. Bioproduction and Anti-inflammatory Activity of Delta-Tocotrienol-Enriched Extracts from Hairy Roots of *Bixa orellana***

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*Bixa orellana*, known as achiote or annatto, is a plant native to South America that has been used as a traditional medicine to treat multiple diseases. The highest natural level of delta tocotrienol, one of the eight E-vitamins, has been found in this species. Recent studies have shown distinct bioactivities for delta-tocotrienol, including anticancer and neuroprotective properties, highlighting its importance in human health. Hairy root cultures of *B. orellana* were established to study the bioactive compounds in these hairy roots and their capacity to produce delta tocotrienol. The hairy roots were cultured in liquid medium under either continuous light or dark conditions for 30 days. Root tissue and culture medium extracts were analyzed for tocotrienols using high performance liquid chromatography. Our results indicate that hairy roots of *B. orellana* produce mainly delta-tocotrienol and trace amounts of gamma-tocotrienol. The anti-inflammatory capacity of the hairy root extracts was assessed using an interleukin-6 assay with adipocytes derived from 3T3-L1 cells. The assays showed that the extracts exhibit anti-inflammatory properties. We propose that hairy root cultures of *B. orellana* are ideal bioproduction systems to study the bioactive compounds of this medicinal plant.

### **O40. Sacha Inchi (*Plukenetia volubilis* Linneo) Oil and Its Effects on Growth and Lipid Profile in Rats**

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The oleaginous content of amazonic seeds from “sacha inchi” (*Plukenetia volubilis* Linneo) plant is a food product with apparent cardio protective properties due to its high omega-3 fatty acid composition. With the purpose of finding the effect of sacha inchi oil in Holtzman rats, isocaloric diets were prepared for forty-five twenty-one-day-old male rats distributed into six groups, in which extra- virgin sacha inchi oil was provided in different proportions and completed with palm shortening as part of the fat composition (17g%) for 30 days: 100% (“A” group, n=8), 0 % (“B” group, n=8), 5% (“C” group, n=8), 50% (“D” group, n=8) , 95% (“E” group, n=8); and 100% of kitchen oil (“Control” group, n=5). Body weight and intake were continually measured and, on day 30th, lipid profile was analyzed. Afterwards, no significant effects on growth or intake were observed. Seric lipids were not statistically different between groups ( $p>0,05$ ); however, total cholesterol and high-density-lipoprotein values (B group:  $93,5 \pm 28,6$ ;  $55,2 \pm 10,6$ ; C:  $86,65 \pm 20,2$ ;  $50,2 \pm 10,5$ ; D:  $80,3 \pm 16,6$ ;  $45,03 \pm 10,2$ ; E:  $74,2 \pm 22,0$ ;  $39,2 \pm 8,3$ ; A:  $68,6 \pm 21,5$ ;  $40,8 \pm 6,7$ ) have diminished in accordance with increasing dietary proportions of sacha inchi oil. Conclusion: Sacha inchi oil has had non-significant developmental and hypocholesterolemic effects in rats, in this experiment.

#### **O41. Extraction of Natural Analgesic Compounds from Plants used Traditionally in Mali: Study of *Pericopsis laxiflora***

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Nowdays, the urge to find new analgesics with less side effects is increasing and plants have always been a source of inspiration for new drugs. In Mali, plants are often used as part of traditional medicine. Traditional healers are taught by their parents how to treat people with plants from surroundings. Therefore, an ethnopharmacological study was conducted on plants used to treat pain in south of Mali. 113 traditional healers were interviewed and 120 plants were recorded. Among them, two plants were most often cited: *Cassia sieberiana* L., mentioned 21 times (roots) and *Pericopsis laxiflora* (Benth) Meeuwen, mentioned 11 times (leaves). Aqueous and methanolic extracts of both plants were screened, with an acid acetic-writhing test on mice, showing a moderate anti-nociceptive activity at 300 mg/kg. Both methanolic and aqueous extracts of *Pericopsis laxiflora* (Benth) Meeuwen displayed significant activities (respectively 51% and 42% of inhibition of abdominal cramps) while the methanolic extract of roots of *Cassia sieberiana* L. induced only 23% of inhibition. Further partitioning with solvents of increasing polarity and column chromatography were conducted. Results led us to purify fractions to isolate active molecules.

#### **O42. An Ancient Seed Revisited: Composition of Pine Nuts (*Pinus pinea* L.) Grown in Chile, Argentina, Turkey, Israel, Italy and Spain**

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Stone pine (*Pinus pinea* L.) is the largest producer of commercial pine nut. It is endemic to the Mediterranean Basin, where pine nuts are part of the Mediterranean diet. In the last decade the demand for pine nuts has raised worldwide due to the growing evidence of their association to a wide range of health benefits. The composition of pine nuts varies among species, depending on geographical and climatic conditions. The aim of the study was to analyze the chemical composition of pine nuts harvested from *P.*

*pinia* L. plantations in Chile (3 zones) Argentina (2 zones), Turkey (4 zones), Israel (3 zones), Spain (1 zone), and Italy (3 zones). Moisture, ashes, protein, lipids, and dietary fiber were determined in all the samples, while vitamin C, phytosterols, tocopherols, phenolic contents (PC) and antioxidant capacity (ORAC) were measured in the Chilean samples. The composition of all pine nuts was nutritionally similar. The predominant component was fat (means of 34.7 to 45.19 g/100 g), followed by protein (means of 32.14 to 36.85 g/100 g). Dietary fiber averaged 12.53 g/100 g, while ashes averaged 4.42 g/100 g. In Chilean nuts, PC varied from 0.27+0.03 to 0.39+0.01 mg GAE/g, vitamin C ranged from 2.37+0.10 to 2.84+0.16 mg/100 g and ORAC ranged from 8.44+0.06 to 8.60+0.07  $\mu$ mol TE/g. The predominant phytosterol was  $\beta$ -sitosterol (1576.55+1.34 to 1948.81+4.63  $\mu$ g/100 g oil), and the main vitamin E was  $\gamma$ -tocopherol (74.70+3.43 to 133.02+3.41  $\mu$ g/100 g oil). Polyunsaturated fatty acids were prevalent (44.85 to 47.94%, leading linoleic), followed by monounsaturated (36.29 to 39.71%, mainly oleic). Funded by FONDEF D11I1134 and CIDAF CID 04/06.

#### **O43. Using Headspace Gas Chromatography for the Determination of Volatile Components and Sensory Profiles in Medicinally Active Plants**

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Essential oils are receiving increased attention due to their various effects on the human central nervous system. The challenge is to find proper methods to analyze essential oils particularly those volatiles emanating from various sources and then captured by the human nose. Static head space chromatography linked to modern mass spectrometers provides very sensitive means of extracting and analyzing volatiles components usually found in low concentrations. The objective of the presentation is to provide examples on the use of Headspace Gas Chromatography for the characterization of volatile components to find new uses and applications of natural plant products. Essential oils (*Lavandin*, *Lavandula x intermedia*), and aromatic plant species (e.g. *Xylopia aethiopica*, *Piper guinense*) were used in this study. A Shimadzu static headspace system linked to a gas chromatograph/Mass Spectrometer (HS/GC/MS, GC2010 Plus/TQ8040) was used in this study. In one of the examples, lavandin essential oil was used to set up aroma evaluation protocols to assess the chemistry of volatile components sensed by the human nose. In spices such as basil and Liberian Non-timber Forest Products, HS/GC/MS was also used to evaluate the chemical diversity of essential oils in small amount of samples in spices and aromatic plants. HS can be used as a fast way to extract and analyze essential oils as compared to more traditional and time consuming extraction techniques such as hydro-distillation. HS was found to be suitable for the analysis of essential oils, particularly to link aroma perception with chemistry of volatile components. HS/GC/MS is a powerful technique to evaluate chemical diversity of plant derived volatiles and to conduct chemosensory studies in the area of essential oil research.



## POSTER PRESENTATIONS

### **P1. Pterostilbene Combined with Epigenetic Agent Possesses Synergy and Attenuation Effect in Prostate Cancer *In Vivo***

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In our previous studies we found that MTA1 expression is significantly increased in prostate-specific Pten-null model, and that potent natural analog of resveratrol, pterostilbene (PTER), exerts its anti-tumorigenic effects by blocking metastasis-associated protein 1 (MTA1)-mediated inactivation (deacetylation) of tumor suppressors. In the current study, we utilized the same mouse model to evaluate the MTA1/HDAC mediated anti-cancer efficacy of combination treatment of PTER and clinically approved HDAC inhibitor, SAHA. We collected 30 prostate-specific luciferase expressing *Pten* knockout (*Pten*<sup>ff</sup>; Rosa26<sup>Luc/+</sup>; Pb-Cre4) male mice and randomized them into four groups: Vehicle control (10% DMSO); PTER (10 mg/kg bw) alone, SAHA (50 mg/kg bw) alone, and PTER + SAHA. Compounds were injected daily, i.p., starting at 8 weeks of age. Mice were sacrificed at week 18. Histopathological (H&E, SMA), immunohistochemical (Ki-67, cleaved caspase-3, CD31) and molecular evaluation (MTA1, p21, p27, Ac-H3, HIF-1 $\alpha$ ) of prostate tissues showed better beneficial effects of combination treatment compared to each agent alone.

### **P2. Antiproliferative Activity of Pectin Extracts Recovered From Banana Peels**

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The banana fruit peels comprise a significant quantity of waste, about 30% of the fruit, and represent an environmental problem due to their large nitrogen and phosphorus contents, as well as their high water content. There is evidence that peels would be a source of high value compounds such as pectins, complex mixtures of polysaccharides containing units of galacturonic acid as the main chain. Nowadays the pectic substances have attracted much interest for their potential use as anticancer agents. In this investigation two pectic extracts obtained from lyophilized banana peel recovered at different temperature and stirring conditions were evaluated. The biological activity of the extracts was performed by a proliferation assay based on the reduction of the resazurin molecule by viable cells. A cancer breast cell line (MCF-7) and a medulloblastoma cell line (SH-SY5Y) were used. The two evaluated extracts showed antiproliferative activity on both cell lines. The average inhibition was for extract I, 24% for the MCF-7 cells and 48% on the SYHY5Y cells and extract II had a 39% of inhibition on the MCF-7 cells and 28% on the SYHY5Y cells. The results suggest that it is possible to develop a commercial process to obtain pectins with antiproliferative activity from banana peel as potential agent chemoprevention.

### **P3. Morin Effects on Airways Defence Reflexes and Allergic Inflammation in the Setting of Experimentally Induced Allergic Asthma**

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**Background:** The aim of experiments was to investigate the effect of a plant polyphenol, 3-hydroxyflavone derivate morin on bronchial hyperreactivity, the cough reflex, ciliary beat frequency (CBF) and inflammation in the setting of experimentally induced allergic asthma.

**Methods:** Using an experimental model of allergic asthma, we evaluated the morin anti-asthmatic potential either after acute administration or long-term (21 days) treatment of OVA sensitized guinea pigs. In light of this fact, we measured the following parameters: the *in vitro* tracheal smooth muscle contraction induced by histamine and CBF, the specific airway resistance (sRaw) to histamine and the sensitivity of a chemically induced cough reflex via an *in vivo* methods; the concentrations of the inflammatory cytokines interleukin IL-4, IL-5, IL-13 in serum and bronchoalveolar lavage fluid (BALF).

**Results:** Acute morin (30 mg p.o.) administration had a comparable antitussive efficiency with codeine, but did not elicit a significant decline in sRaw parameters. Its acute bronchodilatory efficiency did not reach the effect of beta2 agonist salbutamol. Long-term administration of morin (30mg/kg/day) resulted in significant cough suppression, which was by 20% higher in comparison with that of codeine. The long-term sRaw measurements demonstrated that morin was a more potent bronchodilator compared with long-acting beta2 agonist salmeterol. The 21 days treatment of OVA sensitized guinea pigs with morin reduced the levels of IL-4 and IL-13 in the serum and in BALF. This efficiency was comparable with the effect of reference drug, anti-inflammatory acting glucocorticoid budesonide. Long-term morin treatment did not influence CBF values in sensitized experimental animals.

**Conclusion:** We can summarize that long-term morin administration revealed significant antitussive, bronchodilatory and anti-inflammatory effects in the setting of experimentally induced allergic asthma.

**Acknowledgments:** This work was supported by: Slovak Research and Development Agency under the contract No. APVV-0305-12, "Biomedical Centre Martin" ITMS: 26220220187, Grant VEGA 1/0165/14.

### **P5. Rooting Matrix, Tissue Culture Vessels and Acclimatization Treatments Yielded Larger, Faster Growing *Echinacea* Plants**

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*Echinacea purpurea* is a North American native medicinal plant known as purple coneflower. In vitro clones of *Sombrero*® 'Salsa Red' were pre-hardened during Stage III shoot and root development in a modified MS medium gelled with agar, liquid-infused OASIS® IVE growing medium, or agar-infused OASIS® IVE growing medium in vented rigid polycarbonate boxes (OASIS® smart box RV750), or flexible film vessels (OASIS® FFV). After ten days of culture, ventilation was provided to all plantlets in vitro, and after 5 weeks plantlets in flexible film vessels spent 1 week in a greenhouse mist bed in sunlight, while rigid box treatments remained in the laboratory. After 6 weeks in treatment conditions, all plants were potted in peat-based mix (Farfard 3-b) or OASIS® foam root wedges and placed on greenhouse mist bed for two weeks, and then moved to greenhouse nursery for two weeks, and hand-watered as necessary. Plants in all treatments rooted well in vitro (97-100%, with 95% confidence) and the plants from agar were largest and had the most

leaves. During 4 weeks in the greenhouse in peat-based medium, plantlets originally rooted in OASIS® IVE foam grew faster and were larger than the plants from agar. The most leaves were on sunlight acclimatized plants from OASIS® IVE in flexible film vessels planted in peat-based mix, then OASIS® IVE in peat-based mix were better than OASIS® IVE in root wedges, which were better than agar in peat-based mix. The largest plants were from OASIS® IVE in peat-based mix, being larger than OASIS® IVE in root wedges. Plant quality was lowest from agar, and plantlets rooted in OASIS® IVE were better when planted in peat-based mix than root wedges. The OASIS® IVE foam in flexible-film vessels, produced superior quality, when pre-hardened in sunlight and roots were not disturbed during transfer.

## **P6. The Potential Biological Activity of Commercial Samples of Neem (*Azadirachta indica*) Extracts**

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Neem tree (*Azadirachta indica* - Meliaceae), a native plant from Southeast Asia, has been used for thousands of years as traditional medicine in India because of its versatility. It is used to alleviate different medical conditions, including malaria, bacterial and worm infections, and various skin problems. These diverse medicinal properties of neem are associated with the unique chemical composition of its different organs. The objective of this work is to investigate the nematicidal, anti-yeast, antioxidant, and anti-cancer activities of different commercial samples of neem tree.

The nematicidal activity of plant extracts was tested using the free living nematode *Panagrellus redivivus* and anti-yeast activity was assessed using an MTT-method. Total phenolics were quantified using the Folin-Ciocalteu method, and antioxidant activity was quantified using the ABTS radical scavenging assay. The potential anti-cancer cytotoxicity of neem extracts was assessed in WI 38 VA13 human transformed fibroblasts by Trypan Blue Exclusion Assay.

The extracts did not exhibit nematicidal activity against *P. redivivus* and no anti-yeast activity based on these results. Leaves and bark extracts that exhibited high total phenolic and flavonoids content were associated with high antioxidant capacity. Human fibroblasts responded differently to the multiple extracts analyzed at various concentrations and incubation time periods. The results suggest that the high survival rate of *P. redivivus* could be related to the high antioxidant activity that is contributing to the extended lifespan of the nematodes. This research is part of educational experiences in an urban community college setting, enabling students to acquire the critical thinking and research skills necessary to pursue a baccalaureate degree in a science-related discipline.

## **P7. Chemical Composition of Essential Oils from the Pods of Liberian *Xylopiya aethiopica***

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Country spice or Ethiopian pepper (*Xylopiya aethiopica*) is an aromatic evergreen tree native to tropical West Africa. It is mainly used for anti-inflammatory and anti-microbial purposes as well as for cosmetic and culinary purposes. The essential oil chemical composition of this Liberian plant varies between the different anatomical structures which include the seeds, capsules, and entire fruit overall. Through oil distillation and gas chromatography/mass spectrometry, compounds such as  $\alpha$ -pinene,  $\beta$ -pinene, and 1,8 cineole have

been found in different proportions in each parts of the fruit. The objectives of this study were to (i) use static-headspace (HS) GC/MS to compare the chemical composition of essential oils within each part of the fruit, the dried pod (ii) and determine the additional uses of the pods in developing new products. The HS/GC/MS results indicated differences in the chemical volatile compositions in different parts of the pods. The whole intact fruits (pods) emitted volatiles characterized by high levels of  $\alpha$ - and  $\beta$ - pinenes and low levels of 1,8 cineole. The breakdown of the pods in capsules and seeds, revealed that ground capsules showed a similar composition dominated by  $\alpha$ - and  $\beta$ - pinenes and lower levels of 1,8 cineole. The ground seeds were characterized by lower levels of  $\alpha$ -pinene and higher of 1,8 cineole. The volatiles emitted by the whole intact seeds were characterized by high levels of  $\beta$ -pinene and 1,8 cineole. This study provides the potential uses for the various parts of *Xylopiya aethiopicica* based on its potency of aromatic components in its essential oils.

### **P10. *Echinacea purpurea*: Chemical View and Anti-Asthmatic Profile**

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**Background:** Increased interest is observed in new active natural drugs without undesired side effects which are observed at conventional medications. *Echinacea purpurea* has a long history in traditional medicine. Recent trends are focused on its immune-modulatory effects, particularly prevention and treatment of airways diseases.

**Objective:** Verification of therapeutic potency of *E. glycoconjugate* (EC) in induced animal asthma model and comparison of its effect with those of classic bronchodilators, anti-inflammatory and anti-asthmatic drugs.

**Methods:** Alkaline extract of *Echinacea* flowers was neutralized, subsequently extracted with organic solvents, dialyzed and freeze-dried to give the EC. Effect of EC on airways smooth muscle reactivity was studied by *in vivo* and *in vitro* methods (1, 2) with complementary ciliary beating frequency measurement. Anti-inflammatory effect of EC was determined by cytokines levels assessment in plasma and BALF and exhaled NO levels.

**Results:** *Echinacea* conjugate, composed of phenolics, carbohydrates and protein, has molecular mass 10 000. It has a *strong bronchodilator effect* on airways reactivity after mediated contraction of airways, especially at low concentration of histamine (*in vitro*). This effect was confirmed also by changes in basal specific airway resistance measurement (*in vivo*). Measurements of ciliary beat frequency did not show negative impact of EC on mucociliary clearance. EC has reduced exhaled NO levels as well as cytokines levels in plasma and BALF. These facts confirmed its *anti-inflammatory activity*.

**Conclusion:** Our results are important in the context of *Asthma inflammation* because *Echinacea* conjugate has shown both, bronchodilator and anti-inflammatory effects (very efficient in local pulmonary inflammation).

**Acknowledgement:** Study was supported by the EU projects CEKR, CEKR II, BioMed, grant VEGA 1/0165/14, and APVV-0305-12.

### **P11. Evaluation of Oregano (*Origanum vulgare*) Essential Oil on Intestinal Coccidiosis by an Approximation with an Avian Infection Model**

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Some genera within coccidia subclass, such as Isospora, Cryptosporidium, and Eimeria, produce damages to intestinal health in humans and animals. Based on previous studies showing the ability of phenolic compounds to destroy oocysts, we evaluated the effect of oregano essential oil (OEO) on intestinal coccidiosis by applying an avian model. 128 male day old broiler chicks allotted into 16 experimental cages, with 8 chicks each, received one of the following treatments per cage until day 21: T1, standard control diet; T2, standard diet plus 25 ppm OEO. The OEO was extracted by steam distillation and fixed to an inorganic powdered matrix. On day 14 all birds received an oral *Eimeria spp.* mixed inoculum with  $\geq 24 \times 10^5$  non-attenuated oocysts. A Completely Randomized Design with 2 treatments and 8 replications was used. Data were analyzed by Wilcoxon Kruskal-Wallis test with the SAS9.2 software. The applied infection model produced, at day 21, a sub-clinical intestinal coccidiosis being *E. acervulina* the main specie found with clear histological damage evidence. Even though both treatments showed the same frequency of characteristic macroscopic intestinal lesions of *E. acervulina* and *E. maxima* ( $P > 0.05$ ), microscopic evaluations by intestinal frosts showed significant fewer oocysts ( $P < 0.04$ ) and even a percentage of macroscopic positive chicks without any coccidian forms four times greater in the chicks fed the OEO than in the control group ( $P < 0.04$ ). The microscopic evaluation also showed a percentage of chicks positive to coccidia five times greater in the control group than the OEO one ( $P < 0.05$ ). OEO chicks gain 10% more body mass than control chicks but feed intake was not affected; indicating a higher efficiency to transform feed into body mass. These results support the use of OEO in the treatment of intestinal coccidiosis.

### **P12. Milk Thistle (*Silybum marianum*) Extract Anti-Hepatotoxic Application: A Poultry Clinical Intervention**

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Milk Thistle extract has shown applications to support liver health and toxic processes recovery in human medicine. In order to test these attributes for animal production, a local broiler chicken production company with frequent occurrence of hepatotoxic processes was identified and 12 commercial flocks with more than 10,000 chicken each were subjected to a completely randomized experimental design with 2 treatments and 6 replications each, as follows: T1, standard diet; T2, same diet but supplemented with Milk Thistle extract as a feed additive prepared to provide 30 to 40 grams of the extract per metric tonne of feed. All the flocks were naturally subjected to hepatotoxic challenge. During an intervention time of 2 weeks the performance was controlled to identify any change in behavior of flocks. By the end of the second week all naturally dead birds were necropsied to evaluate the presence and severity of liver lesions. All the data were analyzed and the GLM procedure of SAS9.2 was applied. The results showed that in the second week the number of dead birds in T2 dropped to half the number in T1 ( $P = 0.0073$ ). The severity of the lesions found in dead birds from T2 reduced to one third the severity found in the control group ( $P = 0.0001$ ). Also, the number of birds affected by liver lesions reduced from 78 to 29 per flock ( $P = 0.0004$ ) due to the extract fed to T2. The flocks on T1 experienced a reduction in the rate of live weight gain due to the hepatic damage; however under the same challenge, the T2 flocks not only did not reduced their growth rate ( $P = 0.0601$ ) but also showed a higher feed efficiency ( $P = 0.0298$ ) agreeing with the better liver health found in T2 and the important role of the liver in nutritional metabolism.

### **P13. Effect of Oregano (*Origanum vulgare*) Extract on Clinical Enteritis: A *Clostridium perfringens* Avian Model**

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Some species of *Clostridium sp.* such as *C. difficile* and *C. perfringens* can cause intense acute enteritis with diarrhea. Based on the antibacterial effect previously reported for oregano and our previous research with oregano extracts on poultry, an experiment was carried out applying an avian model with *C. perfringens* challenge to evaluate the effect of oregano essential oil (OEO) on clinical enteritis with diarrhea. 192 day old broiler chicks allotted into 24 experimental cages, with 8 chicks each, received one of the following treatments per cage until day 28: T1, control unchallenged chicks; T2, challenged chicks without treatment; and T3, challenged chicks fed 25 ppm OEO. The OEO was extracted by steam distillation and fixed to an inorganic powdered matrix. At days 18, 19 and 20 T2 and T3 chicks received an oral inoculum with  $10^8$  UFC *C. perfringens*. In order to facilitate the invasion of *C. perfringens*, on day 14 T2 and T3 chicks received an inoculum of *Eimeria spp.* with  $\geq 24 \times 10^5$  oocysts. In both cases, T1 chicks received a placebo. A Completely Randomized Design with 3 treatments and 8 replications was used. Data were analyzed with GLM procedure of SAS 9.2 software. The applied infection model produced, at day 28, a clinical picture with 7% feces with desquamated mucosa, 76% watery feces, 55% of the feces containing undigested feed and hyperplasia of lymphoid organs (bursa of Fabricius and spleen;  $P < 0.05$ ). Feeding OEO restore the bursa size and produced the lowest prevalence of feces with undigested feed or desquamated mucosa, and watery feces among the three experimental groups. These results agree with the antimicrobial action of OEO but also support a positive clinical effect.

### **P14. Mechanism of Action of Oregano (*Origanum vulgare*): Integrating Findings on Avian Models**

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Oregano has shown many interesting active properties for human and animal health, so our team has been working in the recent years to have a wider and deeper understanding of its mechanisms of action and how to take advantage of them. The aim of this study was to conduct an analysis with the information collected so far on the action of oregano essential oil (OEO), integrating its mechanisms of action, to identify key attributes for animal feeding. We used the data and findings not only from the previous experiments conducted by our company, but also key findings from other research teams. An integrative model to explain the whole picture of the biological activity of OEO is also proposed. It was found consistent responses on antimicrobial activity, intestinal mucosa health, antioxidant status, and enzyme activity promotion. Underlying gene expression processes were also found. These mechanisms together counteract with a whole effect towards intestinal development and health, reducing nutritional maintenance expenditure and, finally, efficiency of feed utilization. Based on the interactions found, it is concluded that OEO can be used in different ways to support nutritional efficiency and health, depending on actual conditions. Furthermore, some applied forms of use are proposed.

### **P15. Essential Oil Extraction Methods and Inheritance of Major Volatiles in Sweet Basil (*Ocimum basilicum*)**

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The aroma and sensory attributes of sweet basil are impacted by the relative proportions of volatile terpenes and phenylpropanoids present in essential oils. Numerous candidate genes have been identified along the Mevalonic and Shikimic acid biosynthetic pathways, however, little research has focused on the genes associated with the inheritance of these components in segregating populations. This can be attributed in part to the difficulty of accurate, high throughput volatile profiling or phenotyping of plant populations. Volatile extraction methods can drastically affect GC/MS data output and limit throughput and as such headspace analysis and closer connections from fresh to human sensory responses needed as well.

The objectives of this study were to (i) compare hydrodistillation, and static-headspace of methyl chavicol chemotype (MRI) and linalool chemotype (SB22) lines; and (ii) use static-headspace (HS) GC/MS to evaluate inheritance of major basil volatiles in the MRI x SB22 F2 population.

The HS/GC/MS results demonstrated that volatile profiles of MRI and SB22 parent lines were less dominated by the major components methyl chavicol and linalool, respectively, when compared to hydrodistillation. Levels of the main components were lower in HS when compared to hydrodistillation. An F2 population was generated from the MRI x SB22 cross, which demonstrated expected segregation for 1,8 cineole, linalool and methyl chavicol, allowing for analyses of frequency distribution for each component. Although hydrodistillation is considered to be the standard-bearer for essential oil extraction, this study suggests that headspace may be a more accurate representation of human sensory experience and therefore a more appropriate method of volatile phenotyping for inheritance and ultimately QTL analyses. This study provides a framework by which to evaluate segregating basil populations for genetic analyses.

### **P16. Effects of D-004, A Lipid Extract of *Roystonea regia* Fruits, on Men with Benign Prostatic Hyperplasia**

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This work includes two clinical trials conducted in order to compare the effects of D-004, a lipid extract of *Roystonea regia* extract, and *Saw palmetto* extract (SP) in patients with Benign Prostatic Hyperplasia (BPH). In the first phase II study, 61 patients with moderate BPH were double-blindly randomised to D-004 or SP (320 mg/day both) capsules for 4 months. After 2 months on therapy, D-004 and SP decreased mean International Prostate Symptoms Score (IPSS) values versus baseline and this effect increased at study completion, when IPSS decreased 36.0% with D-004 and 34.1% with SP, without differences between groups. Both treatments did not modify prostate size and residual volume post-voiding. These results were confirmed in a second phase III study in 100 eligible patients with mild to moderate BPH. Patients were double-blindly randomised to D-004 or SP (320 mg/day both) capsules for 24 weeks. After 2 months on therapy, D-004 and SP lowered mean IPSS values. This effect increased over the study, IPSS decreasing 70.3% with D-004 and 69.0 % with SP after 6 months on treatment, without differences between groups. Both treatments decreased significantly the residual volume post-voiding as compared to baseline. D-004,

not SP, reduced significantly, but modestly, the prostate size and PSA values versus baseline. No significant between group differences were found. Summarizing D-004 (320 mg/day) was as effective as SP (320 mg/day) for decreasing low urine tract symptoms in men with moderate BPH. Both treatments were well tolerated.

### **P17. Bioproduction and Biosynthesis of Prenylated Stilbenoids in Peanut: Bioactive Compounds for Plant and Human Health**

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Peanut (*Arachis hypogaea*) produces stilbenoids upon exposure to abiotic and biotic stresses. Among these compounds, the prenylated stilbenoids exhibit higher or novel biological activities *in vitro* when compared to their non-prenylated analogs. However, assessment of these bioactivities *in vivo* has been challenging because of their limited availability. Moreover, the biosynthesis of prenylated stilbenoids has not been elucidated. Herein, hairy root cultures of peanut were used to produce prenylated stilbenoids. Co-treatment of peanut hairy roots with elicitors and the metabolic inhibitor clomazone prevented the production of prenylated stilbenoids, suggesting that the prenyl moiety is made via the plastidic isoprenoid pathway. We also characterized the first stilbenoid-specific prenyltransferase from the microsomal fraction of peanut hairy roots. These studies demonstrate the application of hairy root cultures to elucidate the biosynthesis bioactive compounds for plant and human health.

### **P18. A Time Course Analysis of the Effects of Stilbenoids on the Ultrastructure and Viral Populations of Rotavirus-infected Cells**

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Rotavirus (RV) causes severe gastroenteritis in infants and children worldwide. In an effort to prevent the RV pathology, research efforts are focusing on new vaccines and novel therapeutic strategies. To discover the mechanism(s) of action of pathogenesis and host responses to RV infections, our laboratory has demonstrated that trans-arachidin-1 and -3 (t-A1 and t-A3) inhibit RV replication and induce apoptosis/autophagy in a human intestinal cell line, HT29.f8. The objective of this study was to observe the effects of the stilbenoids, t-A1 and t-A3, on the ultrastructure and population diversity in RV-infected cells. A time course study using transmission electron microscopy (TEM) was used to visualize the arachidins effects on cellular ultrastructure and RV particles. Additionally, tunable resistive pulse sensing technology (TRPS) using the qNano system by IZON measured the size distribution of RV particles. The data suggest the arachidins have a significant inhibitory effects on both the apoptotic and autophagic cell death pathways, which appears to effect the maturation of the progeny RV population This implies that t-A1 and t-A3 arrests the development of RV particles at the immature, non-infectious stage of development. Thus, these small molecules have the potential to be development as antiviral therapeutic agents.



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