Natural Health Product Research Society of Canada
In conjunction with the
Canadian Institute of Chinese Medicinal Research

The 11th Annual Conference: From East and West: A Shifting Cultural Landscape of Natural Health Products

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Conference Chair: Paul Shipley
***Scientific Program Committee*:** Siyaram Pandey (Chair), Ed Lui, Emma Guns, Paul Shipley, Stephanie Bennett, John Thor Arnason, Satya Prakash, and Megan Thomas.

WEDNESDAY MORNING - PLENARY

Insights into the Mechanism of Activation of the Cytoprotective Nrf2 Pathway by Phytochemicals

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The Nrf2 transcription factor is emerging as a therapeutic target in the prevention or amelioration of diseases including diabetes, neurodegenerative diseases, autoimmune diseases, arthritis, and cancer. A large number of phytochemicals have been identified over the past 20 years that activate Nrf2, with several showing significant promise in animal disease models. Crucifer-derived sulforaphane has progressed into Phase I and Phase II clinical trials for a variety of conditions including chronic obstructive pulmonary disease and asthma. By binding to the cis-acting regulatory antioxidant response element (ARE), Nrf2 upregulates the expression of hundreds of cytoprotective genes. The proteins they encode participate in antioxidant and anti-inflammatory functions, detoxification, macromolecular repair, and protein degradation, as well as intermediary metabolism. Thus the Nrf2/ARE pathway is a major target of phytochemicals as preventive agents in various diseases. The mechanisms by which phytochemicals activate Nrf2 are still under active investigation. While Nrf2 is regulated by diverse means, an important repressor is the Keap1 protein, to which Nrf2 protein is tightly bound. Keap1 contains a large number of cysteines. A significant number of Nrf2 activators are either electrophilic or are biotransformed to electrophiles, and Keap1 C151 in particular appears to be an important sensor for electrophilic phytochemicals. Various biochemical, biophysical and cell-based studies provide evidence for a model by which covalent modification of C151 by phytochemicals activates Nrf2.

METABOLISM AND CARDIOVASCULAR DISEASE

High-DHA Fish Oil Improves Omega-3 Fatty Acid Status in Red Blood Cells (The Omega-3 Index) and in Whole Blood (The Omega Score) in Adults Taking Statin Medication

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Dietary intake of the omega-3 fatty acids Docosahexaenoic Acid [DHA] and Eicosapentaenoic Acid [EPA] has demonstrated numerous cardiovascular benefits. Red blood cell fatty acid composition has been shown to be reflective of intake of these omega-3 fatty acids. Risk of sudden cardiac death from coronary heart disease can be stratified based on omega-3 levels in

red blood cells [The Omega-3 Index] and also in whole blood [The Omega Score]. Serum fatty acid levels can be quantified to determine both omega-3 [n-3] fatty acid and omega-6 [n-6] fatty acid status. In a randomized, placebo-controlled, double-blind, cross-over study, the effects of a 4-week intervention with high-DHA fish oil on serum fatty acid profiles was examined in 36 adult males and post-menopausal females (aged 60.0 ± 8.9 years, BMI: 29.9 ± 3.3 kg/m2) taking Statin medication. Participants consumed 4 g per day of high-DHA fish oil (four 1050 mg capsules, each containing 620 mg DHA and 150 mg EPA) or placebo for 28 days each, separated by a 4-week washout period. When compared to placebo, treatment with high-DHA fish oil resulted in significant ($P<0.0001$, Tukey-adjusted) increases in serum DHA, serum EPA, omega-3 red blood cell equivalence scores (The Omega-3 Index) and whole blood omega-3 scores (The Omega Score), as well as a significant ($P<0.0001$, Tukey-adjusted) decrease in the n6:n3 ratio. These data demonstrate an improved coronary heart disease risk profile associated with consumption of high-DHA fish oil in adults taking statin medication.

Discovery of a Novel Plant Extract with Anti-obesity Properties

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The overwhelming increase in the prevalence of obesity in recent years represents one of the greatest threats to the health of the developed world. Thus, management of body weight has become one of the top priorities in nutritional and medical research. A series of plant-based extracts were screened for their effects on lipolysis and adipogenesis in vitro using the 3T3-L1 pre-adipocyte cell line. From the in vitro screening, three lead candidate extracts (OHT-33, -83, and -88) were selected to test for their potential anti-obesity effects in vivo. In an eight week trial employing a mouse model of diet-induced obesity, oral administration of low (100 mg/kg BW) and high (500 mg/kg BW) doses of the extracts showed promising anti-obesity effects, without signs of toxicity. A significant ($P < 0.05$) reduction in body weight was evident at week 2 through week 8 for the high-dose OHT-88, with a total reduction of 15.9% compared to the obesity control by the end of the trial. Epididymal fat pad weight was also significantly ($P < 0.05$) reduced by 24.3% in response to the high dose OHT-88 treatment. Additionally, fecal fat excretion was increased significantly ($P < 0.05$) in the high-dose OHT-88 group, compared to the obesity control. The results suggest that OHT-88, an extract from an edible plant, has anti-obesity properties that could be the basis for a natural health product for weight management.

Microalgae: A Potential Source of New Nutraceutical and Functional Food Ingredients

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Microalgae are a diverse group of eukaryotic photosynthetic organisms. They have an extraordinary potential for cultivation as energy crops and are considered as a source of a wide range of interesting products with potential commercial applications. The National Research Council Canada (NRC) launched the Algal Carbon Conversion (ACC) program to develop novel microalgae cultivation and processing technologies with the long-term aim of converting CO2 emissions into renewable fuels and other value-added products. NRC's Natural Health Products (NHP) program is designed to support the pre-clinical product development efforts of Canadian NHP companies. These two programs have enabled NRC to collect, cultured and test a diverse collection of algal strains for potential ingredients for functional foods and nutraceuticals. Here the NRC reports the in vitro anti-inflammatory activity of natural products from a number of microalgal strain. The nitric oxide (NO) inhibitory activity guided purification led to isolation and identification of several active polar lipids including monogalactosylmonoacylglycerols (MGMG),

monogalactosyldiacylglycerols (MGDG), digalactosyldiacylglycerols (DGDG) and betaine lipids, and their structures were elucidated based on spectral analyses. Further study suggested that these polar lipids inhibited the NO production through down-regulation of inducible nitric oxide synthase (iNOS) indicating their possible use as anti-inflammatory agents. HPLC based pigment analysis led to identification of several microalgal species as a potential source of lutein and other pigments currently use in functional food and nutraceutical applications. Moreover, a number of microalgal species were also identified which produce significant quantities of omega-3 fatty acids including eicosapentaenoic acid (EPA).

North American Crataegus as a Cardioprotective Natural Health Product

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The genus Crataegus, known as hawthorn, contains small, berry-producing trees which are found throughout North America, Europe and Asia. Hawthorn natural health products (NHPs) are used to treat heart-related problems such as congestive heart failure, hypertension, angina and arrhythmia. While there is traditional use of hawthorn throughout the northern hemisphere, the natural health product industry has utilized only Eurasian species. This research aimed to determine the viability of two North American species, *C. douglasii* and *C. columbiana*, as ingredients in commercial NHPs by comparing them to two European species prominent medicinally, *C. monogyna* and *C. laevigata*. The differences in the chemical makeup among and within leaf samples from each species were characterized using nuclear magnetic resonance spectroscopy and their antioxidant potential determined using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) based assay. Previous studies have indicated that rutin and its derivatives have the highest cardioprotective activities. We found that the North American species had significantly higher concentrations of rutin and its derivatives. Furthermore, one North American species, *C. chrysocarpa*, demonstrated significantly higher antioxidant capacity than the other species tested. These studies show that the North American species have the potential to be a more effective natural health product than the European species studied.

Anti-obesity Properties of Ursolic Acid

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Ursolic acid (UA) is a pentacyclic triterpene acid and present in many different plants and fruits. It has recently been reported to affect adiposity and obesity. Several studies have shown that UA inhibits pancreatic lipase activity, promotes muscle hypertrophy, and increases brown fat mass, thermogenesis, energy expenditure and lipolysis. However, it is unknown whether and how UA affects adipogenesis that is important in weight management. Therefore, the present study was to determine the effect and mechanism of action of UA on adipogenesis. The 3T3-L1 preadipocytes were differentiated in the presence or absence of UA and then measured for cell proliferation, differentiation, fat accumulation, and the expression of a number of proteins that regulate adipogenic differentiation, fatty acid synthesis and oxidation. The results demonstrated that UA dose-dependently attenuated adipogenesis, decreased the expression of several proteins involved in adipogenic differentiation of preadipocytes. Ursolic acid increased the expression of proteins regulating fat oxidation while decreasing the expression of proteins controlling fat acid synthesis. Further experiments showed that UA increased the phosphorylation of AMP-activated protein kinase (AMPK) and the protein expression of silent mating type information regulation 2, homolog 1 (Sirt1). The anti-adipogenic effect of UA was reversed by the AMPK siRNA, but not by the Sirt1 inhibitor

nicotinamide. Liver kinase B1 (LKB1), the upstream kinase of AMPK, was upregulated by UA. When LKB1 was silenced with siRNA or the inhibitor radicicol, the effect of UA on AMPK action was diminished. The results demonstrate that UA inhibits 3T3-L1 preadipocyte differentiation and adipogenesis through the LKB1/AMPK pathway.

INDUSTRIAL INNOVATIONS IN NATURAL HEALTH PRODUCTS

NHP Quality Analysis and Standardization Research – New Approaches and Technologies

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The Canadian Natural Health Product (NHP) is internationally recognized for its quality and safety and as such quality remains one of the foremost important issues for the industry. Due to the complexity nature of NHPs, quality may vary considerably and industry accepted standards, and industry accepted QC/QA methods require constant improvement and upgrading to maintain Canada's reputation for quality. The Canadian NHP industry is also dealing with product expansion where an increasing number of imported raw materials or functional ingredients are being added to new NHP products thus increases the need for standards and methods. Therefore, new approaches and more integrated technology platforms are becoming more important for assessing NHP quality, including botanical identity, origin, integrity, that will help to counteract challenges with inferior ingredients and/or substitution or adulteration. As an overview of recent advancement in NHP quality analysis, the presentation will highlight some of the new approaches and technologies in literature, including array-based techniques and direct analysis in real time TOF-MS for identification of raw materials, chromatographic and NMR fingerprinting in coupling with chemometrics, quantitative NMR, and bioresponse fingerprinting in product quality control. In addition, NRC will detail its capacity and expertise that enable customized solutions for quality and standardization that will help the Canadian NHP industry remain synonymous with safety and quality.

Breadfruit (Artocarpus altilis) is a Source of High Quality Protein

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Protein deficiency has been observed as a leading cause of malnutrition in the general population as well as child death in the tropics. As a tropical staple, breadfruit shows great potential for supplying high quality protein with sufficient essential amino acids in the developing countries. Current study using HPLC analyzes amino acid contents in 42 breadfruits (*Artocarpus altilis*) and 7 hybrids (*A. altilis* × *A. mariannensis*). Both breadfruits and hybrids contain all of the essential amino acids and are especially rich in phenylalanine, leucine, isoleucine and valine. Significant difference exists between cultivars. Ma'afala protein contains the highest total essential amino acid content compared to protein in other grain and non-grain staples, such as corn, wheat, rice, soybean and potato, etc. Consuming one mature Ma'afala can meet most of the daily requirements for essential amino acids for a preschool child (20kg) or an adult (60 kg). Together these data show that breadfruit cultivars provide protein of higher quality than many plant sources including soy, rice and pea and that Ma'afala flour is a potentially innovative product for manufacture of novel food and health products.

Wasabi as a New Greenhouse Crop for BC and its Potential as a Functional Food and Natural Health Product

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Wasabi or Japanese horseradish (*Wasabia japonica*

[Miquel] Matsu.) is a perennial plant of Japan used as a traditional condiment. Wholesale prices in Japan can be as high as $200/kg for fresh high quality stems and given the demand for more products, could yield an excellent profit for Canadian greenhouse growers. Production is increasing every year with a current production that covers approximately 5 hectares in Canada. Wasabi was traditionally a prized culinary condiment used only in Japan, but is now in vogue in high-end restaurant and sushi bars throughout the world and increasingly served as a value added ingredient to various processed foods. In addition, manufacturers of natural health products are becoming interested in wasabi as a potential novel nutraceutical product. Wasabi belongs to a group of cruciferous vegetables characterized by its high content of glucosinolates secondary metabolites that, upon enzymatic reaction with myrosinase, release bioactive isothiocyanates (ITCs). Allyl-ITC and 6-(methylsulfinyl)hexyl isothiocyanate (6-MITC) are the major ITCs from wasabi, are believed to prevent some forms of cancer by induction of detoxification enzymes such as quinone reductase (QR) and glutathione S-transferase (GST). Air drying and freeze drying methods were evaluated for leaves and stems from three Wasabia japonica cultivars grown in soilless growing system. The phytochemical profiles and ITC contents of the dehydrated products were evaluated. Based on the findings, it was determined that one cultivar met or exceeded the required product specifications for chemical content and stability, as compared to other commercially available materials. The freeze drying method investigated was found to be suitable and will be further studied to support scale up activities and potential commercial launch.

An Introduction to the Green, Clean and Economical Mazza Extraction Technology

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Health conscious consumers are seeking out naturally sourced functional foods and nutraceuticals, especially those that are produced using green technologies that are compatible with organic production and processing systems. This is driving a market demand for nutrients and herbal extracts that is currently estimated at $3.5 billion in North America alone. The Mazza Innovation Ltd. patented Pressurized Low Polarity Water (PLPW) extraction technology is used to create high quality functional ingredients for applications in health products. Applications of the technology enable manufacturers to produce innovative, high quality products that are also competitive in costs savings compared to existing techniques. This presentation will address the value proposition of the Mazza process, especially the high quality products at lower operating costs compared to existing solvent-based technology.

WEDNESDAY AFTERNOON - PLENARY

Healing from Within - Natural Substances Trigger the Endogenous Repair Mechanism

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GINSENG

Reversal of Alopecia in a Mouse Model of Human Alopecia by Daily Dietary Administration of a Proprietary Extract (CVT-E002) of *Panax Quinquefolius* (North American Ginseng)

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Human alopecia is a world-wide affliction affecting approximately 30% of adult males and is frequently observed in post-menopausal females. Decades of study on alopecia have concluded that the condition is the result of an autoimmune attack on the hair follicle growth center (anagen). Current pharmaceutical agents are moderately successful and are beset with significant negative side-effects. In the present pre-clinical study, we describe a side-effect-free agent which has fully reversed alopecia. The C3H strain of mice develops alopecia with age and is the well-established model for human

alopecia. Aging mice (16-18 mo), upon losing all or most of their body hair, were placed on a daily conventional diet (6gm chow/day/mouse) containing CVT-E002 (80mg/day) for the next 32wk (8 mo). These mice began to show body/face hair re-growth after only 2wk with complete re-furring by 25wk. The effect, however, is dose-dependent with 2mg CVT-E002 resulting in only sparse, fine, late-developing hairs. To determine if the presence of CVT-E002 in the daily diet was essential to maintaining the re-grown coats, we removed CVT-E002 from the diet of the re-furred mice after 32wk and converted them to the conventional diet for the next 3-1/2 mo. All such mice had indeed retained their fully re-grown coats. Finally, immune cells in mice fed CVT-E002, were significantly elevated vs control alopecic mice. Thus, CVT-E002 is both an immune cell stimulant in aged mice an a stimulant of the hair growth follicle in alopecia.

Cyto-protective Effects of Extracts of *Panax quinquefolius*.

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Ginseng is known to have anti-oxidative anti-inflammatory properties. In our study we have examined extracts derived from Ontario grown *Panax quinquefolius* for its cyto-protective and immunomodulatory effects in two experimental models. 1. UV-B induced skin photo injury in SKH-1 hairless mice. Three different ginseng extracts (aqueous, alcoholic and polysaccharide) were incorporated into a base cream at different concentrations allowing the delivery of the same total ginsenosides and/or polysaccharides concentrations to the backs of mice and tested against a single UVB irradiation at 300mJ/cm2. Ginseng treatments were given for 3 consecutive days prior to irradiation and examined 1 and 5 days later. Irradiation resulted in elevation in plasma and skin cytokine levels (IL-1, IL-6 & TNF) at both time points, and epidermal disruption and blister formation as well as DNA damage on day 1 and epidermal inflammation on day 5. All ginseng treatments produced protection, but the aqueous extract offered the best result. 2. Cyclophosphamide (CP)-induced immunosuppression in Balb/c mice. We investigated the effect of crude ginseng polysaccharide extract on the toxicity induced by a single sub-lethal dose of CP (300mg/kg i.p.). CP treatment resulted in reduction of phagocytic index, hemagglutination, splenocyte counts, bone marrow cellularity, and plasma cytokine levels (IL-1, IL-6 & TNF). Ginseng extract was administered by oral gavage for 14 days at 50mg/kg and 100 mg/kg body weight, and the first dose was given 2 hr. following CP. The toxic parameters were reduced significantly by ginseng treatment. Delaying ginseng treatment by 3 days did not reduce its effectiveness, suggesting that inhibition of CY bioactivation was not responsible for the observed protection. These data provided evidence supporting the cytoprotective effects of *Panax quinquefolius* and the involvement of different bioactive components. (Supported by Ontario Research Fund, MRI).

Post-Absorption and Metabolism Approach for Drug Discovery from Natural Products: Antidepressant S111

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Many chemical ingredients of herbal medicines are poorly absorbed and therefore do not represent the pharmacological mechanisms of their clinical effects. We proposed that one of better approaches to discovery lead compounds is to study compounds post absorption and metabolism. Compounds found in the blood or target organs after orally taking herbal medicines can be re-synthesized to form a class of novel drugs namely Post Absorption/Metabolism Drugs (PAMDs). Ginseng has been used as a major ingredient in traditional Chinese medicine (TCM) for mood regulation for many years in China. A more recent randomized, controlled trial of ginseng in postmenopausal women also reported improvements in mood and anxiety. In the present study, we tested anti-depression effect of a semi-synthesized aglycone

ginsenoside, S111 that represents one of the post metabolism compounds of ginseng in a series of depression models and clinical studies. Transcriptomics of various brain regions were analysed and compared with that of untreated and fluoxetine, a clinical anti-depressant, treated animals. Our results showed that orally given S111 daily for consecutive 10 days significantly antagonized Reserpine induced various locomotor inhibition in mice. In a 21-day chronic stress induced rat depression model, animals with S111 given daily demonstrated substantial improvement in an open-field test as much as fluoxetine. Furthermore, S111 increase 5-HT and NE levels in the brains of depressed animals. However, unlike fluoxetine, S111 only displayed much less inhibitory effect on reuptake of the monoamines than fluoxitine in vitro, suggesting its anti-depressant effect may be mediated by a different mechanism. The transcriptomic study further showed two distinctive gene expression profiles between S111 and fluoxetine, indicating that the two drugs may have quite different anti-depression mechanisms. S111 caused much less disturbance in the normal animals than that of fluoxetine and S111 had a better therapeutic efficiency. However, some genes were commonly regulated by both S111 and fluoxetine, suggesting that those genes may represent a group of targets that are critical for anti-depression. Currently, S111 is in phase II clinical trial for anti-depression. Our results are the first to show that a metabolic product of ginseng can be potentially developed into a modern drug, which may represent a new class of novel drugs from natural products.

Calcitriol Enhances PPD Mediated Anticancer Activity in Prostate Cancer Models In Vitro: Pharmacodynamic Based Interaction

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Background: The potential role of vitamin D metabolites (calcitriol) and ginseng extracts (PPD) in prevention/treatment of Prostate cancer (PCa) has gained much attention in recent years. We evaluated the anticancer activity of calcitriol and PPD, and their combinations on two well characterized human PCa cellines (androgen dependent LNCaP and androgen independent C4-2). We hypothesized that calcitriol and PPD combination significantly would sensitize their respective anti-cancer efficacies. Methods: The effects of the treatments on PCa cell viability and proliferation rate were evaluated by MTS and Brdu assays, respectively. We compared the IC20 and IC50 of PPD treated alone and in a combination in presence or absence of endogenous androgen. Then, we determined the mechanisms as follows: The protein expression levels of the genes those are known to control cell cycle (cyclin D1 and cdk2); apoptosis (Bcl-2, Bax, and caspase-3), androgen receptor, PSA and vitamin D receptors were also examined using WB techniques. Results: PPD treatment alone inhibited LNCaP and C4-2 cell growth with an IC50 of 41 and 53 µM, respectively. However, in combination treatment the IC50 were reduced to 23 and 16 µM, respectively. Adding 10 nM calcitriol at clinically relevant concentrations to PPD significantly lowered tumor cell proliferation rate with greater inhibition was seen in Css containing media. Preliminary results show that PPD significantly upregulates VDR expressions, while calcitriol further enhances PPD ability to induce caspase-3 and inhibit cyclin D and cdk2 levels. Conclusions: Calcitriol sensitizes PCa cells to PPD anticancer mediated effect by enhancing PPD ability to induce apoptosis, reduce cell proliferation and retard cell regulation perhaps due to an increase in VDR levels. Our findings suggest that there is potential clinical value in combining PPD with calcitriol for the treatment of human PCa as could limit calcitriol toxicity by using lower concentrations that enhance overall treatment efficacy.

Ginsenosides Inhibit 1α,25-dihydroxyvitamin D3 Metabolism by CYP3A4 in Human Liver and Intestine in vitro

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The beneficial effects of vitamin D3 are exerted through 1α,25-dihydroxyvitamin D3 [1α,25(OH)2D3], the dihydroxy metabolite of vitamin D3. Hepatic and intestinal biotransformation of 1α,25(OH)2D3 and modifiers of metabolic capacity could be important determinants of bioavailability in serum and tissues. The purpose of the present study was to investigate the potential of ginsenosides and their aglycones to block hepatic and intestinal inactivation of 1α,25(OH)2D3, which is the most potent ligand of vitamin D receptor. In vitro biotransformation reactions were initiated with NADPH regenerating solutions following initial preincubation of pooled human hepatic or intestinal microsomal protein or human recombinant CYP3A4 supersomes with 1α,25(OH)2D3 or midazolam. Formation of hydroxylated metabolites of 1α,25(OH)2D3 or midazolam was analyzed using liquid chromatography-mass spectrometry. Co-incubation of 1α,25(OH)2D3 with various ginsenosides (Rg1, Rh2, aPPD, aPPT and total ginsenosides) led to differential inhibition (30-100%) of its hydroxylation. Follow up inhibition studies with aPPD and aPPT at varying concentrations (0.5-100μM) led to up to 91-100% inhibition of formation of hydroxylated metabolites of 1α,25(OH)2D3 thus preventing inactivation of active vitamin D3. The IC50 values of aPPD or aPPT for the most abundant hydroxylated metabolites of 1α,25(OH)2D3 ranged from 3.3 to 9.0μM in human microsomes. The inhibitory mechanism of aPPD or aPPT for CYP3A4-mediated biotransformation of 1α,25(OH)2D3 was competitive in nature (apparent Ki: 1.7-2.9μM). In summary, our results suggest that ginsenosides, specifically aPPD and aPPT, inhibit the CYP3A4-mediated catabolism of active vitamin D3 in human liver and intestine, potentially providing additional vitamin D-related benefits to patients with cancer, neurodegenerative and metabolic diseases.

NHP REGULATIONS AND INDUSTRY

Health Claim Opportunities and Standards of Evidence for Natural Health Products in the Canadian Regulatory Framework

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The Canadian regulatory framework for Natural Health Products [NHPs] provides health claim opportunities for many NHP substances, including plant material(s) and/or extracts, animal materials(s) and/or extracts, vitamins, minerals, essential fatty acids, amino acids probiotics, and synthetic duplicates. Some NHP substances have been used for many years in Traditional and/or Herbal medicine practices, and the safety and efficacy of these substances has been established by Health Canada. Subsequently, access to "pre-cleared" information [PCI] for both Traditional and general modern health claims allows for a more timely regulatory pre-market review. Health Canada pre-cleared information in support of health claims is publicly available in several forms, including ingredient monographs, product monographs, and abbreviated labeling standards. In the absence of PCI, health claim opportunities still exist for modern health claims, and/or new combinations of NHP substances; however, a more rigorous pre-market review will be conducted by Health Canada for these products. In the wake of regulatory modernization in the NHP sector, requirements for substantiation of product safety and efficacy correlate to the level of claim submitted. This session will provide an overview of the health claim opportunities for NHPs in the current Canadian regulatory framework, and will discuss standards of evidence for successful product licencing initiatives.

GMOs and Natural Health Products - Impacts and Implications

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An increasing number of Canadians are becoming more conscious of the presence of Genetically Modified Organisms in consumable products. Although labeling, negative or positive is not mandatory the number of products that claim "GMO Free" is on the rise. How does this impact the Natural Health Product industry? Recent discussion have identified concerns and has highlighted some contention in definitions and raised further questions. If a formulation contains a GMO component is it still natural? Is there a tolerance, does zero still exist for some ingredients? If its Organic is it GMO free? With the Non GMO Project and the Whole Foods initiative many brand holders are apprehensive about meeting the standards that may be imposed. Further these programs are neither regulated or standardized and have implications on trade, cost of sourcing ingredients, specifications and finished product testing. There are many advocate groups that are lobbying and/or protesting the technology in itself as well as the presence of these products in the Canadian market place. Others are demanding mandatory labeling. Despite the activities of these groups Health Canada do not perceive a risk to the health of Canadians and between substantial equivalence and novel food notifications the number of GMOs will only increase.

Progress in Development of Resources to Assess Dietary Supplement Exposures

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Researchers, practitioners, policy makers, and regulators need data in order to estimate total nutrient intakes for population-based nutritional status assessment and planning. Such estimates are often derived from national nutrition surveillance of the population, such as the National Health and Nutrition Evaluation Survey (NHANES) administered by the U.S. Centers for Disease Control and Prevention (CDC). Reliable intake data can provide accurate estimates of those whose total nutrient intakes are below the Estimated Average Requirement (EAR) or above the Upper Limits (UL) for particular nutrients and are therefore at dietary risk. Reliable information of this type can help epidemiologists link nutrient intakes with disease outcomes and make comparisons across research studies to ensure that estimates are comparable study to study. When used in combination with food composition tables provided by the United States Department of Agriculture, nutrition surveys allow estimation of nutrient intake from food, but recent information indicates that large segments of the U.S. population regularly use dietary supplements and therefore acquire a significant proportion of their nutritional intake from supplements. Tools such as dietary supplement label databases and mobile applications to assist consumers capture non-food nutrient intake will supplement food composition databases and provide researchers and others with more accurate estimates of population wide nutrient status.

What's in your "Natural" Supplement? Application of NMR Spectroscopy to Natural Products

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Natural products such as herbal remedies have been increasing in sales with annual revenue of 5.11 billion dollars in the United States alone. This has raised concerns as surges in adulteration of natural products with other materials have been detected in the market. In some cases, this is due to accidental species misidentification, but in other cases, non-disclosed materials were added to increase product efficacy. This illegal practice can be detrimental to

the natural product industry and extremely dangerous to the health of the consumer. Due to the diversity and complexity of chemical compounds within botanical materials, a demand exists for methods to understand the composition of natural products for proper quality control and authentication. In this study, an NMR-based screening tool was applied to natural products. A targeted and non-targeted NMR screening for adulterated dietary supplements will be described. This approach shows the simultaneous analysis of natural products which involves 1) Identification of known components, impurities and adulterants in reference spectrum database, 2) Quantification using a calibrated NMR spectrometer and 3) Multivariate modeling for product classification. With the increasing demand for robust high-throughput analytical methods, an automated NMR-based screening tool is a powerful approach for validating the identity, purity, strength, and composition of many natural products.

Regulatory Update: Clinical Research in the Canadian NHP Sector

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Innovation is evident in the Canadian Natural Health Product (NHP) sector, with unique ingredients and health products being commercialized on a regular basis. Some NHPs provide novel combinations of established ingredients, while other NHPs seek to market new ingredients not yet investigated in human participants. Regardless of a health product's ingredient profile, clinical data must be provided to Health Canada to support product safety and efficacy in order to attain market access. In the absence of pre-cleared information or existing published clinical data in support of a product's claim(s) and safety profile, a sponsor can pursue product-specific research via a clinical trial application [CTA] to the Natural Health Products Directorate [NHPD] of Health Canada. The CTA process constitutes the formal regulatory framework by which a sponsor can communicate to Health Canada a proposed study protocol, as well as quality documentation on proposed investigational product(s). Health Canada must provide Notice of Authorization [NOA] prior to any clinical study commencing on a NHP in Canada, with the exception of post-market studies. Recently, as a result of regulatory modernization, the NHPD modified its approach to classification of CTAs to be centred about the principles of self-care. CTAs for products whose indications fall outside of the premise of self-care will be allocated to the Therapeutic Products Directorate (TPD) or the Biologic and Genetic Therapies Directorate (BGTD) by the NHPD. This presentation will provide an overview of the current clinical trial regulations within the Canadian regulatory framework, and provide a summary of recent Health Canada initiatives, including updated serious adverse event [SAE] reporting and the online clinical trials database.

STUDENT ORAL PRESENTATIONS

Incorporation of the Natural Non-protein Amino Acid Beta-methylamino-L-alanine (BMAA) into Protein

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N-β-methylamino-L-alanine (BMAA) is a naturally occurring neurotoxic non-protein amino acid produced in cyanobacteria. Recent results indicate that BMAA can be incorporated into proteins during synthesis and analyses of extracted proteins from various sources have detected BMAA. We hypothesized that BMAA is incorporated into protein and investigated this hypothesis with a cell-free protein synthesis system. Protein was produced when all 20 protein amino acids were included, when BMAA was added to the mixture and when BMAA substituted was in place of 8 individual limiting amino acids. BMAA was detected in the synthesized protein when it was added to the standard protein amino acids and when serine, glutamate, alanine, proline phenylalanine, isoleucine, threonine or glutamine was limiting. Denaturation released about half of the BMAA

from the synthesized proteins indicating that protein associated BMAA was both incorporated into the protein via a peptide bond and also non-covalently associated with the tertiary protein structure. The preliminary studies had used the standard E. coli DNA template while follow-up studies were done using an equivalent amount of full DNA template isolated from human autopsy brain samples. The human DNA incorporated BMAA into human proteins at significantly higher rates than was observed with the bacterial template. Together, these data support the hypothesis that the non-protein amino acid BMAA is inserted into protein through errors in protein synthesis.

Repellent and Insecticidal Activities of Three Lavender Essential Oils on the Fruit Fly *Drosophila melanogaster*

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Plant extracts are widely used for controlling pests. The essential Oils (EOs) extracted from three different lavender cultivars *Lavandula angustifolia*, Lavandula x intermedia cv GROSSO (GROSSO), and Lavandula x intermedia cv OKANAGAN (P1) were tested for their insecticidal and/or repellent effects on adult fruit flies. The EO composition of each lavender was determined by gas chromatography-mass spectrometry (GC-MS). *L. angustifolia* oil was characterized by relatively high concentrations of linalool and linalool acetate and relatively low levels of 1,8-cineole, camphor, and borneol. GROSSO oil was characterized by moderately high linalool and linalool acetate, and increased relative percentages of 1,8-cineole, camphor, and borneol. P1 oil was characterized by high concentrations of linalool, 1,8-cineole and borneol, and low levels of linalool acetate and camphor. In contact toxicity assays, *L. angustifolia* was the most toxic oil, and was significantly more toxic than P1 oil. In the fumigation trials no difference was found between the three EOs. Using high throughput repellency assay chamber the repellency of each lavender essential oil was determined. The Spatial Activity Index (SAI) showed that all three oils are repellent, but only *L. angustifolia* and P1 oils were significantly repellent. Generally, all of the EOs showed strong toxicity and repellent effects. These results suggests that Lavender EOs have the potential to be used as plant-based natural products for insect pest control in organic agriculture.

Effect of Pomegranate Fruit Extract on Testosterone-induced Benign Prostatic Hyperplasia in Rats

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Benign prostatic hyperplasia (BPH) affects most men after the age of 50 years. Inflammation and oxidative stress are thought to play an important role in the pathology of BPH along with hormonal and apoptotic changes. Pomegranate contains a variety of polyphenolic compounds that have been studied in a medley of diseases for their anti-oxidant, anti-inflammatory, pro-apoptotic and phystoestrogenic actions. The present study investigated the effects of Pomegranate Fruit Extract (PFE) 25, 50 and 100 mg/kg on testosterone-induced BPH in rats. BPH was induced in 4 groups by injecting testosterone 3 mg/Kg S.C for 4 weeks, 3 groups received PFE concurrently at doses of 25, 50 and 100 mg/kg. Animals were sacrificed 72 hours after the last dose and prostate weight, prostate weight/body weight ratio, serum prostatic acid phosphatase level were assessed along with histopathological study of the prostate tissue. Furthermore, reduced and total glutathione, SOD, CAT, MDA were measured to assess oxidative stress level in the prostatic tissue. Also, inflammatory status was assessed by quantifying COX-II, iNOS, and Nf-ƙB expression in the epithelial cells Immunohistochemically. All PFE doses decreased levels of oxidative stress parameters, COX-II and iNOS gradually with increasing the dose. PFE showed a therapeutic effect in testosterone-induced BPH, this effect was directly proportional to the dose of PFE used. It could be concluded that oxidative stress and

inflammation play an important role in development of BPH, and that PFE is able to offer a protection against testosterone-induced BPH through its antioxidant and anti-inflammatory properties. Also, there are further ongoing IHC studies investigating the effect of PFE on hormonal pathways (AR,ER α, and ER β) as well as apoptotic pathways (Phospho AKT and Ki-67).

THURSDAY MORNING - PLENARY

Mechanistic Investigation of Polyphenol Metabolites Bioactivities in Alzheimer's Disease

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Alzheimer's disease (AD) is a major neurodegenerative disease of aging, affecting an estimated 5.3 million individuals in the U.S. (Alzheimer's Association 2012). Current FDA-approved treatments for AD have only modest symptomatic effects and are not disease-modifying. Recent studies in our laboratory demonstrated that dietary supplementation with a specific grape-derived polyphenolic preparation (GP) significantly improves cognitive function in a mouse model of Alzheimer's disease (AD). GP is comprised of the proanthocyanidin (PAC) catechin and epicatechin in monomeric (Mo), oligomeric, and polymeric forms. In this study, we report that following oral administration of the independent GP forms, only Mo is able to improve cognitive function and only Mo metabolites can selectively reach and accumulate in the brain at a concentration of ~400 nM. Most importantly, we report for the first time that a biosynthetic structurally identified epicatechin metabolite, 3'-O-methyl-epicatechin-5-O-β-glucuronide (3'-O-Me-EC-Gluc), one of the PAC metabolites identified in the brain following Mo treatment, promotes basal synaptic transmission and long-term potentiation (LTP) at physiologically relevant concentrations in brain region associated with memory function through mechanisms associated with cAMP response element binding protein (CREB) signaling. Our studies suggest, for the first time, that biosynthetic 3'-O-Me-EC-Gluc and other brain-targeting PAC metabolites may causally promote learning and memory. This novel evidence provides support for further characterization of synthesized, brain penetrating, grape derived polyphenol metabolites as a potential treatment strategy in AD and other forms of dementia.

TRADITIONAL MEDICINE AND PHARMACOGNOSY

Challenges and Solutions for the Quality Control of Natural Health Products

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Standardization and efficient quality control are the most important issues in the natural health products industry today and require world-wide attention, action, and resolution. After analyzing a number of commercial products including some of the most popular options currently in the market, it is evident that major quality control problems plague the industry. Such issues include: the incorrect labelling of plant species, the lack of detectable active compounds present, lowered concentrations, and extracts spiked with related compounds in a bid to meet the standards they claim. The adulteration of some of the most popular botanical extracts, *Ginkgo biloba*, *Lonicerae japonicae*, and more will be discussed. Evidently, finding a consistent HPLC profile for the identification of raw herbs and botanical extracts is becoming widely crucial. Many attempts have been made to construct a HPLC profile that identifies the plant species and ensures the botanical extract profile is comparable to the raw herb, but the key answer to successful testing lies in the quantity of raw materials. Large amounts of authentic plant sample are required to set up a consistent HPLC profile, due to the complications in simultaneously analyzing many different compounds from a formulated botanical product. Therefore, the interference between compounds brings great difficulty to the testing of formulated products using the already established analytical method designed for the single herb. To shorten the retention time and enable different compounds to be analyzed in a formulated product, the UHPLC or RRLC (Rapid Resolution Liquid Chromatography) method was introduced, providing a high-speed and high-resolution alternative. For instance, using

RRLC, the retention time for ginsenoside evaluation in different Ginseng species can be significantly reduced to 3-10 minutes. This significant gain in speed coupled with the ability to profile formulated herb products with over four different authentic plant species makes the RRLC method one of the most sophisticated methods available for the identification and evaluation of formulated herbal products. As the result of extensive research and studies conducted on standardization and analytical methodology development for botanical extracts, we believe the updated analytical techniques are sufficient in the quality control of formulated products. The above mentioned research and development of *Rhodiola rosea*, *Panex notoginseng*, and *Ganoderma lucidum* has been used in the preparation of monographs and standard reference extracts for United States Pharmacopoeia and these results have been adopted as industry standards.

Modernization of Chinese Medicine: A Case Study with Ginseng at OGIRC

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The recent resurgence in popularity of Traditional Chinese Medicine has led to a movement in modernization of this traditional medicine. Different approaches and strategies have led to the advancement in knowledge pertaining to cultivation, processing, production, characterization, and formulation as well as better understanding of medicinal property and evidence for clinical practice. In this presentation, we will use the R&D program at the Ontario ginseng Innovation and research Consortium (OGIRC) as a case study. We have developed a multi-partnership and multi-disciplinary program that has provided high quality raw herb with good traceability mechanism to produce high quality extracts according to GMP guidelines. The phytochemically-characterized extracts have been examined by a multi-disciplinary team in pharmacology to elucidate the cellular and molecular activity of this multi-action herb. Moreover, novel value-added products have been developed with advanced processing technology. A longer term goal is to improve the quality of this herb; and the use of plant biotechnology for cultivar development has yielded promising results. The operational frame of this approach will be discussed with specific outcome as examples and the identification of challenges encountered. (Funding from Ministry of Research and Innovation)

Significance of Bone Morphogenetic Proteins for Understanding of Traditional Chinese Herbs in Treatment of Liver Disease

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Purpose: Bone morphogenetic proteins (BMPs) are a group of growth factors belonging to the superfamily of transforming growth factor beta. Our previous findings showing significantly increased BMP4 levels in bile duct ligated livers and increased expression of BMP4 mRNA in activated hepatic stellate cells, which are responsible for the development of fibrosis in the liver. Several traditional Chinese herbs have been identified to reverse liver fibrosis. However, the mechanism(s) of these herbs in the treatment of liver fibrosis remains to be investigated. Methods: By employing hepatic stellate cell line (LX-2 cells) and hepatoma cell line (Huh-7 cells), we investigated the regulation of BMP4 expression with several components of these herbs. BMP4 expression was evaluated by RT-PCR and Western blot analyses and WST-1 proliferation reagent determined cell proliferation. Results: We employed saikosaponin-d (SSd) and paeoniflorin (PF) extracted from the bupleurum and *Paeonia lactiflora* to treat LX-2 and Huh-7 cells respectively. SSd significantly inhibited LX-2 cell proliferation and PF inhibited Huh-7 cell proliferation, respectively. Although BMP4 did not affect both LX-2 and Huh-7 cell proliferation, both SSd and PF reduced BMP4 expression in both cells. Moreover, BMP4 promoted hepatic stellate cell trans-differentiation as shown in elevated level of alpha smooth muscle actin (alpha-SMA). SSd inhibited stellate cell trans-differentiation (decreased alpha-SMA level). Furthermore, SSd also increased the level of BAX in LX-2 cells. Conclusion: BMP4 could be the growth factor

involved in the regulation of hepatic stellate cells and hepatoma cells that is perturbed by this treatment.

Development of Innovative Therapeutics from Traditional Chinese Herbal Medicine in Treating Human Cancer Diseases

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Traditional Chinese herbal medicine (TCM) has been accumulating huge amount of experiences in treating different kinds of human diseases including cancer and improving health situation in clinical practice. Development of innovative therapeutics with traditional herbal medicine is very necessary and meaningful. Through both clinical trial and experimental research, the present studies investigated the therapeutical effects of the traditional Chinese medical herbs on human lung cancer. It discovered that several specific Chinese medical herbs have therapeutic effects on human advanced lung cancer and the Lewis lung cancer in animal models. The results showed that the medical herbs could inhibit the growth of lung tumor, decrease the disease symptoms and improve the living quality and health situation of the host with lung cancer. It demonstrated that the potential cellular immunological mechanisms are associated with improving the host immune system function by these medical herbs. Using the system of culturing K562 cells, a p53 deficient erythroleukemia cell line, the studies determined that the specific medical herb, Hex (Astragalus membranaceus), could stimulate human adult globin gene expression, induce terminal differentiation and apoptosis of erythroleukemia cell lines. Combination of Chinese medical herbs and the extraction with western medical drugs and specific monoclonal antibodies has indicated significant synergic therapeutical effects in treating some malignant cancer diseases such as melanoma. It may provide a rationale and potential for developing new strategies for targeting therapeutic intervention in cancer diseases.

Liuwei Dihuang, a Promising Anti-Obesity Herbal Formula of Traditional Chinese Medicine

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Overweight and obesity have become a world epidemic and imposed heavy burden to the health care systems and significantly affect the quality of human life. Although a number of programs and products have been developed and used, success is marginal. It is strongly demanding to develop new products that are efficacious and safe. In this regard, a classic formula of traditional Chinese medicine, Liuwei Dihuang (LWDH), has shed a light. In the past few years, we have conducted a series of studies to determine the efficacy and mechanism of action of this herbal formula on body weight, visceral fat mass, energy metabolism, energy expenditure, and metabolic phenotypes in an obese-prone rat model. The first study showed that LWDH concentrated pills at a dose of 3,500 mg/kg/d significantly decreased weight gain and food intake, and also reduced serum triacylglycerols, nonesterified fatty acid (NEFA), leptin and insulin levels. Liver function testing revealed no adverse side effects after 10 weeks of treatment. In order to identify the active component(s), ethanol and water extracts were obtained. The chemical analysis revealed that water and ethanol extracts contained similar bioactive compounds but concentration is quite different. Further animal studies demonstrated that the both extracts markedly decreased weight gain and improved metabolic phenotypes in obese-prone rats at doses that were only 10-30% of the concentrated pills, and the efficiency of ethanol extract was doubled relative to the water extract. Moreover, the both extracts significantly increased fat and carbohydrate oxidations, energy expenditure and relative efficiency of fat oxidation for energy expenditure. The results of these three animal

studies have demonstrated that LWDH, especially ethanol extract has great potential to be further developed into a natural product for the prevention and treatment of obesity and its related metabolic phenotypes.

Abietane –Type Diterpenoids from Plectranthus barbatus and their Antiprotozoal Activity

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Plectranthus barbatus (Lamiaceae) is a species native to Tropical Africa, Asia and Australia. It is most commonly used in traditional medicine for treatment of liver diseases and intestinal disorders. Genus Plectranthus is a rich source for abitane phyllocladanes, ent-kaurenes and a seco –kaurenes diterpenes. Previous screening for more than 100 plants, including *P. barbatus*, growing in Arabian Peninsula for their antiplasmodial, antileishmanial and antitrypanosomal activities showed promising antiprotozoal activity. Chromatographic separation of the n-hexane extract of *P. barbatus* led to the isolation of five abietane-type diterpenes, dehydroabitane (1), 5,6-didehydro-7-hydroxy-taxodone (2), taxodione (3). 20-deoxocarnosol (4) and 6,11,12,-trihydroxy -7,20-epoxy-8,11,13-abietatriene (5). The structures of the isolated diterpenes were determined using spectroscopic methods. The plant extract and the isolated compounds were tested for their antimalarial activity against *Plasmodium falciparum*, for their antileishmanial activity against Leishmania infantum and for their antitrypanosomal activity against *Trypanosoma cruzi* and *T. brucei*. To assess selectivity, cytotoxic activity was determined against MRC-5 cells. Compound (2) was found to be the most active and selective one against all tested parasites particularly *P. falciparum* and *T. brucei* with IC50 values of 9.2 and 1.9 µM and showed good selectivity with SI-values of 10.4 and 50.5, respectively. Compounds (3), (4) and (5) showed high antiprotozoal activity against all strains with IC50-values between 5.6 and 30.1 µM but with high cytotoxicity. Meanwhile, compound (1) showed only a weak antiplasmodial activity (IC50: 123.7 µM) and was found to be inactive against *L. infantum*, *T. cruzi* and *T. brucei* at the highest concentration tested.

Workshop: Cyanobacteria & BMAA

Cyanobacteria in the Dietary Supplement and Natural Health Product Marketplace

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Worldwide, algae and cyanobacteria have been wild harvested or cultivated as a food source and treatment for various physical ailments for thousands of years. In western cultures, certain fresh water cyanobacteria have been a source of food for about 30 years, in particular *Spirulina* (*Arthrospira*) *platensis* and *Spirulina maxima*. Beginning in the 1980s, another species, Aphanizomenon flos-aquae, was adopted for similar uses. These are rich in proteins, vitamins, essential amino acids, minerals, and essential fatty acids. Consumers of cyanobacteria report many putative positive effects such as mental clarity, increased energy, blood and colon cleansing, increased focus, improved digestion, increased eye health, and healthier joints. Consumer use and interest have stimulated research to verify nutritional efficacy and potential health benefits. Chemical composition of cyanobacteria is subject to variation by habitat, cultivation process, harvest procedure, control for contaminating species, processing to preserve nutrients, and storage conditions. Commercial operations may use closed fermentation, concrete ponds, or harvest from wild lakes. Co-occurrence of toxigenic species or toxigenic varieties of target species is an issue that must be addressed by manufacturers as various natural toxins are known to be produced by cyanobacteria. Going forward, CGMP controls and scientifically valid methods for determination of a diverse group of structural

classes will be needed, including those for paralytic shellfish poisoning congeners, non-protein amino acids, peptide microcystins, and others. This talk will provide an overview of cyanobacteria sold as dietary supplements and their toxins, as well as identifying areas where there are methods and where methods are needed.

BMAA in Environmental Samples: The Big Picture

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Beta-N-methylamino-L-alanine, BMAA, is a neurotoxic amino acid produced by cyanobacteria and present in a variety of environmental samples. It has been found in organisms around the globe using multiple analytical techniques. BMAA has been implicated as an environmental risk factor for neurodegenerative disease and BMAA-induced neurotoxicity has been demonstrated to occur through multiple processes. Sources of human exposure include dietary ingestion and the inhalation of toxins through aerosolization and airborne particles. Further research is necessary to protect public health and understand the chronic effects of BMAA exposure.

Challenges and Opportunities in the Detection and Quantification of BMAA

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Accurate detection and quantification of BMAA in complex environmental and food samples remains challenging. Potential pitfalls include appropriate analyte extraction, derivatization, complete chromatographic separation of biologically-occurring isomers, and extensive optimization of detection parameters. Additional challenges are associated with method validation, including data analysis methodologies, analyte recovery, and precision of the methods. There is a need for a comprehensive collaborative inter-laboratory trial to resolve method issues for food, water and environmental samples.

Analytical Methods and Reference Materials for Cyanobacterial Toxins

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Cyanobacteria are found in marine and freshwater environments worldwide. Some species produce potent toxins called cyanotoxins that present serious threats to wild animals, pets, livestock and people. Cyanotoxins include hepatotoxins such as microcystins and cylindrospermopsins and neurotoxins such as anatoxins, saxitoxins and beta-N-methylamino-L-alanine (BMAA). The main route of exposure to humans is through contaminated drinking water supplies and breathing in aerosols. Another potential means of exposure could be through algal dietary supplements. Sensitive multi-toxin detection methods are required for the screening and quantitation of cyanotoxins in water, algae and dietary supplement samples. In this presentation, our efforts to develop and validate methods for the detection and quantitation of cyanotoxins will be presented. Our methods are based on either hydrophilic interaction liquid chromatography (HILIC) or reversed phase liquid chromatography (RPLC) combined with tandem mass spectrometry. A major problem in the field has been the lack of availability of certified reference materials (CRMs) for instrument calibration and validation of method performance. Our efforts to develop methods and CRMs will be reviewed first. Then our results to date on a feasibility study for preparing a freeze-dried cyanobacterial RM and future plans for a CRM will be presented.

Marine and Fresh Water Toxins Analysis AOAC Task Force

James Hungerford.
Co-chair of AOAC Task Force and GR Marine and Freshwater Toxins Applied Technology Center, FDA Bothell, WA, USA.

Thursday Afternoon

IMMUNITY AND NEURODEGENERATIVE DISEASE

Ubisol – Q10 as an Effective Treatment for Halting Neurodegeneration in Environmental and Genetic Susceptibility Models of Parkinson's Disease

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Parkinson's disease (PD) is a prevalent neurodegenerative disorder that arises due to progressive loss of dopaminergic neurons in the substantia nigra region of the brain. Past studies suggest that PD could be caused by a combination of both genetic and environmental factors, such as loss-of-function mutations and environmental toxins such as paraquat and rotenone. Though the primary mechanism of PD remains unclear, evidence shows that oxidative stress is one of the most important factors leading to the disease pathology. We used a water soluble formulation of CoQ10 (Ubisol – Q10) and demonstrate its effectiveness in protecting neurons both in vitro and in vivo. Our paraquat rat model demonstrates slow progressive loss of dopaminergic neurons as seen in patients suffering from sporadic PD and the DJ-1 deficient MPTP toxin mice model represents genetic susceptibility to PD. We show that Ubisol-Q10 is efficient in preventing neurodegeneration when administered prophylactically and therapeutically and prolonged treatment is required to sustain neuroprotection in the PQ rat model. Since DJ-1 deficiency has been shown to increase susceptibility to the toxin MPTP, we have shown that prophylactic treatment with Ubisol – Q10 prevents neurotoxicity and hence prevents PD. This formulation is required in much lower doses to provide neuroprotection and is efficient in crossing the blood brain barrier. The antioxidant properties of CoQ10, along with the formulation's increased bioavailability and ability to stabilize the mitochondria provides efficient neuroprotection in both the models. Ubisol – Q10 is already FDA GRAS approved and can be produced for testing in human clinical trials.

Anti-inflammatory Effects of an Omega-3 Supplement Depend on the Ratio of Desirable (omega-3 and PUFAs) to Undesirable Fatty Acids (Saturated and Trans Fatty Acids)

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Evidence from numerous studies indicates that omega-3 fatty acids (w3 FAs) reduce inflammation and decrease risk of several diseases. This has led to significant spurt in the use of these supplements. However, w3 FAs are highly prone to oxidation and are also susceptible to form trans fatty acids (tFAs) during the high temperature refining process. The supplements could also contain saturated fats (SFAs). These undesirable fatty acids could have detrimental effects on bioactivity of the supplements. The current study was undertaken to compare chemical composition and anti-inflammatory effects of Afinity Omega Health which contained 68% w3 FAs with 2 other commercial w-3 products, Product A containing 64% w3 FAs, Product B containing 30% w3s. The products were tested by an independent research lab at the National Institute for Nanotechnology/NRC under blind conditions. The results demonstrated that the Afinity product was 5 times more effective than either of the other products in reducing TNF secretion from human macrophages, THP-1 cells. The anti-inflammatory effect was not found to correlate with the amount of EPA and DHA. Chemical analysis showed that the ratio of desirable fatty acids (w3 FAs and other PUFAs) to undesirable fatty acids (SFAs and tFAs) was 7 times greater in Afinity product than product A and 30 times greater than Product B. The results indicate that tFAs or SFAs could have detrimental

effects on anti-inflammatory effects of w3 FAs. In conclusion, overall ratio of desirable to undesirable FAs appears more important in predicting anti-inflammatory effects of a w-3 supplement.

Eremophenolide Compounds Inhibit Human Mast Cell Activation and Reduce Allergic Inflammation

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Introduction: Sesquiterpenes are a diverse family of biologically active plant compounds that have been used to treat infection, inflammation and cancer. Clinical trials in allergic rhinitis patients have suggested that sesquiterpenes reduce symptoms associated with allergic inflammation. In this study, we investigated the effect of novel eremophilane-type sesquiterpenes isolated from Petasites Japonicus in in vivo inflammatory mouse models and in vitro human mast cell models of allergic inflammation. Methods: 95% ethanol extracts from P. Japonicus were separated into petroleum ether, chloroform and n-butanol soluble fractions. The effect of these fractions on mouse models of passive cutaneous anaphylaxis (PCA), capillary penetrance induced by histamine, and delayed-type hypersensitivity (DTH) was measured and their effects on human mast cell degranulation, [Ca2+]i flux and mediator release was determined in vitro. Results: Compared to control, the chloroform soluble fraction inhibited heterogeneous PCA, histamine-induced capillary permeability, and DTH. This fraction contained an eremophilenolides compound, 6β-angeloyloxy-3β,8-dihydroxyeremophil-7(11)-en-12,8α-olide (compound F1). Two novel compounds, F1a and F1b, were synthesized and tested for their ability to inhibit human mast cell activation (LAD2). F1a and F1b inhibited antigen- and compound 48/80-induced LAD2 cell degranulation by 52% and 61% ($p<0.05$) and F1a inhibited antigen-induced [Ca2+]i flux. F1a and F1b also inhibited LAD2 production of tumor necrosis factor (TNF) by 33% and 75%, respectively, and monocyte chemoattractant protein-1 (MCP-1) by 72% and 77%, respectively ($p<0.05$). Conclusion: Eremophilenolides suppressed in vitro human mast cell activation and in vivo allergic reactions, indicating that these compounds may be utilized as potent agents for the treatment of allergic inflammation.

Cortisol Lowering Properties of Betulinic Acid and Medicinal Plants Containing It

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This study reports for the first time the cortisol lowering properties betulinic acid and two medicinal plants containing it, *Souroubea sympetala* and *Platanus occidentalis*. The plants have have previously been reported to have antianxiety properties and betulinic acid was identified as the active principle. The cortisol-lowering effect of betulinic acid and *S. sympetala* was investigated in an in vitro and in vivo model in rainbow trout. Both leaf extract and BA significantly lowered cortisol in response to an adrenocorticotropic hormone (ACTH) challenge in vitro and a net restraint assay in vivo. The in vitro study suggested direct inhibition of cortisol in head kidney cells rather than action through the cascade of responses in the hypothalamus-pituitary-adrenal (HPA) stress axis. Similarly in rats, methyl betulinate and *Souroubea sympetala* reduced corticosterone levels in rats subjected to restraint but had no effect on cortisol in control animals.

Workshop: Genetic Tools for the Identification of Botanicals

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Natural health products encompass a broad spectrum of goods that can contain ingredients ranging from vitamins and minerals to botanicals and animal-derived products. Manufacture of these products begins with demonstration of ingredient identity and proceeds to assuring strength, purity, and composition of the ingredients and finished products. In the case of botanical products, these efforts range from assuring that biomass is properly identified and herbarium specimens cataloged to determining that quantitative measurements of phytochemicals are accurate and precise. In the natural health product world, botanicals and their contained mixtures are considered the active ingredient (AI). Unlike single-chemical AIs, botanicals are variable because their composition depends on genotypic and phenotypic variation, geographical origin, weather exposure, harvesting practices, and processing. Complicating matters, while a large number of botanicals and parts thereof are in the marketplace in the form of relatively unprocessed dried pieces, powders, and tea cut biomass, various highly processed botanical AIs in the form of concentrates, metabolites, constituents, and extracts are also in the natural health product supply chain. These are essentially manufactured materials the nature and composition of which are defined by the processes used in their manufacture. Different proprietary processes used on biomass that is nominally the same will predictably result in AIs that are chemically and biologically different from each other and will likely be devoid of useful genetic material. Relatively unprocessed ingredients that are not AI (excipients, lubricants, binders) may also have botanical origins and are also present in finished products. The degree to which ingredients are processed prior to incorporation into finished products will thus dictate the tools that can be used in affirming identity. Morphological taxonomy can be used to identify relatively unprocessed materials (whole plants, tea cut pieces), while newly emerging DNA techniques can be used to identify materials that have undergone a bit more processing (finely powdered biomass). Organic solvent extracts used as raw materials will require techniques such as phytochemical fingerprinting (HPTLC, LC/MS, NMR) for identification purposes. This workshop will provide an overview of the challenges of botanical ID with respect to safety and regulatory compliance; discuss traditional approaches to botanical identification (including chemical fingerprinting and metabolomics approaches), and describe advances in the DNA technology and the utility of emerging techniques for the identification of plants and plant derived finished products in commerce.

FRIDAY MORNING - PLENARY

EPA/DHA Omega-3 for Health and the Prevention/Management of Chronic Disorders: Update 2014

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DHA (docosahexaenoic acid) is a physiologically-essential omega-3 fatty acid found at relatively high levels in the brain and retina of the eye where it mediates optimal cognitive functioning and visual acuity, respectively. Recent clinical trials have shown striking health benefits for newborn infants (lower frequency of very-low birth weights, lesser days in intensive care, others) through increasing DHA intakes by supplementation to well above typical N. Am. dietary intakes in mothers during pregnancy. Higher levels of DHA in breast milk via increased maternal intakes and DHA inclusion in formula have been associated with improved cognitive and visual outcomes in infants while reducing bronchial inflammation. DHA supplementation has been found to improve reading ability in school children with abilities well below average. The omega-3 in the form of LNA (alpha-linolenic acid) from plant food sources is converted with rather limited efficacy to DHA in humans. The current intake of DHA via fish/seafood in North America/elsewhere is well below target intakes as recommended for optimal health by various agencies worldwide. The omega-3 fatty acids from fish/fish oils in the form of EPA (eicosapentaenoic acid) plus DHA docosahexaenoic acid)have been found to offer major health benefits including the prevention and complimentary management of

chronic disorders. These benefits include the favourable modification of several risk factors for cardiovascular heart disease (marked lowering of blood lipid as triglyceride, moderate blood pressure-lowering, anti-thrombotic and anti-arrhythmic effects, others). For those taking cholesterol-lowering medication or having suffered a heart attack or stroke, supplementation with EPA/DHA has been found to lower serious cardiac events and stroke recurrence, respectively, on follow-up. EPA/DHA supplementation exhibits anti-inflammatory effects (eg, reduced joint pain in those with rheumatoid arthritis) and offers benefit in exercise-induced asthma, certain mental disorders, age-related disorders (such as dry-eye syndrome) and other conditions (incl. breast cancer very recently) based on population and clinical studies. Due to the low intake of fish/seafood in North America, there is a wide gap between current and target intakes of EPA/DHA for optimal health care. EPA/DHA supplementation via nutraceuticals or functional foods can also provide a marked lowering in health care costs as part of a preventive health care strategy if encouraged by governmental and health authorities.

NHP CHEMISTRY & QUALITY CONTROL

Application of UPLC-QTOF-MS in MSE Mode for the Rapid and Precise Identification of Alkaloids in Goldenseal (*Hydrastis canadensis*) and Bioassay

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Goldenseal has a long history of use in North American folk medicine and today has emerged as one of the 20 most popular herbal supplements used worldwide. The alkaloids present in goldenseal are believed to be the main bioactive compounds with various pharmacological effects including antibacterial, antifungal, and anticancer activities. The most abundant alkaloids in goldenseal are berberine, hydrastine and canadine. To date, a detailed understanding of the alkaloid composition of goldenseal has been lacking due to limitations in analytical technique and the availability of standards. Here we describe a new application of ultra-performance liquid chromatography coupled with an electrospray ionization quadrupole time of flight mass spectrometry operating in MSE mode (UPLC/Q-TOF-MSE) for the sensitive, fast and effective characterization of alkaloids in goldenseal. This approach allowed identification of alkaloids using a cyclic low and high collision energy spectral acquisition mode providing simultaneous accurate precursor and fragment ion mass information. A total of 45 compounds were separated and 40 of them characterized including one new compound and 7 identified for the first time in goldenseal. The spectral data obtained using this method is comparable to those obtained by conventional LC-MSn. However, the UPLC/Q-TOF-MSE method offers high chromatographic resolution with structural characterization facilitated by accurate mass measurement in both MS and MS/MS modes in a single analytical run; this makes it suitable for the rapid analysis and screening of alkaloids in plant extracts. The cytotoxicity of berberine, hydrastine and canadine was evaluated against A549, DLD-1 and WS1 cell lines. The results showed that berberine was the most active compound.

Metabolomics as an Exploratory Tool for Natural Health Products Research?

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Classically, understanding for the underlying chemistry governing NHPs has stemmed from targeted phytochemical analysis, which aims to quantify known compounds with biological activity through extraction, separation and detection methods. This approach is necessary to ensure quality and safety of NHPs, however it does not permit one to explore the 'unknowns' present in a sample. New technologies in metabolomics allow for the qualitative and quantitative analysis of all of

the metabolites present within a biological sample. Using an untargeted metabolomics approach, we explored the phytochemical complexity of cranberry (*Vaccinium macrocarpon* Ait.) and two commercial cranberry products. Multivariate and univariate statistical approaches were applied in conjunction with logical algorithms such as significant ion generation and synthetic biotransformation. Using this approach, a wide spectrum of flavonol and anthocyanin derivatives were detected in whole cranberry and commercial products, and suggests that the medicinal efficacy of cranberry is far more complex than once thought.

Characterization of a New Shrimp Oil Produced in New Brunswick, Canada

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Shrimp oil produced in the Acadian Peninsula, New-Brunswick, is a new product coming from the industrial transformation of shrimp (*Pandalus borealis*). While shrimps are harvested and transformed for human consumption, their by-products are further processed to extract the oil in a sustainable way. Being a new product, the chemical profiles of this shrimp oil have been studied using an array of analytical methods, ranging from HPLC-DAD to Raman spectroscopy. Shrimp oil harbours a dark red colour due to the presence of astaxanthin, and its characteristics will be presented. Total fatty acids profile (TFAP) and lipid class analyses were performed. Triglycerides with good levels of omega-3 and -6 fatty acids were established as the shrimp oil's main components. Other notable ingredients will be presented. In conclusion, the shrimp oil produced in New Brunswick, Canada presents many qualities entitling it as a prospective functional ingredient for natural health products. Funding sources: Atlantic Innovation Fund, Atlantic Canada Opportunity Agency, New Brunswick Innovation Foundation, National Research Council of Canada's NHP Program, Natural Sciences and Engineering Research Council of Canada.

Characterization of Astaxanthin Fatty Acid Esters from Shrimp Oil by LC-DAD and LC-ESI-HRMS

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Astaxanthin is an important carotenoid associated with potent antioxidant activity that provides beneficial effects to the immune system and contributes to the prevention of cardiovascular diseases. Astaxanthin and its natural formed esters are usually present in low concentrations and are often found in complex biological matrices. As part of NRC's efforts in developing value-added products from fishery processing residues, this study presents an analytical method for characterization of astaxanthin esters from shrimp oil using high pressure liquid chromatography on an HALO C8 media with DAD (detection at 476 nm) and ESI-HRMS detection. Characterization of astaxanthin and its esters was based on the accurate mass measurement performed in positive ion mode on a LC-ESI Orbitrap Exactive MS. Preliminary data showed a total of 31 peaks identified as astaxanthin derivatives in shrimp oil, among which 3 are in the free form, 10 are mono-esters and 18 are di-esters. Complete characterization of the other fatty acid esters of astaxanthin is ongoing. In conclusion, structural information of astaxanthin and its fatty acid esters in shrimp oil is provided using LC-DAD and LC-ESI-HRMS, which should aid in the standardization and quality control of shrimp oil ingredients used in functional foods or

natural health products. Funding source: Atlantic Innovation Fund; Atlantic Canada Opportunity Agency (ACOA); National Research Council of Canada's Natural Health Product (NHP) Program. Keywords: Shrimp Oil; Astaxanthin fatty acid esters; LC-DAD; LC-ESI-HRMS.

Improvement of Lavender Essential Oil (L. x intermedia cv Grosso): In vitro Genetic Modification of an Important Natural Health Product

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Lavenders (Lavandula) are essential oil producing members of the Lamiaceae family used in alternative and traditional medicine, and in particular aromatherapy, for the treatment of anxiety and other nervous disorders, depression and insomnia among others. Quality and bioactivity of a given essential oil and its relevant applications are defined by its terpenoid composition. Particular constituents such as camphor and 1,8-cineole favour use in the treatment of inflammatory disorders and alternative medicine, while those rich in linalool and linalyl acetate are valued for their pleasant scent and are used extensively in aromatherapy. The most widely grown commercial cultivar of lavender is the hybrid L. x intermedia cv Grosso (Grosso), which produces as much as six times more oil as related species, and has high levels of linalool, linalyl acetate, camphor and 1,8-cineole. In order to enhance oil quality, we have developed a protocol for the regeneration of Grosso and applied this protocol with chemical mutagenesis to produce oil mutants. Through screening of leaf essential oil, we have identified several unique mutant chemotypes. These plants represent potentially valuable new commercial cultivars, and provide important tools for studying the regulation of monoterpene biosynthesis. To identify the mutations responsible for the observed phenotypes we will employ forward genetic screening using next-generation RNA sequencing.

Application of Fourier Transform Near-Infrared (FT-NIR) Spectroscopy for Detecting Economically Motivated Adulteration in Raw Materials

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Botanicals in short supply, or those that are expensive; or new to the market are targets for Economically Motivated Adulteration (EMA). EMA occurs with substitution of the material with similar materials which may or may not cause undesired side effects. Nevertheless, substitution of similar but less expensive materials results in a product falling short of label claim. Dilution with maltodextrin, silicon dioxide and other "processing" aids may not be as harmful when ingested as other forms of EMA, but also result in a product falling short of label claim. Conformity Index algorithms that compare the deviation of an FT-NIR spectrum of a new material to established limits can be used to screen for EMA. The average spectrum of multiple scans/lots of a material known to be acceptable is calculated. Then, standard deviation of the absorbance values for that data set is calculated and the difference between the spectrum of an unknown sample and the reference is then compared at each wavelength. An absolute deviation is then weighted by the corresponding standard deviation on the respective wavelength and a conformity index value is calculated. In this study adulteration of Echinacea, cocoa and bilberry with maltodextrin was investigated. Conformity index plots shows that FT-NIR was able to detect adulteration with maltodextrin to at least the 1% level in those ingredients. Melamine adulteration in milk powders was also investigated and FT-NIR was able to detect down to lower levels, below 0.1% melamine adulteration, due to the sharp band for melamine at 6810cm-1.

NHPS AND CANCER

Complexity of Natural Health Products: Good or Bad? Long Pepper Extract Versus Piperlongumine in Cancer Therapy

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Natural health products (NHPs) have great promise in cancer research. The complexity and pharmacological properties of NHPs pose a difficulty in establishing a specific target and mechanism of action of many NHPs. However, this complexity might be advantageous, as multiple components act in synergy to target multiple pathways in cancer cells. Long pepper, from the Piperaceae family, has been used for centuries for various diseases. Several species of long pepper have been identified, extracts of which have various benefits; with reports indicating their effectiveness as pain and inflammatory suppressants and in the treatment of lung diseases and fevers. Some identified compounds in long pepper include piperines and piperlongumine (PL), which show effects on metabolic activities and oxidative stress response in cancer cells, respectively; however, the effective concentration of piperlongumine is high. We evaluated the anticancer potential of long pepper extract (water and ethanolic) in cancer cells. Interestingly, the ethanolic extract (PLX) showed high selective anticancer efficacy, in several human cancer cells, at very low doses. HPLC analysis of this extract indicated very low amounts of PL, suggesting that in addition to PL, other phytochemicals might be responsible (in synergy) for the observed cytotoxicity. PLX induced oxidative stress, mitochondrial potential collapse and apoptosis in cancer cells. Excitingly, oral administration of PLX reduced the growth of human colon cancer xenotransplants in nude mice. These results are proof that NHPs as complex mixtures, that could be delivered orally, presents better and safer options to conventional purified compounds for cancer treatment.

Fractionation and Activity Analysis of Dandelion Root Extract; Extensive Study of Efficacy and Mechanism of Cell Death Induction in Cancer Cells

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Substantial research has been carried out to delineate the mechanism of action and the medicinal components of natural extracts for clinical use, especially for the field of cancer; however, many complex extracts, which have high anticancer activity, are yet to be fully understood. At this juncture, finding a safer, non-genotoxic treatment for cancer remains a challenge. Our group is studying the anticancer properties of various natural compounds and extract, including dandelion root extract (DRE), which has shown unprecedented efficacy and specificity in inducing death receptor-mediated extrinsic apoptosis in several human cancer cells. The objective of this study was to deconvolute the activity of the complex mixture of DRE and identify the bioactive component(s), in both the active aqueous and ethanolic extracts. Three bioactive components have been identified; alpha-amyrin, beta-amyrin and lupeol. These components have shown better efficacy, when used in combination than alone. Furthermore, extensive analysis of the mechanistic pathways revealed that DRE is able to target multiple cell death pathways (extrinsic, intrinsic, as well as pro-death autophagy), in order to induce programmed cell death, specifically in cancer cells. We hypothesize that the multiple bioactive components of DRE work together in synergy to exploit the multiple vulnerabilities target and destroy cancer cells. These exciting findings of the study will provide scientific proof for an unprecedented mode of cancer treatment, one that uses a natural extract with few possible side effects and will bring us one step closer to understanding the important role NHPs play in the fight against cancer.

Novel Curcumin Analogues with Improved Anti-cancer Activity and Synergistic Effect in Combination with Piperlongumine

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Turmeric spice has been used in traditional medicine for centuries. The active ingredient of turmeric has been identified as curcumin, a polyphenol that has been investigated in many health avenues. It has been found to exhibit anti-inflammatory, hypoglycemic, antioxidant, and antimicrobial properties. Curcumin has also emerged as a potential chemopreventative and anti-cancer agent in many cancer cell lines. Unfortunately, curcumin is metabolized and eliminated very readily, resulting in low bioavailability, leaving a need for alternative options. This has led to our research project, which involves evaluating the anti-cancer effects of novel curcumin analogues, with potentially increased bioavailability. We have evaluated ten novel analogues, which were synthesized by Dr. Wang's lab at Wenzhou Medical College. Some of these compounds have been found to have anti-cancer activity in multiple cancer cell types, including breast cancer, colon cancer and osteosarcoma. The EC-50 of these compounds with respect to cytotoxicity to cancer cells has been found to be many fold lower than curcumin. Importantly, the curcumin analogues exhibit selectivity, as similar tests with non-cancerous cells show minimal cytotoxicity. In addition, combinatorial studies with the curcumin analogues and piperlongumine (a natural compound from Long Pepper) have yielded some exciting results and show a synergistic effect in inducing cancer cell death. The mode of action of these compounds in terms of inducing different forms of cell death is currently being investigated. In conclusion, the preliminary results of these novel analogues are very promising and we hope this project may lead to an improved cancer treatment.

Aqueous and Ethanolic Extracts of Neem Leaf as Inducers of Apoptosis in Cancer Cells

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In Canada, 187,600 people were diagnosed with cancer last year. The number of cancer patients diagnosed each year continues to grow; this increase in cancer incidence comes with an increase in deaths associated with cancer. Current chemotherapies are mostly genotoxic, causing harsh side effects due to non-selectivity towards normal cells. Our group analyzes the anti-cancer effects of natural products (NHPs), in order to find better effective and safe alternatives. We have previously demonstrated the efficacy of several NHPs, including dandelion root extract (DRE) and long pepper extract as potential anti-cancer agents. These interesting findings prompted further research into other natural products such as Neem (*Azadarichta Indica*), which has been used in Ayuverdic medicine for centuries. The objective of the study was to determine the possible anticancer potential of Neem extracts and to evaluate its possible mode of action. Our results indicate that both ethanolic and aqueous extracts of Neem were indeed effective in inducing apoptosis in leukemia and colon cancer cells. Further analysis with leukemia cells has suggested that Neem extracts have a caspase-independent mechanism of action, as caspase activation was not essential for apoptosis induction. Furthermore, an increase in the production of reactive oxygen species (ROS) was observed in isolated mitochondria from leukemia cells, indicating that the mitochondria are a potential target of this extract. Future works are required to further delineate the pathways involved in Neem induced apoptosis, as well as the bioactive component(s). These findings suggest the potential of Neem extracts as safer alternatives to conventional chemotherapy.

Piperlongumine Enhances the Anti-cancer Effect of Natural Pancratistatin and Synthetic Analogs

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The rapid cell growth and proliferation associated with cancer has been partly explained by the process of mitochondrial remodelling associated with the shift towards glycolytic metabolism. The mitochondrial remodelling confers a proliferative advantage and an acquired resistance to apoptosis. Additionally, cancer cells have also been shown to possess high basal levels of reactive oxygen species (ROS) as a result of these mitochondrial alterations and an associated increase in metabolic activity. As a result, cancer cells are predicted to be more dependent on ROS stress response mechanisms. Our research has found that the natural compound pancratistatin (PST) and synthetic PST analogues induce apoptosis in many cancers including osteosarcoma, glioblastoma, neuroblastoma, breast cancer, melanoma, prostate cancer, leukemia, and colorectal cancer cells by mitochondrial targeting in 2D and 3D culture. Specifically, they caused mitochondrial membrane potential collapse, and with isolated mitochondria led to increased production of ROS, and caused release of apoptogenic factors independent of p53 status. The natural compound piperlongumine (PL) has been shown to inhibit glutathione S-transferase pi 1 (GSTP1) and its associated defence mechanism against ROS accumulation. When these pro-oxidative PST analogues were used in combination with PL, an enhanced anti-cancer effect was produced. Furthermore, these analogues were able to reduce growth of human tumours in immunocompromised mice. Importantly, these analogues exhibited minimal toxicity in noncancerous cells and in mice. Hence, we present novel strategies targeting mitochondrial and oxidative vulnerabilities in cancer cells with PST and PST analogues combined with PL as potentially safe and effective alternative to current chemotherapeutics.

Holy Basil Extract - A Natural Antioxidant and Ayurvedic Medicine that Triggers Caspase-independent Apoptosis in Human Cancer Cells

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Throughout history, cancer has proven to be an incredibly difficult disease to overcome and treat. While there are many forms of chemotherapeutic drugs available today, very few of these have the ability to target cancer without toxic repercussions. In light of this, it is prudent to investigate the possibility of new selective and non-toxic remedies that could serve as alternatives to current chemotherapeutic strategies. Natural health products have proven themselves to be very promising in this regard, and have provided remedies for countless diseases in the past and in present society as well. Specifically, the Indian herb Tulsi (*Ocimum Sanctum*), commonly known as 'Holy Basil' has been shown to possess multiple health benefits for a number of diseases, including cancer; however, the details of anticancer effectiveness and mechanism are not fully understood. This study highlights the ability of aqueous and ethanolic extracts of Holy Basil to halt the proliferation of leukemia and colon cancer cells in a dose- and time-dependent manner. These extracts induce apoptosis in both cancer cell types, by targeting the mitochondrial vulnerability in cancer cells. Interestingly, the apoptosis induced by Tulsi extracts is independent of the caspase-mediated pathways. Mitochondrial reactive oxygen species (ROS) levels indicate that the aqueous extract possesses antioxidant properties, whereas the ethanolic extract showed pro-oxidant properties. With these results, it is clear that Holy Basil could represent a noteworthy and potentially safer alternative to current chemotherapy agents.

POSTER ABSTRACTS

Treatment with Extracts of *Uncaria tomentosa* Promotes Apoptosis in the Human Breast Cancer Cell Line, MCF7

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Uncaria tomentosa, commonly known as cat's claw, is a medicinal plant native to Peru, which has been used traditionally for the treatment of various inflammatory disorders and cancer. Some studies have shown that treatment of with *Uncaria tomentosa* promotes repair of cellular DNA in patients treated with chemotherapy drugs, preventing mutations and cell damage. Treatments with *Uncaria tomentosa* also inhibits inflammatory responses by inhibiting the proliferation of T and B lymphocytes, and decreasing the production of pro-inflammatory cytokines (IL-1, IL-6, and TNF-α). We are currently examining the effects of *Uncaria tomentosa* extracts on the growth of MCF-7 cells, a human breast cancer cell line. Our results have shown that treatment of MCF-7 cells with *Uncaria tomentosa* extracts inhibits their proliferation and promotes cell death in a dose–dependent manner. Further, extracts produced by boiling the ground roots in 70% ethanol are much more effective than extracts produced by boiling in water. *Uncaria tomentosa*-ethanol extracts potently induce cellular apoptosis as measured by changes in cell morphology and DNA fragmentation (TUNEL assays) within 24 h of treatment. We are continuing to identify the mechanism by which *Uncaria tomentosa* causes MCF-7 cell death to determine if it is a potential treatment for patients with breast cancer.

GC/MS Guided Terpenoids Isolation from Essential oil of Big Sagebrush (*Artemisia tridentata* Nutt)

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Larvacidal Big Sagebrush (*Artemisia tridentata* Nutt) was extracted using custom made pressure extracting device. GC/MS testing of the extracts designated artemiseole, eucalyptol, camphor and an unknown terpenoid. This unknown terpenoid is partitioned by methanol steam distillation and isolated by silica gel column. The structure of this terpenoid is still under investigation.

Structural and Biological Evaluation of Novel Anti-cancer Compounds

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Cancer therapy has traditionally featured small molecular drugs that target vital cellular processes including DNA replication and cell division. Paclitaxel, a natural compound purified from the bark of the Pacific yew tree, is one of these traditionally used chemotherapies. It has been shown to preferentially target rapidly dividing cancer cells by inhibiting depolymerisation of tubulin during mitosis. Despite the prominence of Paclitaxel in current chemotherapeutic regiments, its mechanism of mitotic inhibition has toxic consequences for noncancerous cells and results in

numerous deleterious side effects. The effective cancer-killing properties of paclitaxel coupled with the presence of severe side effects has prompted academic interest in identifying alternative chemotherapeutic agents that exploit cancer-specific vulnerabilities. Our research has identified two novel compounds by screening a pharmacophore library of 500 compounds. These compounds were synthesized through a simple De novo process by chemists at McMaster University. These two compounds were effective in reducing cell viability of cancerous cell in a time and dose dependent manner. They inhibit cell proliferation and induce apoptosis in various leukemia and osteocarcoma cell lines. The efficacy of these compounds is similar to paclitaxel at certain concentrations, however, despite approaching the cancer-killing capability of paclitaxel, the cytotoxic effects are minimal in noncancerous cells. Thus, the preliminary data suggests that these novel compounds can be a safer alternative to toxic chemotherapeutic agents used currently.

Uncaria Tomentosa as an Apoptosis Inducer in Melanoma B16- BL6 Cell Line

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Uncaria tomentosa, commonly known as Cat's claw, is a medicinal herb found in South America. It has been traditionally used in the treatment of many inflammatory disorders and has an antioxidant and anti-inflammatory properties. We have previously shown that *Uncaria tomentosa* can have anti-inflammatory activities on leukocytes. We are now investigating the effect of the *Uncaria tomentosa* as an anti- cancer therapy. We have shown that Uncaria tomentosa can inhibit the growth of the cell cultures and can induce apoptosis in the murine melanoma cell line B16- BL6. Extractions of Uncaria with 70% ethanol were more efficient at inducing apoptosis than water extraction. Apoptosis inducer was expressed on early 24h but almost all cells in the ethanolic extraction of Uncaria were inducing apoptosis by 72h. Uncaria caused an increase in DNA fragments (TUNEL assay), caspase-3 cleavage, sub G1 peaks in flow cytometry, and apoptotic morphology. Our experimental results indicate that *Uncaria tomentosa* can effectively kill melanoma cancer cells in vitro, in dose dependent manner, by enhancing apoptosis.

The Effect of *Nigella sativa* on Murine Melanoma B16-BL6 Cell Line

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Nigella sativa commonly known as black seed. It belongs to the botanical family of Ranunculaceae. *Nigella sativa* is used in traditional medicine in the Middle East and some Asian countries, as a treatment for many diseases including fever, asthma, and several types of cancer. A large number of in vitro and in vivo studies investigated that *Nigella sativa* has pharmacological properties such as, anti-inflammatory, antioxidant, and anticancer properties. Over the recent years there has been growing interest in natural products including *N. sativa* due to its promising anti-cancer effects. We have performed several in vitro studies to determine the effect of *N. sativa* on the growth of the malignant B16-BL6 melanoma cell line and non-malignant cell line. We have shown that treatment with N. sativa in ethanolic extract has significant inhibition of both malignant and non-malignant cell proliferation. Also, treatment with *N. sativa* in aqueous extract can reduce the growth of malignant cell proliferation while having lesser effects on non-malignant cell proliferation. We also demonstrated that *N. sativa* when extracted with ethanol can induce apoptosis in treated B16-BL6 cell line while *N. sativa* extracted in aqueous solution is much less effective. Furthermore, we will characterize the active components of the *Nigella sativa* that have an anti-cancer effects.

Lactobacillus reuteri Bacteria Shows an Anti-proliferative Effect on Colon Cancer Cells and Resistance to Simulated Intestinal Fluid While Producing Short Chain Fatty Acids Production

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Purpose. Few investigations had shown a potential effect of *Lactobacillus reuteri* in colonic health and specifically a potential biotherapeutic effect in CRC. The goal of this study is the screening number of L. reuteri strains, according to their SCFAs production and resistance to simulated intestinal fluids and the characterization of their inhibitory effect on colon cancer cells. Methods. *L. reuteri* strains were compared according to their production of SCFAs in the conditioned media (CM) and on their effect on cancer cell growth (Caco-2). The composition of SCFAs and lactic acid produced by bacteria in CM was used to prepare pure SCFAs mixtures. The anti-proliferative and apoptotic effect of the probiotic supernatant (PS) and conditionned medium (CM) towards colon cancer cells SW-480 was investigated compared to normal colon cells CRL-1831. Results. The results showed that the production of SCFAs was strain dependent and that some L. reuteri strains produced the higher amount of total SCFAs and inhibited the best Caco-2 cancer cell proliferation in vitro. SCFAs formulations corresponding to these strains were also the best at inhibiting CRC growth too. Selected probiotic treatments showed inhibitory effects cancer cells but not on normal cells and produced significantly more PA than other bacteria use as control. Conclusion. Some *L. reuteri* strains were considered PA-producer bacterium and have a better potential to be an effective agent in biotherapeutic strategy for cancer prevention.

Chemotaxonomy and Phytochemical Identification in Crataegus by Nuclear Magnetic Resonance Spectroscopy-based Metabolomics

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Crataegus, more commonly known as hawthorn, is a genus of flowering, fruit-bearing, small trees that is native to northern temperate zones, including areas of North America, Europe, and Asia. Hawthorn-derived products have practical applications, most importantly in traditional medicine and natural health products (NHP). Hawthorn NHPs are used as an alternative treatment for various cardiovascular ailments including congestive heart failure, angina, arrhythmia, and hypertension. NHPs of Crataegus have shown promising benefits for adjunctive therapy in treatment of chronic heart failure in previous literature. However, taxonomical confusion in the genus, termed the "Crataegus problem," is a problem resulting from minimal morphological variation between species and genetic complexity due to polyploidy with gametophytic apomixis in some Crataegus species. The aim of this research is to employ 1H nuclear magnetic resonance spectroscopy (NMR)-based metabolomics in order to develop a sophisticated method for chemotaxonomic investigation of various North American Crataegus species with simultaneous phytochemical characterization and quantification with emphasis on compounds thought to have positive impact on human health. This research will provide a method, using sophisticated sets of 1H NMR spectral data, to better elucidate relationships between North American Crataegus species—helping to simplify the Crataegus problem by providing strong chemical evidence for taxonomic purposes. By working out what is likely to be, or not to be, a unique species of hawthorn, and also revealing the composition of medicinal phytochemicals in some North American hawthorn species, this research benefits taxonomy and hawthorn-based NHP quality, consistency, and research and development.

Labeled Composition of Dietary Supplements (DS) with "Energy" in the Product Name in the Dietary Supplement Label Database (DSLD)

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Released in 2013, the Dietary Supplement Label Database (DSLD) contains complete label information on DS sold in the US. DSLD currently contains >25,000 labels, with 1000 labels being entered monthly. Objective: To profile dietary ingredients (DI) with and without Daily Values (DV) in DS sold as energy products. Methods: The "Quick Search" option in DSLD identified dietary supplements with "energy" in the product name. DI composition as declared within the Supplement Facts panel was compiled from information in DSLD.The LanguaL® Dietary Supplement Thesaurus was used to categorize the DI with and without DVs in these energy products. Results: 157 DS in DSLD had "energy" in the product name, (126 in non-liquid (mainly pills) and 31 in liquid [serving size >1 fl oz] form). Of the non-liquid forms, 52 of 126 (41%) declared caffeine on the label and 43 of 52 (83%) provided the amount of caffeine. Of the liquid forms 30 of the 31 (97%) and 9 of the 30 (30%) did so respectively. Conclusions: Manufacturers generally declare the amounts of DI with Daily Values (DV) within the Supplement Facts panel. The amounts of non-DV dietary ingredients are seldom declared. These DI are often components of "proprietary" blends. Healthcare practitioners will need other sources of information if they want to know amounts of these DI patients or clients are consuming. Healthcare practitioners should also know that these products can provide significant amounts of vitamins. Many energy products contain water-soluble vitamins at levels that far exceed (e.g., >1000%) recommended daily values.

The Dietary Supplement Label Database (DSLD)

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Dietary supplements (DS) are significant sources of nutrients and other bioactive substances. About half of adult Americans and a third of American children regularly consume DS. Objective: To develop a public database containing label information from virtually all dietary supplement products offered for sale in the US, with a Web-based interface providing ready access to the data. Method: An ad hoc Federal Working Group provides guidance on the collection, classification, and handling of information from DS labels. Results: Launched in June 2013, the database will capture most of the more than 55,000 different DS labels in the US marketplace. DSLD currently contains >25,000 labels, with 1000 labels entered monthly. DSLD reflects label contents and is available at http://www.dsld.nlm.nih.gov/dsld/. The name, form, and amount(s) of active and inactive ingredients, information about the manufacturer/distributor of products, label claims, warning statements, and percent daily value are captured, along with a photograph of the label. Simple and enhanced search options are available. Users can search for specific terms in any DS label field. DSLD is designed for easy use and also provides links to databases such as MedlinePlus®, PubMed® and NIH fact sheets. At present there is an option to download single labels to Excel. Conclusion: The DSLD captures label information not included in existing DS composition databases. It can serve as a useful resource for population-based surveys and other epidemiological studies. The DSLD is also a useful resource for healthcare professionals and consumers. The DSLD will be updated regularly to incorporate changing labels.

Release 2 of the Dietary Supplement Ingredient Database (DSID): National Estimates for Children's and Adult Multivitamins

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The Dietary Supplement Ingredient Database (DSID) is a federal initiative to provide analytically-derived estimates of ingredients in dietary. Release 2 provides data for children's multivitamins (MVMs), updates adult MVM estimates and is available at http://dsid.usda.nih.gov. Representative adult and children's MVMs were identified and purchased from multiple market channels. These supplements were analyzed for their vitamin and mineral content with certified reference materials. Mean percent differences from label were calculated for each nutrient and compared to labeled levels using regression analysis. The relationship between label and analytical content for each nutrient was identified as either linear (n=12) or quadratic (n=5). Mean % differences from label averaged 1- <10% above label for eight nutrients (zinc, phosphorus, magnesium, copper, iron, vitamin B-6, niacin, manganese); 10 to <20% above label for 5 nutrients (thiamin, folic acid, vitamin B-12, riboflavin, and calcium;); 20 to <30% above label for 3 nutrients (iodine and vitamins A and E) and >30% above label for vitamin D. Data estimates were linked to children's MVM products reported in the National Health and Examination Survey and can be used by researchers to more accurately quantify nutrient intake from dietary supplements.

Phytochemical Control of Humankind's most Deadly Enemy

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Mosquitoes are insect vectors for some of the most economically damaging diseases to humankind including yellow fever, Dengue fever, and malaria. We are investigating plants that produce a wide range of active phytochemicals that could be developed for control of the yellow fever mosquito A. aegypti, with limited or no environmental impact. A bioassay was developed to test larvicidal activity of plant extracts in aquatic environments. In standardized assays, 4th instar larvae of Aedes aegypti were exposed to various concentrations of plant-derived extracts for 24 hours. At the end of the 24-hour exposure period the mortality of larvae was determined. In proof of concept experiments, pentane and methanol extracts of Artemisia tridentata were evaluated using a minimum of 7 concentrations ranging from 1 to 3,400 mg/L. The lethal concentrations for 50 percent mortality (LC50) were determined using a PROBIT analysis. The data from these studies demonstrated the efficacy of Artemisia oils for controlling mosquito larvae and indicates a new direction for development of natural insecticides.

In Vivo Assessment of the Therapeutic Effects of Lyophilized Saliva Extract (LSE) from Leech (*Huridinaria Manillensis*) on PC3 Tumor Model in Nude Mice

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Introduction. Ancient Egyptian, Indian, Greek and Arab physicians used leeching for treating a wide range of diseases thousands of years ago. Recently, a large number of peptides and proteins have been

identified and characterized in leech saliva extract, such as anti-thrombin agents, metastatic inhibitors and anti-microbials. Currently, leech therapy is established as an important tool in microsurgery and reconstructive operations having demonstrated superior clinical outcomes for the optimal salvage of grafted tissues and amputated digits. Methods. In the current study we have evaluated, for the first time, the in vivo efficacy of Lyophilized Saliva Extract (LSE) from the tropical leech (*Huridinaria Manillensis*) in PC3 tumor model in nude mice representing androgen-unresponsive prostate cancer. We used 4 groups of male nude mice, six mice per group, which were subcutaneously administered with 0.5 mg/kg or 1 mg/kg LSE, Taxotere (15 mg/kg) as positive control or vehicle, respectively. Results. The data demonstrates that there is a significant decrease in the tumor growth profile of PC3 xenografts with either Taxotere or LSE (1 mg/Kg) treatment compared to the vehicle-treated control mice. Interestingly, there was no significant difference between the anti-tumor activity of Taxotere and LSE (1 mg/Kg). In addition, there was a significant decrease in the body weight of Taxotere-treated mice compared to that of the vehicle- and LSE-treated mice indicating that LSE treatment was less toxic than Taxotere in mice. Conclusion. LSE (1mg/Kg) has a significant anti-tumor activity as demonstrated in the PC3 prostate cancer xenograft model with no apparent side effects, as compared to Taxotere.

Tanshinone IIA Isolated from *Salvia miltiorrhiza* Induces Apoptosis in Human Oral Cancer KB Cells through a Mitochondria-dependent Pathway

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Tanshinone IIA (Tan IIA), an active phytochemical in the dried root of *Salvia miltiorrhiza* Bunge, has shown an anti-proliferative activity on various human cancer cell lines including nasopharyngeal carcinoma cells. However, the effects of Tan IIA on human oral cancer cells remain unknown. This study aimed to investigate the anti-proliferative effects of Tan IIA on human oral KB cells and explored the possible underlying mechanism. Treatment of KB cells with Tan IIA significantly suppressed cell proliferation/viability and induced cell death in a dose-dependent manner using Sulforhodamine B colorimetric assay. Analysis of cell morphology showed that the Tan IIA-induced growth inhibition of KB cells was associated with induction of apoptosis. Cell cycle analysis indicated exposure to Tan IIA resulted in cell cycle arrest at G2/M phase in KB cells using flow cytometry. The dissipation of mitochondrial membrane potential observed by using flow cytometry and the expression of caspases, and PARP monitored by immunoblotting analysis indicated that the induction of apoptosis by Tan IIA in KB cells was mediated through the mitochondria-dependent caspase pathway. These observations suggest that Tan IIA could be a potential anti-cancer agent for oral cancer.

In-vivo Hepatoprotective and Antioxidative Activities of *Glycosmis pentaphylla* Leaves Against CCl4-induced Oxidative Hepatocellular Damage

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The present study was conducted to evaluate the hepatoprotective and antioxidative potential of hydroalcoholic extract of Glycosmis pentaphylla leaves (GpE) and its fractions (chloroform, ethyl acetate & n-butanol). The toxicant CCl4 (1 ml/kg in olive oil; sc) was administered on 4th and 5th day to induce oxidative hepatotoxicity in rats. The hepatoprotection of the test drugs at various dose levels where compared to the reference drug silymarin (50 mg/kg). The pre-treatment of rats with higher dose of GpE (400 mg/kg) and EAf (150 mg/kg) for 7 days produced a significant ($p<0.05$) dose dependent hepatoprotective and antioxidative affect; evident by decreased levels of serum (AST, ALT, ALP & TB) and tissue TBARS and increased levels of serum (TP & ALB) and reduced tissue GSH level. Furthermore, the histological examination provided the supportive evidences that strongly demonstrate antioxidative affect on hepatocytes and restoring their normal functional

ability. The present study scientifically validates the free radical-scavenging property to be one of the mechanisms of hepatoprotection and justify the traditional medicinal claims attributed to this plant. Additional studies are in progress to elucidate bioactive constituent/s and their concentration in the plant.

Pterostilbene Inhibits the Matrix Metalloproteinase and the Urokinase Signaling Systems in Human Prostate Cancer Cells via Alterations in Cellular Signal Transduction Pathways

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Pterostilbene is an antioxidant in blueberries. Previously, wild blueberry (*Vaccinium angustifolium*) extracts were shown to inhibit in a dose & time dependent manner MMP-2 & MMP-9 activity in DU145 human prostate cancer cells in vitro. This study examined the effects of pterostilbene (PT) on MMP & urokinase activities in DU145 cells. PT (50 ug/ml)(6h) inhibited MMP-2/-9 activity. PT decreased cellular viability ~10% post 6 h. exposure. PT treatment resulted in increased TIMP-1/-2 & in decreased EMMPRIN & RECK protein levels. PT treatment decreased uPA & uPAR and increased PAI-1/-2 protein expression levels. PT treatment resulted in increased expression of pERK-1, pERK-2 & ERK1 protein levels. PT decreased p-p38 & p38 protein levels. No apparent change in either ERK2, JNK1 & JNK2 or p-JNK2 protein expression occurred in response to PT. PT did increase p-JNK1 protein levels. Treatment of cells with PT also resulted in increased protein expression levels of p-Akt & P-I-3 kinase p85 with no apparent change in either Akt or P-I-3 kinase p110 protein expression levels. Treatment of DU145 cells with PT (50 ug/ml) for 6 hours resulted in increased JAK1, STAT3 & STAT4 protein expression levels and decreased JAK2 & STAT1 protein levels with no apparent change in either JAK3 or STAT2 protein levels. These results suggest that pterostilbene (a blueberry "bioactive") has the ability to inhibit the expression of MMP-2/-9 & urokinase activities via alterations in cellular signal transduction mechanisms in DU145 cells. (Telus Motorcycle Ride for Dad (PEI Division) Prostate Cancer Research Fund)

Screening and Profiling for Enhanced Alkamide Production in Echinacea

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Alkylamides produced by *Echinacea purpurea* and E. augustifolia appear to be responsible for the antifungal and immunomodulatory effects of preparations and extracts of these herbs, and are being used as markers of their efficacy. Different activities are observed with the many alkylamides produced by Echinacea; for example diynoic alkamides show the greatest antifungal and cell wall disruption activities. We are pursuing multiple approaches to enhance yields of these compounds. Using in vitro techniques we are exploring the effects of environmental variables, and potential inducers of alkylamide synthesis. This work is also utilizing hairy root cultures produced from inoculation with a variety of Agrobacterium rhizogenes strains, and includes the de novo regeneration of new plantlets from these root cultures. Outside of axenic culture we are also preparing to screen differing genotypes, under a variety of conditions for variations in alkylamide production.

Paper Spray Ionization Mass Spectrometry for Analysis of *Crocus sativus* (Saffron)

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Paper Spray Ionization (PSI) is a method for separation and introduction of complex sample matrices into mass spectrometers. In PSI, the sample is applied to a piece of filter paper and a voltage (3.5 kV) applied. The position of tip from

the mass spectrometry cone (90o) and the distance between the cone and paper tip (5 mm) are important factors that be affect the ionization efficiency and/or peak intensity of analytes. The advantages of PSI are the limited sample preparation required, the potential for detection and quantification of metabolites that are not detected in electrospray ionization-based systems and the capacity to analyze whole tissues. In proof of concept experiments, we analyzed whole anthers and extracts of *Crocus sativus* for identification of know metabolites and metabolomics profiles. These data provide evidence of the potential of the technique for applications in food and natural products research.

Shrimp Oil Lowers Blood Glucose Levels and Improves Glucose Tolerance in Diet-induced Insulin Resistant Rats

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The objective of this study was to determine the anti-diabetic effect of shrimp oil in diet-induced insulin resistant rats. Male Wistar rats were divided into 5 groups. One group was fed an AIN-93G diet (normal control) and the other four were fed a high-fat (28%) and high fructose (20%) diet and provided with 20% fructose drink water for 14 weeks. The rats on the high-fat high-fructose diet were treated with 0 (insulin resistant control), 5, 10, and 20% of shrimp oil in the diet by replacing the same amount of lard. Fasting or semi-fasting blood glucose was measured every other week. Oral glucose tolerance tests (OGTT) were performed after 8 and 13 weeks and insulin tolerance tests (ITT) conducted after 6 and 12 weeks, respectively. Body weights were obtained weekly and food intake daily. It was found that the replacement of lard with 20% shrimp oil significantly lowered fasting and semi-fasting blood glucose levels. Rats treated with 20% shrimp oil also showed significant improvements of oral glucose tolerance and insulin response. Shrimp oil did not affect the body weight or food intake. The results demonstrate that the replacement of lard with shrimp oil is beneficial to glucose homeostasis and insulin function in rats with high-fat and high-fructose diet-induced insulin resistance. The research was supported by Atlantic Canada Opportunities Agency (Atlantic Innovation Fund grant 193594) and NRC’s Natural Health Products Program.