ABSTRACTS

"Reflections"

The 9th Annual Conference of the Natural Health Products Research Society of Canada

May 22nd to 25th, 2012

Delta Grand Okanagan

Kelowna, British Columbia, Canada

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Speaker Abstracts

Opening Plenary
Tuesday, May 22nd – Evening
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"My Journey with ginseng to inspire, innovate and discover"
Edmund MK Lui

Lui, Edmund M.K., Department of Physiology and Pharmacology Medical Sciences Building, Room 216 The University of Western Ontario London, Ontario, Canada, N6A 5C1

In spite of a surge in the use of herbal supplements in the 90s and early 2000s, the overall health supplement market has remained fairly stable in recent years. A current lack of understanding of the effects of some supplements and guidelines for their appropriate use are causing concern. Research in this area and product quality is needed for consumer confidence. However, there is a lack of funding support from conventional resources and there is no concerted effort and mechanism to stimulate collaboration between private sector and academia to address this issue. I will take this opportunity to share with you my recent experience with ginseng research that shows there are opportunities, but we need to take steps to face many challenges. Ontario is the world's largest producer of American ginseng and it is very important to the bioeconomy. There are many challenges and uncertainty facing the industry. In 2008, we established an Ontario-wide network (Ontario Ginseng Innovation and Research Consortium – OGIRC) through a $20.8 million operating grant from the Ontario Research Fund of the Ministry of Research and Innovation ($6.9 million), private industries, government laboratory and five partner academic institutions in the province of Ontario to investigate new technologies for ginseng agriculture and product development. I will emphasize on the work that was needed to mobilize scientists from different disciplines to engage in this interdisciplinary research and inspire scientists with not much prior knowledge and interest in botanical medicine research to be committed and to convince the private sector partners to contribute to the research. A progress report of our accomplishments and solutions to our challenges will be described

Past Presidents Plenary
Wednesday, May 23rd – Morning
Okanagan room

"Applications of Metabolomics to Medicinal Plants"
Susan J. Murch

Murch, Susan J., Chemistry University of British Columbia Okanagan Campus Kelowna, BC, Canada, V1V 1V7

The use of plants as sources of medicines and other valuable phytochemicals is widespread but the vast majority of the estimated 30,000 compounds in each individual plant tissue have never been isolated, identified or described. Traditional bioassay-guided fractionation usually begins with an active extract and proceeds through several chemical isolation and purification steps but activity is slowly lost through the process and in the vast majority of extracts, activity is completely lost before a compound is identified. There are two possibilities. Either the active principle is not stable in all of the various chemical matrices or more than one compound is required for the activity. Metabolomics can be used for compound discovery and to understand synergy in natural products since the full chemical composition of a complex active extract can be defined. In our research group, we are using metabolomics approaches to traditional medicines to identify unique "lead" compounds that are present in active bioassay fractions but missing from non-active fractions. The key to making this approach successful is the production of standardized active plant extracts and statistical methods to evaluate data quality and eliminate false discovery. This approach will discover novel bioactive compounds with limited chemical stability, lead to greater understandings of synergy and develop new ways of studying interactions of medicinal plants with human metabolism.

Lipids and Vitamin D
Wednesday, May 23rd – Morning
Okanagan Room

Opening Plenary

"Vitamin D is the Answer!--What's the Question?"
Carole A Baggerly

Baggerly, Carole A., Carole A Baggerly c/o GrassrootsHealth PO Box 234208 Encinitas, CA 92024

Prevention of preterm births to breast cancer are being reported to be affected by higher serum levels of vitamin D (100 nmol/L and higher). D*action is an international vitamin D intervention project with more than 10,000 participants. The project is being run by GrassrootsHealth, a non-profit public health promotion organization. Its target is to do bi-annual screening of vitamin D serum levels and track health outcomes to see what can be documented about large populations. To date, some key findings are about safety (much higher doses are safe) and what actually happens to the serum level with higher intakes. This presentation will address some of the data to date in the study plus share some public health strategies that are working.

"Cytochrome P450 3A4-mediated microsomal biotransformation of 1α,25-dihydroxyvitamin D3 in mouse and human liver: drug related induction and inhibition of inactivation"
Emma Guns

Tomlinson Guns, Emma S; Deb, Subrata and Adomat, Hans H, Vancouver Prostate Centre Jack Bell Research Centre 2660 Oak Street Vancouver, BC V6H 3Z6

Vitamin D3 activity is mediated via its dihydroxy metabolite, 1α,25(OH)2D3. Hepatic inactivation of 1α,25(OH)2D3 by cytochrome P450 (CYP) enzymes could be an important determinant of bioavailability in serum and tissues. In the present study, we investigated the comparative biotransformation of 1α,25(OH)2D3 in mouse and human liver microsomes and determined the effects of commonly used drugs on the inactivation of 1α,25(OH)2D3. To compare the effects of clinically important glucocorticoids on hepatic inactivation of 1α,25(OH)2D3, adult male CD-1 mice were treated with either vehicle (50% ethanol), dexamethasone (80 mg/kg/day) or prednisone (80 mg/kg/day) for three consecutive days by intraperitoneal injection. Following incubation of 1α,25(OH)2D3 with mouse liver microsomes (MLM), the hydroxy metabolite formation pattern and the extent of substrate depletion were similar in vehicle- or prednisone-treated mice, whereas treatment with dexamethasone led to emergence of additional metabolites and increased substrate depletion, as determined by liquid chromatography/mass spectrometry. The metabolite formation profile in vehicle-treated mice was different than that of human liver microsomes (HLM). Studies using specific CYP chemical inhibitors showed that CYP3A isoforms are responsible for the microsomal biotransformation of 1α,25(OH)2D3 in MLM. Co-incubation of 1α,25(OH)2D3 with commonly used drugs (e.g. ketoconazole, tamoxifen, ritonavir or clarithromycin) led to approximately 60-100% inhibition of CYP3A4-mediated inactivation of 1α,25-(OH)2D3 in HLM. In

conclusion, a species-based difference was identified between CYP3A-mediated hepatic microsomal metabolism of 1α,25(OH)2D3 in humans and mice and we have shown that commonly used drugs could affect vitamin D homeostasis.

"Lipid Replacement Therapy a glycophospholipid formulation with NTFactor, NADH and CoQ_{10}, significantly reduces fatigue in long-term intractable chronic fatiguing illnesses and chronic Lyme disease"
Garth Nicolson

Nicolson, Garth L., Settineri, R., Ellithorpe, R., Dept. of Molecular Pathology Institute for Molecular Medicine P.O. Box 9366 S. Laguna Beach, CA 92652

Background: A preliminary open label study was initiated to determine if a combination oral functional food supplement containing a mixture of phosphoglycolipids (NTFactor) [1], coenzyme Q_{10} and microencapsulated NADH (ATP Fuel) could affect fatigue levels in long-term patients with intractable fatigue. Methods: 58 patients (30 females, 28 males, av. Age 55) used the functional food ATP Fuel, each day for 60 days. Participants were patients with chronic fatigue syndrome (n=30), chronic Lyme disease (n=17) or other fatiguing illnesses (fibromyalgia syndrome or Gulf War illness, n=16). Participants had been symptomatic for >17 years, had seen >15 physicians and had taken >35 drugs or therapies without resolution of fatigue. Subjects were told to maintain normal activities and diet during the trial. Fatigue was scored using the Piper Fatigue Scale (PFS), a validated instrument that measures four dimensions of fatigue [2]. Results: Participants in the study responded to the combination test supplement, showing a 30.8% reduction in overall fatigue within 60 days ($P < 0.001$). Regression analysis of the data indicated that reductions in fatigue were gradual, consistent, and occurred with a high degree of confidence ($R2 = 0.960$). The regression analysis data suggested that further reductions were likely if the participants had continued the supplement beyond the 60-day trial. Analysis of fatigue subcategories indicated that significant improvements ($P < 0.001$) were present in every fatigue subcategory. Patients with the most severe forms of fatigue responded slightly better than those with milder fatigue, independent of their medical diagnoses. Conclusion: The combination supplement ATP Fuel, containing NTFactor, microencapsulated NADH and CoQ_{10}, was a safe and effective method to significantly reduce fatigue in long-term chronic illness patients with intractable chronic fatigue [3]. References: 1. Nicolson GL, Settineri, R. Lipid Replacement Therapy: a functional food approach with new formulations for reducing cellular oxidative damage, cancer-associated fatigue and the adverse effects of cancer therapy. Funct Food Health Dis 2011; 4: 135-160. 2. Nicolson GL, et al. Lipid Replacement Therapy with a glycophospholipid-antioxidant-vitamin formulation significantly reduces fatigue within one week. J Am Nutraceutical Assoc 2010; 13(1): 10-14. 3. Nicolson, G.L., Settineri, R. and Ellithorpe, E. Lipid Replacement Therapy with a glycophospholipid formulation with NADH and CoQ_{10} significantly reduces fatigue in intractable chronic fatiguing illnesses and chronic Lyme disease. Int J Clin Med 2012; in press.

"Novel probiotic formulation for managing cholesterol: clinical investigation of BSH-active *Lactobacillus reuteri* NCIMB 30242 yogurt formulation in hypercholesterolemic adults"
Satya Prakash

Jones M.L. , Martoni C.J., and Prakash S., Biomedical Technology and Cell Therapy Research Laboratory Department of Biomedical Engineering and Physiology Artificial Cells and Organs Research Centre Faculty of Medicine, McGill University 3775 University Street, Montreal, Quebec, H3A 2B4, Canada.

In quest to develop new heart health formulation we have designed and investigated the cholesterol lowering efficacy of a microencapsulated BSH-active *Lactobacillus reuteri* NCIMB 30242, yogurt formulation taken twice per day over 6 weeks, in hypercholesterolemic adults. A total of one-hundred fourteen subjects completed this double-blind, placebo-controlled, randomised, parallel-arm, multi-center study. This interventional study included a 2-week wash-out, 2-week run-in, and 6-week treatment period. Subjects were randomized to consume either yogurts containing microencapsulated *L. reuteri* NCIMB 30242 or placebo yogurts. Over the intervention period, subjects consuming *L. reuteri* NCIMB 30242 containing yogurts attained significant reductions in LDL-C of 8?92% (P=0?016), TC of 4?81%, (P=0?031), and non-HDL-C of 6?01% (P=0?029) over placebo, and a significant absolute change in apoB-100 of -0?19 (P=0?049) mmol/l. Serum concentrations of TAG and HDL-C were unchanged over the course of the study. Present results show that consumption of BSH active *L. reuteri* NCIMB 30242 yogurt is efficacious and safe for lowering LDL-C, TC, apoB-100, and non-HDL-C in hypercholesteromic subjects. The efficacy of BSH active L. reuteri NCIMB 30242 yogurts appears to be superior to traditional probiotic therapy and akin to that of other cholesterol lowering ingredients. Details of these and other studies will be discussed.

Plenary
Wednesday, May 23rd – Afternoon
Okanagan Room

"Why and How to Develop Traditional Herbal Medicine to Meet Unmet Clinical Needs"
Yung-Chi Cheng

Cheng, Yung-Chi, Yale University School of Medicine, New Haven CT 06520

Based on historical claims of many different traditional herbal medicines, a number of unmet health needs by current mainstream medicine could be helpful by those traditional medicines. Those unmet needs include the prevention and treatment of complicated diseases or symptoms such as metabolic diseases, autoimmune neuronal diseases and cancer as well as improvement of functions of individuals. In order to verify those claims, Consistent preparations of herbal medicine to perform credible clinic trials in providing evidence of the claims is critically important. The source of herbs is important for making high quality and consistent preparations of herbal medicine. Well-designed clinical studies to be accepted world-wide are also important for establishing the clinical efficacy, safety, and dosage to be used. A study of the interaction of herbal medicine and conventional drugs is important since the efficacy of conventional drugs could be compromised or enhanced by herbal medicines. Understanding the mechanisms of action and active compounds involved for each mechanism from herbal medicine will be helpful for better use or for the advancement of traditional herbal medicine. This presentation will be focused on those above mentioned key points. This work is supported by grant #1PO1CA154295-01 from NIH/NCI, USA. This abstract was also presented for the Plenary Lecture of The Pharmaceutical Society of Korea (PKS) in Jeju Island, Korea, April 19, 2012.

The Chemistry of Natural Health Products
Wednesday, May 23rd – Afternoon
Cascade/Cassiar Room

Opening Plenary

"Natural products from *Angelica archangelica* in Iceland"
Sigmundur Gudbjarnason

Gudbjarnason, Sigmundur, SagaMedica

Natural health products have been developed following extensive research on bioactive phytochemicals in *Angelica archangelica* in Iceland. The product SagaPro was developed for treatment of noctura or frequent urination at night and was marketed 2005. In 2011 SagaPro underwent a clinical trial carried out by an independent company Encode. The results of this double blind,

placebo controlled trial showed that SagaPro is safe and effetive in treatment of nocturia. The product SagaPro is effective in treatment of overactive bladder in men and women. A second product is SagaMemo made from a mixture of Angelica tincture and a tincture from Geranium sylvaticum. These tinctures have anticholinesterase activity and the mixture shows synergy. SagaMemo is used to enhance memory and reduce the risk of dementia. SagaMedica ehf. is the company developing these and several other natural health products. The company is also developing products that could be effective in treatment of cancer. An extract of the leaves contains natural products that prevent breast cancer cells from develpoing tumors in mice. The furanocoumarins in seeds prevent proliferation of several types of human cancer cells in tissue culture. The products SagaPro and Angelica tincture have been used together to arrest tumor growth in people with prostate cancer or inoperable sarcoma. These products do not cure cancer by removing the tumors but they prevent further growth of the cancer and the patients can lead a normal life.

"Characterization of Antioxidant Capacity from Fruits with Distinct Anthocyanin Biosynthetic Pathways"
Elham Hesseini Beheshti

Beheshti, Elham H. and Guns, Emma S.

"Essential Oil from the common herb Oregano may be an effective Antimicrobial Agent"
Giuseppe Cannillo

Cannillo, Giuseppe, Salerno, Antonio, Via Paolucci 3 Corato, Bari 70033 Italy

During recent years, plant essential oils have come more into the focus of phytomedicine. Their widespread use has raised the interest of scientists in basic research of essential oils. Especially, the antimicrobial and antioxidant activities of essential oils as well as their potential anti-cancer activity have been investigated in recent years. Essential oils are volatile, natural, complex compounds characterized by a strong odour and are formed by aromatic plants as secondary metabolites. Due to their bactericidal and fungicidal properties, pharmaceutical and food uses are more and more widespread as alternatives to synthetic chemical products to protect the ecological equilibrium. Oil from the common herb oregano may be an effective treatment against dangerous, and sometimes drug-resistant bacteria. Two studies have shown that oregano oil and in particular carvacrol, one of oregano's chemical components appear to reduce infection as effectively as traditional antibiotics. There has been growing interest in the utilization of nanoemulsions in the food, beverage, and pharmaceutical industries because they have a number of potential advantages over conventional emulsions for certain applications. Nanoemulsions have the ability to greatly increase the bioavailability of highly lipophilic substances encapsulated within them. Our research has found that by creating nanoemulsions we were able to enhance the antimicrobial activity of Oregano essential oil against many pathogenic bacteria, and have also reduced the cellular toxicity of the essential oil by creating nanoparticles, suitble for human ingestion.

"Identification of mosquito (*Aedes aegypti*) deterrent compounds from male inflorescence of breadfruit (*Artocarpus altilis*)"
Max Jones

Jones,A.M.P., Klun, J.A, Cantrell, C.L., Ragone, D., Chauhan, K.R., Brown, P.N., and Murch, S.J., 50 Stone rd. E Guelph, ON N1G 2W1

The dried male inflorescences of breadfruit (*Artocarpus altilis*, Moraceae) are traditionally burned in communities throughout Oceania as a smudge to repel flying insects such as mosquitoes. The present study was conducted to identify the chemicals responsible for mosquito deterrent activity. A series of crude extracts were evaluated, and the most active, the hydro-distillate, was used for further chemical analysis and bioassay-guided fractionation. The hydro-distillate and all resulting fractions displayed significant deterrent activity. Exploratory GC-MS analysis revealed more than 100 distinctive peaks, and more than 30 compounds were putatively identified, including a mixture of terpenes, aldehydes, fatty acids, and aromatics. A systematic bioassay-directed study using adult Aedes aegypti females identified several saturated fatty acid, capric, undecanoic, and lauric acid, as the primary deterrent compounds. A synthetic mixture of commercially obtained fatty acids present in the most active fraction, and individual fatty acids tested alone, were all significantly more active than N,N-diethyl-m-toluamide (DEET). These results provide support for the efficacy of this traditional practice and indicate the potential of male breadfruit flowers as a value added agricultural product and of fatty acids as alternative mosquito repellents.

"Detection Of Anti-Inflammatory Triterpenoid Saponins From The Kenyan Medicinal Plant *Ruellia prostrata*"
Christine Ong'ayo Wangia

Wangia, C.O., Kanui, T.I., Waweru, P.M., Wakori, E.W.T., Kareru, P.G., Mount Kenya University Department Of Pharmacology P.O. Box 342-01000 Thika-Kenya

Ruellia prostrata is a perennial creeper with widespread medicinal uses including anti-arthritic activity. We conducted both organic and aqueous extraction with the ultimate object of characterizing the plant's bioactive compounds. Sequential organic extraction of the ground whole plant material with chloroform, dichloromethane (DCM), petroleum ether (PE) and methanol was by maceration, while aqueous extraction was by boiling. The extracts were subjected to phytochemical analysis for the presence of terpenoids, steroids and saponins. Thin layer chromatography (TLC) and Fourier transform infrared (FTIR) spectroscopic analyses were performed on the crude drugs. TLC results showed six spots in the methanol extract, two spots in both the chloroform and DCM extracts and one spot in the PE extract. FTIR spectra revealed the following spectra; PE; C-Hstr at 2920 and 2851 cm-1, C=C-Cstr aromatic at 1460 cm-1 and C-O str at 1377 cm-1. DCM extract; O-Hstr at 3393 cm-1, C-H str, C-H str (2924 and 2855 cm-1); C=Cstr (1628 cm-1) and C-O-C at 1042 cm-1. Chloroform extract; C-H str (2920 and 2851 cm-1) and C=C str (1655 cm-1). Methanol extract; O-H str at 3339 cm-1, C-Hstr (2855 cm-1); C=Cstr (1628 cm-10 and C-Ostr (aromatic, at 1389 and 1269 cm-1). Characteristic peaks for saponins are; O-H absorption (3265-3393 cm-1), C-H absorption ranging from 2851-2924 cm-1, C=C absorbance (1628-1655 cm-1) and oligosaccharide linkage (C-O-C) absorption due to sapogenins (1036-1042 cm-1). The IR spectra confirmed presence of saponins in all the crude drugs, and thus *R. prostrata* was confirmed to contain saponins,

which may confer its biological activity. KEY WORDS: *Ruellia prostrata*, saponins, glycosides, anti-inflammatory, FTIR spectra, biological activity.

"Evaluation of FT-NIR as a Tool for Optimizing Nutraceutical Dry Powder Blending"
Robert Cocciardi

Kradjel, Cynthia, Cocciardi, Robert, Lee, Sunghee, Soderberg, Alexander, 30 Rue de Syracuse, Candiac, QC, J5R 0B6

In Nutraceutical dry powder blending, re-work and long blend times can inflate cost and affect profit margin. Uniform blending can be challenging when formulas use powders with dissimilar particle size and densities. This study investigates the use of Fourier transform near infrared (FT-NIR) for testing the identity of raw materials and for optimizing dry powder blending. Raw materials of 15 different fruit and vegetable powders were evaluated. Samples were poured into glass vials and placed onto the integrating sphere window for measurement in diffuse reflectance. An identity test model was developed by calculating the Euclidean distance between the different groups using vector normalized spectra. The selectivity value (S), the ratio of the distance between average spectra of two groups to the sum of their cluster radii, was used to evaluate their degree of discrimination. A value for S>2 at the 99% confidence level was obtained for 11 out of 15 raw materials using the entire NIR spectral region. A sub-library for cranberry, pomegranate, raspberry and strawberry resulted in a value for S>2 at the 99% confidence level when using principal component analysis. Reference spectra of 40 50/50 kale spinach blends were compared to spectra taken during the blending process. A conformity index, CI< 3.0, at each spectral data point determined the end point of the process. Valid, fit for purpose FT-NIR identity test methods can be developed for raw materials used in fruit and vegetable blends and manufacturers can reliably monitor the end point of the blending process.

Complementary Therapy and Diet
Wednesday, May 23rd – Afternoon
Okanagan room

"The Effect of Diet and Inflammation on Cancer"
Gerald Krystal

Ho, Victor W., Hamilton, Melisa J., Bennewith, Kevin L., Lam, Vivian, Lin, Ann H-A., and Krystal, Gerald, Terry Fox Laboratory British Columbia Cancer Research Centre 675 West 10th avenue Vancouver, B.C. V5Z 1L3 Canada

Since cancer (CA) cells are typically far more dependent on glucose than normal cells for their growth and survival, we asked if we could lower blood glucose levels sufficiently through diet changes alone to significantly slow tumor growth. Specifically, we compared the effects of low carbohydrate (CHO), high protein diets to a Western diet on the growth of tumors in mice and found they significantly slowed their growth. Strikingly, in a mouse model of breast CA, nearly 50% of the mice on a Western diet had tumors by 1 year of age whereas none were detected in mice on the low CHO diet. Moreover, whereas only 1 mouse on the Western diet reached a normal life span, more than 50% of the mice on the low CHO diet reached/exceeded it. This was associated with weight gain in the Western- but not the low CHO-fed mice and this was interesting since obesity triggers chronic inflammation, which increases the risk of CA. With regard to inflammation, our immune system is comprised of an innate and an adaptive (acquired) immune component. The innate component dictates the type of adaptive immune response evoked via the intensity/makeup of the cytokine "storm" it generates. In contrast to chronic inflammation, acute inflammation, especially if it triggers a robust anti-viral response, leads to tumour regression and we hypothesise that a simple blood-based assay that quantifies the nature/intensity of the cytokine "storm" will predict the susceptibility of individuals to CA and we will discuss preliminary results with this assay.

"Omega fatty acids and choline: Micronutrients for brain development and health"
Sheila Innis

Innis, Sheila, Department of Pediatrics, University of British Columbia, Room 179, 950 West 28th Avenue Vancouver, BC V5Z 4H4

"Salvestrols and CYP1B1 - a Natural Team Combating Cancer Naturally"
Dan Burke

Burke, Dan, Professor M. D. Burke, CARE Biotechnologies, Charnwood Science Centre, 103 High Street, Syston, Leicester LE7 1GQ UK

Salvestrols are one of the most recently discovered groups of phytonutrients and are found in many fruits, vegetables and herbs in our normal diet. They are a fine example of natural anticancer

prodrugs and depend for their efficacy on activation by a human enzyme, CYP1B1, which is intrinsic to cancer cells. CYP1B1 is a characteristic of all types of human cancer. It is so highly overexpressed in cancer cells and so minimally expressed in critical normal tissues, that in most instances CYP1B1 cannot even be detected in normal cells. As a result, as evidenced in human cell culture, Salvestrols are able to kill cancer cells without harming normal cells. Salvestrols, once activated, are both cytotoxic and cytostatic. They can kill cancer cells by stimulating apoptosis. It is probable that, historically, Salvestrols have been an important contributor to dietary protection against cancer. Unfortunately the modern Western diet is grossly deficient in Salvestrols. A main cause of this deficiency is probably the routine treatment of commercial crops with synthetic agrochemical fungicides. This is because Salvestrols are a tiny subgroup of the large class of antipathogen phytochemicals called phytoalexins, and are accordingly produced by plants mainly in direct response to fungal infection. Fruit and vegetables that have been grown in strict accordance with organic principles can have much higher level of Salvestrols. However, for various other reasons, a diet of commercially obtained fruit and vegetables, even if largely organic, is unlikely to provide a consistently adequate intake of Salvestrols.

"Curcumin reduces neoplasms in colitis-associated cancer via effects on ILK, beta-catenin, cyclin D, NF-kappa B and PKB activation"
Kiran Assi

Assi, K and Salh, B, Jack Bell Research centre Rm 436a 2660 oak Street Vancouver BC, V6h 3Z6

Aims: Curcumin is known to possess several anti-inflammatory and anti-neoplastic properties, and has been shown to attenuate inflammation in murine colitis models as well as human IBD. However its role in colitis-associated cancer is presently unclear. Methods: We utilized a well -characterized model for studying curcumin's effects on colitis-associated cancer; an initial dose of azoxymethane was followed by 3 cycles of 7 days 2.5% DSS in the drinking water, alternating with 14 days off. Neoplasms were counted and size measured by microscopy; immunohistochemistry was performed to assess expression of ILK, ser473PKB, beta catenin, cyclin D, NF kappa B nuclear localization; HCT116 cells were used to investigate potential mechanisms. Results: There were 11.2 (+ 4.5) tumors/mouse greater than 2mm in diameter in the control group as compared with 3.4 (+ 2.5) tumors/mouse in the curcumin-treated group ($p<0.001$). This was accompanied by significant reductions in the levels of expression of ILK ($p<0.01$), ser473PKB ($p<0.01$), and nuclear localization of cyclin D ($p<0.001$), NF kappa B ($p<0.01$) and beta-catenin ($p<0.01$). Exposure to curcumin reduced the level of ILK expression in HCT116 cells, which was significantly reduced by prior exposure to the proteosomal inhibitor MG132. Conclusions: Curcumin reduces neoplasm frequency in cancer-associated colitis via interruption of Wnt signaling as well as reducing levels of ILK (and downstream ser473PKB) expression. This is achieved in part through activation of the proteosome. This compound may have a role in cancer chemoprophylaxis in patients with chronic IBD.

Past Presidents Plenary
Thursday, May 24th – Morning
Okanagan room

"Natural Health Products and Diabetes: Show me the Evidence!"
Vladimir Vuksan

Vuksan, Vladimir, Department of Nutritional Sciences and Risk Factor Modification Centre, St. Michael's Hospital, Faculty of Medicine, University of Toronto and Toronto, ON, Canada

Today, approximately 220 million people live with diabetes with the incidence steadily on the rise. The growing popularity of dietary supplement including medicinal herbs and use in addition to prescription medicines in the management of diabetes has driven a surge of a wide array of products on the market. Despite indications for their purported efficacy in regulating diabetes, there is a disconcerting lack in clinical evaluation of existing natural health products for their perceived health benefits and potential risks associated with use. At present, limited evidence steaming from clinical trials exists investigating the safety and efficacy of products that may affect blood glucose control and associated complications of diabetes. A brief overview of popular products offered in Canada along with data on clinical efficacy to date will be discussed. Some of the treatments presented will include available evidence on cinnamon, chromium, fiber, oily-grain, herbs such as ginseng and conjugated linoleic acid, among others. In conjunction with conventional medicine for the adequate treatment of diabetes in some patients, supplementation with some evidence-based complementary dietary and herbal remedies, along with lifestyle factors, may generate greater success in controlling the disease and associated risk factors in these affected individuals.

Standards of Evidence for Functional Foods and Natural Health Products
Thursday, May 24th – Morning
Okanagan Room

Opening Plenary

"Developing Quality Specifications for Natural Health Products: an Evidence Based Approach"
Paula N. Brown

Brown, Paula N. (1,2); Finley, Jamie P. (1); Le, Cuong H. (2,3); Murch, Susan J. (2) (1) Natural Health & Food Product Research Group, Centre for Applied Research and Innovation, British Columbia Institute of Technology, 3700 Willingdon Avenue, Burnaby, British Columbia, Canada, V5G3H2 (2) Chemistry, University of British Columbia, Okanagan Campus, 3333 University Way, Kelowna, British Columbia, Canada, V1V 1V7 (3) University of Victoria - Genome BC Proteomics Centre, Vancouver Island Technology Park, #3101-4464 Markham Street, Victoria, British Columbia, Canada, V8Z 7X8

The demand for natural health products continues to grow as North Americans discover the potential health benefits, turn to alternative forms of medicine and seek to improve the quality of their lives. Despite evidence indicating dietary intake of *Vaccinium macrocarpon* Aiton (cranberry) may have health benefits, investigators have been challenged to turn epidemiological observations into concrete information about the role of specific cranberry phytochemicals in disease prevention. Many specifications for quality in use today were originally designed to allow buyers and sellers to judge "quality" according to some pre-arranged characteristic, are not necessarily reflective of product efficacy. An evidence based quality assurance approach would ensure that substances being measured are relevant to the target botanical and not simply surrogate markers of quality. Using Vaccinium macrocarpon Aiton (cranberry) as an example, two different approaches to phytochemical characterization, quantitative measurements of anthocyanins in cranberry fruit and metabolomics profiling coupled with chemometric analyses, are compared. Considerations of method rigor extend from analytical method validation to quality assessments of multivariate data models. Different approaches for identifying metabolites of significance, such as the area under the receiver operating characteristic (ROC) curve and the significance analysis of microarrays (SAM) statistic, will be discussed. Together the data and associated statistical analyses demonstrate the potential for mass spectrometry-based metabolomics to serve as tool for characterization of natural health products.

"Paradoxical Immunomodulating Effect of North American Ginseng Aqueous Extract"
Chike Azike

Azike, Chike G, Department of Physiology and Pharmacology University of Western Ontario London, Ontario N6A 5 C1, Canada

The objective of this study is to examine the paradoxical immuno-modulatory effect of North American (NA) ginseng Aqueous (AQ) extract. Nitric oxide (NO) production in culture by pulmonary alveolar macrophages isolated from adult rats treated orally with 125mg/Kg AQ extract

for 3 or 6 days, were used to evaluate the influence of ginseng on immune function. Both treatments resulted in significant increase in NO production as compared to cells from non-terated control group. Moreover alveolar macrophages isolated from rats treated with ginseng for 6 days showed a reduction in NO response to Lipopolysaccharide (LPS) infectious-inflammatory challenge in culture; this observed effect perhaps reflects an immuno-suppressive effect of orally administered ginseng. This ex vivo anti-inflammatory effect could be replicated in in vitro study which showed similar desensitization of LPSI response in murine RAW 264.7 macrophages by pretreatment with AQ or crude polysaccharide (PS) fraction for 24 hr prior to LPS challenge.

"Quantitative Analysis of Aloe Vera by NMR, Automated Using the Assure-RMS Software"
Kim Colson

Markus, Michelle A.; Colson, Kimberly L. and Gafner, Stefan, Bruker BioSpin 15 Fortune Drive Billerica, MA 01821

Aloe vera is a medicinal plant with a wide range of uses from topical application to soothe burns to oral consumption to aid digestion. It is added to a wide range of health and beauty products. Analyzing Aloe vera extract in detail, components include glucose, acetylated mannose polymers, and malic acid. As the material ages, degradation products include acetic acid, lactic acid, formic acid, and fumaric acid. Common additives include the preservatives sodium benzoate and potassium sorbate. Depending on the formulation, other additives such as glycerol may be present. A 1H-NMR spectroscopy-based method was previously developed to quantitate some of these components [1]. We have implemented and extended this method within the Assure-RMS software package to provide automated analysis of Aloe vera samples. The automated analysis will be described, first presenting the readily quantitated components, emphasizing the features of the spectra of these components that lend themselves to robust analysis. Then more problematic components will be examined. Strategies to improve the quantitation, including additional data and more sophisticated analysis, will be discussed. References: [1] Jiao et al. (2010) J. of the AOAC International, Vol. 93, p 842-848.

"Standards of Evidence for Schedule A Diseases"
Sharan Sidhu

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Scientific evidence is used to substantiate health claims for non-traditional natural health products (NHPs). Not all evidence is created equally and evidence is graded into levels of "strength" where clinical trial data would be the strongest form of evidence. Evidence requirements are stipulated by the product and the depth of the claim. Where a claim for treatment of a specific condition may require clinical evidence a more non-specific claim for example "for the maintenance of good health" may be supported by lower levels of evidence. Although there is some very unarguable data on the use of natural sources and their derivatives; if aimed at the general public, NHPs cannot claim treatment or cure for Schedule A diseases. This class includes diabetes, cancer, depression, asthma and hypertension and although prevention claims can be made, the standards of evidence are as rigorous as a treatment or curative claim. Animal models and data generated from small clinical studies, serve as background information and are insufficient for schedule A disease preventative

claims. The strongest form of evidence comes from well-designed, appropriately analysed, prospective Phase III intervention studies, followed by other forms of clinical trials. NHPs can also fall into the Professional Use Only category, which is currently in development by NHPD and could possibly include treatment or curative claims for Schedule A diseases. The standards of evidence would still apply and in its entirety the evidence must support the safety and efficacy of the NHP for the recommended conditions of use.

"LC-SPE-NMR/MS for the structure elucidation of natural products"
Markus Godejohann

Godejohann, Markus, Bruker Biospin GmbH Silberstreifen 4 76287 Rheinstetten

The coupling of chromatography to NMR and MS spectroscopy is a very effective tool for the characterisation of unknown natural products. Post column solid phase extraction enables the enrichment of chromatographic peaks and offers the advantage of elution with pure organic deuterated solvents. This allows fast dereplication of already known structures and opens the path for ab initio structure elucidation using the NMR and MS data acquired. Software tools are available to generate structures which are consistent with the NMR data provided. Examples of succesfull application of this approach will be presented.

"Clinical Research Investment in the Natural Health Products and Functional Foods Sector"
Krista Coventry

Coventry, Krista J., Nutrasource Diagnostics Inc. 120 Research Lane, Suite 203 University of Guelph Research Park Guelph, Ontario, Canada N1G 0B4

The highly competitive marketplace for natural health products and functional foods has driven stakeholder interest in clinical research. There are many reasons to consider a clinical trial, including desire for product-specific safety and/or efficacy data, compliance with regulatory requirements for health claims, to gain a competitive advantage in the marketplace, and/or to support expansion into new markets. Traditionally, clinical trials were sourced solely to support the safety and efficacy of a medicinal ingredient or combination of medicinal ingredients. However, regulatory agencies are now seeking this high level of specificity and research more regularly when evaluating health claims for natural health products and functional foods. Furthermore, with an increased interest in personalized nutrition and wellness, today's consumers are demanding support for product health claims. Given the significant investment and expertise required to design and execute a successful clinical trial, many stakeholders are seeking guidance and resources. This session will provide an overview of clinical trials for natural health products and functional foods and will provide insight into considerations for clinical research in this sector.

Natural Health Products and Neurodegenerative Diseases
Thursday, May 24th – Morning
Cascade/Cassiar room

"Effects of a traditional Cree anti-diabetic medicine on glucose tolerance and cognitive function in a mouse model of Alzheimer's Disease"
Carolina Cieniak

Cieniak, Carolina; Ahmed, Fida; Granger, Matthew W.; Taylor, Matthew W.; Juzwik, Camile; Haddad, Pierre S.; Foster, Brian C.; Arnason, John T.; Bennett, Steffany A.L., University of Ottawa 20 Marie Curie, Room 283 (Gendron Hall) Ottawa ON K1N6N5

Type II diabetes (T2D) may be a risk factor for Alzheimer's Disease (AD), where AD patients have elevated insulin levels and decreased insulin sensitivity. However, the exact mechanism linking the two disease states remains to be elucidated. AD09, a plant used by the Cree of Eeyou Istchee in the treatment of diabetes, was used to treat TgCRND8 transgenic mice ectopically expressing human amyloid precursor protein with Swedish (KM670/671NL) and Indiana (V717F) mutations. TgCRND8 mice and their non-transgenic littermates were fed the ethanolic extract of AD09 at a concentration of 250 mg/kg daily for two months. The learning and memory response of the mice was then evaluated using the Morris Water Maze followed by a glucose and insulin challenge to measure their metabolic response. We found that AD09 may alter the ability of Tg mice to learn, as measured by escape latency and search strategy, while having no impact on vehicle-fed and NonTg animals. This effect may be mediated by AD09's ability to alter insulin sensitivity in Tg mice (Supported by CIHR TGF-96121 and MOP6286 to JTA and SALB).

"Problems with Detection and Quantification of beta-Methylaminoalanine (BMAA) in NHPs"
W. Broc Glover

Glover, W. Broc Murch, Susan J., University of British Columbia Okanagan Campus Kelowna, British Columbia, Canada, V1V 1V7

Beta-methylaminoalanine (BMAA) is a naturally occurring non-protein amino acid biomagnified from cyanobacteria to plants to animals to humans through several food chains. Since many different cyanobacteria product BMAA, the potential for BMAA contamination of the cyanobacterial NHPs sold as Spirulina should be investigated. Spirulina is incorporated into a wide range of NHPs juices to supplements, powders and extracts as a "green protein" marketed as a healthy alternative to animal-based proteins. Spirulina is produced in large ponds or greenhouse-style growing systems as a mixed cyanobacterial culture primarily containing 2 species, Arthrospira platensis and Arthrospira maxima, and may also contain other species. While some labs have consistently reported detection of BMAA in many species of cyanobacteria, other labs have not detected BMAA leading to confusion, speculation and controversy. BMAA analyses are complicated by insufficient sensitivity, diversity in sample matrices, and a failure to detect BMAA at the predicted values in mass spectra. Therefore, the first requirement for determination of whether

BMAA is a contaminant in Spirulina is the development of a rugged, accurate method for detection and quantification in cyanobacteria and NHPs. Our research shows that BMAA is incorporated into proteins and can form a series of metal adducts and complexes. Therefore, methods designed to quantify only free BMAA at the molecular mass produce false negative data in cyanobacterial samples. A comparison of 3 methods showed both positive and negative identification of BMAA in the same NHP product indicating that an optimized, rugged method is required to ensure safety and quality of Spirulina products.

"Halting the progression of parkinsonian neurodegeneration by water soluble formulation of CoQ_{10}"

Krithika Muthukumaran

Muthukumaran, Krithika., Facecchia, Katie., Laframboise, Alyson., Sandhu, Jagdeep K., Sikorska, Marianna., Smith, Jessica., Harrison, Kate., Keshen, Corrine., Lopatin, Daniel., Cohen, Jerome., Weinstock, Shelly., Miller, Harvey., Lathier, Patricia., Pandey, Siyaram Department of Chemistry and Biochemistry, 401, Sunset Avenue, Windsor, Ontario Canada N9B 3P4

Oxidative stress is one of the most important factors leading to neurodegeneration and hence the pathophysiology of diseases, such as Parkinson's disease (PD) and Alzheimer's. It has been shown that exposure to environmental toxins such as paraquat, a commonly used herbicide, can lead to an increase in the incidence of PD. An effective therapeutic that could halt the progression of neurodegeneration has not been available so far. We use a water soluble formulation of CoQ_{10} (WS-CoQ10) and have tested its ability to protect neurons both in vivo and in vitro. The in vitro results have shown that this drug is effective in protecting neurons from neuronal toxins. Prophylactic studies show that WS-CoQ10 helps protect dopaminergic neurons in rodents subjected to toxic insult (MPTP in wild type and transgenic DJ-1 mice, paraquat in rats). It has also been shown that WS-CoQ10 prevents progression of neurodegeneration in both rodent models of PD post injury. We evaluated the neuroprotective effects in terms of behavioural recovery and the number of tyrosine hydroxylase positive neurons in the substantia nigra region of the brain. The results suggest that our water soluble formulation has better bioavailability and therapeutic efficacy and can be used in the treatment of PD. It is effective in lower doses than the other formulations of CoQ_{10} currently available in the market. It is already FDA GRAS approved and can be produced for testing in human clinical trials.

"Liuwei Dihuang (LWDH), a traditional Chinese medicinal formula, protects against beta-amyloid toxicity in transgenic *Caenorhabditis elegans*"
Junzeng Zhang

Sangha, Jatinder S.1, Sun, Xiaoli 2,3,4, Zhang, Kaibin 2,3, Ji, Xiuhong 2, Wang, Zhimin 3, Wang, Yanwen 2, Zidichouski, Jeffrey 2, Prithiviraj, Balakrishnan 1,*, Zhang, Junzeng 2,** 1 Department of Environmental Sciences, Nova Scotia Agricultural College, Truro, NS, B2N 5E3, Canada 2 Institute for Nutrisciences and Health, National Research Council Canada, Charlottetown, PE, C1A 4P3, Canada 3 Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, 100700, P. R. China 4 School of Traditional Chinese Medicine, Capital Medical University, Beijing, 100069, P. R. China1 Department of Environmental Sciences, Nova Scotia Agricultural College, National Research Council Canada 550 University Avenue, Charlottetown, PE C1A 4P3

Liuwei Dihuang (LWDH), a classic Chinese medicinal formula, has also been used to improve or restore declined functions related to aging process and geriatric diseases, such as impaired mobility, vision, hearing, cognition and memory. Recently, studies had revealed the beneficial effects and some possible mechanisms of LWDH in protecting or improving learning performance, cognition and memory in animal models, although the mechanisms of action are still largely unknown. As such, the effects and possible mechanisms of LWDH in protecting beta-amyloid related paralysis were studied in invertebrate model *Caenorhabditis elegans*. Chemical profiling of LWDH-WE (water extract) and LWDH-EE (ethanol extract) revealed the presence of different bioactive components in LWDH-EE as compared to LWDH-WE. Among various polar components identified in the extracts using HPLC and NMR, LWDH-WE was rich in monosaccharide dimers and trimers whereas total phenolic compounds including gallic acid and paeonol were higher in LWDH-EE (60.43±3.56 mg) than LWDH-WE (14.97±0.36 mg) per gram as gallic acid equivalent. In vitro studies revealed higher DPPH radical scavenging activity with LWDH-EE extract than LWDH-WE. When tested against in vitro aggregation of Abeta, both LWDH-WE and LWDH-EE were not effective. However, in vivo studies with transgenic *C. elegans* strain CL4176, with inducible expression of Abeta with temperature upshift, showed that Abeta-induced paralysis phenotype was strongly alleviated with LWDH-EE treatment. Interestingly, beta-amyloid deposits in *C. elegans* (CL2006), which constitutively express Abeta, were low with LWDH-EE at 4th day but the difference was less evident at 8th day after treatment. In addition, in vivo production of reactive oxygen species (ROS) was reduced in LWDH-EE treated worms than the untreated worms that correlated with increased sod3::GFP fluorescence. Real time gene expression analysis revealed that expression of transgene amy1 was reduced whereas hsp16.2 was increased several fold at 15 h after temperature up shift. Analysis of transgenic worms with hsp16.2::GFP revealed increased levels of fluorescence with LWDH-EE post 2 h thermal stress suggesting for a role of heat shock proteins in stress alleviating properties of LWDH that might be important in lowering beta-amyloid toxicity. Taken together, these results suggest that LWDH extracts alleviate beta-amyloid induced toxicity, in part, through increased heat shock proteins, reduced ROS, and antioxidant activity. Key words: Liuwei Dihuang (LWDH); beta-amyloid toxicity; *Caenorhabditis elegans*; Alzheimer's disease.

Natural Health Products and Cancer
Thursday, May 24th – Afternoon
Okanagan room

Opening Plenary

"Lipid Replacement Therapy: a Functional Food Approach for Reducing Cancer-Associated Fatigue and the Adverse Effects of Cancer Therapy"
Garth Nicolson

Nicolson, Garth L., Department of Molecular Pathology Institute for Molecular Medicine P.O. Box 9355 S. Laguna Beach, CA 92652

Background: Cancer-associated fatigue and the chronic adverse effects of cancer therapy can be reduced by Lipid Replacement Therapy (LRT) using a membrane lipid-antioxidant-vitamin mixture given as a food supplement [1,2]. Recent clinical trials using cancer and non-cancer patients with chronic fatigue have shown the benefits of LRT in reducing fatigue and restoring mitochondrial electron transport function [3, 4]. In addition, LRT reduced the frequency and severity of adverse effects of chemotherapy, resulting in improvements in incidence of fatigue, nausea, diarrhea, impaired taste, constipation, insomnia and other quality of life indicators [5]. Objective: To examine the effects of NTFactor®, a functional food, on fatigue in cancer and non-cancer patients with moderate to severe fatigue. Methods: Patients (n=67, av age=57.3) diagnosed with various levels of chronic fatigue severity received an oral mixture of membrane glycophospholipids, vitamins and minerals (NTFactor®; Physician's Advanced Formula, Nutritional Therapeutics, Inc.) for one week. Fatigue was assessed by the Piper Fatigue Scale before, during and after patients received supplements [6]. Results: Using the Piper Fatigue Scale there was a 36.8% reduction ($p<0.001$) in fatigue in one week. There was no difference between the response of males and females, and no adverse effects of the supplement occurred during the study. Conclusion: LRT with NTFactor® appears to be a useful, nontoxic method to reduce fatigue in patients with or without cancer. References: 1. Nicolson GL. Lipid replacement/antioxidant therapy as an adjunct supplement to reduce the adverse effects of cancer therapy and restore mitochondrial function. Pathol Oncol Res 2005; 11:139-144. 2. Nicolson GL. Lipid replacement therapy: a nutraceutical approach for reducing cancer-associated fatigue and the adverse effects of cancer therapy while restoring mitochondrial function. Cancer Metastasis Rev. 2010; 29(3): 543-552. 3. Agadjanyan M, et al. Nutritional supplement (NTFactor) restores mitochondrial function and reduces moderately severe fatigue in aged subjects. J Chronic Fatigue Syndr 2003; 11(3):23-26. 4. Nicolson GL, Ellithrope R. Lipid replacement and antioxidant nutritional therapy for restoring mitochondrial function and reducing fatigue in chronic fatigue syndrome and other fatiguing illnesses. J Chronic Fatigue Syndr 2006; 13(1):57-68. 5. Colodny L, et al. Results of a study to evaluate the use of Propax to reduce adverse effects of chemotherapy. J Am Nutraceutical Assoc 2000; 2(1):17-25. 6. Nicolson, GL, et al. Lipid Replacement Therapy with a glycophospholipid-antioxidant-vitamin formulation significantly reduces fatigue within one week. J Am Nutraceutical Assoc 2010; 13(1):10-14.

"Evaluating The Efficacy Of Dandelion Root Extract as an Anticancer agent In Highly Aggressive and Resistant Cancers"
Pamela Ovadje

Ovadje, Pamela. Ma, Sabrina. Nicoletti, Leah. Hamm, Caroline. Pandey, Siyaram, Department of Chemistry & Biochemistry University of Windsor 401 Sunset Avenue, Windsor, ON N9B 3P4

According to the Canadian cancer statistics for 2011, every hour of every day, an average of 20 people will be diagnosed with some type of cancer and eight people will die from it. At this point, it is still a challenge to find a safe and non-toxic form of chemotherapy in the treatment of cancer. Dandelion extracts have been used for centuries for the treatment of various diseases, ranging from diarrhea to hepatitis. We have previously shown the effectiveness aqueous extract of dandelion root (DRE) in actively inducing programmed cell death selectively in Jurkat cells. Further studies in our lab have shown that DRE efficiently reduced viability and induced selective apoptosis and autophagy in aggressive cancer cells, including chronic myelomonocytic leukemia, pancreatic cancer, melanoma and colon cancer, with no toxicity to noncancerous cells. Apoptosis was induced by the rapid activation of the death-receptor mediated pathway of apoptosis, where we observed rapid activation of caspase-8, followed by caspase-3 activation. In addition, mitochondrial membrane potential collapse, increased ROS production and induction of pro-death autophagy was observed. More importantly, we have studied the effect of DRE in Peripheral blood mononuclear cells (PBMCs) isolated from newly diagnosed leukemia patients. DRE efficiently induces apoptosis in a dose and time dependent manner in ex-vivo samples, without affecting PBMCs from healthy volunteers. Furthermore, oral administration of DRE inhibited the growth of human colon cancer and melanoma xenotransplants in immunocompromised mice. These results present a novel non-toxic alternative to conventional chemotherapy.

"Parthenolide's anti-leukemic stem cell activity is enhanced by the inhibition of dipeptidyl peptidases 8 and 9"
Paul Spagnuolo

Spagnuolo, Paul A., Hurren, Rose, Gronda, Marcela, Maclean, Neil., Schimmer, Aaron D., Health Science Campus University of Waterloo 10 Victoria Street South Kitchener, Ontario N2G 1C5

Parthenolide (PTL) is the bioactive component of the medicinal plant, Feverfew, and is sold as an herbal extract for the treatment of migraines. It induces specific toxicity to leukemia stem cells; however, PTL also activates cell protective effects that limit its clinical application. Therefore, we sought to identify agents that synergistically enhance PTL's stem cell cytotoxicity. Using a high-throughput combination drug screen, we identified the oral hypoglycemic, vildagliptin, which synergized with PTL to induce death of the leukemia stem cell line, TEX (combination index (CI) = 0.36 and 0.16, at EC 50 and 80, respectively; where CI < 1 denotes statistical synergy). The combination of PTL and vildagliptin reduced the viability of cells from acute myeloid leukemia patients but had no effect on the viability of normal human peripheral blood stem cells. The basis for synergy was independent of vildagliptin's primary action as an inhibitor of dipeptidyl peptidase (DPP) 4. Rather, using chemical and genetic approaches we demonstrated that the synergy was due to inhibition of the related enzymes DPP 8 and 9. In summary, these results highlight DPP 8 and 9 inhibition as a novel chemosensitizing strategy in leukemia stem cells. Moreover, these results suggest that the combination of vildagliptin and PTL could be useful for the treatment of leukemia.

"Selective Cytotoxicity in Aggressive Human Cancers Induced by *Piper longum* Extract"
Siyaram Pandey

Ma, Dennis., Tremblay, Phillip., Tuffley, Ian., Gupta, Manika., Ma, Sabrina., Ovadje, Pamela., and Pandey, Siyaram., Dept. of Chemistry and Biochemistry University of Windsor 401 Sunset Ave. Windsor, ON N9B 3P4

Nature has long provided an abundance of products used for the treatment of countless ailments. In contrast to most chemotherapeutics for cancer therapy, many natural extracts could offer safe and effective treatment without harsh side effects. The *Piper longum* plant has been used in traditional medicine to treat various ailments. In this study, we reveal Piper longum extract (PLE) to possess potent anti-cancer properties. PLE induced apoptosis selectively in multiple cancer cell types as seen with nuclear condensation and externalization of phosphatidylserine. Such apoptotic induction by PLE was not observed in noncancerous cells. PLE-induced-apoptosis in cancer cells was caspase-independent as the Z-VAD-FMK, a broad spectrum caspase inhibitor was not able to inhibit PLE-inducedcell death. PLE-induced apoptosis was caused by oxidative stress as the anti-oxidant N-Acetyl-L-cysteine was able to partially rescue cancer cells from PLE. Release of the apoptogenic factors such as apoptosis inducing factor (AIF) and endonuclease G (EndoG) from isolated cancer cell mitochondria and mitochondrial membrane potential dissipation in whole cells induced by *Piper longum* extract suggests mitochondrial targeting by this natural extract. Furthermore, we showed efficacy of PLE in inhibiting tumor growth in an in vivo model. PLE was found to decrease the growth of human colon tumor xenografts in immunocompromised mice. Thus, we illustrate PLE to potentially be an efficacious and safe option for cancer therapy.

"Treatment with flaxseed oil (*Linum usitatissimum*) induces cellular apoptosis in B16-BL6 cancer cells"
Alison Buckner

Buckner, Alison L., Buckner, Carly A., Lafrenie, Robert M., 4236 Marlene Court Hanmer, ON P3P 1E1

The high concentration of fiber-based lignans and omega fatty acids contribute to the classification of flaxseed oil as a functional food. Flaxseed oil is often taken by cancer patients undergoing chemotherapeutic treatments because of its unusually high levels of omega-3 fatty acids. We have examined the anti-cancer effects of flaxseed oil by studying its direct effects on cancer cell growth and cell death in vitro. A total of seven different oils containing high concentrations of omega fatty acids, including flaxseed, olive, sunflower, canola, sesame, peanut and grapeseed oils were characterized by HPLC and GC/MS analysis for fatty acid profiles and used to treat cells. Treatment with flaxseed oil was associated with a rapid slowing of growth by the aggressive murine melanoma cell line B16-BL6. Treatment of B16-BL6 with each of the other characterized oils showed no significant changes in cell growth. DNA fragmentation gels, fluorescence microscopy and detection of Caspase and PARP cleavage by Western blotting of cellular proteins have shown that the treated cells are undergoing apoptosis. Additional experiments have indicated a potential mechanism for the activity of flaxseed oil in the induction of apoptosis. Our results suggest that flaxseed oil may contain a specific agent that can aid in the optimization of anti-cancer treatment.

“TBA”
Mary Hardy

Hardy, Mary

Industry: The Herbal Perspective
Thursday, May 24th – Afternoon
Cascade/Cassiar room

"NHP Industry - The Herbal Perspective"
Barbra J Johnston

Johnston, Barbra J., 300-2130 Leckie Place Kelowna BC

A snapshot of a small companies transition from home based business virtually unregulated to a 30,000 square foot fully regulated facility with 50 employees. Our success and challenges working with local herb growers, the NPN process and competing in a non-enforcement environment. How we have been able to market and gain momentum from the Canadian regulations in the US and overseas. How and why as a company we feel it is important to be part of the solution to the challenges of the regulations not part of the problem.

"Challenges of Formulating and Researching with Changing NHPD rules"
Terry Willard

Willard, Terry, 2613 14st Sw, Calgary AB, Canada, T2T 3T9

I have been formulating and researching in the international Natural Food industry since 1975. While each country has their own specific laws and rules, I have found the last several years to be some of the most challenging and frustrating for the Canadian market place. The Standards of Evidence (SOE) is an ever-moving target. I have been on Federal government committees since the late 1980's, and presently a member of NHP-PAC. I can now say with some degree of trepidation that there appears to be a bit of light at the end of the tunnel. My lone term focus has been on multi-ingredient formulas, mostly of botanical and fungal origin. These are areas that seem to be the most challenging. In reviewing some of the trends and research that are used for other countries, which ones are rich for us to look at for the future Canadian market?

"Marketing the Science of NHPS's – Challenges with Fact & Fiction"
Gary Leong

Leong, Gary, Jamieson Labs. 4025 Rhodes Dr. Windsor, ON. N8W 5B5

Commercial success with a Natural Health Product is a staged process that involves many different parties at different points. Researchers, Supplier, Manufacturers, Regulators, Laboratories, Retailers and Customers; they all play a role in what products are in demand and which ones are available in the Canadian Market. It is a dynamic, ever changing environment that startles the worlds of breakthrough science and snake oil showmanship. The challenges and opportunities of operating within this environment will be discussed.

"Using a 3x3 to Understand Botanical Natural Health Products"
Steven Dentali

Dentali, Steven J., American Herbal Products Association 8630 Fenton Street, Suite 918 Silver Spring, MD 20910 USA

This presentation will briefly introduce the work of the American Herbal Products Association (AHPA) and cover basic botanical product issues required to properly comprehend the universe of botanical materials. Three basic concepts will be introduced in order to describe botanicals from their raw material stage to their finished products. These concepts focus on 1) the botanical material stage, 2) botanical ingredient degree of purification, and 3) the relationship between constituents and extract potency. Each of these three concepts will be understood as differentiated into three subcategories so that a comprehensive framework from which to understand and discuss botanical materials used industrially can be provided. The first concept will clarify the separate botanical forms of crude raw materials, ingredients, and finished products. The second concept will explore the continuum from crude extracts to purified chemicals by providing the three basic types of extracts, which are largely determined by their complexity. The final concept will explain the three possible relationships between identified constituents and the bioactivity of the extracts containing them. Understanding this "three by three" approach of basic concepts, which permeate the industrial use of herbal materials, will help users more knowledgeably navigate the field of natural health products.

"Quality Control Challenges in Dietary Supplements: Accidental and Intentional Adulteration of Botanical Raw Materials, Herbal Extracts, and Essential Oils"
Mark Blumenthal

Blumenthal, Mark, Founder & Executive Director, American Botanical Council; Editor, HerbalGram & HerbClip, American Botanical Council PO Box 144345 Austin, TX 78714

The trade of botanical ingredients for the production of herbal drugs and phytomedicines, dietary supplements, and natural cosmetics is global, with supply and quality issues in one geographical region affecting other areas. Chemical complexity of botanicals requires added quality control diligence for raw material suppliers and manufacturers. In recent years there have been numerous cases of accidental misidentification of botanical materials due to nomenclatural confusion, lack of adequate quality control measures, etc. Also, there have been persistent cases of inadvertent contamination with heavy metals, agricultural chemicals, excessive microbial load, excessive solventlevels in extracts, etc. But there is also the disturbing trend of intentional adulteration -- economically motivated adulteration (EMA) -- as well as the"spiking" of extracts with undisclosed lower-quality and lower-cost ingredients. This also includes the spurious and illegal addition of activepharmaceutical ingredients (conventional pharmaceutical drugs), e.g., sildenafil in products masquerading as dietary supplements for erectile dysfunction and sibutramine in weight-loss products. Additionally, it is possible that solvents used in the production of botanical extracts may exceed residual levels deemed appropriate by domestic and international authoritative bodies. These are areas of great importance to healthcare practitioners, particularly those who routinely recommend dietary supplements to their patients. This presentation reviews many of these quality

control challenges and notable cases of safety concerns and economic fraud created by them as is being compiled by the American Botanical Council, the American Herbal Pharmacopoeia, and the National Center for Natural Products Research at theUniversity of Mississippi in a non-profit consortium called the ABC-AHP-Botanical Adulterants Program. There will also be information on solvents and solvent residues as documented in a new publication on solvents from ABC.

"Ensuring Safety, Traceability, Qality and Ethics from the Field and Forest to the Finished Product"
Connie Kehler

Past Presidents Plenary
Friday, May 25th – Morning
Okanagan room

"Revolutions in Natural Health Product Research: Past, Present, and Future Paradigms"
Allison McCutcheon

McCutcheon, Allison R. 4118 West 12th Avenue Vancouver, BC V6R 2P6

The Structure of Scientific Revolutions (Thomas Kuhn, 1962) is widely regarded as one of the most important scientific treatises of the 20th century and a landmark in intellectual history. Kuhn asserted that major changes in scientific fields do not come about gradually through patient, orderly investigations by established researchers but rather that revolutionary breakthroughs occur when conventional theory-embedded methods fail to solve new problems. Citing as examples the dramatic changes in world view that Copernicus, Galileo, and Einstein invoked, he described these rapid transformations in scientific thought as paradigm shifts. In 1997, the largest demonstration of democracy in Canadian history catalyzed the formation of Health Canada's Natural Health Product Directorate (NHPD) and the subsequent enactment of the NHP Regulations. After five years of comprehensive NHPD stakeholder consultations on research priorities and obstacles, a consensus on strategies to facilitate significant improvements in NHP research was developed. Scientists from academia, industry, and government founded the NHP Research Society to foster the changes needed to enable and promote scientifically meaningful research. Although NHP research has proliferated over the past ten years, scientists have soldiered on in their orderly, reductionist approach and evidence that the field is building towards a revolutionary cusp remains elusive. Will enough momentum be gained to make a genuine paradigm shift to a balanced melding of western scientific thought and traditional cultural medicine? What progress has really been made and what are the most promising future directions? The expert opinions of leaders in the NHP field were polled for answers to these questions and to identify the most noteworthy research advances over the past decade. A captivating synthesis of their responses will be showcased in this presentation utilizing the engaging new Prestize platform.

Natural Health Product Regulation
Friday, May 25th – Morning
Cascade/Cassiar room

Opening Plenary

"Health Product Interactions"
Brian C. Foster

Foster, Brian C., 278 D Dalehurst Drive Nepean, Ontario K2G 4J5

Substances within all health products (biologics, drugs, foods and natural health products) have the potential to interact with other health products. The interaction may be either pharmacokinetic or pharmacodynamic in nature resulting in decreased, increased (boosted, pharmacoenhanced) or unchanged activity. The reversible or irreversible interactions occur when substances within health products compete for the same receptor, metabolic enzyme or transport protein active sites or shunt the substance and their metabolites through other pathways. The interaction may be associated with the pharmacologically active agent or another substance such as an excipient or botanical constituent such as the furanocumarins present in grapefruit juice and other foods. Health products can affect more than enzyme and protein such that clinically relevant interactions adversely affecting the safety and efficacy of health products are possible, particularly in populations on multiple products or with polymorphisms affecting disposition. The presentation will examine the potential of health products to affect the metabolism of human cytochrome P450 isoforms and transport, in vitro models for interaction studies, and how in vitro results may translate to the clinical situation.

"Regulatory Challenges for Natural Health Products in Food Format"
Krista Coventry

Coventry, Krista J., Nutrasource Diagnostics Inc. 120 Research Lane, Suite 203 University of Guelph Research Park Guelph, Ontario, Canada N1G 0B4

Innovation is at an all-time high in today's competitive wellness market, as companies try to develop a niche for their unique health products. There has been a particular increase in product development in the functional food and beverage category. Many of these innovative food and beverage products contain ingredients and/or make claims which result in their classification as Natural Health Products in Canada. These "food-type Natural Health Products" continue to pose a unique regulatory challenge for stakeholders seeking access to the Canadian market, as they must conform to the regulations for Natural Health Products. Health Canada provided formal guidance for industry in 2009 with the publication of the "Classification of Products at the Food-NHP Interface: Products in Food Format". However, in the NHPD Status of Submissions Quarterly Report (2011-Q2), Health Canada indicated that review of these Natural Health Products in Food format had been halted. Industry has since anticipated a transition of several or all categories of "Natural Health Products in food format" over to the regulatory framework for food. The first transition was realized recently in the energy beverage category in October of 2011, with further

Health Canada guidance pending. This session will provide insight and perspective on Natural Health Products in food format and will discuss regulatory challenges for industry during this transition.

"The Regulation of Natural Health Products (NHPs) in Canada"
Scott Sawler

Sawler, Scott, Natural Health Products Directorate Health Products and Food Branch, Health Canada 2936 Baseline Road, A.L. 3303B Ottawa, ON CANADA K1A 0K9

Health Canada's responsibility with respect to Natural Health Products (NHPs) is to assess products intended to be sold in Canada to assure that they are safe, effective, of high quality and are labelled appropriately to enable informed choice by consumers. The Natural Health Products Regulations set out the standards and requirements for the sale of NHPs in Canada, and post market monitoring of NHPs helps assure the safety of products available to consumers. Health Canada employs risk based strategies to establish the appropriate level of oversight for NHPs in Canada – resources are focused on higher risk products. Health Canada is striving to reduce the administrative burden for bringing NHPs to market in order to facilitate better access to these products and support industry innovation.

"Challenges in the Authorization of Clinical Trials Involving Natural Health Products"
Robin J. Marles

Marles, Robin J., Robin J. Marles, Ph.D. Director, Bureau of Clinical Trials and Health Sciences Natural Health Products Directorate Health Products and Food Branch, Health Canada 2936 Baseline Road, A.L. 3302C Ottawa, ON CANADA K1A 0K9

Clinical trials involving natural health products (NHPs) in Canada are subject to the Natural Health Products Regulations. Quality is one of the most problematic issues observed during our assessment of almost 400 NHP clinical trial authorization applications. Multiple botanicals, e.g. for Traditional Chinese Medicine studies, must each be assessed. Botanicals have more than one active constituent or marker and clinical trials may involve standardized, highly concentrated extracts. These issues make assessment of identity, purity, and potency, and thus safety and likelihood of efficacy, very complex. Other challenges encountered include insufficient data for chemical contaminants (e.g. heavy metals, solvent residues, pesticides, and mycotoxins), stability of the investigational product throughout the trial period, proprietary manufacturing information, and the potential for interactions. Most NHP clinical trials are for serious health conditions. This gives rise to concerns regarding the appropriateness of the clinical protocol and its statistical power, progression from preclinical studies to Phase III designs without adequate human dosing and safety studies. Another challenge is comparing proposed interventions to standards of care with respect to the likelihood of efficacy for serious health conditions that will require the product to be prescription-only if market authorization is obtained. With careful planning, NHP clinical trial serious adverse reactions can be minimized but when they happen, investigators must understand their assessment and reporting requirements. Fortunately, there are government and academic sources of guidelines for good clinical practices and reporting specifically for NHP interventions.

"U.S. Dietary Supplement Regulations- An Update"
Joseph M. Betz

Betz, Joseph M. Fabricant, Daniel S., NIH Office of Dietary Supplements, 6100 Executive Blvd., Ste. 3B01, Bethesda, MD, 20892 USA Center for Food Safety and Applied Nutrition U.S. Food and Drug Administration 5100 Paint Branch Parkway College Park, MD 20740 USA

The Dietary Supplement Health and Education Act (DSHEA) of 1994 amended the U.S. Federal Food, Drug, and Cosmetic Act. Passage of the DSHEA assured consumer access to supplements, defined dietary supplements as a special category of foods, and established a regulatory Framework enforced by the Food and Drug Administration (FDA). Various estimates put the total number of products on the U.S. market at around 55,000, with a total value of approximately $25-29 billion US. Under the Act, companies are prohibited from selling a Dietary Supplement (DS) that is toxic or unsanitary, makes false or unsubstantiated claims, claims to cure, treat, mitigate, or prevent disease, contains a new dietary ingredient without premarket notification, fails to be made according to current Good Manufacturing Practice (cGMP), is not labeled properly, or fails to meet label claim. The manufacturer is also responsible for reporting all serious adverse event reports they receive to the FDA. In addition to DSHEA, the Dietary Supplement and Nonprescription Drug Consumer Protection Act requires manufacturers to report serious adverse events to FDA, and the Food Safety Modernization Act (FSMA) required FDA to publish New Dietary Ingredient (NDI) guidance for industry. In contrast to the regulatory framework for Natural Health Products, the DSHEA provided for limited premarket review, no premarket approval, and no product registration for DS. Continuing marketplace surveillance along with passage of the FSMA and the beginning of full cGMP enforcement have led to increased regulatory activity over the past several years. Since 2008, there have been over 400 recalls of dietary supplement products tainted with active ingredients in FDA-approved drugs or their analogs. In addition, of the more than 270 GMP inspections conducted since 2008 more than 25% have resulted in the issuance of "Official Action Indicated" (OAI) notifications by inspectors. These notices mean that inspectors will recommend regulatory and administrative actions. This talk will provide a brief review of recent regulatory activities, an update on the number of serious AER's and provide an overview of the draft NDI guidance published by FDA in 2011.

"International Perspective of How NHP Research Partnerships are Changing: Researchers, Industry, Consumers and Governments"
Michael J. Smith

Smith, Michael J.

"Production of NHP's in the GMP Environment"
John Baker

Baker, John, John D. Baker, M.Sc.,P.Ag General Manager Bioniche Botanicals 231 Dundas Streeet East Belleville, ON K8N 5J2

Many of the materials used to create Natural Health Products (NHP's) are botanical. These materials originate from farms, forests, oceans and growth environments that contribute to a wide range of potential contamination problems. Establishing good procurement practices that mitigate the levels of contamination is the first of several gates that help protect the integrity of the finished NHP. Once the raw material has passed inspection and met specification, the job of converting the active herbal ingredients to a stable effective ethical NHP involves many gates (checks and balances) before it can be released for sale. Even though the NHP-GMP system appears onerous, it provides a level of quality assurance that protects both the manufacturer and consumer.

Metabolism and Diabetes

Friday May 25th – Morning
Okanagan room

Opening Plenary

"The fruits of our forests: Local dietary interventions to reduce the burden of diabetes in Canadian aboriginal communities"
Cory Harris

Harris, Cory S., Haddad, Pierre S., Egeland, Grace & Johns, Timothy, Centre for Indigenous Peoples' Nutrition and Environment School of Dietetics and Human Nutrition 21,111 Lakeshore Road Ste. Anne de Bellevue, Quebec H9X 3V9

The toll of type 2 diabetes on aboriginal Canadians continues to increase, with people developing the disease and its complications at a higher rate and a younger age than people of non-native descent. Because diabetes prevention and management in aboriginal communities are often complicated by geographical, cultural and socioeconomic factors, the need for effective, locally accessible and culturally acceptable therapeutic options intensifies. Local plant biodiversity, which contributes to traditional diets and healing systems across the country, represents a promising source of dietary interventions that uniquely meet this need. Focusing on berries, this presentation draws on interdisciplinary evidence to support the multi-dimensional anti-diabetic and health-promoting potential of traditional plant foods. Nutritionally, berries are not only rich in nutrients commonly lacking from aboriginal diets but provide fibre and a low glycemic load to help manage blood sugar levels. Pharmacologically, berries contain a wide range of non-nutrient secondary metabolites with established biological effects, including hypoglycemic, insulinomimetic, antioxidant and anti-inflammatory activities that may slow the development of both diabetes and related vascular

complications. Unlike other fruits and vegetables, berries grow abundantly throughout Canada and are a ubiquitous component of traditional diets. As such, berry-based interventions would be widely accessible and culturally appropriate. They would also align well with models of aboriginal health, benefit from traditional knowledge and promote physical activity as well as community engagement. The presentation concludes by considering issues of safety, efficacy in vivo, and sustainable harvest – concerns that must be addressed prior to promoting berries as an anti-diabetic intervention.

"Natural Products for the Prevention of Diabetes and Metabolism among the Bangladeshi Immigrants to the United States of America: First Hand Information from the Bronx Borough of New York City"
Md. Ariful Haque Mollik

Mollik, Md. Ariful Haque, 39-30, 59th St., Apt. C7, Woodside, New York 11377 United States of America

In the United States of America, natural products use is most prevalent among the immigrants. Very few studies have been conducted in the Bangladeshi immigrants to the United States of America on natural products knowledge. Therefore a gap in knowledge exists. This study deals with the field observations recorded on therapeutic applications of natural products used in diabetes and metabolism by the Bangladeshi immigrants of the Bronx borough in New York City. Fieldwork was carried out from November 2011 to February 2012. Semi structured interviews and guided field-walk methods were used to gather information on natural products used by the Bangladeshi immigrants. Along with natural products, information was also collected on natural products parts used, formulations, and dosages. Information on physiochemical as well as pharmacological activity studies on these natural products (if any) was obtained from several data bases. These natural products names included *Apis cerana* Fabricius, *Ocimum tenuiflorum* L., *Nigella sativa* L., *Aconitum napellus* L., *Agaricus campestris* L., *Labeo rohita* F. Hamilton, *Ophicephalus striatus* Bloch, *Lactuca sativa* L., *Persea americana* Mill., *Plantago major* L., *Glycyrrhiza glabra* L., *Olea europaea* L., *Corchorus capsularis* L., *Ipomoea aquatica* Forssk., *Cocos nucifera* L., *Allium sativum* L., *Solanum melongena* L., *Citrus maxima* (Burm.) Osbeck, and *Camellia sinensis* (L.) Kuntze. Information on home-grown use of natural products has led to discovery of many medicines in use today. It is important that modern scientific studies be conducted on these natural products towards isolation and identification of compounds through which diabetes and metabolism can be effectively treated.

"Anti-Diabetic Potentials of Ethanol and Water Extracts of 17 Plants Used by the Eeyou Istchee Cree First Nations of Northern Quebec"
Nan Shang

Shang, Nan 1,2, Musallam, Lina 1,2, Vallerand, Diane 1,2, Walshe-Roussel, Brendan 2,3, Arnason, John T. 2,3, Haddad, Pierre S. 1,2 1Natural health products and metabolic disease laboratory, Department of pharmacology, University of Montreal, Montreal, Quebec Canada H3T1J4, 2Canadian Institutes of Health Research Team in Aboriginal Antidiabetic Medicines and Montreal Diabetes Research Center, Montreal, Quebec Canada H3T1J4, 3Medicinal Plant and Ethnopharmacology laboratory, Department of biology, University of Ottawa, Ottawa, Canada K1N 6N51Natural health products and metabolic disease laboratory, Department of pharmacology,

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Traditional preparations are often based on hot water extracts (HWE), while phytochemical procedures rely on organic solvent (often, ethanol; EE). We thus compared these two extraction methods on the anti-diabetic potential of 17 Cree plants in two relevant bioassays: stimulation of glucose transport in muscle cells, and inhibition of hepatic glucose production. Our results showed that some of the HWE lost their antidiabetic activity completely (in both assays) in comparison to EE, some partially lost their activity, while one plant (AD01) retained similar potential in both assays. Using western blot, we investigated whether EE and HWE of AD01, AD08, AD09, W2 and W7 activated similar or different pathways involved in glucose homeostasis in these bioassays. In C2C12 muscle cells, EE of AD01, AD08 and AD09 stimulated glucose uptake by activating AMPK pathway and increasing Glut4 expression level. While AD01 HWE and EE exhibited similar activities, HWE of AD08 completely lost its effect on all parameters. Interestingly, HWE of AD09 activated Akt pathway instead of AMPK to increase glucose uptake, albeit to lesser extent as compared to its EE counterpart. In the liver, all 5 plant HWE and EE extracts activated AMPK pathway, which is involved in inhibiting hepatic glucose production. In addition, two plant extracts (AD09 and W2) also activated Akt pathways. Therefore, the method of extraction is a significant determinant of the biological activity of medicinal plants at the molecular level. Changes in the quality and quantity of extract components related to extract require further experimentation.

"Elucidation Of Hepatic Glucose Homeostasis And Bioassay-Guided Fractionation Of Fermented Blueberry Juice"
Abir Nachar

Nachar, Abir(1,2); Vinqvist, Melinda(3); Musallam, Lina(1,2); Vuong, Tri(4); Kalt, Wilhelmina(3); Matar, Chantal(4); Haddad, Pierre S.(1,2) 1.Natural Health Products and Metabolic Diseases Laboratory, Department of Pharmacology, Université de Montréal, Montreal, Quebec. 2.Montreal Diabetes Research Center, Montreal, Canada. INAF :Institut des nutraceutiques et des aliments fonctionnels. 3.Food chemistry, Agriculture and Agri-Food Canada, Government of Canada. 4.Department of Nutritional Sciences, Faculty of Health Sciences, University of Ottawa, Ottawa, Canada

BACKGROUND: The liver plays an essential role in glucose homeostasis where Glucose-6-phosphatase (G-6Pase) is the rate limiting enzyme in gluconeogenesis pathway and glycogen synthase (GS) in glycogenogenesis pathway. Recent studies reported that fermentation of blueberry juice (Vacciniumangustifolium; FBBJ) by Serratia vaccini bacterium possess strong antidiabetic potential both in vivo and in vitro. The purpose of this project is to elucidate the effects of FBBJ on hepatic glucose production and storage and to identify the active principles responsible for these activities. METHODS: FBBJ was fractionated using standard chromatography procedures. Confluent hepatic H4IIE and HepG2 cell lines were treated with maximal non-toxic concentrations of FBBJ, fractions or compounds. G-6Pase and GS activities were measured using the glucose oxidase method and a radioactive assay, respectively. RESULTS: Insulin inhibited G-6Pase activity by 67% and increased GS by 2 folds as compared to DMSO control. Seven polyphenolic fractions were obtained from FBBJ fractionation (FBBJ.F1 – F7). FBBJ and its fractions FBBJ.F1 and FBBJ.F2 inhibited G-6Pase by 31%, 45% and 51% while activating GS by 2, 2.4 and 4 folds, respectively, as compared to DMSO. Further analysis of the active fractions yielded 4 compounds;

compound 4 (45.5μM) decreased G-6Pase activity by 54% and increased GS by 2 folds. CONCLUSIONS: FBBJ antidiabetic activity involves modulation of hepatic glucose homeostasis. Bioassay-guided fractionation identified potential active compound, which will help standardize FBBJ.

"The Impact of Natural Health Products on Oxidative Stress and Inflammatory Responses *in vitro* and *in vivo*"
Mohsen Amin

Amin, Mohsen*, Ellen, Richard P.*, Arnason, John T.**, Walshe-Roussel, Brendan**, Amin, Gholamreza***, and Glogauer, Michael* * Faculty of Dentistry, Matrix Dynamix Group, University of Toronto, Toronto, Canada ** Faculty of Science, Department of Biology, University of Ottawa, Ottawa, Canada *** Faculty of Pharmacy, Department of Pharmacognosy, University of Tehran, Tehran, Iran

Plant extracts as natural health products (NHPs) contain bioactive phytochemicals that may exert anti-inflammatory and anti-oxidative activities in biological systems. Such products have been used in traditional and alternative medicine practices for many centuries but the current literature is not as rich in basic mechanistic investigation as the history of usage of the products. This project investigated the use of bioactive natural products (turmeric, ginger, green tea and their purified bioactive components, curcumin and 6-gingerol) as an intervention to diminish neutrophil responses in inflammation models. There is a growing body of knowledge that exaggerated neutrophil recruitment to the site of inflammation and production of peroxidases and reactive oxygen species aggravates tissue damage. Therefore, we hypothesize that the natural products used in this study will prevent the accumulation of neutrophils, decrease activation of neutrophils as measured by release of proinflammatory cytokines, and exert anti-oxidative activities. Total anti-oxidant assay (OxiSelectTM) and a sub-dermal air pouch mouse model were used to test the anti-oxidative and anti-inflammatory effects of ethanolic extracts and phytochemical standards in vitro and in vivo. All the products showed strong dose-dependent anti-oxidant activities compared to uric acid as positive control. The plant extracts, ginger and turmeric as well as their bioactive components, 6-gingerol and curcumin inhibited the neutrophil responses in the air pouch LPS-induced inflammation model as measured by the number of neutrophils attracted to the site of inflammation and myeloperoxidase activities. In addition, plant extracts were profiled using RP-HPLC-DAD and marker compounds of interest were quantified. Natural health products used in this study showed convincing anti-inflammatory properties which make them potential candidates to be investigated in more details with *in vitro* and *in vivo* models.

"Curcumin : Potential as a Hepatoprotective Molecule Using a Novel Drug Delivery System"
Neeraj Khullar

Khullar N, Singh N, Kakkar V, Kaur IP, Dept of Biotechnology, Panjab University, Chandigarh, India

Oxidative stress is believed to play a key role in the pathogenesis of liver diseases. The curcumin molecule holds promise as a therapeutic agent in the treatment , exhibiting powerful antioxidant and

anti-inflammatory effects. However, its use as a hepatoprotectant is restricted due to its poor bioavailability. Hence, curcumin loaded solid lipid nanoparticles (C-SLNs) were prepared and evaluated, and their efficacy was compared to free drug and a standard hepatoprotective agent in an in vivo model. C-SLNs were tested for their pharmacodynamic potential in various groups of rats and the alleviation of biochemical and histopathalogical changes by various treatments were evaluated post induction. Hepatoprotective activity was evaluated by measuring oxidative stress in liver homogenates. In addition, a detailed histopathological analysis of the liver tissue was also performed. Results indicated significant attenuation of oxidative stress by C-SLNs as compared to the free drug and a standard drug. Further, C-SLNs also improved the architecture of liver in comparison to the free drug and standard drug. We can conclude from the study that in the treatment of liver diseases, C-SLNs hold promise as effective therapeutic agents.

"Caffeic acid methyl and ethyl esters activate AMPK, mobilize PGC1-a and increase glucose uptake in cultured skeletal muscle cells"
Hoda M. Eid

Eid, Hoda M., Martineau, Louis C., Haddad, Pierre S., 2900 Edouard montpetit, Montreal, Canada

Derivatives of caffeic acid are widely distributed in the plant kingdom with substantial quantities in coffee, wheat and oats. In a previous study, twenty caffeic acid derivatives were screened for increasing glucose uptake in vitro. Only two compounds (caffeic acid methyl ester (CAME) and caffeic acid ethyl ester (CAEE) potently stimulated glucose uptake and AMPK pathway without inducing toxicity. Peroxisome proliferator-activated receptor gamma coactivator-1a (PGC-1a) is a transcriptional coactivator that plays a key role in energy metabolism. Recent studies linked the activation of AMPK to PGC-1a subcellular localisation and activation. To determine the optimal concentration and incubation period, differentiated C2C12 cells were incubated with incremental doses of the two compounds (12.5, 25, 50 and 100 uM) for 1h, 6h and 18 h and glucose uptake and activation of AMPK were assessed. We report that CAME and CAEE stimulated glucose uptake in a time- and dose-dependent manner with maximum activity at 100 uM after 18 h incubation. To investigate the effect of CAME and CAEE on PGC-1a, differentiated C2C12 cells were separately treated with the two compounds and the cytoplasmic (C) and nuclear (N) fractions were prepared and immunoblotted using antibodies against PGC-1. Both compounds significantly increased the nuclear abundance of PGC-1a after 18 h treatment. CAME and CAEE may hence improve glucose uptake by promoting the translocation of PGC1-a from the cytosol to the nucleus through an insulin-independent mechanism involving AMPK. The results of the present study support the potential of CAME and CAEE for the treatment of diabetes.

Poster Abstracts
Wednesday 5 pm to 7 pm
Shuswap/Pennask/Skeena room

"Comparative efficacy of *Hemidesmus indicus* and *Bauhinia variegata* against sodium arsenite induced toxicity in *Mus musculus*"

Surjyo Jyoti Biswas

Biswas, SJ and Bandopadhyay, TK, Dr. Surjyo Jyoti Biswas Assistant Professor Department of Zoology, Midnapore College, Midnapore, West Bengal, India-721101

Background: In continuation to our early works on the efficacy of some plant extracts in the amelioration of arsenic induced toxicity in mice, we felt the necessity of testing the comparative efficacy of two plant extracts commonly available in West Bengal against sodium arsenite induced oxidative stress taking into consideration several biomarkers of toxicity and cytology. *Bauhinia variegata* Linn (Family: Caesalpinaceae, BV) grows as a medium-sized, deciduous tree and traditionally used in bronchitis, leprosy, tumours and ulcer and its extracts have been found to have antibacterial and antifungal activity, on the other hand *Hemidesmus indicus* (Family: Asclepiadaceae, HI) known as Anantamul, is a slender twining shrub occurring over greater part of India and has wide pharmacological properties viz. blood purifier, anti-hepatotoxicity, anti-thrombotic, renoprotective, anti-inflammatory, anti-diarrhoeal and anti-enterobacterial activities. Chronic arsenic (As) poisoning from groundwater was first reported in West Bengal in 1982 and since then, As-related health problems in the Indo-Gangetic plains have been reported by different research groups and the element has been classified as a group I carcinogen to humans based on strong epidemiological evidence (IARC 1987, NRC 2001). Orthodox treatment regimens such as DTPA and DMSA have been unsuccessful to treat arsenicosis, further they have varying efficacy and harmful side effects of its own. In such a scenario there is need for proper screening of these ethnic medicinal herbs that can ameliorate the toxicological changes and hence the present investigation was conducted. Methods: Twenty five animals (*Mus musculus*) were randomized into two groups of 5 and 20 mice each and were treated as below for 2 months: Group I: no treatment (control), Group II: arsenic treated, as sodium arsenite 200 ppm in drinking water. After 2 months, arsenic exposed mice were divided into four groups of five mice each and given following treatment consecutively 20 days. Group II a: Feeding of sodium arsenite to mice was continued for rest 20 days at 200ppm, Group II b: Mice were fed chronically fed 1:20 alcohol to distilled water ('vehicle'); Group II c: 150mg/kg leaf extract of *B. variegata* (oral), continued once daily. Group II d: 150mg/kg leaf extract of *Hemidesmus indicus* (oral), once daily for 20 days. Observation: Feeding of leaf extracts to Group II c and d mice considerably reduced genotoxicity and modulated favorably some marker enzymes when compared to suitable controls ($p<0.05$ to $p<0.001$). However the modulation were better in BV fed series when compared to HI ($p<0.01$ to $p<0.001$) with regard to enzymological and cytogenetical endpoints. Conclusion: More studies *in vivo* are required to verify the underlying mechanisms of better potentiality of BV and its mode of action thereby further validate its potential to be used against arsenic intoxication.

"Clarification of the risk based approach to the standards of evidence for natural health products"
Laurie Chapman and Semir Omar

Chapman, L and Omar, S, Natural Health Products Directorate Health Canada 2936 Baseline Road Ottawa, ON K1A 0K9 AL 3302C

The standards of evidence for the safety and efficacy of natural health products in Canada have been clarified for both applicants and assessment officers. Building on the 2002 framework and the recommendations of the natural health product program advisory committee, the standards of evidence have been expanded and detailed to reflect four main "risk profiles". Each risk profile dictates minimum evidence requirements, the key requirements for evidence adequacy and incorporates the characteristics of the evidence. The levels of risk are proportionate to the burden of proof placed on applicants to provide valid evidence of high quality to support safety and efficacy. A continuum of evidence adequacy has been developed based on the risk profile of the product and/or its ingredients. The elements of the evidence that are examined include how the evidence reflects totality of evidence, how the evidence matches the recommended conditions of use, how the evidence matches the ingredient form, how the evidence is generalized to the recommended conditions of use, and how the evidence and/or conditions of use demonstrate that risk has been mitigated. Based on the recommendations of the natural health product program advisory committee, the standards of quality for natural health products have also been revised to improve clarity and to harmonize with other international standards and requirements. Revisions include emphasis on the confirmation of identity and potential techniques to support identification as well as additional guidance on purity, stability and periodic testing (skip testing). This presentation will briefly discuss the proposed changes to the standards of evidence for safety, efficacy and quality of natural health products.

"Capsaicin inhibits the growth of human oral KB cells through apoptotic pathways"
Mu-Kuan Chen

Chen, Mu-Kuan, Lu, Wei-Cheng, Wang, Che-Wei, Department of Otorhinolaryngology, Head and Neck surgery, Changhua Christian Hospital 135 Nanhsiao Street, Changhua, 500, Taiwan

Capsaicin, a pungent phytochemical in a variety of red peppers of the genus Capsicum, has been shown an anti-proliferative activity on various human cancer cell lines. However, the mode of cell death depends on the cell type. Here, using human oral KB cancer cell lines, we evaluate the effects of capsaicin on proliferation and apoptosis, and investigate the mechanisms by which the apoptotic process occurs. The treatment of KB cells with capsaicin decreased cell viability and induced cell death in a dose-dependent manner. The effect was also observed in trypan blue exclusion assay and morphological analysis. Cell cycle analysis indicated capsaicin exposure resulted in Sub-G1 DNA accumulation in KB cells. Further, The dissipation of mitochondrial membrane potential observed by using flow cytometry and the expression of caspase 3, 8, 9, and PARP monitored by immunoblotting analysis indicated that the induction of apoptosis by capsaicin in KB cells is involved in the apoptotic caspase pathways. Overall, these observations demonstrate that capsaicin inhibits the growth of human oral KB cancer cells and triggers apoptosis mediated through the caspase pathway, thereby suggesting that capsaicin could be an anti-cancer agent for oral cancer.

"Use of Flash Chromatography & q-NMR to Develop Chemical Reference Materials for Analysis of Products Derived from Kava"
Darson Du

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Kava (*Piper methysticum* G. Forst. [Piperaceae]) is a culturally important plant of the South Pacific used in community ceremonies and for treatment of headache, rheumatic disorders, and minor injuries. Medicinal products derived from kava were widely available by early 1900, with a number of clinical trials providing evidence for efficacy. These studies helped increase kava's popularity until isolated European reports of possible liver damage began to emerge in late 2000. Subsequent studies and reviews examining kava's safety identified gaps in scientific knowledge. It was acknowledged commercial production of kava dietary supplements differed from traditional preparation, often including plant parts not typically used. In this study a method for purifying targeted phytochemicals, purported to be hepatotoxic, from *P. methysticum* leaves, stem peelings, and roots was developed. Fractionation, guided by GC/MS monitoring of expected masses, was performed using a Reveleris flash chromatography system. Three compounds, tentatively assigned as flavokavain A, B, and C were isolated from root materials and qNMR confirmed high purity flavokavain A and B. The compound assigned "flavokavain C" was inconsistent with the known structure; further NMR analysis identified 4',7-dimethylnaringenin, not previously described in *P. methysticum*. From leaves and stem peelings pipermethystin was isolated and structurally confirmed. Currently there is no physical or chemical quality specification for commercial manufacture of kava products and it is essential that suitable quality specifications are developed. These isolated compounds will be used in future analytical work comparing the phytochemical profiles of *P. methysticum* traditional preparations and commercial products.

"Inhibition of intestinal alpha-glucosidase by an extract from an edible plant"
H. Stephen Ewart

Ghanam, Khadija Kumar, T. Sunil Chavan, Jayanthi Deshpande, Jayant Ewart, H. Stephen, OmniActive Health Technologies (Canada) Ltd. 550 University Ave Building 28 (NRC-INH) Charlottetown, PE C1A 4P6

Elevated blood glucose following a meal (postprandial hyperglycemia) is a hallmark of diabetes mellitus. Inhibitors of intestinal alpha-glucosidase retard glucose absorption into the bloodstream and these have been shown to be an effective means of blunting hyperglycemia. Unfortunately, the alpha-glucosidase inhibitor drugs currently available have associated gastrointestinal side-effects that have limited their use. There is growing interest in the development of alpha-glucosidase inhibitors from food sources, with milder side-effects, that could be used as nutraceuticals for maintaining blood sugar levels in the normal range. We are screening various spices, berries, grains, legumes, and other edible plants for alpha-glucosidase inhibitory activity. One water-soluble extract (OHT-04)

was found to inhibit rat intestinal alpha-glucosidase with an IC50 of approximately 5 μg/ml. The major effect of OHT-04 was on V_{MAX} with little effect on K_M, suggesting a non-competitive inhibition of the enzyme. Further purification and structural characterisation of the components responsible for the activity are in progress.

"Lack of effect of pomegranate fruit extract on hepatic expression and activity of cytochrome P450 enzymes in LNCaP xenograft model"
Emma S. Guns

Deb, Subrata, Chin, Y. Mei, Kim, Catrina, Adomat, A. Hans and Guns, Emma S., Vancouver Prostate Centre at VGH 2660 Oak Street Vancouver, BC, Canada V6H 3Z6

Pomegranate fruit extract (POM) has been identified as a promising dietary supplement with potential anti-tumor effects demonstrated in human prostate cancer models as well as in limited number of clinical studies. Mouse xenograft model of LNCaP human prostate cancer is commonly used in anti-cancer studies with POM. Literature reports suggest that POM has the potential to alter cytochrome P450 (CYP) expression and activity in vitro, which can modulate the biological effects of pomegranate as well as of co-administered drugs. The purpose of the present study was to investigate the effects of POM on expression and activities of CYP isoforms in LNCaP xenograft model. Tumors were grown in athymic nude male mice (6-8 weeks) by subcutaneous inoculation of 2x106 LNCaP cells in two dorsal sites. Mice with tumors measuring ~70-100 mm3 were treated with vehicle (propylene glycol/ethanol/water mixture) or pomegranate fruit extract (POMELLA™) (50 mg/kg/day; p.o.) for four weeks. Livers were quickly excised following euthanization and were used for preparation of microsomes by differential ultracentrifugation. Hepatic CYP protein levels were measured by immunoblot analysis. A liquid chromatography-mass spectrometry-based cocktail assay was developed to analyze the CYP marker activities in mouse liver microsomes. Hepatic expression of CYP1A2, CYP2A, CYP2B, CYP2C and CYP3A proteins were unaffected following treatment with POMELLA™. Similarly, hydroxylation of acetanilide (CYP1A2), coumarin (CYP2B), tolbutamide (CYP2C), bufuralol (CYP2D), lauric acid (CYP4A), triazolam (CYP3A) and dealkylation of pentoxy resorufin did not change in hepatic microsomes. In summary, our results suggest that treatment with POM does not modulate the hepatic metabolic capacity of mice bearing human LNCaP prostate cancer cells.

"A Low Carbohydrate, High Protein Diet Combined With Celebrex Markedly Reduces Mouse Mammary Metastases"
Victor Ho

Ho, Victor W., Hamilton, Melisa J., Dang, Thi Ngoi Ha, Bennewith, Kevin L., and Krystal, Gerald, 13-300 Krystal Lab 675 West 10th Avenue, Vancouver, BC, Canada V5Z 1L3

A majority of tumour cells, compared to normal cells, are more dependent on glucose for survival, exhibiting increased glucose uptake and glycolysis. We, therefore, asked if blood glucose (BG) can be significantly lowered through diet modification to slow cancer progression. Specifically, we compared the effects of isocaloric low carbohydrate (CHO), high protein diets (e.g., 15% CHO, 58% protein, 26% fat) to a conventional Western-like diet (i.e., 55% CHO, 23% protein, 22% fat) on the progression of ectopically-implanted or spontaneously-arising tumours in mice. We found

that both implanted mouse and human tumours grew slower in low-CHO-fed mice, an effect that was enhanced by the COX-2 inhibitor, Celebrex. Importantly, there was no significant weight difference between the tumour-bearing mice on a Western and low-CHO diets, and the low-CHO-fed mice exhibited lower BG, insulin, and lactate levels, which suggest reduced glycolysis in low-CHO-fed mice. In a HER-2/neu-induced mouse mammary cancer model, tumour penetrance in 1-year old mice on a Western diet was nearly 50%, whereas no tumours were detected in low-CHO-fed mice of the same age. This was associated with weight-gains in Western diet-fed but not low-CHO diet-fed mice. Furthermore, since metastasis-promoting lactate is produced through the glycolytic breakdown of glucose, which our data suggest is reduced by low-CHO diets, we asked if our low-CHO diet, with or without Celebrex (at 1g/kg), could significantly reduce the metastasis of 4T1 mammary tumour cells. Our results indicate that the number of lung metastases was dramatically reduced in mice fed our low-CHO diet combined with Celebrex.

"Nanoencapsulation: A New Trend in Delivering Botanicals Health Products"
Helmi Hussien

Hussien, Helmi H., Marles, Robin J., and Khadem, Shahriar, Bureau of Clinical Trials and Health Sciences, Natural Health Products Directorate, Health Products and Food Branch, Health Canada, 2936 Baseline Road, Ottawa, Ontario, Canada KIA 0K9

Natural health products are valuable sources of bioactive compounds with the potential to treat or prevent many serious chronic ailments, such as cancer and heart disease. However, low solubility and poor absorption have been significant barriers to their further development as drugs. Nanoencapsulation of phytochemicals is one of the most important recent developments to enhance bioavailability by changing their pharmacokinetics. Nanoencapsulation involves forming active substance-loaded particles with diameters ranging from 1 to 1000 nm, where medicinal botanical substances such as limonene or lutein are entrapped within a semi-permeable matrix. Applications of nanoencapsulation include controlled release, particle coating, taste masking, stabilization, and improvement of shelf life. Labile compounds such as fatty acids, vitamins, antioxidants, hormones, and coenzymes, which may be sensitive to any number of factors, can lose biological activity when unprotected and the resulting decomposition products may lack the desired biological functions or even be toxic. Encapsulating unstable compounds can protect them from undesirable changes while retaining their pharmacological efficacy. Novel technologies have been developed to nanoencapsulate a multitude of bioactive ingredients in dietary supplements. It is expected that such nanoencapsulation product engineering practices will become very profitable in the near future given the enhanced functional properties and stability of these new formulations of botanicals.
Acknowledgements: Thanks go to our colleagues in the Bureau of Clinical Trials and Health Sciences, Natural Health Products Directorate, Health Canada.

"Colorectal caner anti-proliferative potential of lactobacillus probiotic excretory products: in vitro study"
Imen Kahouli

Kahouli I., Tomaro-Duchesneau C., Malhotra M., Saha S., Jamali M.A., and Prakash, Satya, 2615 Rue centre H3K1K2 (QC) Montreal Canada

Colorectal cancer (CRC) is the second leading cause of death worldwide. The available CRC treatments includes surgery, chemotherapy and others. However, they have limitations. Probiotic cells have been show to have implication in CRC. Particularly, lactic acid bacteria (LAB) have been shown to mitigate CRC symptomes/biomarkers. Many *Lactobacillus* strains were tested for their therapeutic and chemopreventive effects in CRC. The goal of this study is to evaluate the potential anti-cancer effect of *Lactobacillus acidophilus* excretory products in CRC. Thus, the effect of the probiotic bacterial supernatants on the proliferation of colon cancer cells was investigated. For the preparation of the probiotic supernatant, the pellet of live bacterial cells was collected form a 16h MRS-bacterial culture. Then, the probiotic supernatants were prepared at a concentration that corresponds to 108 probiotic cell/ml. Then, they were added with the cell medium and incubated with colon cancer cells for 72 hours. Results showed the probiotic supernatant extracted from active live bacterial reduced SW-480 cell viability up to 58 % compared to the control. We investigated cell apoptosis and found an increased up to 67% in treated cells. In addition, the supernatant extracted from dead probiotic cells reduced SW-480 cell viability up to 32 % and increased apoptosis up to 41%. This work suggests that live active *Lactobacillus* excretory products have potential in inhibiting colon cancer cell proliferation and CRC prevention in general. Further research in identifying molecules, mechanisms of action and animal testing will be needed.

"Rheumatoid Arthritis Treated with Plants: Consequences from a Cross-Sectional Investigation within Jessore District of Bangladesh"
Md. Ariful Haque Mollik

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Today a person is very much preoccupied. Various types of body-pains are common afflictions affecting people throughout the world. A more debilitating type of pain arises from rheumatoid arthritis, which is believed to affect at least 5% of the world's population, mostly the elderly. Since regular use or over-use of various pain-killer drugs may have side-effects, an alternative route to treat pain is through use of plants provided by the traditional health practitioners (THPs) and which are generally believed to be without any side-effects. We accordingly conducted an investigation amongst the THPs of Jessore district within Bangladesh to gather information on plants used to treat rheumatoid arthritis. In-depth information regarding plants type, preparation of medicines, ailments for which they are used, dosages, and side effects if any, were obtained from the THPs. All plant samples were later identified at the Bangladesh National Herbarium. The plants used to treat rheumatoid arthritis included *Calotropis gigantea* (L.) Dryand., *Santalum album* L., *Nigella sativa* L., *Zingiber officinale* Roscoe, *Cynodon dactylon* (L.) Pers., *Cissus quinquangularis* Chiov., *Tagetes erecta* L., *Lawsonia inermis* L., *Bambusa bambos* (L.) Voss, *Aerva sanguinolenta* (L.) Blume, *Musa sapientum* L., *Clerodendrum indicum* (L.) Kuntze, *Brassica napus* L., *Curcuma longa* L., *Azadirachta indica* A.Juss., *Persicaria maculosa* Gray, *Olea europaea* L., *Ocimum tenuiflorum* L., and *Aphanamixis polystachya* (Wall.) R.Parker.

Taken together the above plants provide effective remedies for the population such that they do not have to visit modern medicinal practitioners. Therefore the plants used as traditional healthcare system need urgent conservation.

"Simultaneous estimation of (-) Epicatechin, beta Boswellic acid, Diosgenin in herbal medicine by High Performance Thin Layer Chromatography"
Bhavesh H Patel

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A simple, sensitive, rapid, reproducible thin-layer chromatographic method was developed and validated for quantitation of (-) Epicatechin, beta-Boswellic acid and Diosgenin in antiarthritic herbal formulation. The stationary phase silica gel G60F254 was selected for separation and the sample was developed using a mixture of n-Hexane: Chloroform: Acetone: Formic acid in the ratio of 4: 1.6:4:0.6 v/v/v/v as mobile phase. The analytical markers were quantified at 585 nm after spraying with anisaldihyde-sulphuric acid as spraying reagent. The linearity range for (-) Epicatechin, beta-Boswellic acid and Diosgenin were 100-600 ng/band with average recovery of 99.78, 99.59 and 99.73 and correlation coefficient of 0.997, 0.997 and 0.996 respectively. The limit of detection and limit of quantitation values were found to be16.76, 23.48, 7.83 and 50.78, 71.16, 23.74 respectively. The formulation was quantified and found to be 5.18, 2.41 and 0.02 % w/w respectively. The developed method was successfully applied for the assay of market formulations containing *Acacia catechu*, *Boswellia serrata*, *Trigonella foenum-graceum* extracts.

"Metabolomic Examination of *Streptomyces* species Using Nuclear Magnetic Resonance Spectroscopy and Multivariate Statistical Analysis"
Derek Rollo

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Streptomyces is a soil-inhabiting genus of Gram-positive aerobic bacteria of family streptomycetaceae, order actinomycetales and class acinobacteria. *Streptomyces* spp. contributes about two thirds of all microbially produced antibiotics due to their extensive secondary metabolism. Different spp. produce relatively unique secondary metabolites that are bioactive as antibiotic, antifungal, insecticidal, anti-neoplastic, anti-parasitic, and immunosuppressant drugs. Two-dimensional J-resolved nuclear magnetic resonance (NMR) spectroscopy can be coupled with principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) to facilitate metabolomic examination of different spp. This approach was applied for the examination of ethyl acetate extractions of cultured *S. laurentii*, *S. nodosus*, *S. coelicolor*, *S. parvulus* and one unknown species. Principal components were computed to represent variance within the sample groups. Interpretation of the PCA and PLS-DA statistical models resulting from processed NMR data allowed for a comparison of similarities and differences between the Streptomyces spp. Loadings plots were examined in order to identify peaks that contributed to variance in the models. These were then compared to known bioactive secondary metabolites to determine if they had a significant impact on the observed variance. Additional sources of variance, which were not readily identifiable, were

examined to determine if they might be unknown compounds that could represent novel secondary metabolites. NMR coupled with MVA is intrinsically useful for chemotaxonomic purposes. Furthermore, it allows a method for screening unique compounds from newly discovered *Streptomyces* spp., promoting the discovery of putative bioactive metabolites.

"Potential role of probiotics for the inhibition of *S. mutans* and *C. albicans*"
Shyamali Saha

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Dental caries (DC) is a widespread disease; similarly, oral candidiasis (OC) is a dental disease which is on the rise. Streptococcus mutans is the primary causative organism of DC with its contribution to the initiation of disease by lowering oral pH. *Candida albicans* is also found in late caries and is the causative organism of OC. An in vitro assay was designed to evaluate the potential of *L. reuteri* NCIMB 701359, *L. reuteri* NCIMB 701089, *L. reuteri* NCIMB 11951, *L.reuteri* NCIMB 702656, *L. reuteri* NCIMB 702655, *L. fermentum* NCIMB 5221, *L. fermentum* NCIMB 2797, *L. fermentum* NCIMB 8829, *L. acidophilus* ATCC 314, *L. plantarum* ATCC 14917 and *L. rhamnosus* ATCC 5310 for prevention/treatment of DC and OC. *S. mutans* NCIMB 702062 and *C. albicans* ATCC 11006 were incorporated in molten agar at concentrations of 0.5% (v/v) and 1% (v/v), respectively. 100ul of tested solutions were incorporated in pre-formed wells in agar plates. Zones of clearance were observed following an incubation period of 48 hours at 37°C with 5%CO_2, demonstrating inhibition. All the tested probiotics demonstrated inhibition of *S. mutans* and *C. albicans*. Further optimization was performed using four concentrations of the tested probiotic strains. Following optimization, *L. fermentum* NCIMB 2797 required the least number of cells, $1.33x10^7$ cells for the inhibition of *S. mutans* and $9.07x10^6$ cells for *C. albicans* to demonstrate a 1mm of zone of clearance. This research demonstrates that live probiotic cells have potential to be used for the prevention/treatment of oral diseases such as DC and OC.

"Synthesis and Characterization of Ginsenoside Rg1-loaded PPF Microspheres"
Mehrnaz Salarian

Salarian, Mehrnaz. Lui, Edmund., Charpentier, Paul A., Faculty of Engineering The University of Western Ontario London, Ontario, Canada, N6A 5B9

Poly(propylene fumarate), a promising biodegradable material for treating skeletal defects, was synthesized using a two step procedure. To encourage the healing process and stimulate osteogenesis, an angiogenic agent, ginsenoside Rg1 can be incorporated into PPF matrix. PPF microspheres encapsulating ginsenoside Rg1 were synthesized using a water-in-oil-in-water (W1-O-W2) double-emulsion-solvent-evaporation technique. The morphology and size of the microspheres were characterized by scanning electron microscopy (SEM) and ZEISS light microscopy. The mean diameter of the prepared PPF microspheres was 40 um. Released ginsenoside was quantified by UV-Vis spectrophotometer, and drug release profiles were studied. Angiogenecity of ginsenoside Rg1 before and after release was confirmed by the assay of tube formation in EA.hy926 cell line derived from the fusion of HUVEC with the A549 human pulmonary adenocarcinoma cell line. PPF

microspheres developed in this study are promising to serve as vehicles for controlled drug delivery and tissue engineering.

"Controlled Release and Anti Inflammatory Effect of Ginseng Extracts from Microsphere Hydrogel Combination System"
Raziye Samimi

Samimi, Raziye. Lui, Edmund. Charpentier, Paul A., Faculty of Engineering The University of Western Ontario London, Ontario, Canada, N6A 5B9

Panax quinquefolius is a medicinal plant used in traditional herbal medicine grown in North America mainly for its roots. North American ginsenosides have been extracted from North American ginseng root using ultrasound assisted extraction method and identified by HPLC-UV and SFC-ELSD. Also, a novel drug delivery system for ginseng extracts have been developed which could achieve a controlled release of nutraceutical agents in an oral delivery system. in vitro release behavior of these herbal extracts have been investigated. The inhibitory effect of ginseng extracts (before and after encapsulation in drug carrier) have been tested using murine macrophages showing potential for anti-inflammatory preparations.

"Using NMR Metabolomics to Profile Leaf Extracts of Hawthorn Species"
Cynthia Shum

Shum, Cynthia[1], Brown, Paula N.[2], Dickinson, Timothy A.[3], Shipley, Paul R.[1]

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Hawthorn, *Crataegus* spp. is commonly used in natural health products (NHPs) for preventing and treating cardiovascular diseases. Fruits, leaves and berries of hawthorn are considered the most potent parts of the plant. Phenolic compounds derived from hawthorn are thought to contribute significantly to its therapeutic effects. NHPs are made from a mixture of plant parts and are standardized to a variety of bioactive compounds but the plant parts used and their quantities are rarely defined.

The main objective of this project was to determine if variance existed between the leaf profiles of *Crataegus* species. It will be determined if the compounds that strongly influence variance are known cardioprotective compounds, allowing us to determine if these important compounds vary more between than within species.

Leaf samples of *C. wilsonii*, *C. heldreichii*, *C. monogyna*, *C. pinnatifida*, *C. hupehensis*, *C. chrysocarpa*, *C. maximowiczii* and *C. almaatensis* were analyzed using one-dimensional proton and two-dimensional J-resolved nuclear magnetic resonance (NMR) spectroscopy coupled with multivariate analysis by principal component analysis (PCA). Scores plots showed greater separation between than within

the species, in both the whole leaf metabolome and the phenolics region. Through examination of loadings plots, we found separation between the whole leaf metabolome to be primarily contributed by sugar and fatty acid profiles. Chlorogenic acid, vitexin and/or its derivatives were identified by loadings plots as the main contributors to variance within the 6-10 ppm region, where peak signatures represent the presence of flavonoids and other phenolic compounds shown to have cardioprotective properties *in vitro*. Analysis of the NMR spectra confirmed the presence of these compounds as contributors to variance.

"The production of ferulic acid by *Lactobacillus fermentum* NCIMB 5221 can benefit human health"
Catherine Tomaro-Duchesneau

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Ferulic acid (FA) is an antioxidant compound known to neutralize free radicals, such as reactive oxygen species (ROS). These free radicals have been shown to be involved in DNA damage, cancer and aging, contributing to the pathogenesis and development of a number of inflammatory diseases. The development of an FA oral therapeutic has been attempted, but its activity is hampered by its absorption in the small intestine followed by its quick excretion. Interestingly, colonic microbial enzymes have been shown to produce FA. In this research, selected *Lactobacillus* strains were screened for FA production by Ferulic Acid Esterase (FAE), as determined by the release of FA from a natural substrate, ethyl ferulate (EFA). The presented work successfully screened for FAE active bacteria using a qualitative precipitation assay on MRS-EFA agar. The production of FA, from the FAE positive strains, was then quantified by high-pressure liquid chromatography. The most active FAE bacterial strain from those screened, in terms of FA production, was shown to be *Lactobacillus fermentum* NCIMB 5221, producing 0.168+/-0.001mg/mL FA, following 48 hours of incubation in 0.296mg/mL EFA. The total antioxidant capacity of the strain was also investigated using a QuantiChrom Antioxidant Assay kit measuring the reduction of Cu2+ to Cu+. L. fermentum NCIMB 5221 had a production of 509.58+/-13.23uM Trolox-equivalent antioxidant activity. This work opens up future potentials for the use of a synergistic formulation of *L. fermentum* NCIMB 5221 with its intrinsic microbial FAE activity for therapeutic applications in the form of a functional food and health product.

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