

#### **CONFERENCE ABSTRACT BOOK**

OPEN ACCESS

# Abstracts of the 19<sup>th</sup> Annual Conference of the Natural Health Products Research Society of Canada: New Frontiers in NHP Research

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#### **ABSTRACT**

With their 19th Annual Conference, the Natural Health Products (NHP) Research Society of Canada celebrates 20 years of excellence in supporting research and evidence-based policy and product development in Canada and around the world. The general theme of the Conference is thus appropriately entitled "New Frontiers in NHP Research: Celebrating 20 Years of Innovation". Indeed, it is in a forward-looking stance that the Conference showcases state-of-the art innovations in NHP research from the academic, industry and governmental perspectives, as is the tradition of the Society. Scientific sessions cover NHP applications in cancer, cardiometabolic disease, and aging as well as food derived NHPs and industry innovations. Panel discussions are another trademark of the Annual Conference and address inconsistencies in NHP lab testing, the role of Traditional Herbal Medicine in NHP research, and new developments in NHP analysis. Finally, government and industry representatives offer perspectives on national and international regulatory frameworks.

**KEYWORDS:** NHP Research Society of Canada; natural health products; food; traditional medicine; cancer; cardiometabolic disease; aging; dietary supplements; medicinal plants; functional foods



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#### **Conference Abstracts**

#### **Oral Presentations in NHPs in Cardiometabolic Diseases**

#### Plant Bioactives to Alleviate Chronic Inflammatory Diseases: The Role of the **Gut Microbiota**

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In this presentation I will provide growing evidence that plant polyphenols and other bioactives can help prevent and even in some cases reverse metabolic diseases. I will show that berry polyphenols blunt metabolic inflammation and alleviate obesity-linked type 2 diabetes, fatty liver diseases and even cancer through their action on the gut microbiota. Clinical evidence for the beneficial impact of a polyphenol-rich extract from the Amazonian berry camu camu will also be presented. I will further show that substituting sucrose for maple syrup and other natural sweeteners can prevent cardiometabolic risk factors in both animal models of obesity and humans with overweight, in association with changes in the gut microbiota.

#### Red Beetroot Improves Metabolic Disorder and Beneficially Modulates Intestinal Microbiota

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Prebiotics are compounds that cannot be digested by the human body but can be utilized by intestinal microbiota to induce health benefits. Red beetroot (RB) is rich in fibers and betalains, compounds with interesting prebiotic potential. The aim of this study was to evaluate the prebiotic potential of RB and its effects on metabolic health in a murine model of obesity.

To achieve this, Wistar rats were fed with three different diets for eight weeks: 1) a high-fat, high-sucrose westernized diet (WD), 2) WD with RB (6% powder), and 3) a conventional CHOW diet. Supplementation with RB significantly (RB vs W, p < 0.05) modified the composition of the microbiota, mainly through the stimulation of *Lachnospiraceae*, Ruminococcaceae and Roseburia, and tended to increase microbial abundance (p = 0.09). However, RB did not affect short-chain fatty acid production. While RB did not impact rat glycemia, it significantly reduced peak insulin production and tended (p = 0.06) to lower insulin resistance (HOMA-IR). Additionally, RB significantly reduced hepatic steatosis score, and induced hepatic beneficial transcription factors, thereby influencing steatosis (PPARa, PPAR $\beta$ , p < 0.05) and antioxidant control (Nrf2, p = 0.06). Furthermore, RB supplementation significantly reduced small-intestine permeability and the colonic transcription of genes associated with metabolic disorders, colorectal cancer (CRC) and inflammation (Nrf2, PGC1α, SREBP1, NF-κB). Our study demonstrated RB health benefits related to glucose metabolism, liver health, and its action against metabolic disorders and CRC. Additionally,



the results showed that RB modulates microbiota composition, although further studies are necessary to determine if RB constitutes a prebiotic rich food, as the utilization of its compounds by the microbiota could not be demonstrated.

#### Bittering Herb to NHP: Endocannabinoid Degrading Enzymes Inhibition by Hop (Humulus lupulus) Extracts and their Constituents

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The human endocannabinoid system (ECS) consists of endogenously produced cannabinoids (endocannabinoids) that interact with the cannabinoid receptors and are rapidly degraded, primarily by two enzymes: FAAH and MAGL. Therefore, inhibition of FAAH and/or MAGL can increase the concentration of the endocannabinoids and enhance ECS activation. Furthermore, FAAH and MAGL inhibition could slow the production of downstream prostaglandins, thus reducing inflammation. Inhibition of these enzymes have been shown to reduce anxiety, PTSD, and neuroinflammation. Traditionally used medicinal plants might modulate the ECS and account for some of their therapeutic properties. Hops (Humulus lupulus, Cannabaceae), were traditionally used to treat anxiety, spasms, and insomnia – similar to medical cannabis. However, hops are cultivated mainly as the bittering agent in beer. Hops have unique secondary metabolites, such as (co- and ad-) humulone, (co- and ad-) lupulone, and xanthohumol. Ethanolic extracts of hops (n = 24) were tested on FAAH and MAGL inhibition in a dose-response manner. The IC<sub>50</sub> were then correlated to the extract's chemical components. CoLupulone was negatively correlated to FAAH IC<sub>50</sub> values (i.e., more potent) and positively correlated to MAGL IC $_{50}$  values (i.e., less potent). Furthermore, AdHumulone content was positively correlated to FAAH IC<sub>50</sub> (i.e., less potent) and showed no relationship to MAGL IC<sub>50</sub> values. The prenylated chalcone, xanthohumol, in the plant extract is not significantly correlated to the IC<sub>50</sub> of FAAH nor MAGL. Hops are easily accessible and there are hundreds of varieties available thanks to the brewing industry. Therefore, a hop-based Natural Health Product could be produced that modulates the endocannabinoid system and reduce inflammation without cannabis.

#### The CIHR Team in Aboriginal Antidiabetic Medicines: Celebrating 20 Years of Successful Partnership Between Modern Science and Traditional Medicine Pierre Haddad<sup>1</sup>

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In 2003, we instated the Canadian Institutes of Health Research (CIHR) Team in Aboriginal Antidiabetic Medicines (TAAM). The CIHR-TAAM brought together Indigenous Traditional Knowledge Holders (TKH), Indigenous community researchers as well as a multidisciplinary group academic scientists coming from the fields of ethnobotany, phytochemistry, pharmacology, toxicology, public health and even community psychology. Initially, the team used over 60 different in vitro bioassays and in vivo animal models to assess the antidiabetic, antioxidant, anti-inflammatory, cytoprotective and herb-drug interaction potential of a series of 17 Boreal plant species emanating from traditional Indigenous pharmacopeia. A comprehensive prioritization of the top 10 plans was then carried out, presented to the TKH and compared with their own use/respect/experience with the plants. In a second set of qualitative studies, perceptions, barriers and facilitators toward the use of Traditional Medicine and contemporary healthcare were probed in community users, TKH, health professionals and health administrators. Thematic NVivo analysis of interviews identified six categories of key influencing factors, namely personal, relational, cultural, structural, policy and treatment ones. Finally, the most recent studies concerned community lifestyle interventions for diabetes prevention and care that was based on Indigenous culture; more specifically, traditional food, traditional medicine and cultural physical activity. Qualitative analysis examined the cultural relevance of the interventions as well as the impact on the wellbeing of participants. This was accomplished in four Indigenous communities of Quebec and British-Columbia with variable size, research capacity and healthcare organization. Overall, our studies confirm the soundness of relying on Indigenous Traditional Medicine for diabetes care.



### The Use of Bioactive Probiotics as a Novel Strategy for Cardiovascular Disease and **Metabolic Syndrome**

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The recent widespread influence of western lifestyle and diet has led to a global epidemic of metabolic syndrome, warranting the need for novel interventions. Metabolic syndrome is a cluster of complex risk factors that predispose an individual to cardiovascular disease, diabetes and stroke. An imbalanced gut microbiota is associated with metabolic syndrome and low-grade inflammation, a key characteristic of cardiometabolic dysfunction.<sup>2</sup> Supplementation with probiotic biotherapeutics is an attractive option to restore intestinal homeostasis. We have assessed the role of ferulic acid esterase (FAE)-active Lactobacillus fermentum NCIMB 5221 (Lf5221) on intestinal inflammation using HT-29 intestinal epithelial cells (IECs). Lipopolysaccharides (LPS) initiate a strong immune response via the NF-κB factor in HT-29 cells, causing cell injury and ultimately apoptosis. Our results show that exposure to LPS decreases IEC viability by 19.3%, compared to non-treated cells. However, co-incubation with Lf5221 modulates the immune response likely through NF-κB inhibition, leading to 116.9% increased viability. We also measured the release of pro-inflammatory interleukin (IL)-8 by HT-29 cells pre-treated with IFN-γ and LPS, in the presence or absence of bacterial cells. Our findings reveal an 89.7% decrease in IL-8 expression in IECs co-treated with Lf5221, compared to control. Finally, investigation of Lf5221 individually and combined with other probiotics in diabetic and obese models of Drosophila melanogaster demonstrated its ability to improve several key metabolic risk factors, such as rescuing diet-induced weight gain to the levels of nontreated controls.<sup>3</sup> Overall, our findings suggest endotoxin-protective and anti-inflammatory benefits of a FAE-active probiotic. The use of the presented probiotic as a translational therapeutic for a next generation metabolic syndrome and cardiovascular therapy will be presented.

### **Oral Presentations in NHPs in Healthy Aging**

#### Natural Health Product Research in Aging and Neurodegenerative Diseases: Trends and Promises

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At the core of human existence lies a profound and intricate relationship with the plant world. Throughout our lives, we will rely on plants to breathe, eat, and heal, and Indigenous teachings across the globe recognize sacred properties to culturally symbolic medicinal plants. During this talk, we will explore the traditional and contemporary uses of herbal remedies to promote healthy aging and attenuate symptoms of neurodegenerative disorders. We will discuss conceptual and methodological approaches used in our laboratory to understand the biological mechanisms underlying the pro-health benefits of plant-based natural products. More specifically, our interest lies in the ability of these molecules to promote defense mechanisms in the aging brain by targeting non-neuronal brain cells, as these cell types regulate neuroinflammatory and neurotrophic processes involved in neuron survival. Experimental approaches to address these complex questions rely on cutting-edge technologies, such as induced pluripotent stem cells and the generation of 3D microfluidic and organoids-based modeling platforms. These exciting technologies - and others - hold promises to create more complex disease models that recapitulate the cytoarchitecture and dynamic cell-cell interactions characteristic of the human brain. Applying these tools to natural health product research in aging and neurodegenerative disorders has the potential to facilitate new discoveries and inform on their safety, efficacy and mechanisms of action.



### Interim Report on a Living Review of Systematic Reviews of Natural Health Products and Natural Therapies in the Prevention and/or Treatment of COVID-19

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Attention to self-care, including use of natural health products escalated during the COVID-19 pandemic. The World Naturopathic Federation (WNF) coordinated a series of rapid reviews to synthesize existing evidence on natural health products (NHPs) for COVID-19 and upper respiratory tract inflections to help synthesis emerging evidence. Subsequently, a living review of systematic reviews aims to investigate the types and volume of research pertaining to NHPs and therapies as they relate to the prevention and/or treatment of COVID-19 and post-COVID syndrome. A monthly search for published peer-reviewed systematic reviews of the topic was initiated May 2022 and is ongoing. Using a systematic keyword search strategy with clear inclusion and exclusion criteria, a summary of the types of studies included, the overall outcome and treatment focus were assessed. A total of 225 systematic reviews encompassing 5,636 studies of randomized controlled trials (49.8%, n = 112), observational studies (21.3%, n = 48), clinical studies (20.4%, n = 46), and other studies (12%, n = 27) were included. Of those, 28.9%(n = 65) of the systematic reviews focused on prevention, 67.6% (n = 152) on treatment, and 3.1% (n = 8) on post-COVID. The natural health products reviewed included herbal medicine, vitamins, minerals, other natural health products, and other therapies, with 83.5% (n = 188) of all systematic reviews stating a positive outcome and beneficial potential of the natural treatment or therapy investigated. This living systematic review concludes that there is a growing interest in research pertaining to natural health products and therapies with respect to the prevention of COVID-19 infections and addressing disease severity and mortality, especially in adjunct to conventional medical intervention. Nonetheless, there is a lack of high-quality evidence and consistency in outcome reporting across the large breadth of natural treatment and management options.

### The Prenylated Flavonoids Cannflavins Demonstrate Dual Inhibition of the **Pro-Inflammatory Pathway**

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Hemp-derived NHPs demonstrate diverse therapeutic properties, but cannabinoids and THC don't account for the potency of the entire extract. We identified prenylated flavonoids, namely cannflavins, present in extremely low concentrations in planta, that demonstrate significant anti-inflammatory properties. At Canurta we developed the analytical capabilities to screen cannabis sativa germplasms for cannflavins We also leverage proprietary technology to extract these polyphenols from hemp. Here, we break down the mechanism of action of cannflavins in the inhibition of pro-inflammatory pathways. On a molecular level, the inflammatory stimulus activates the two main branches that cause inflammation: leukotrienes (5-LO) and prostaglandins (PGES). Cell-free *in-vitro* enzymatic assays using mPGES-1 (prostaglandin E2 synthase-1) enzyme from the human cell line A549-derived microsomes demonstrates that cannflavins directly inhibit the prostaglandins pathway (mPGES-1). Separately, cannflavins inhibited the other inflammatory branch pathway (leukotrienes) in a 5-LO cell-free assay using human monocytes, and performed similarly to a commercial leukotriene inhibitor. To translate the *in-vitro* assays, we evaluated the anti-inflammatory efficacy of cannflavins on an in-vivo acute respiratory distress syndrome (ARDS) model by LPS-induced altered respiratory function in mice.



#### Mechanisms of Neuroprotection by Combined Formulation of Water-Soluble CoQ10 and Ashwagandha Root Extract

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Alzheimer's Disease (AD) is one of the most common neurodegenerative disorders that is primarily characterized by a decline in memory and cognition, and in most cases ultimately fatal. Pathological features of AD include the formation of amyloid-β plaques, neurofibrillary tangles composed of tau protein, and the loss of neurons in the hippocampus. Past studies have found that AD is associated with the improper functioning of multiple cellular mechanisms such as mitochondrial dysfunction, increased oxidative stress, inhibition of autophagy and proteasome activity, and increased inflammation through the activation of microglia. Although the specific cause of AD development is unknown, both environmental and genetic influences are hypothesized. Current treatments for AD are developed to target and reduce symptoms, however the progression of the disease is inevitable. Due to the multi-faceted nature of the disease, it is important that treatment options take a comprehensive approach. Certain natural health products (NHPs) that are safe for long-term use have been found to possess neuroprotective properties and potentially target the mechanisms implicated in AD. It is hypothesized that using a combinatorial treatment of two NHPs, Ubisol-Q10 and a water-soluble formulation of Ashwagandha, the progression of AD can be mitigated by resolving the previously mentioned mechanisms. Using a double transgenic mice model of AD, brain tissues were preserved and sectioned from different treatment groups. Markers of a variety of biochemical mechanisms were analyzed and compared through the immunofluorescent staining of tissues. At present, results show a decrease in amyloid-beta plaques, oxidative stress, and inflammation, and an increase in astroglial activation. Future experiments will look at synaptic and axonal health of neurons, and mitochondrial stability. This project examines the potential efficacy of NHPs as a treatment for AD.

#### Towards a Better Understanding of the Chronobiotic Effects of Pro-Anthocyanidins Sarra Beji<sup>1,2</sup>, Mathilde Mouchiroud<sup>1,2</sup>, Yves Desjardins<sup>3,4</sup>, Mathieu Laplante<sup>1,5</sup>, Frédéric Picard<sup>1,2</sup>, André Marette<sup>1,5</sup>, Denis Richard<sup>1,5</sup>, Olivier Barbier<sup>2,3</sup>, Andréanne Michaud<sup>1,3,4</sup>

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Consumption of a high fat diet (HFD) damages normal biological rhythms, leading to circadian disruptions and metabolic dysfunctions. Circadian rhythms are regulated by a central clock, but also intrinsically by clocks expressed in each organ. The synchronization of these clocks is achieved in response to zeitgebers (time givers). However, how these zeitgebers act synergistically and whether substances with chronobiotic properties may have the potential to resynchronize altered rhythms and improve metabolism is less known. Study objectives are: 1) To determine the synergistic impact of different zeitgebers on the intrinsic clocks of metabolic organs; and, 2) To evaluate the chronobiotic potential of pro-anthocyanidins (PACs) from berries in a HFD-induced obesity model. We first evaluated the interaction between diet, temperature, and PACs. Male C3H/HeJ mice were fed a HFD for 12 weeks. During the last 4 weeks, they were exposed to either 10°C (cold) or 30°C (warm) and received a daily gavage of PACs (0.2 mg/g) at ZT2. Tissues were then collected at ZT4, ZT8, ZT12, ZT16, ZT20 and ZT24. Food intake, body weight and composition, as well as blood glucose, insulin and triglyceride levels were measured. The faecal content of bile acids was also evaluated. The expression of metabolic genes and genes involved in the molecular circadian clock were measured. Consumption of a HFD diet led to desynchronizations in peripheral clocks, which could have contributed to alterations in metabolic organs and impaired glucose metabolism. PACs improved glycemia without significantly affecting food intake and body weight and had a significant impact on the molecular circadian clocks of different metabolic organs. Furthermore, temperature and PACs influenced different bile acid metabolites. PACs could improve glucose metabolism through their ability to act as chronobiotics capable of resynchronizing molecular clocks independently of temperature.



#### <u>Oral Presentations in Natural Products and Bioactive Compounds in Food</u>

### Polyphenols: Natural Biomolecules in Alzheimer's Disease, a Multi-Target Perspective

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Alzheimer's disease (AD) is a common neurodegenerative disease that brings a heavy burden to patients, families, and society. Although newly approved drugs (ducanumab, lecanemab ...) brought new hope for treating AD, these drugs are still controversial. Finding natural bioactive compounds from plants, fruits, vegetables and beverages to prevent or to treat AD has a long history and has achieved a lot of brilliant results. Many natural bioactive compounds with anti-AD potential are polyphenolic compounds such as flavonoids, proanthocyanidins which are widely found in fruits, vegetables and in some beverages (tea, chocolate, wine...) and plants. These properties of polyphenolic compounds are not only limited to their antioxidant activity but they could also target β-aggregation, neurofibrillary tangles, autophagy and mitochondrial function, as well as in cerebral insulin resistance. Furthermore, to date, a harmful dose has not been described. However, more research is needed to fully understand the mechanisms by which these compounds exert their effects, safety and efficacy in preclinical and clinical studies. Recently, polyphenol-containing nanoparticles have also attracted extensive research attention due to their antioxidation property, higher solubility and stability and thus have shown great promise in the preparation of polyphenols for therapeutic delivery. We will focus on reviewing the protective effect of polyphenols from fruits, vegetables, extra virgin olive oil and in AD.

#### **Enzymatic Depolymerization of Algal Polysaccharides and Evaluation of Prebiotic Effect**

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Algal polysaccharides such as alginate and carrageenan are common food ingredients widely used around the world. Oligosaccharides derived from these biopolymers are shown to have prebiotic effect and promote growth of beneficial gut bacteria. Here we report on some of the preliminary findings in enzymatic depolymerization of algal polysaccharides and the prebiotic effect of the algal oligosaccharides. In our study, 3 algal polysaccharides were prepared from macroalgae Ascophyllum nodosum (AN), Chondrus crispus (CC), and a microalga Botrycoccus braunii (BB), and tested along with 4 commercial algal polysaccharides; kappa-carrageenan, fucoidan, agarose and laminarin. Commercial cellulase, glucanase, and kappa-carrageenase, as well as agarase, carragenase, and porphyranase prepared in-house were screened for their depolymerization activities on algal polysaccharides. TLC, LC-HRMS and NMR were used to assess the enzyme activity and also to further characterize the oligosaccharides. Two bacterial strains (Lactobacillus acidophilus LA-5 and Bifidobacterium animalis subsp. Lactis) had been cultured for the preliminary prebiotic activity testing under anaerobic conditions. Commercial fructooligosaccharides (FOS) and inulin were included as positive controls. Depolymerization screening showed that the enzymes were able to work on most of the polysaccharides tested, except for the one prepared from AN, and its main component, fucoidan from a commercial source. Based on this, oligosaccharides from agarose, CC polysaccharides, kappa-carrageenan, BB polysaccharides and laminarin were prepared and tested. The preliminary results showed that oligosaccharides derived from agarose, CC, BB, laminarin and kappa-carrageenan promoted the growth of *L. acidophilus* and *B. animalis*, implying their potential application as prebiotic ingredients for gut health.



### The Use of Bioactive Ginger Extract as a Novel Microbiome-Targeting Therapeutic **Agent for Cardiovascular Disease**

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Inflammatory conditions closely correlate with the development and progression of cardiovascular disease (CVD). A disturbance to the intricate relationship between the intestinal microbiota and heart, known as the heart-gutaxis, plays a pivotal role in CVD. Notably, intestinal dyshomeostasis may increase lipopolysaccharide (LPS) production and alter immune signaling, thereby compromising the intestinal barrier and promoting a systemic low-grade inflammatory state. Zingiber officinale (ginger), possesses diverse constituents that may combat inflammation and oxidative stress. We have investigated the effects of bioactive ginger extract (GE) on the production of pro-inflammatory interleukin-8 (CXCL8) and on cellular metabolic activity using the HT-29 intestinal epithelial cell line. Cells were pre-treated with IFN-γ followed by LPS, with or without the addition of GE at various concentrations (4, 6, 8, 10 and 16 mg/mL). Results demonstrate remarkable reductions in CXCL8 production in the highest dosage group, with 89.79%, 99.26%, and 84.23% decrease for the pre-, co-, and pre and co-treatment groups, respectively. Similarly, cells treated with 10 mg/mL of GE showed a decrease of 97.86% in the combined treatment group. We also found GE treatment increases intestinal cell viability by 31.93% and 41.18% at concentrations of 6 and 8 mg/mL, respectively, in LPS-treated cells. Overall, our findings demonstrate that ginger effectively counters IFN-γ/LPS-induced CXCL8 elevation and cell damage in intestinal epithelial cells. Ginger's ability to reduce CXCL8 levels and enhance cellular viability shows promise for a gut-targeted remedy for gut microbial disturbance associated with CVD. Understanding the heart-gut-axis opens new therapeutic avenues for addressing CVD. The application of ginger as a novel therapeutic agent for CVD will be presented.

#### A Cranberry Extract Improves Performance Markers in Trained Endurance Runners Francis Parenteau<sup>1</sup>, Veronica Furno Puglia<sup>1</sup>, Sabrina Manolakos<sup>1</sup>, Amanda Laplante<sup>1</sup>, Karim Mehdi<sup>1</sup>, Andreas Bergdahl<sup>1</sup>

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Purpose: To determine the effect of a polyphenol-rich cranberry extract on lactate production and muscle oxygenation in trained endurance runners.

Methods: 15 trained endurance runners (9 males, 6 females) consumed a cranberry supplement for a period of 28 days. The athletes performed a 1500m time trial followed by a 400m time trial, at baseline and after the chronic consumption of cranberry. Blood lactate was measured pre-test and at 1- and 3-minutes post-test, while muscle oxygen saturation was measured throughout the time trials using near-infrared spectrometry. Cohen's effect sizes (ES) were used to estimate the impact of the cranberry supplement on lactate production and deoxygenation and reoxygenation rates. Data are reported as baseline versus post-intervention means ± standard deviation.

Results: The cranberry supplement moderately altered the lactate response after the 400m time trial, both at 1 minute  $(11.90 \pm 0.97 \text{ vs } 10.57 \pm 0.90 \text{ mmol}; ES 0.55)$  and 3 minutes  $(11.09 \pm 0.90 \text{ vs } 10.49 \pm 0.87 \text{ mmol}; ES 0.30)$ , but not for the 1500m time trial. For the rate of muscle deoxygenation, a moderate effect was observed for the 1500m time trial  $(-5.84\%/s \pm 2.14\%/s \text{ vs} - 4.11\%/s \pm 1.89\%/s; \text{ES} - 0.53)$ , whereas only a small effect was measured for the 400m time trial  $(-5.67\%/\text{s} \pm 2.89\%/\text{s} \text{ vs} - 5.03\%/\text{s} \pm 2.38\%/\text{s}; ES - 0.19)$ . Muscle reoxygenation rate following the 1500m time trial showed a large effect (1.99%/s  $\pm$  0.79%/s vs 2.54%/s  $\pm$  1.09%/s; ES -0.91), unlike for the 400m time trial (3.06%/s  $\pm$ 1.99%/s vs 2.50%/s  $\pm 1.03\%$ /sec); ES 0.23).

Conclusion: Consumption of a cranberry supplement for 28 days is sufficient to buffer the lactate response to exercise during a 400m time trial and improve muscle oxygenation metrics during a 1500m time trial.



#### Investigation of Cannabinoid Content In Cannabis Beverage Products and Related Risk of CYP450 Inhibition

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Cannabis is available to Canadian consumers as flower than can be smoked or vaporized and, since 2019, in the form of edible products. Among newly emerging cannabis edible products, beverages are increasing in popularity and diversity. These products typically contain extracts of cannabis flower that can contain tetrahydrocannabinol (THC) and cannabidiol (CBD) at different ratios. Cannabis edibles have a regulated limit of 10mg of THC per serving, with no limit for CBD content. With different volumes of beverages available, this creates a range of concentrations of cannabinoids. If labelled cannabinoid content is not representative of actual cannabinoids present, risk of toxicity, therapeutic failure or unexpected outcomes increases. Moreover, both THC and CBD have inhibitory potential towards major cytochrome P450 (CYP450) enzymes, which presents risks for interactions with concomitantly used medications. This is exacerbated with the high concentrations of CBD in beverages having the potential for inhibition to CYPs 3A4, 2C9, and 2C19 among others. Consumers may be uncertain how to select products safely and reliably, especially consumers with medical conditions or those taking prescription drugs. To date, there has been no evaluation of cannabinoid content variability in cannabis beverage products. This research was undertaken with the goal of informing safe use and quality control of non-solid cannabis edible products. This study aimed to characterize major cannabinoid content (THC and CBD) in beverages available through the Ontario Cannabis Store (OCS). This work investigates consistency of cannabinoid content in a collection of commercial beverages through quantification of THC and CBD by High Performance Liquid Chromatography. The inhibitory potential of these beverages was assessed using CYP450 microsome assays and modelled relative to cannabinoid content. This work provides information on quality control and safety of these emerging cannabis products to inform public use, manufacturing practices, and regulatory oversight.

#### **Oral Presentations in NHPs in Cancer**

#### Metabolic Modulation by Avocado-Derived Bioactives Improves Cancer Outcome Paul Spagnuolo<sup>1</sup>, Matt Tcheng<sup>1</sup>

<sup>1</sup>University of Guelph, Guelph, ON, Canada

Avocados are rich in macronutrients and bioactive molecules. Avocatin B (Avo) is a 1:1 ratio of two 17-carbon polyhydroxylated fatty alcohols (avocadyne: AYNE and avocadene: AENE) found in avocado pulp (the part we eat) and pit (the part we discard). Work by our group and others show that Avo imparts impressive bioactivity in modulating metabolism, selectively killing cancer cells, restoring insulin sensitivity, and neutralizing viruses. Mechanistically, Avo and AYNE are potent inhibitors of fatty acid oxidation (FAO; i.e., the breakdown of fat for energy) and impart selective toxicity toward acute myeloid leukemia (AML) cells. Specifically, AYNE (anti-AML EC50: 2.5 μM) suppresses clonogenic growth of patient-derived AML cells with no effect on normal hematopoietic cells. Further, AYNE (100mg/kg twice weekly for 5 weeks) reduced patient-derived AML cell engraftment in the bone marrow of immune-deficient mice. To identify a molecular target, AYNE treated cells were immune-precipitated with antibodies against each intra-mitochondrial enzyme involved in long chain FAO (e.g., very long acyl-CoA dehydrogenase (VLCAD; step 1) and the alpha and beta subunits of the mitochondrial trifunctional protein: HADHA, HADHB; steps 2-4). Immunoblotting and LC/MS analysis confirmed that AYNE co-eluted with VLCAD, but not with HADHA or HADHB, confirming a direct physical interaction between AYNE and VLCAD; activity of VLCAD was also inhibited. To expand on in vivo findings and increase target validation, mice were injected with patient-derived AML cells and after 8 weeks treated with AYNE. Human AML cells were then extracted from femurs and purified. In human cells, VLCAD activity was reduced and VLCAD protein melting point shifted, an indirect drug-binding measure, as measured by immunoblotting. This demonstrates that AYNE binds to and inhibits VLACD in vivo. In summary, these results highlight VLCAD as a target of food-derived AYNE and further suggest the potential clinical utility of avocado-derived bioactives.



#### Extracts Piper Longum and Camellia Sinensis Induce Selective Apoptosis in Human Glioblastoma Spheroid Cultures and Glial Cancer Stem Cell Populations

Karolina Konior<sup>1</sup>, Darcy Wear<sup>1</sup>, Siyaram Pandey<sup>1</sup>

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Glioblastoma (GBM) is the most aggressive and common form of brain cancer, where standard chemotherapies have limited effectiveness and impose severe adverse effects due to non-specific cell targeting. In addition to noneffective treatments, GBM has self-renewing cancer stem cells referred to as glial stem cells (GSCs) responsible for drug resistance, malignancy, and relapse. Natural health products Piper longum (LPE) and Camellia sinensis (STE) have shown selective induction of apoptosis in U-87 Mg GBM cancer cells, alone and in combination with chemotherapies. This study investigates the efficacy of these extracts and their interaction on a three-dimensional glioblastoma model, to better observe cell-to-cell and cell-matrix interactions. Our results demonstrated morphological cell disintegration and a decrease in spheroid microtumor growth after 72-hour STE and LPE-treated cells. Furthermore, spheroid cultures demonstrated enhanced anti-cancer activity in chemotherapeutic temozolomide (TMZ) when combined with LPE or STE in comparison to that of TMZ alone, suggesting synergistic herb-drug interactions. A population of U-87 Mg GSCs was identified using biomarkers CD44, CD133 and ALDH1. Stem cell sensitivity was tested against the extracts during 72 and 120 hr treatment periods, where STE and LPE significantly reduced the percentage of stem cells in comparison to the control, with STE being more selective towards GSCs than LPE. These results indicate STE's and LPE's potential as a novel, non-toxic therapeutic treatment against cancer relapse and chemotherapeutic drug resistance.

### Synthite Tea Extract (Camellia sinensis) Induces Cell Death in Triple Negative Breast Cancer Stem Cells and Enhances the Efficacy of Common Chemotherapeutics

Abby Raad<sup>1</sup>, Siyaram Pandey<sup>1</sup>

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Triple negative breast cancers (TNBC), such as the MDA-MB 231 cell line, are the most aggressive type of breast cancer. Current chemotherapeutics employ nonselective mechanisms of action, making them toxic and unsuitable for longterm use. With around 40% of TNBC patients relapsing after treatment with chemotherapeutics, there is a need to develop safer and more effective anti-cancer therapeutics. Cancer stem cells (CSCs) are a tumour-initiating and self-renewing population of cells within a tumour, thus risking therapy failure or relapse. Treatments targeting CSCs may therefore improve patient prognosis. Camellia sinensis, or White Tea, is a Natural Health Product that has been studied in the past for its selective anti-cancer activity. More studies are needed to validate its use in modern cancer therapy. This study aims to elucidate the ability of Synthite Tea Extract (STE), a formulation of White Tea provided by Synthite Industries, to induce cell death in MDA-MB 231 CSCs. Interactions between STE and current chemotherapeutics were also investigated. CSCs were identified with the expression of CD44 by fluorescence microscopy. Scratch wound healing assays were conducted to analyze migratory response of cells. STE significantly reduced the percentage of CSCs, and there was no significant difference in the anti-CSC activity of STE and paclitaxel treatments. Wound closure distance was significantly greater in cells treated with STE, STE-paclitaxel, and STE-cisplatin combinations compared to either chemotherapeutic alone, suggesting synergistic herb-drug effects. Our findings suggest STE is a promising candidate to develop a well-tolerated integrative therapy that provides breast cancer patients with long-term care.

### Exploring The Anti-Cancer Potential of Synthite Tea Extract in Melanoma Treatment: Apoptotic Efficacy and Synergistic Interactions with Chemotherapy

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Melanoma, a cancer of melanocytes, is one of the most prevalent forms of skin cancer around the world. In modern medicine, common treatments for melanoma include chemotherapy, surgery, and radiation therapy. However, these



approaches come with several issues. Major concerns include the non-selective nature of these tissues, resulting in toxicity to normal cells, and their decreased efficacy as melanoma progresses into its later stages. A growing number of studies suggest that natural herbal extracts may have potential as an alternative and supplemental anti-cancer agent. In this study, Synthite Tea extract (STE – supplied by Synthite Industries Ltd.), an unprocessed fresh tea extract from Camellia sinensis, is evaluated for its apoptotic ability in multiple human melanoma cell lines. Additionally, the interaction between STE and temozolomide (TMZ), the standard chemotherapeutic used for treating melanoma, is investigated. The results show that STE induces apoptosis in multiple human melanoma cell lines in a dose- and time-dependent manner. Moreover, the STE and TMZ combination treatment show a slight synergistic effect when combined. Results also suggest that STE has the potential to effectively disrupt the spheroid growth in 3D spheroid cultures. Future studies should investigate the effect of STE in-vivo through mouse xenograft models, understand the impact of the individual compounds in STE, and observe effects on cancer stem cells.

### Anticancer Natural Health Products including Black Maitake Mushroom Extract and their Interaction with Standard Chemotherapy and Sensitivity of Cancer Stem Cells to NHPs: Possibility of Supplementary Therapeutics and Prevention of Relapse

#### Siyaram Pandey<sup>1</sup>

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Natural extracts and bioactive compounds have been used for medicinal purposes for centuries by various traditional practices including Ayurveda and traditional Chinese medicine and KEMPO. Some of the most common anticancer drugs like Taxol, vincristine, etoposides and doxorubicin are derived from natural extracts. Treatment of metastatic and aggressive cancers using current chemotherapeutics including adjuvant therapies with multiple compounds lead only to limited success, but severe adverse effects. Some of the Natural health products (NHPs) have very potent anticancer activity and offer a promising option compared to common treatments. Natural extracts have multiple compounds that could target multiple pathways to induce cell death in selectively in cancer cells. It has been shown that certain NHPs are effective in targeting oxidative and mitochondrial vulnerabilities of cancer cells. Studies also showed that these NHPs are non-toxic, well tolerated and effective in reducing growth of tumor xenografts in immunocompromised mice. Black Maitake mushroom (*Grifola frondose*) extract has been extensively studied for its nutritional, immunomodulating, and anti-cancer activity in the past. However, interaction of these NHPs with current chemotherapeutic drugs is not known. Furthermore, the sensitivity of cancer stem cells to these NHPs is also not investigated. We have shown positive interaction of NHPs (including long pepper, green tea, rosemary and black maitake mushroom extract) with chemo and their effectiveness in inducing cell death in cancer stem cells. These results could lead to the development of safe and effective treatment alongside standard chemo regiments as supplements for cancer patients.

### <u>Oral Presentations in NHP Industry Innovations</u>

### The Use of Bioactive Probiotics as a Novel Strategy for Cardiovascular Disease and **Metabolic Syndrome**

Jacqueline L. Boyajian<sup>1</sup>, Susan Westfall<sup>1</sup>, Rahul Thareja<sup>1</sup>, Ahmad Abosalha<sup>1</sup>, Paromita Islam<sup>1</sup>, Sabrina Schaly<sup>1</sup>, Karan Arora<sup>1</sup>, Satya Prakash<sup>1</sup>

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The recent widespread influence of western lifestyle and diet has led to a global epidemic of metabolic syndrome, warranting the need for novel interventions. Metabolic syndrome is a cluster of complex risk factors that predispose an individual to cardiovascular disease, diabetes and stroke. An imbalanced gut microbiota is associated with metabolic syndrome and low-grade inflammation, a key characteristic of cardiometabolic dysfunction. Supplementation with probiotic biotherapeutics is an attractive option to restore intestinal homeostasis. We have assessed the role of ferulic acid esterase (FAE)-active Lactobacillus fermentum NCIMB 5221 (Lf5221) on intestinal inflammation



using HT-29 intestinal epithelial cells (IECs). Lipopolysaccharides (LPS) initiate a strong immune response via the NF-κB factor in HT-29 cells, causing cell injury and ultimately apoptosis. Our results show that exposure to LPS decreases IEC viability by 19.3%, compared to non-treated cells. However, co-incubation with Lf5221 modulates the immune response likely through NF-kB inhibition, leading to 116.9% increased viability. We also measured the release of pro-inflammatory interleukin (IL)-8 by HT-29 cells pre-treated with IFN-γ and LPS, in the presence or absence of bacterial cells. Our findings reveal an 89.7% decrease in IL-8 expression in IECs co-treated with Lf5221, compared to control. Finally, investigation of Lf5221 individually and combined with other probiotics in diabetic and obese models of Drosophila melanogaster demonstrated its ability to improve several key metabolic risk factors, such as rescuing diet-induced weight gain to the levels of nontreated controls. Overall, our findings suggest endotoxin-protective and anti-inflammatory benefits of a FAE-active probiotic. The use of the presented probiotic as a translational therapeutic for a next generation metabolic syndrome and cardiovascular therapy will be presented.

#### Peptide Profile and Bioavailability of Collagen Hydrolysates Used for the Treatment of Osteoarthritis

#### Christina Larder<sup>1</sup>

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Osteoarthritis (OA) is the most common joint disorder, with a social and financial burden that is expected to increase in the coming years. Due to limited treatment options, patients often resort to supplements, such as collagen hydrolysates (CHs). CHs have demonstrated positive results in clinical trials, such as decreased joint pain, increased mobility, and structural joint improvements. The functional components of CHs are bioactive peptides (BAPs). However, there are significant knowledge gaps regarding the digestion, bioavailability, and bioactivity of CH-derived BAPs, and how different CH products compare in that regard. Our objective was to investigate two types of CHs; a low molecular weight formula (Genacol "CH-GL") and a Generic CH ("CH-OPT"). Methods were developed to measure BAP content after CH in vitro digestion, and their peptide profiles were assessed. Digesta were applied to an innovative HIEC-6/HepG2 co-culture to determine BAP bioavailability. Greater tri-peptide content (Gly-Pro-Hyp, Pro-Hyp-Gly) was released following CH-GL digestion compared to the CH-OPT (p < 0.05). All peptides assessed (Gly-Pro, Hyp-Gly, Ala-Hyp, Pro-Hyp, Gly-Pro-Hyp) were bioavailable (>10%), except for Gly-Pro-Hyp from the CH-OPT (p < 0.05). Notable hepatic effects were observed with CH-GL treatment. Additionally, Pre- and Postdigestion peptide profiles differed between CHs. The efficacy of Genacol CHs in previous clinical work is likely due to its unique peptide profile and BAPs bioavailability. This work also provides an innovative approach for examining the bioavailability of dietary supplements following digestion and first-pass metabolism.

### Dietary Supplements to Reduce Symptom Severity and Duration in People with SARS CoV-2: A Double Blind Randomized Controlled Trial

#### Dugald Seely<sup>1</sup>

<sup>1</sup>Canadian College of Naturopathic Medicine, Toronto, ON, Canada

Coronavirus Disease 2019 (COVID-19) has caused morbidity, hospitalization, and mortality worldwide. Despite effective vaccines, there is still a need for effective treatment of COVID-19, especially for people in the community. We sought to evaluate whether a combination of vitamin C, vitamin D3, vitamin K2, and zinc is an effective treatment option for outpatients diagnosed with COVID-19. Participants were randomized to receive either vitamin C (6g), vitamin D3 (1000 units), vitamin K2 (240 mcg), and zinc acetate (75 mg) or placebo daily for 21 days and were followed for 12 weeks. An additional loading dose of 50,000 units vitamin D3 (or placebo) was given on day one. The primary outcome was participant-reported overall health using the EuroQol Visual Assessment Scale summed over 21 days. Secondary outcomes included health status, symptom severity, symptom duration, incidence of delayed return to usual health, frequency of hospitalization, and mortality. 90 patients (46 Control, 44 Treatment) were randomized. The study was stopped prematurely due to insufficient capacity for recruitment. The mean difference (Control - Treatment) in cumulative overall health was -37.4 (95% CI -157.2 - 82.3), p = 0.53 on a scale of 0-2100. No clinically or statistically significant differences were seen in any secondary outcomes. In this



double blind, placebo controlled, randomized trial of outpatients diagnosed with COVID-19, the dietary supplements vitamin C, vitamin D3, vitamin K2, and zinc acetate showed no clinically or statistically significant effects on the documented measures of health compared to a placebo when given for 21 days. Termination due to feasibility limited our ability to demonstrate the efficacy of these supplements for COVID-19. Further research is needed to determine clinical utility.

#### GenBioChem® Triple Fingerprinting Technology for Ensuring Quality Natural Health Products (NHP) - Identity, Purity, Potency, and Consistency from Batch to Batch Jacqueline Shan<sup>1</sup>

<sup>1</sup>PBG BioPharma Inc, Leduc, Alberta, Canada

The NHP industry has certainly come a long way in the last two decades, with its benefits largely outweighing its disadvantages. However, some significant obstacles still remain, including concerns surrounding the standardization of NHPs, the assurance of reliable and consistent ingredients that meet consumer needs and expectations as well as regulatory requirements.

Based on years of research, product development, and commercialization on various plant and marine-based natural products and ingredients, scientists at PBG BioPharma® Inc. have established a GenBioChem® Triple Fingerprinting Technology platform to address the need for standardization and identification of NHPs and ingredient materials. GenBioChem® Triple Fingerprinting Technologies are analytical techniques used to help standardize ingredients and finished products in terms of identity, purity, potency, and traceability. The three techniques include:

- 1. Genomic analysis This technique identifies the genetic markers of NHPs to confirm the ingredient(s) species of origin(s). It helps ensure that the correct ingredients have been used and can identify potential adulterants.
- 2. Chemical fingerprinting This technique separates the chemical constituents of NHPs to identify their chemical profile. It helps establish the identity of the product and can detect contaminants or impurities.
- 3. Biological analysis This technique studies and analyzes the biological or pharmacological activities of NHPs and their bioactive ingredients by way of laboratory and clinical studies. It helps establish consistent measurements of active ingredients, providing improved dosage accuracy and patient outcomes.

Finally, Good Manufacturing Practices (GMP) are essential in ensuring NHPs are consistently produced according to quality standards. in batch-to-batch consistency and ensuring that the required active ingredients and specifications established are met consistently.

### <u>Oral Presentations in Regulatory Opportunities for NHP Research</u>

### NHP Regulation Modernization Update

#### Lisa Lange<sup>1</sup>

<sup>1</sup>Natural and Non-Prescription Health Product Directorate, Health Canada, Ottawa, ON, Canada

Health Canada's Natural and Non-Prescription Health Products Directorate will be presenting on key regulatory modernization initiatives that are advancing the Self-Care Framework, as well as the requirements for licensing of natural health products and authorization of clinical trials. In particular, we will provide a status update on the key regulatory initiatives underway that are designed to help strengthen the Natural Health Products program and support a risk-based approach to regulatory oversight for all self-care products as well as an overview of the standards of evidence and clinical trials. The objective is to support a shared understanding between the regulator and the research community on key regulatory modernization projects underway in order to enable increased opportunities for collaboration between Health Canada and the Natural Health Product Research Society of Canada.



#### Global Regulation of Natural Health Products: Where does Innovation and Informed **Choice Fit?**

#### Michael Smith<sup>1</sup>

<sup>1</sup>University of Mississippi, Oxford, MS, USA

Consumers and practitioners alike continue to include Natural Health Products within their health care options. The quantity of information now available as well as an increased multicultural and cosmopolitan approach to health and healing has meant that herbal medicine sector is now truly global with an increased role of online sales and products often manufactured in a country away from where are purchased. While the market may be international, this is often not the case for regulations. In addition to challenges posed by this global market place, regulators must manage national and regional expectations in fulfilling their primary mandate of protecting the consumer from harm. This has resulted in a very complex regulatory milieu globally of differing definitions, frameworks and approaches. This can provide both opportunities as well as challenges for all parts of the herbal medicine community, notably those conducting clinical research, consumers, and industry. Using specific examples this presentation will describe at a high level the most common approaches taken by various jurisdictions in the regulation of herbal medicines. Recognizing the desire for a consistent global approach or "harmonization" of regulations, key barriers and opportunities will be explored with examples of best-case approaches identified. Specific attention will be given to aspects related to innovation and the development of novel products together with what needs to be in place to support consumers in making an informed choice about whether to include or not include these products within their health care options.

### Updates on Canadian Regulation of Natural Health Products - An Industry **Perspective**

#### Sonia Parmar<sup>1</sup>

<sup>1</sup>Canadian Health Food Association, Toronto, ON, Canada

The natural health product industry is facing new and upcoming regulation which is threatening innovation, jobs, and competitiveness in the sector. This presentation will provide the industry's perspective on regulatory hot topics in Canada, including self-care, cost recovery, labelling, CBD and innovation.

#### **Poster Presentations**

#### **Are THC Levels Artificially High on Canadian Cannabis Product Labels?**

Mohammed Albadry<sup>1</sup>, Seamus Riordan-Short<sup>2</sup>, Ryan Hayward<sup>2</sup>, Thomas Farleigh<sup>3</sup>, Rob O'Brien<sup>2</sup>, Paula Brown<sup>1</sup>

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<sup>3</sup>Vivariant Laboratories, Mississauga, ON, Canada

Currently the Cannabis Act has strict limits for the amount of THC allowed in each class of product with the exception of dried or fresh cannabis which has no legislative limit for THC content. Recently there have been reports that cannabis products sold through the legal channels in Canada are making inflated total THC content claims. A study comparing the reported total THC content on labels to the total THC content found by laboratory testing on post-market products was conducted. Ten products were purchased through the BC Cannabis Store system and tested by an ISO 17025 certified testing laboratory. All 14 products were sent blinded for interlaboratory testing. All three laboratories employed their own validated analytical methods for cannabinoid determination. The interlaboratory test results were in agreement with %CVs ranging from 0.5 – 7.3%. The total THC content found in commercial samples were significantly lower than reported on the labels, ranging from 20 - 46% below label claim. These results suggest there an issue with inflated THC levels being reported product labels. Interestingly, the inter-laboratory test comparisons indicate the issue is not necessarily with the laboratory testing itself or analytical methods. Given that there is a known natural inherent variance of cannabinoid levels across cannabis plants and that



harvest, processing and packaging can result in a loss of potency, the issue may be attributed to a lack of prescriptive guidance on sampling and testing practices for finished goods. Sampling plans should be used to guide representative testing and testing should be conducted post-packaging not post-harvest to ensure the reported values on labels are reflective of the finished product being sold.

#### Aconitine Poisoning from Ingestion of Product Labeled as Sand Ginger in British Columbia

Michael Chan<sup>1</sup>, Mohammed Albadry<sup>1</sup>, Lorraine McIntyre<sup>2</sup>, Emily Newhouse<sup>3</sup>, David McVea<sup>2</sup>, Dennis Leong<sup>4</sup>, Raymond Li<sup>4</sup>, Arnold Fok<sup>3</sup>, Derek Song<sup>3</sup>, Nikita Sahaturna<sup>2</sup>, Debra Kent<sup>4</sup>, Paula Brown<sup>1</sup>

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Aconitum, commonly known as Monkshood or Wolfsbane, is a genus of over 250 species of herbaceous plants within the Ranunculaceae family, many of which are extremely poisonous. Although Aconitum roots have been used in traditional medicines, due to the presence of highly toxic alkaloids they must be properly prepared and dosed to ensure their safe consumption. Sand ginger (Kaempferia galanga L.) is plant within the family Zingiberaceae whose ground root is commonly used as a spice in Asian cooking. When ground, both sand ginger and Aconitum roots can appear similar. Two individuals presented to Burnaby hospital in British Columbia with symptoms of cardiovascular irregularity, dizziness, and vomiting after having eaten a meal made with "sand ginger powder". Based on the circumstances and reported symptoms emergency physicians and BC Poison Control suspected aconitine poisoning. Public health officials investigated and traced the product to a local retailer where samples were collected and submitted to BCIT for testing. The presence of toxic Acontium alkaloids were detected using liquid chromatography with mass spectral detection. The particularly high concentration of alkaloids detected suggested the material was predominantly derived from Aconitum species. Public health advisories were released, and public health officials coordinated the removal of the product from the market. The results of this case study demonstrate how communication and coordination between physicians, poison control, public health and research labs can effectively address and respond to public health hazards.

### Synthite Tea Extract and Long Pepper Extract Selectively Induce Cell Death and Improve the Efficacy of Chemotherapeutics in Prostate Cancer Cells

Hannah Drew<sup>1</sup>, Siyaram Pandey<sup>1</sup>

<sup>1</sup>University of Windsor, Windsor, ON, Canada

The lack of treatment specificity in conventional chemotherapies often results in negative side effects, making them unsuitable for usage over long periods of time. Natural health products have exhibited anticancer effects and can be utilized as pharmaceuticals due to their safety and high tolerance for consumption. The potential anticancer effectiveness of white tea (Camellia sinensis) and long pepper (Piper longum) extracts, as well as their interactions with prostate cancer and conventional chemotherapies, remain unexplored despite previous reports of their efficacy. If positive interactions are exhibited, the consumption of these two extracts may hold promise for reducing druginduced side effects and enhancing targeted anticancer effects when used in combination with chemotherapeutic agents. It was demonstrated that both Synthite tea extract (STE) and long pepper extract (LPE) exhibit anticancer effects individually and in combination with one another. Extracts individually and in combination showed enhanced induction of oxidative stress and targeting of mitochondria in cancer cells. Further, it was noted that the addition of STE and LPE with the chemotherapeutic Taxol displayed enhanced induction of apoptosis when compared to individual chemotherapy and extract treatments alone in prostate cancer cell lines PC-3 and DU-145.



#### Acute Effects of Moringa Oleifera Leaf Infusion on Endurance Sports Performance Dona Dbedinhessi<sup>1</sup>

<sup>1</sup>UQAM, Montreal, QC, Canada

Results from a recent survey show that 87% of elite athletes are using supplements in Canada. Moringa Oleifera (MO) is a tropical plant from which roots, seeds and bark are used both as foods and in traditional medicine. Hence, MO was previously shown to exert antioxidant, anti-inflammatory and analgesic properties. On the other hand, MO's ergogenic properties remain to be investigated. The aim of the present study was to evaluate the impact of MO on sports performance. To do so, we conducted a study where we compared the time taken by participants to perform a physical task (20km time trial on a stationary bike) before and after a 7-deay treatment with MO leaf infusions. The study was conducted on 21 participants (12M, 9F, Age: 26.7±4.9 years old, Weight: 73.0±16.6 kg, Height: 169.6±9.5 cm, VO2max: 43.9±8.1 ml/kg/min) at a power of 114.1±42.8 Watts and a ventilatory threshold of 1+10% (SV1+10%). Heart rate (HR), the perception of exertion (RPE) and the blood lactate level were also measured during the experimentation. Before and after MO treatment no significant difference were noted for the time to complete the 20km task ( $50.3\pm20.4$  vs  $49.8\pm19.9$  min, p = 0.150, d = 0.3), HR ( $\Delta$  186.7 $\pm$ 8.8 vs 188.5 $\pm$ 7.9 bpm, p = 0.133, d = -1.0), RPE (14.3 $\pm$ 3.1 vs 14.4 $\pm$ 2.6, p = 0.831, d = -0.0) and lactate (7.8 $\pm$ 4.6 vs 7.9 $\pm$ 3.8 mmol/L, p = 0.949, d = -0.0) levels. The present study is still being carried out in order to reach the pre-determined number of participants required to reach statistical power. Although statistical significance was not reached, the present results indicate that MO treatment could provide an important beneficial impact with regards to sports performance. Hence it is important to complete the study and clarify the mechanisms through which MO could exert its ergogenic effects in the future.

### **Determination of Anthocyanins in Elderberry Products using Ultra High** Performance Liquid Chromatography with Ultraviolet Detection

Hazrah Moothoo<sup>1</sup>, Ronan Yu<sup>1</sup>, Ying Liu<sup>1</sup>, Andrew Thomas<sup>2</sup>, Wendy Applequist<sup>3</sup>, Paula Brown<sup>1</sup>

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European Elder (Sambucus nigra L. subsp. nigra) fruit has been consumed as a food product and traditionally used in herbal medicine to alleviate symptoms associated with the common cold and influenza. American Elder (Sambucus nigra subsp. canadensis (L.) Bolli) fruit has not been researched to the same extent as S. nigra; however it has been reported to contain similar anthocyanins such as cyanidin-3-O-sambubioside-5-Oglucoside, cyanidin-3,5-di-O-glucoside, cyanidin-3-O-(2-O-β-D-xylopyranosyl)-β-D-glucopyranoside, and cyanidin-3-O-glucoside while cyanidin-3-O-β-[6"-O-E-p-coumaroyl-sambubioside]-5-O-β-glucopyranoside has only been reported in S. canadensis. Consumer use of Elderberry products has risen exponentially in the last few years. The marketplace has seen a boost of these products in a variety of formats such as dried berries, tinctures, syrups, extracts, and capsules. The method presented here has been developed, optimized and validated to quantify key anthocyanins in Sambucus dried berries and finished products according to AOAC International Single Laboratory Validation guidelines. The anthocyanins were extracted using acidified aqueous methanol with chromatographic separation achieved in 20 minutes using ultra high performance liquid chromatography with an ultraviolet diode array detector. The method presented is a useful tool for industry stakeholders needing to differentiate between S. nigra and S. canadensis to screen raw materials and finished products, evaluate elite crop breeding selections, and/or test products for targeted clinical research.

#### Analysis of Phenylpropanoids, Flavonoids and Flavonol Glycosides in Natural Health & **Food Products**

Ben Ollinger<sup>1</sup>, Mohammed Albadry<sup>1</sup>, Ronan Yu<sup>1</sup>, Paula Brown<sup>1</sup> <sup>1</sup>BC Institute of Technology, Burnaby, BC, Canada

Phenylpropanoids and flavonoids are specialized plant secondary metabolites involved in plant defense to biotic or abiotic stresses. Their biosynthetic accumulation may be constitutive and/or induced in response to external stimuli



and can play a role in plant signaling to drive plant defense responses. Although their biosynthesis and regulation are not fully understood, these phytochemicals have been found to be very useful for humankind, possessing a wide range of potentially therapeutic activities. Phytochemical comparisons both within and across plant species are often made with the suggestion that health benefits could be afforded to consumers through consumption of products rich in these compounds. To support research into the potential health benefits, we report the development of a method for determining 14 closely related phenylpropanoids, flavonoids and flavonol glycosides in natural health & food products. Using elderberry as a model for a phytochemically rich and diverse ingredient, we have demonstrated ultra-high performance liquid chromatography with ultraviolet detection can be used to accurately separate and quantify the 14 target compounds in a variety of different consumer product formats.

### An Evaluation of the Efficacy of Water-Soluble Natural Health Products as a Treatment for Mechanisms Implicated in Parkinson's Disease

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Parkinson's disease (PD) is a common neurodegenerative disease that is characterized by a loss of dopaminergic neurons and Lewy bodies in the substantia nigra region of the brain. Traditional treatments of PD include Levodopa which slows the progression of PD but can cause adverse side effects with long-term usage. Natural health products (NHPs) are a proposed treatment option that circumvents the negative side effects associated with traditional treatments and may prevent the progression of Parkinson's disease as well as alleviate associated symptoms. Previous studies demonstrate that NHPs such as Ubisol-Q10 and Ashwagandha root extract possess neuroprotective and anti-inflammatory capabilities, which may target mechanisms implicated in PD. It is hypothesized that Ubisol-Q10 and Ashwagandha may prevent PD by stabilizing mitochondrial function, inducing autophagy, and decreasing oxidative stress and inflammation. Additionally, a combinatorial treatment of Ubisol-Q10 and Ashwagandha is hypothesized to be most effective due to the complex nature of PD. PD was induced in rat models through paraquat injections, and brain tissue was preserved and sectioned into 5 different treatment groups. The degree of oxidative stress, autophagy, inflammation, and activation of neurotrophic growth factors in the treatment groups was examined through immunofluorescent antibody staining and microscopic imaging. Results demonstrated that Ubisol-Q10 and Ashwagandha increased neuroprotection and decreased oxidative stress and inflammation when compared to the untreated group. Enhanced mitochondrial function and synaptic branching was also observed in the combinatorial group. Future studies quantifying tyrosine hydroxylase-positive neurons in the substantia nigra via a stereological analysis may provide greater insight into using NHPs as a potential treatment for PD.

### Achillea millefolium L. Liquid Phases In Vitro Enzyme Inhibition Pierluca Pica<sup>1</sup>, Cory Harris<sup>1</sup>

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Achillea millefolium L., commonly known as yarrow, has been a part of the ethnobotany of North America for centuries. It is used in home remedies for pain relief or the treatment of ailments including the common cold. Yarrow contains alkylamides which are exogenous ligands of the endocannabinoid system, which can help reduce tissue inflammation through cell signalling and enzyme inhibition. This work aims to better characterize the phytochemical drivers behind this plant's anti-inflammatory potential by inhibiting fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), two membrane associated members of the serine hydrolase family of enzymes, which degrade endocannabinoids. The initial steps required phase separation of the plant roots, leaves, and flowers into aqueous and organic phases to be tested in inhibition assays. It was hypothesized that the flowers' aqueous phase would be the most potent inhibitor of these enzymes since it contains the most phytochemical diversity, including phenolic compounds and alkylamides. Results showed that organic extracts showed the greatest extent of inhibition of all samples tested, with complete inhibition of FAAH activity. High performance liquid chromatography (HPLC) analysis was used to determine the phytochemistry of aqueous and organic fractions to identify which biomolecules present in each phase were potentially driving enzyme inhibition. The next step in identifying the main phytochemical drivers behind anti-inflammation activity of yarrow is further fractionation of groups of phenolics and



alkylamides to identify new compounds and then performing a dose response with some of these compounds for FAAH and MAGL. Following this, future work will proceed in an EndoC-βH1° cell-line to test for anti-inflammation and anti-oxidation activity of fractions.

### **Encapsulation of Fruit Powders using Spray-Drying and Spray-Freeze Drying to Improve Stability and Functionality**

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This study investigates the encapsulation of fruit powders to prevent post-processing losses (such as mold formation and loss of bioactives). Strawberry, orange, and mango were used as fruit sources, and both spray-drying and sprayfreeze drying techniques were employed to produce the encapsulated fruit powders. Various core and wall materials were examined for encapsulation of the fruit powder. Encapsulation core materials include maltose, maltodextrin dextrose equivalent (DE) 4 - 7, and maltodextrin DE 16.5 - 19.5. Wall materials chosen were HP-MC and whey protein isolate. Core and wall ratios were studied by adding HP-MC and maltodextrin DE 4-7. The results indicated that encapsulating strawberries with maltodextrin DE 4-7 and HP-MC at a ratio of 4:1 yielded optimal powder characteristics and retained bioactive compounds. Similarly, strawberry encapsulation with 5% maltodextrin and spray drying inlet temperature of 185°C showed optimal bioactive compound retention. Another important consideration is the process yield; encapsulation with soy protein isolate increased the powder obtained after the spray drying. Encapsulation with whey protein isolates added weight to dry orange juice powders, significantly improving the quantity of powder obtained. Spray-freeze drying shows a more porous structure, which explains the enhanced re-dispersibility in deionized water. These findings contribute to understanding fruit powder encapsulation methods and can have implications for developing fruit powders with improved stability and functionality.

### Effect of Extraction Methods for Producing Seaweed Salt from the Brown Seaweed Macrocystis pyrifera

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This study aimed to explore effective extraction methods for producing seaweed salt from the brown seaweed Macrocystis pyrifera. The research investigated various factors that impact the yield and mineral composition of seaweed salt, specifically focusing on two extraction methods: solid-liquid extraction using a shaking water bath and ultrasound-assisted extraction using an ultrasonic bath. The study also examined different extraction times (ranging from 5 to 60 minutes) and temperatures (20°C, 40°C, and 60°C). The potassium, sodium, magnesium, and calcium contents were quantified using inductively coupled plasma optical emission spectroscopy. The findings revealed that both extraction methods resulted in seaweed salt with high potassium content, low sodium content, and a favorable sodium-to-potassium ratio. Notably, the shaking water bath extraction method exhibited significantly higher salt yield (p < 0.05) compared to the ultrasound bath method. However, the ultrasonic bath method yielded seaweed salt with significantly higher magnesium and calcium contents (p < 0.05). Taking salt yield and energy efficiency into consideration, the optimal extraction conditions were determined to be extraction using a shaking water bath at 20°C for 30 minutes and extraction using an ultrasound bath at 20°C for 15 minutes.

#### Discrepancies Between Labeled and Actual THC Levels in Cannabis Products Sophie Thanasack<sup>1</sup>, Mohammed Albadry<sup>1</sup>, Hazrah Moothoo<sup>1</sup>, Paula Brown<sup>1</sup>

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The accurate labeling of Tetrahydrocannabinol (THC) content in cannabis products required under the Canadian Cannabis Regulations is crucial for public health and consumer safety. Recent studies have revealed significant disparities between labeled and actual THC levels in marketed products across North America. A series of products were purchased from a government recreational Cannabis store in British Columbia to assess the extent of the



problem. First, replicate products from the same lot were purchased and tested using a validated analytical method (OMA 2018.10) to evaluate within-lot variance and to compare the values to label claim. Secondly, a product with varying weights was purchased and the buds were separated by size based on their measured diameter. These group's weights were recorded and HPLC was used to determine if bud size had a significant impact on THC content. The results of this study show that sample selection has an impact on THC content. Further, products that undergo handling during the packaging process are likely to lose potency, so it is important testing be completed on the actual finished product, rather than prior to packaging to ensure labels accurately reflect consumer products. Without specific sampling and testing guidance, the labels of cannabis products do not accurately reflect the potency of the purchased product, so it is buyer beware.

#### **Conflicts of Interest**

The authors declare that they have no conflicts of interest.

#### **Authors' Contributions**

PSH: Chair of the Program and Local Planning committee, made the call for abstracts on behalf of the NHPRS, reviewed the abstracts for acceptance, and contributed to the copyediting and review of the publication.

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