In vitro Antioxidant and Antibacterial Activity of Twenty-One Northern Ontario Medicinal Plants

A thesis presented to

The Faculty of Graduate Studies

of

Lakehead University

by

MOHAMMAD HAIDER HASSAN

In partial fulfillment of requirements
for the degree of
Masters of Science in Biology

May 9th, 2013

Abstract

Aboriginal communities in the northern Ontario region utilize an abundance of locally grown medicinal plants. However, no prior documentation or phytochemical studies on the northern Ontario medicinal plants existed in literature. This prompted me to exploit the ethnobotanical resources in this region towards the study of antibacterial bioactivity and alleviation of oxidative stress. Oxidative stress plays a fundamental role in the pathogenesis of many major human illnesses, such as cancer, cardiovascular diseases, diabetes and Alzheimer's syndrome. Also, infectious diseases are a major concern in our society due to the advent of multiple drug resistant strains of bacteria that cause millions of mortalities worldwide.

From the accumulated list of 48 northern Ontario medicinal plants, I selected 21 plants based on their documented anticancer, antibacterial, antioxidant, antidiarrheal and anti-inflammatory properties. These plants were separated into leaf, flower, stem and root tissues and extracted with ethanol. In total, 43 extracts were assayed for antioxidant and antibacterial activity in this study. The antioxidant activity was evaluated through the DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt), ORAC (Oxygen radical absorption capacity), and EC_{50} (half maximal effective concentration) assays. The total phenolic content of medicinal plants was also determined. The antibacterial activity was determined through the hole-plate diffusion and minimum inhibitory concentration (MIC) assays on *Bacillus cereus*, *Escherichia coli*, *Micrococcus luteus, Mycobacterium avium* subsp. *avium*, *Paenibacillus alvei* and *Aeromonas caviae* bacteria. The crude extract was fractionated through manual liquid chromatography (LC) into five fractions of varying polarity using a mixture of hexane: ethyl acetate: methanol solvents and assayed for inhibitory activity

in the MIC assay. Also, a few plants were shortlisted and studied for more detailed antibacterial activity through minimum bactericidal concentration (MBC) and time-kill analyses.

In the antioxidant assays, all plant extracts exhibit some level of activity, however, a few were exceptional. The extracts of *Cornus canadensis*, *Ledum palustre*, *Prunella vulgaris, Arctostaphylos uva-ursi*, and *Apocynum androsaemifolium* L. from the *Cornaceae, Lamiaceae, Ericaceae* and *Apocynaceae* families, respectively, display the highest antioxidant activity and total phenolic contents. In the antibacterial assays, plants from the *Asteraceae, Apocynaceae, Cornaceae* and *Ericaceae* families display the highest activity. Particularly, the leaf and/ or flower extracts of *Xanthium strumarium*, *Anaphalis margaritacea, Arctostaphylos uva-ursi*, *Apocynum androsaemifolium* L., *Cornus canadensis, Solidago canadensis* and *Grindelia squarrosa* exhibit high inhibition diameters and low MIC values. Also, for the majority of extracts, an increase in bioactivity was observed in the medium polarity LC fraction, relative to the crude. Particularly, the medium polarity fraction of *Anap. margaritacea* flower exhibits MIC values in the range of 0.08 -1.25 mg/ml against all six bacteria tested. The crude extract of *Anaphalis margaritacea* flower also displays MBC values in the range of 0.16 - 5 mg/ml against *A. caviae, M. luteus, P. alvei* and *B. cereus* bacterium and demonstrates complete extermination within eight hours of incubation. Overall, this investigation provides evidence for the application of these medicinal plants towards the treatment of infectious and oxidative stress related diseases in Native Aboriginal communities.

Acknowledgments

Throughout my master's work, Dr. Wensheng Qin has been a solid pillar of support, encouragement and research stimulation. He has also been a source of concrete criticisms and new ideas that have made me strive for excellence. I really appreciate Dr. Qin with full gratitude for taking me as a student and making my future brighter. I also want to sincerely thank Dr. Justin Jiang for his incredible attention to detail, simplicity and honest criticisms. Without his input, I firmly believe that my work would be unsatisfactory by a mile. I also want to sincerely thank Dr. Baoqiang Liao for being part of my committee and providing support throughout my masters. Overall, I believe that this project couldn't have been possible without the help of Ms. Erika North. Her assistance in collection and identification of the medicinal plants was central to my work.

I also want to thank Dr. Mehdi Dashtban and Dr. Miranda Maki for being a constant stream of wisdom in the lab and role models that I tried to emulate. I really appreciate all their help in sharing their expertise on conducting better experiments with proper controls. Most people have a boring and unsociable attitude towards research. Through my experience in Dr. Wensheng's lab, I couldn't disagree more. It's the usual parties to celebrate a graduating student, an uncanny sense of humor from Tyler Drawson, the incredible help from Dr. Miranda, or the unintentionally funny comments from Dr. Yuanyuan Hong that made life bearable. I have come to learn that science is more than research. It's the act of communicating, conducting research, striving for excellence, group work and imagination that define this dynamic discipline.

At last, I want to thank the Department of Biology and the Biorefining Research Institute (BRI) for providing proper facilities to conduct my research.

Table of Contents

List of Tables

List of Figures

Abbreviations

AML: Acute myeloid lymphoma

ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt

AAPH: 2,2'-Azobis(2-methylpropionamidine) dihydrochloride

AUC: Area under the curve

Bcl protein: B cell lymphoma protein

CFU: Bacterial Colony Forming Units

CDK: Cyclin dependent kinase

COX-1: Cytochrome C oxidase-1

DPPH: 2,2-diphenyl-1-picrylhydrazyl

ET: Electron transfer methods

ETOAc: Ethyl Acetate

EC_{50}: Half maximal Effective Concentration

GLOBOCAN: Global Cancer

HAT: Hydrogen Atom Transfer methods

Hex: Hexane

IC_{50}: Half maximal inhibitory concentration

LC: Liquid chromatography

ORAC: Oxygen Radical Absorption Capacity Assay

MBC: Minimum Bactericidal Concentration

MeOH: Methanol

MDA: Malondialdehyde

MRSA: Methicillin-resistant *Staphylococcus aureus*

MSSA: Methicillin-sensitive *Staphylococcus aureus*

MIC: Minimum inhibitory concentration (Concentration that entirely inhibits bacterial/ fungal growth)

MIC_{50}: Half maximal minimum inhibitory concentration

MIC_{90}: Concentration that inhibits 90% of bacterial growth

NIR: Near-infrared radiation

NPHS: National population health survey

PUFA: Polyunsaturated fatty acid

ROS: Reactive Oxygen Species

TCM: Traditional Chinese medicine

TBA: Thiobarbituric acid

TBARS: Thiobarbituric acid-reactive substance assay

WHO: World health organization

ZOI: Zone of Inhibition

5-LO: 5-lipoxygenase

TE / gdw: Trolox Equivalence per gram dry weight

GAE/ gdw: Gallic Acid Equivalence per gram dry weigh

CHAPTER #1

General Introduction: Northern Ontario Medicinal Plants

Haider M. Hassan

ABSTRACT

Majority of scholarly investigations conducted in the 20th century have provided the incentive for establishing plants as sources of diverse phytochemicals. With infectious and oxidative stress related diseases causing millions of mortalities worldwide and the advent of multiple drug resistant strains of bacteria, the discovery of new bactericidal and anticancer agents is crucial. Hence, presented here is a novel list of 48 northern Ontario medicinal plants that may be a source of antifungal, antibacterial, anticancer and/or antioxidant phytochemicals. A total of 2 ferns and allies, 2 sedge and grass, 6 trees, 4 shrubs, 1 vine and 33 herbs were identified. These plants were accumulated through interviews with native Elders and a survey of ethnobotanical literature on northern Canadian species of medicinal plants. I also present a critical review of their potential constituents, medicinal properties, and analysis of four promising plants (Skullcaps, devils club, St. John's Wort and evergreens). Skullcaps and St. John's Wort are model plants with documented anticancer, antibacterial and antifungal bioactivities. However, a considerable gap in ethnopharmacological data was found for species of skullcaps (*Scutellaria galericulata, parvula* and *lateriflora*) and St. John's Wort (*Hypericum mutilum, majus, canadense*) growing in the northern Ontario region. This finding provides promising incentives in the ethnopharmacological community for medicinal research in this region.

1. Introduction

Global Cancer (GLOBOCAN) statistics estimated a global count of 12.7 million new cancer cases and 7.6 million cancer related mortalities to have occurred in 2008; approximately 56% of the cases and 64% of the deaths occurred in economically developing countries (Jemal et al. 2011). According to an estimate by Ames et al. (1995), one in four deaths in America are due to cancer. Propagation of oxidative stress is one of the major causes of cancer, which is generated through an imbalance between reactive oxygen species (ROS) and antioxidant molecules. The chronic increase in ROS is known to amplify genetic instability, which triggers cell transformation and tumor progression. Also, many studies indicate that an increase in ROS results from stimulation of the cell cycle progression by growth factors, or mutations that activate the tyrosine kinase pathways (Shumacker et al. 2006). As such, the discovery of novel antioxidant agents from the plant and synthetic sources has received renowned attention.

Infectious diseases are also a major problem, particularly in the developing countries. Worldwide, one in three deaths result from an infectious or communicable disease (Lopez 2006). This problem can be traced to the evolution of multiple drug resistant strains of pathogenic bacteria due to a concentrated use of existing antimicrobial drugs (Ahmad et al. 1998). These issues have created immense clinical problems in the treatment of infectious diseases.

The agricultural industry also suffers from the advent of microbial infestations on crops (Hadacek & Greger 2000). Some estimates claim that plant pathogens cause an approximate 20% reduction in crop yield per year worldwide (Oerke et al. 1994). As such, with increasing world population (Gomiero et al. 2011), and limited land for agricultural growth, the use of fungicides and bactericides remain an integral part of agriculture and food protection.

1.1. Natural Products as Promising Aspects

Medicinal properties of various plant extracts have been documented since the 5th century B.C. It is estimated that more than two-thirds of current drugs are derived from plant sources (Coe & Anderson 1996). Between 1981-1982, 60% all drugs developed to combat oxidative stress originated from plant sources (Newman & Cragg 2007). In the case of infectious diseases, that number reached 70%. Furthermore, a scrutiny of medical indications by the source of compounds has demonstrated that natural products and related drugs are used to treat 87% of all categorized human diseases, including antibacterial, anticancer, anticoagulant, anti-parasitic, and immunosuppressant agents, among others (Newman et al. 2003). Between 2001 and 2005, 23 new drugs from plant origins were introduced to treat diseases such as cancer, fungal infections, bacterial infections, diabetes, atopic dermatitis, Alzheimer's syndrome, and genetic diseases such as tyrosinemia and gaucher's disease (Lam 2007). Despite the tremendous success of drug discovery from natural sources, the pharmaceutical industry has retracted its investigation of plants as sources of novel chemicals (Coe & Anderson 1996; Farnsworth & Morris 1976; Lam 2007).

There are major economic incentives for the discovery of natural products. Herbal therapy is a way of life in almost 80% of the people in rural areas, especially those in Asia, Latin America and Africa (Shale et al. 1999). According to a World Health Organization (WHO) survey, about 70 - 80% of the total world population depends on herbal remedies as a source of their primary health care (Chan 2003). It is estimated that in U.S., approximately 30% of the population uses $13 billion worth of alternative or herbal remedies per year (Keen et al. 1994). A relatively recent telephone survey by the National Population Health Survey (NPHS) of 11, 424 Canadian adults reported a 15% usage of natural products within the prior 2 days (Singh &

Levine 2007). Another survey of the Canadian breast cancer patients found that 25% reported usage of herbal medicines (Smith & Boon 1999). The great demand for herbal products, and the diminishing forestry in Northern Ontario, should incentivize the pharmaceutical industry and research community to investigate plants in this region for sources of phytochemicals for the treatment of oxidative stress related and infectious disease.

2. Traditional Medicinal Plants of Northern Ontario

2.1. Northern Ontario Medicinal Plants with Potential Bioactive Substances

There has been considerable research in Canada on medicinal plants collected by Aboriginal First Nations individuals (Westfall & Glickman 2004). Jones et al. (2000) tested 18 medicinal plants, used by First Nations peoples in eastern Canada, for their anti-fungal properties against opportunistic human pathogens. They discovered that 13 plants contained anti-fungal properties, and that medical knowledge held by First Nations Peoples significantly correlates with the laboratory findings. Fraser et al. (2007) assessed 36 medicinal plants from two Cree communities (Whapmagoostui and Mistissini) for antioxidant activity via 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and their ability to protect human low-density lipoprotein from oxidation. Upon comparison of antioxidant activity of these 36 medicinal plants with 16 extracts not utilized for medicinal purposes, a positive correlation was found between the established traditional knowledge of Cree Elders and the radical scavenging activity of these plants. Essentially, plants used by Native Indians towards the cure of certain illnesses have more therapeutic potential than a randomly chosen sample (Arnason et al. 1981).

The list of northern Ontario medicinal plants (**Table 1**) was compiled through interviews with native Elders and a survey of ethnobotanical literature (Argus et al. 1999; Arnason et al. 1981; Bryan et al. 1993; Chevallier 1996; Etkin 2008; Hälvä & Craker 1996; Heatherly 1998; Li 2000; MacKinnon 2009; Mowrey 1990; Small & Catling 1999; Willard et al. 1992)**.** The criteria for plant selection was based on the North American First Nations ethnobotanical medicinal plant trend (Jones et al. 2000), which states that plants used towards the treatment of burns, cuts, infections, diarrhea, and mouth conditions are likely to contain antibacterial substances. Plants used towards the treatment of oxidative stress related diseases (i.e. cancer, inflammation) were also included in the list. A total of 2 fern and ally, 2 sedge and grass, 6 trees, 4 shrubs, 1 vine and 33 herbs were accumulated with reference to their conventional medicinal names (**Table 1**). Overall, 105 different species of plants are listed due to the existence of various species within the conventional nomenclature. The potential secondary metabolite(s) of plants were identified via a rigorous literature analysis of their medicinal properties. No prior herbal research has been documented for northern Ontario; as such, this is the first reporting of medicinal plants in this region.

3. Research on Medicinal Plants of Northern Ontario

Northern Ontario constitutes 87% of the land mass allocated for the province of Ontario, but houses only 6% of the provincial population. Unlike urban centers, it is renowned for investment in forestry and many industries resort to forestry resources for economic stability and diversification (Duinker et al. 1991). This region has a large diversity of plant species used in traditional aboriginal communities for various medicinal incentives. Described here are a few examples of medicinal plants from northern Ontario that have attracted considerable interest in the ethnobotanical community.

3.1. Skullcaps (*Scutellaria* sp.)

Scutellaria (*Lamiaceae* plant family) genus contains approximately 350 species (Shang et al. 2010). It is widely distributed in mountains of temperate and tropical regions including Europe, North America and South Asia. They range from 0.05 - 1 m in height and have been used for thousands of years in the traditional Chinese medicinal (TCM) practices (Shang et al. 2010). Skullcaps are copiously noted for anti-proliferative, anti-cancer, antibacterial and antiviral bioactivities. *Scutellaria baicalensis* (Huáng Qín Täng) is one of the 50 fundamental herbs in Chinese medicine (Yin et al. 2004) and a significant quantity of literature documents its anti-cancer activity (Kumagai et al. 2007; Parajuli et al. 2009; Scheck et al. 2006; Shang et al. 2010; Ye et al. 2002). Ye et al. (2002) demonstrated that *S. baicalensis* is cytotoxic at half maximal Inhibitory Concentration (IC_{50}) of 1.1, 0.9, 0.52, 0.82 and 1.1 mg/ml to squamous cell carcinoma, breast cancer, hepatocellular carcinoma, prostate carcinoma, and colon cancer, respectively. There was a strong dose-dependent inhibition of proliferation in all cell lines tested. Scheck et al. (2006) correlated its bioactivity to the presence of phenolic compounds Baicalein and Baicalin (**1a** and **1c**, respectively) and concluded a possible synergistic viability when used in concert with other chemotherapeutic agents. Kumagal et al. (2007) associated the anti-proliferative effect of *S. baicalensis* to mitochondrial damage, modulation of anti-apoptotic family of genes (Bcl), increased level of cyclin dependent kinase (CDK) inhibitor $p27^{KIP1}$ and decreased level of proliferation stimulatory c-myc gene. Among other species in the genus, *Scutellaria barabata* has also been shown to exhibit strong *in vitro* anti-cancer activity (Cha et al. 2004; Kumagai et al. 2007; Shang et al. 2010; Shoemaker et al. 2005; Yin et al. 2004; Yu et al. 2007). Despite the documented success of this herb in treating cancer, research on northern Ontario species of *Scutellaria (S. galericulata, S. parvula* and *S. lateriflora*) is vastly lacking.

This situation presents a promising opportunity for researchers towards discovery of potential anticancer agents.

Scutellaria sp. also exhibit antimicrobial properties. Sato et al. (2000) isolated flavonoids from *Scutellaria barbata* that displayed selective toxicity towards methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin sensitive *S. aureus* strains (MSSA). The most potent component was found to be apigenin (**1b**) with minimum inhibitory concentration (MIC) of 3.9-15.6 µg/ml against MRSA and MSSA. Baicalin, an antibacterial, anticancer, anti-HIV and anti-inflammatory flavonoid representing 5% of the dry weight of *S. baicalensis*, also displayed a half maximal minimum inhibitory concentration (MIC_{50}) and MIC_{90} of 1.04 and 1.30 mg/ml, respectively, against ten strains of *Helicobacter pylori* (Wu et al. 2008). The myriad signature bioactivities recorded for this herb, promotes the *Scutellaria* species as interesting subjects of ethnopharmacological research.

3.2. St. John's Wort (*Hypericum* sp.)

St. John's Wort is a well-studied perennial herb that grows widely in Europe, Western Asia, North Africa and America (Gupta & Möller 2003). It is also known as amber, Klamath weed, millepertuis, rosin rose, or Tipton's weed (Rowe and Baker 2009). St. John's Wort was utilized in ancient native traditions for over 2000 years as a spiritual plant to alleviate sickness, misfortune, anxiety, depression and served as a topical treatment for superficial wounds and burns (Gupta & Möller 2003; Rowe and Baker 2009). Active ingredients from *Hypericum perforatum* have claimed antibacterial, antidepressant, antiviral and anticancer properties. Also, the anti-depressant activity of this herb is well debated, and has been attributed to the inhibition of norepinephrine, serotonin and dopamine synaptosomal uptake in many double blind studies

(Carpenter 2011; Gupta & Möller 2003; Müller et al. 1997). However, Rapaport et al. (2011) refutes this result due to a high response measurement of the placebo dose, relative to St. John's Wort extract. Attention has been allocated to hyperforin (**2**) and hypericin (**3**) as the active ingredient behind this property (Barnes et al. 2001).

Hyperforin (**2**), a natural phloroglucinol isolate, is also reported to have anticancer activity. Merhi et al. (2011) discovered that hyperforin (**2**) inhibited the growth of AML cell lines (U937, OCI-AML3, NB4, HL-60) by inducing apoptosis in a time and concentration-dependent manner. The normal blood cells were not affected in the treatments. This activity has been attributed to the suppression of Cytochrome C Oxidase-1 (COX-1) and 5-Lipoxygenase (5-LO) activity, key enzymes in the formation of pro-inflammatory eicosanoids (Albert et al. 2002). Further, hyperforin has been shown to act synergistically with hypericin (**3**) in its inhibitory effect on leukemic cell growth (Hostanska et al. 2003). The antibacterial property of hyperforin (**2**) has also been observed only at high concentrations, and its low potency makes it unlikely to be used towards this aspect.

Preparations of *H. perforatum* are available at pharmacies, herbal medicine and health-food stores. It is considered one of the best selling herbal products. Due to the success of this herb, it is surprising that many species in this genus have not been well researched (i.e. *H. mutilum, H. majus, H. canadense*). *H. multilum* and *H. canadense* are species of St. John's Wort found in northern Ontario. Though antibacterial activity has been reported for the *H. multilum* extract (Carlson et al. 1948), the literature is significantly outdated and lacking in aptitude. This presents a promising opportunity for contemporary research.

3.3. Devils Club (*Oplopanax horridus*)

Devil's Club (*Oplopanax horridus*), native to Thunder Bay, is considered one of the most important spiritual and medicinal plants to indigenous communities within its affinity. It is a large shrub located in cool moist forests of western North America and is known for its large palmate leaves and erect, woody stems covered in brittle spines. Devil's club is well-researched for its hypoglycemic, antibacterial, and antioxidant properties. Kobaisy et al. (1997) studied the anti-microbial properties of polyyne (**4**) isolated from the root bark of Devil's Club. The isolate exhibited significant anti-*candida*, antibacterial and antimicrobial activity, with an MIC of 10µg/ml against *Mycobacterium tuberculosis* and isoniazid-resistant *Mycobacterium avium* in a disc diffusion assay. These results are in correlation with findings that the inner bark of Devil's Club has been used by the indigenous people to cure tuberculosis (Thommasen et al. 1990). Devils Club is also noted for anti-proliferative, antioxidant and anti-viral activities. McCutcheon et al. (1995) studied anti-viral activity of inner bark methanolic extract of Devil's Club and found it partially inhibited the Herpes virus type 1. Tai et al. (2006) documented anti-proliferative activity of inner bark extract against K562, HL60, MCF7 and MDA-MB-468 cancer cell lines. The ethanolic extracts exhibited synergistic effects when combined at non-inhibitory concentrations with non-cytotoxic concentrations of camptothecin or paclitaxel. The anti-proliferative activity of Devils Club extracts may be correlated to its strong antioxidant profile.

Devils Club has been unanimously quoted in the Native community to relieve diabetic symptoms. Large et al. (1938) demonstrated that at extract concentrations of 0.1, 0.2, and 0.25 c.c. per lb. of body weight, a rapid reduction in blood sugar from 35 to 70 mg. per 100 c.c. was observed. On the contrary, Thommasen et al. (1990) reported an absence of hypoglycemic activity when administered to an insulin-dependent diabetic patient, a newly diagnosed non-

insulin-dependent diabetic, and two healthy individuals in a closely monitored study. With these apparently contradictory data, further investigations are necessary to evaluate the hypoglycemic property of this plant.

Also, considering the range of bioactivities, it is surprising that very few publications report on the isolation and characterization of a bioactive compound from Devil's club. More research needs to be geared towards this aspect.

3.4. Evergreens

Evergreen plants have a long leaf lifespan, and are active in all seasons (Moore 1980). They encompass a wide array of trees and shrubs, including conifers, gymnosperms and angiosperms. Evergreen trees, perennials and herbs have been used for the treatment of infection, cancer or toothache alleviation in native communities. Prior to winter, a sappy substance called "pine gum" is harvested from Juniper, Jack pine, Balsam Fur, Spruce, Tamarack or other conifers and rubbed as a paste on wounds to prevent infection. This paste is also rubbed on teeth to mitigate toothaches. The root of evergreen plants is harvested in the winter, extracted with water, and applied on superficial wounds or cuts to prevent infection.

Clubmoss (*Lycopodiaceae* family) is a low growing, non-flowering, spore producing vascular plant that covers approximately 80% of the soil surface in many grassland communities of the northern mixed prairie of North America (Romo 2010). Clubmoss species have been used in Chinese medicines as tea or poultice for the treatment of amnesia, contusion, swelling, schizophrenia and hematuria (Bai 1993). *Lycopodium varium* from New Zealand exhibited insecticidal activity (Ainge et al. 2002). Orhan et al. (2007) tested the antibacterial activity of *Lycopodium clavatum* and found that it inhibited all the bacteria tested with an MIC range of 4-

64 µg/ml. Their study also revealed antifungal and antiviral properties from this species. Further, many alkaloids displaying a wide array of bioactivities have been isolated from club mosses. Huperzine A (**5**), lycopodine (**6**), serratezomines, carinatumins, and complamandine are among a few alkaloids isolated that exhibit insecticidal, antibacterial, anti-acetylcholinesterases and induction of neurotropic factor secretion activity, respectively (Choo et al. 2007; Kubota et al. 2009; Morita et al. 2005; Orhan et al. 2007). Huperzine A also exhibits anticholinesterase activity and is presently in phase II clinical studies for the treatment of Alzheimer's disease in elderly patients (Rafii et al. 2011). It has been used for the treatment of myasthenia gravis, dementia and senile memory improvement (Yu et al. 1986). However, lycopodine is the major alkaloid found in clubmosses (Orhan et al. 2007).

4. Perspectives

There has been considerable research on the isolation of bioactive substances from medicinal plants, their efficacy against diseases, potential targets, architectural characterization, and therapeutic properties. Several plant-derived drugs have been introduced in the market, and there is significant evidence to support continued research on their extraction and isolation. For example, the famous anticancer drug Paclitaxel was isolated from pacific yew (*Taxus brevifolia*) and is now administered in the treatment of breast, lung and ovarian cancer (Jemal et al. 2011). Past experiences have taught us that plant phytochemicals possess a broad range of bioactivities against bacteria, fungi, cancer and other diseases. New procedures and advanced separation techniques may lead to the discovery of novel chemical entities previously neglected. Overall, many plant compounds remain untapped resources in medicine; as such, it is essential to

investigate their therapeutic properties and chemical structures. In accordance, based on the abundant medicinal plant resource and the great knowledge in utilizing these plants by the First Nation People in northern Ontario, there is great potential for drug discovery in this region.

5. Thesis Objectives

Through a literature survey, no prior research on northern Ontario medicinal plants was identified. This presented me with a unique opportunity for ethnopharmacological research in this region for the treatment of infectious and oxidative stress related diseases. Hence, the objective of this thesis is to evaluate the *in vitro* antioxidant and antibacterial activity of twenty-one northern Ontario medicinal plants. The medicinal plants were collected from the Thunder Bay region from a list of forty-eight northern Ontario medicinal plants (**Table 1**). The antioxidant activity is to be determined through DPPH, ABTS and ORAC assay and correlated with the total phenolic contents of medicinal plants. The antibacterial activity is to be determined through the hole-plate diffusion and minimum inhibitory concentration (MIC) assays against environmental isolates of *Escherichia coli, Mycobacterium avium* subsp. *avium, Micrococcus luteus, Aeromonas caviae* and *Paenibacillus alvei* bacterium. To elucidate the chemical characteristics of plant metabolites, crude extracts are to be further separated into varying fractions via gradient solvent manual liquid chromatography and analyzed in the MIC assay. Also, a few highly bioactive crude extracts are to be shortlisted and analyzed for the minimum bactericidal concentration (MBC) and time-kill analysis in order to provide incentive for future clinical investigations.

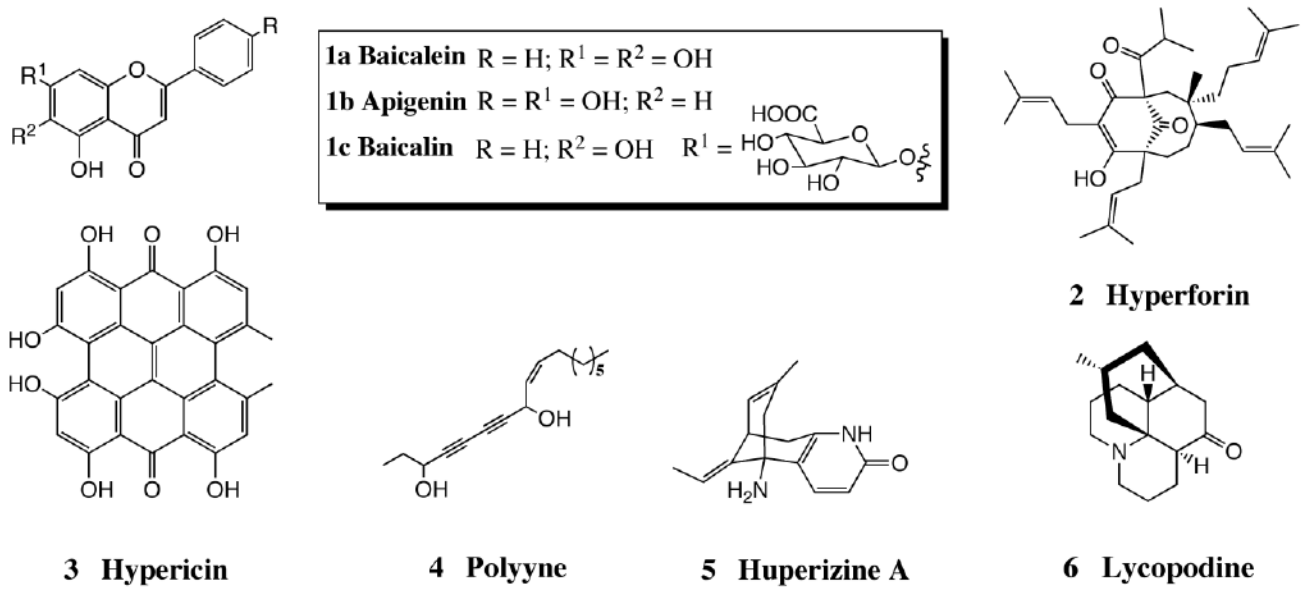

Figure 1: Chemical structures of bioactive compounds isolated from medicinal plants: Baicalein (**1a**), apigenin (**1b**) and baicalin (**1c**) isolated from the roots of *Scutellaria baicalensis*; Hyperforin (**2**) isolated from the aerial parts of *Hypericum perforatum*; Hypericin (**3**) isolated from the entire *Hypericum perforatum* plant; Polyyne (**4**) isolated from the root bark of *Oplopanax horridus*; Huperzine A (**5**) and lycopodine (**6**) isolated from the aerial parts of *Lycopodium clavatum*.

Table. 1. List of 48 Northern Ontario medicinal plants.

a. *Fillicinophyta*

Conventional Name	Plant Family	Botanical Name	Medicinal Properties	Potential Constituent(s)
Clubmosses	Lycopodiaceae: Clubmoss	*Lycopodium annotinum, L. clavatum, L. complanatum, L. dendroideum, L. inundatum, L. lucidulum, L. sabinifolium, L. selago, L. sitchense, L. tristachyum, L. x zeilleri*	Antifungal, antibacterial, antiviral (Orhan et al. 2007); relieve spasms, increase urine flow, estrogenic; reduce pain, fever, inflammation; insecticidal (Ainge et al. 2002; Ibrahim et al. 2001)	Lycodine type alkaloids (Nagai et al. 2005), lycopodine alkaloids (Orhan et al. 2007), tetracyclic alkaloids (Yin et al. 2006), clavine alkaloid (Wink & Schneider 1990), huperizine A (Orhan et al. 2007)
Common Horsetail	Equisetaceae: Horse tail	*Equisetum arvense L., E. fluviatile L., E. palustre L., E. pretense Ehrh., E. sylvaticum L., E. variegatum.*	Teas used to treat gout, gonorrhea, stomach problems, bronchitis, tuberculosis and infection (Feresin et al. 2003), antioxidant (Amarowicz et al. 2004)	Antioxidant phenolics and proteins (Nagai et al. 2005), saponins, alkaloids (Abascal & Yarnell 2008)

b. *Plantae*

Conventional Name	Plant Family	Botanical Name	Medicinal Properties	Potential Constituent(s)
Juniper	Cupressaceae: juniper	*Juniperus communis L., J. horizontalis*	Treatment of pneumonia, fever, colds, coughs, rheumatic joints, inflammation, diarrhea; antimicrobial (Matovic et al. 1996)	Tannins (Matovic et al. 1996), alkaloids , terpenoids, flavonoids, sterols (Kumar et al. 2010; Wink 1987)
White Spruce	Pinaceae: pine	*Picea glauca, P. mariana*	Used to treat infection (Jøhnk et al. 2005), insect bites, cuts (Jøhnk et al. 2005), scrapes	Camphor (Roy & Bergeron 1990), polyphenolics (Ralph et al. 2006), β-sitostarol (Dreikorn 2000), monoterpenes, 4-ally-lansinol (Ibrahim et al. 2001)
Mossy Cup Oak	Fagaceae: beech	*Quercus macrocarpa*	Used to treat diarrhea, toothaches, skin infections, cuts, sore throats, burns; has antiviral and antibacterial properties (Güllüce et al. 2004)	Tannins (De La Rosa et al. 2001), phenolic acids (Cantos et al. 2003), polyphenols (Scalbert & Haslam 1987)
Balsam Poplar	Salicaceae: willow	*Populous balsamifera* L., *P. tremuloides, P. grandidentata*	Used to treat diarrhea, fevers, skin problems, worms, inflammation; antimicrobial, antifungal (Isaeva et al. 2010; Mathes 1963)	Monoterpenes (Bryant et al. 1992), sesquiterpenoids (Mattes et al. 1987), phenolic glycosides (Mattes et al. 1987), flavonoids (Isaeva et al. 2010), tannins (Schimel et al. 1996)
White Elm	Ulmaceae: elm	*Ulmus americana* L.	Used to treat inflammation, diarrhea, burns, heartburn, antibacterial (Lee et al. 1992), antifungal (Burden & Kemp 1984)	Cerato ulmin (Richards & Takai 1988), phenolics (Witzell & Martín 2008), sesquiterpenes (Burden & Kemp 1984)
Horse Chestnut	Hippocastanaceae: Buckeye	*Aesculus hippocastanum*	Used to treat blood circulatory problems and varicose veins; has anti-edema, anti-inflammatory, and free radical scavenging properties (Lou et al. 2004); antimicrobial (Fant et al. 1999)	Flavonoids (Kapusta et al. 2007), saponins (Benthin et al. 1999), triterpenoid glycoside (Loew & Kaszkin 2002), polyphenols (Lou et al. 2004), antimicrobial proteins (Fant et al. 1999)

c. Magnoliophyta

Conventional Name	Plant Family	Botanical Name	Medicinal Properties	Potential Constituent(s)
Quack Grass		*Elymus repens* L.[2]	Antiseptic, laxative: used to treat fever, syphilis, jaundice, swollen and rheumatic limbs, chest	DIBOA, vanillin, β-hydroxybutyric-, 4-hydroxycinnamic-, ferulic-, vanillic-, syringic- and

	Poaceae: grass		pain, poor eyesight; affects crop development and reduce crop yields (An et al. 2005)	protocatechuic acids (An et al. 2005); allelopathic aglycans (Hagen, 1989)
Sweet Grass		*Hierochloe odorata*	Treat coughs, fever, venereal infections (Mohagheghzadeh et al. 2006)	Coumarin (Small & Catling 1999), antioxidant (Kumar et al. 2010), 8-dihydroxycoumarin (Krishnaiah et al. 2007)

d. Conipherophyta

Conventional Name	Plant Family	Botanical Name	Medicinal Properties	Potential Constituent(s)
Devil's Club	Apocynaceae: dog bane	*Oplopanax horridus* [Z]	Hypoglycemic (Large et al. 1938), antibacterial (Kobaisy et al. 1997), antiviral (Tai et al. 2006), antioxidant (McCutcheon et al. 1995); used to treat diabetes, colds, bronchitis, pneumonia; anticancer (Sun et al. 2010)	Polyynes (Kobaisy et al. 1997); sesquiterpenoids (Small & Catling 1999); hydrophobic anticancer metabolites (Sun et al. 2010); diynes (Copp 2003); phenolic glycosides (Huang et al. 2011)
Dogwood or Bunchberry	Cornaceae: dogwood	*Cornus canadensis* L. [Z], *C. alternifolia, C. rugosa Lam., C. stolonifera*	Anti-inflammatory, fever-reducing, pain killer; used to treat diarrhea, dysentery and burns, infections (Post & Urban 1995)	Antioxidant phenolics (Mulabagal & Tsay 2004; Tanaka et al. 2003)
Common Bearberry	Ericaceae: heath	*Arctostaphylos uva-ursi*	Used to treat urinary tract infections, diarrhea, bladder problems, bronchitis, bleeding, cystitis (Abascal & Yarnell 2008); has antiseptic effects, anti-bacterial (Betoni et al. 2006; Puupponen-Pimiä et al. 2001), antioxidant properties (Amarowicz et al. 2004)	Phenolic glycoside arbutin, tannins (Small & Catling 1999), antibacterial polyphenol (Betoni et al. 2006), arbutin (Abascal & Yarnell 2008)
Prince's Pine	Pyrolaceae: wintergreen	*Chimaphila umbellate* [Z]	Antimicrobial; used to treat infections, fevers, colds, sore throats, coughs, backaches, stomachaches, bladder problems, cystitis (Abascal & Yarnell 2008)	Antifungal compound (Galván et al. 2008), arbutin (Abascal & Yarnell 2008)

e. Anthophyta

Conventional Name	Plant Family	Botanical Name	Medicinal Properties	Potential Constituent(s)
Common Hops	Cannabaceae: hemp	*Humulus lupulus* [2]	Antibacterial; used to relieve tumors, pneumonia, wounds, toothaches, fevers, painful swellings, boils; anti-HIV (Yazaki et al. 2009)	α acids, β acids, prenylated chalcones (De Keukeleire et al. 2003); prenylated flavonoids, humulone and lupulone, phloroglucinol derivatives (Yazaki et al. 2009)

f. Magnoliphyta

Conventional Name	Plant Family	Botanical Name	Medicinal Properties	Potential Constituent(s)
Skunk Cabbage	Ariceae	*Symplocarpus foetidus* [2]	Treatment of epileptic seizures, applied to wounds, rheumatism; potentially antifungal (Edilmesi 2002)	Antifungal nitro compounds (Edilmesi 2002)
Sweet Flag	Alismataceae: water plantain	*Acorus calamus* L. [2]	CNS-depressent, anti-inflammatory, antioxidant, antispasmodic, memory enhancing, antidiarrheal, anti-helmenthic; insecticidal (Varma & Dubey 1999)	Phenylpropanes, monoterpenes, sesquiterpenoids, β-asarone (Small & Catling 1999); phenolics (Gurevitch et al. 2002)
Wild Onions and Wild Chives	Liliaceae: lily	*Allium schoenoprasum* L., *A. stellatum*	Used for treatment of cuts burns, insect bites, stings; Antibacterial, antiviral, antifungal (Kumari et al. 2009)	Saponins (Benkeblia 2004), phenolics (Barile et al. 2007)
Spreading Dog Bane	Papocynaceae: dog bane	*Apocynum androsaemifolium L., A. cannabinum L.*	Used to treat headaches, insomnia, constipation, indigestion, rheumatism, liver disease, syphilis; Antitumor properties (Murthy et al. 2011)	Indole alkaloids (Murthy et al. 2011), monoterpenoid indole alkaloids (Mishra et al. 2006), latex (Jing-Yan & Zhao-Pu 2010)
Wild Ginger	Aristolochiaceae: birthwort	*Asarum canadense* L.	Used to treat coughs, stomach problems, fever, gas, stomach upset and rashes; Antibacterial, antifungal properties (Yarnell & Abascal 2008)	Anti-adhesion compounds (Yarnell & Abascal 2008), chalcone and flavanol glycosides (Iwashina & Kitajima 2000); aristolochic acid alkaloids, borneol, α-pinene terpineol, ellagic acid (Arnason et al. 1981)
Swamp and Common Milkweed	Asclepiadaceae: milkweed	*Asclepias incarnate* L., *A. syriaca* L.	Used for treating blindness; stomachaches, asthma, bowel problems, rheumatism, intestinal worms; Milky sap applied to cuts and burns to infections and	Latex containing surface cardenolides (Wittstock & Gershenzon 2002)

			irritations (Wittstock & Gershenzon 2002)	
Common Yarrow	Asteraceae: sunflower	*Achillea millefolium* L.	Antiseptic, antibacterial (Vasinauskiene et al. 2006), anti-inflammatory, anti-spasmodic; used to treat diarrhea, urinary tract infections (Woods-Panzaru et al. 2009)	Isovaleric acid, camphor, azulenes, dehydromatracaria ester (Arnason et al. 1981); lactones, sterols, flavonoids (Arnason et al. 1981)
Black-eyed Susan and Wild Goldenglow		*Rudbeckia hirta L, R. laciniata* L.	Used to treat inflammation, indigestion, sores, snakebites, swellings, earaches; antimicrobial (Luczkiewcz & Cisowski 2001)	Polyacetylenes (Arnason et al. 1981); flavonoids, phenolic acids, anthocyanins (Luczkiewcz & Cisowski 2001); sesquiterpene lactones, pulchelin E (Luczkiewicz et al. 2002)
Common and Rough Dandelion		*Taraxacum ceratophorum, T. officinale*	Antimicrobial, lower sugar and cholesterol level, anti-inflammatory, immune stimulant; treat liver, urinary tract issues (Woods-Panzaru et al. 2009)	Sesquiterpene lactones (Michalska & Kisiel 2003), phenolics (Hudec et al. 2007); Taraxacin, taraxerin, taraxerol, taraxasterol, inulin, gluten, gum, potash, choline, levulin, putin (Small & Catling 1999)
Gumweed		*Grindelia squarrosa*	Used to treat bladder inflammation caused by fungi or food (Hoffmann et al. 1993)	Alkaloids (Hazlett & Sawyer 1998), diterpenes, polyphenolics (Hoffmann et al. 1993)
Pearly Everlasting		*Anaphalis margaritacea*	Used to treat swollen mucus membranes, paralysis; have anti-inflammatory, astringent effects; antimicrobial (Borchardt et al. 2008)	Polyacetylenes, pentaynes (Borchardt et al. 2008)
Canadian Goldenrod		*Solidago canadensis, S. multiradiata*	Used to treat rheumatism, neuralgia, headaches, sore throat, kidney stones, ulcers; contains antioxidant and antiseptic substances (Abascal & Yarnell 2008)	Flavonoids, glycosides, saponins (Abascal & Yarnell 2008); phenolic acids (Buchsbaum et al. 1984)
Cocklebur		*Xanthium strumarium* L.	Antibacterial, antifungal, antimalarial (Cerdeiras et al. 2007)	Carboxyatractyloside (Cutler 1985); 8-*epi*-tomentosin, xanthanolides (Park et al. 2001)
Sweet Coltsfoot		*Petasites frigidus*	Inhibits bacterial growth; used to treat inflammation, swelling, burns, sores and skin diseases; anti-	Pyrrolizidine alkaloids (Smith & Culvenor 1981)

			carcinogenic	
Chamomiles		*Matricaria maritime* L., *M. matricarioides*	Antibacterial (Pastirova et al. 2004), anti-ulcer, antiviral, anti-inflammatory (Pastirova et al. 2004)	Sesquiterpenes, polyacetylenes, flavonoids, coumarins (Pastirova et al. 2004)
Blue Cohosh	Berberidaceae: barberry	*Caulophyllum thalictroides*	Used to treat pelvic inflammatory disease, colic, sore throat, inflammation, fevers, infections (Gottshall & Lucas 1949)	Quinolizidine alkaloids (Roy & Bergeron 1990)
Marsh Yellow Cress	Brassicaceae: mustard	*Rorippa palustris*	Used to treat inflammation, infection, anemia, bronchitis (Bussmann et al. 2008)	Isothiocyanates (Ishimoto et al. 2000), tropane alkaloids (Brock et al. 2006), glucosinolates (Gurevitch et al. 2002)
Shepherd's purse		*Capsella bursa-pastoris*	May have anti-cancer substances (Daniel 2006)	Flavonoids, glucosinolates, saponins, volatile oils and sulfur containing compounds (Daniel 2006); tropane alkaloids (Brock et al. 2006)
Leaf Mustard and Wild Turnip		*Brassica juncea, B. rapa* L.	May have anti-cancer substances (Daniel 2006); tonic for fevers, croup, asthma, headaches; antibacterial (Arnason et al. 1981)	Tropane alkaloids (Jing-Yan & Zhao-Pu 2010), glucosinolates (De La Rosa et al. 2001)
Bittercress and Cuckooflower		*Cardamine parviflora* L., *C. pensylvanica, C. pratensis* L.	Used to treat fevers, colds, sore throats, headaches, heart palpations, chest pains, gas, stomach upset, lack of appetite; may contain anti-cancer substances (Daniel 2006)	Glucosinolates (Gurevitch et al. 2002), tropane alkaloids (Brock et al. 2006)
Sundews	Droseraceae: sundew	*Drosera anglica, D. intermedia, D. linearis, D. rotundifolia*	Antibacterial, antiviral, antifungal, anticancer, used to treat coughs, aryngitis, pertussis, tracheitis, catarrh, tuberculosis, asthma, chronic bronchitis, ulcers, insecticidal (Bekesiova et al. 1999; Pareek et al. 2005)	Polyphenolics (Bekesiova et al. 1999); naphthoquinones, plumbagin, flavonoids (Pareek et al. 2005)
Gentians	Gentianaceae: gentian	*Gentiana linearis, G. amarelle; Gentianopsis crinite, G. detonsa*	Used to treat fever, indigestion, jaundice, skin diseases, heartburn; Antimicrobial properties (Dorman & Deans 2000)	Secoiridoid glucosides--gentiopicroside (Mulabagal & Tsay 2004)
Common Geranium	Geraniaceae: geranium	*Geranium bicknellii*	Antiseptic (Dorman & Deans 2000), anti-diuretic; used to treat	Terpenoids, phenylpropanoid eugenol (Dorman & Deans 2000);

			toothaches	tannins and polyphenols (Scalbert & Haslam 1987)
Northern St. Johns Wort	Gutteriferae: St. Johnswort	*Hypericum mutilum, H. perforatum, H. majus, H. ellipticum, H. canadense*	Anti-inflammatory, antibacterial (Dall'Agnol et al. 2003); used to treat diarrhea, worms, coughs, depression, tuberculosis, tumors, cuts, ulcers, neurological disorders (Murch et al. 2003), cancer (Murch et al. 2003)	Hypericin, pseudohypericin, hyperforin (Murch et al. 2003); phenolics, chlorogenic acid, quercitrin, quercitin, rutin, apigenin-7-O-glucoside (Çırak et al. 2007)
Heal-all	Lamiaceae: mint	*Prunella vulgaris*	Antibacterial, antiviral, anti-inflammatory (Jirovský et al. 2007)	Rosmarinic acid, phenolic acids (Jirovský et al. 2007); ursolic acid, oleanolic acid (Wink 1987)
Skullcaps		*Scutellaria galericulata* L., *S. lateriflora* L., *S. parvula*	Used to treat anticancer (Scheck et al. 2006); anti- inflammatory (Smith & Culvenor 1981); antioxidant, anticonvulsant, antibacterial, anti viral (Shang et al. 2010)	Flavonoids (Abascal & Yarnell 2008); baicalin, baicalein, wogonin (Murch et al. 2003), diterpenes (Shang et al. 2010), phenophorbide (Fajer et al. 1992), apigenin (Sato et al. 2000)
Flax	Linaceae: flax	*Linum perenne* L., *L. usitatissiumum* L.	Used to prevent breast, prostate and colon cancer (Hemmati et al. 2007)	Justicidin B, glycosides of 7-hydroxyjusticidin B (Hemmati et al. 2007); lignans, aryldihydronaphthalene (Hemmati et al. 2007); podophyllotoxin (Hemmati et al. 2007)
Fireweeds	Onagraceae: evening primrose	*Epilobium angustifolium* L., *E. ciliatum, E. leptophyllum, E. palustre* L.	Used to treat inflammation, burns, boils, sores, rashes, mouth ulcers, yeast infections (Iwashina & Kitajima 2000)	Flavonoids, myricetin, sitosterol (Small & Catling 1999); tannins (Arnason et al. 1981), other phenolics (Romani et al. 2002), 3-*O*-D-glucuronide (Romani et al. 2002)
Sorrels	Oxalidaceae: wood sorrel	*Oxalis acetosella* L., O. *stricta* L.	Used to treat inflammation, diarrhea, urinary tract infections, sprains, boils and pimples, traumatic injuries, infections (Feresin et al. 2003)	Benzoquinones, phenols (Feresin et al. 2003); oxalic acid (Wink 1987)
Bloodroot	Papaveraceae: poppy	*Sanguinaria canadensis* L. z	Used to treat vomiting, diarrhea, gas, stomachaches, ulcers, tuberculosis; antibacterial, antifungal, anti-inflammatory, antioxidant, antitumor (Arnason et al. 1981)	Sanguinarine alkaloids (Arnason et al. 1981), Benzophenanthridine alkaloids (Arnason et al. 1981)
Plantains	Plantaginaceae	*Plantago major* L., *P.*	Used to treat	Iridoid glycosides aucubin

	: plantain	*media* L.	inflammation, diarrhea, toothaches, headaches, bronchitis, sore throats, laryngitis, coughs, tuberculosis, infections (Jelager et al. 1998)	and catalpol (Fajer et al. 1992)
Knotweeds and Smartweed	Polygonaceae: buckwheat	*Polygonum archoreum, amphibium* L., *P. hydropiper* L., *P. lapathifolium* L., *P. pensylvanicum* L., *P. punctatum, P. scabrum, P. vivparum* L.	Known for treating various types of cancer (Yildirim et al. 2003); used to treat diarrhea, fever, chills, stomach pain, kidney problems, heart trouble, bleeding problems, antiseptic (Sato et al. 2000), antibacterial (Datta et al. 2000)	Phenolcarboxylic acids, flavonoids, anthraquinones, stilbenes (Nonaka et al. 1982); sesquiterpene acid (Datta et al. 2000)
Pitcher Plant	Sarraceniaceae : pitcher plant	*Sarracenia purpurea* L.	May have anticancer, antiviral, antimicrobial properties (Etkin 2008)	Coniine alkaloids (Carlson et al. 1948); triterpenes, phytosterols, sesquiterpenes (Etkin 2008)

[z] *Rare plant species. This information was provided by Erika North, herbarium curator and contract lecturer at Lakehead University.*

CHAPTER 2

Antioxidant Activity of Twenty-One Northern Ontario Medicinal Plants

Haider M. Hassan

ABSTRACT

There is a considerable demand in the pharmaceutical industry for natural antioxidants due to their low toxicity and protective effects in the human body. However, phytochemical research in the northern Ontario region is scarce, despite an abundance of medicinal plants utilized in Native Aboriginal communities. The present study investigates the *in vitro* antioxidant activity and total phenolic contents of 43 extracts from twenty-one northern Ontario medicinal plants. The antioxidant activity was evaluated through the DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt), ORAC (Oxygen radical absorption capacity), and EC_{50} (half maximal effective concentration) assays. All extracts exhibit some level of antioxidant activity. The extracts from *Cornus canadensis*, *Ledum palustre*, *Prunella vulgaris, Arctostaphylos uva-ursi*, and *Apocynum androsaemifolium* L. display a high phenolic content and antioxidant capacity. The highest antioxidant activity is observed for the *Cornus canadensis* leaf extract with a value of 14.00 ± 0.45 µg/ml, 177.95 ± 3.95 µM TE/gdw, 172.67 ± 2.80 µM TE/gdw and 359.82 ± 0.70 mg GAE/ 10 gdw for EC_{50}, DPPH assay, ABTS assay and phenolic content, respectively. Overall, the observed bioactivities of these plants correlate with their usages in traditional medicinal practices in native aboriginal communities.

1. Introduction

Oxidative stress plays a critical role in the pathogenesis of many age related diseases, such as Alzheimer's syndrome, cardiovascular diseases and cancer (Sohal & Weindruch 1996; Schumacker 2006; Markesbery 1997; Ou et al. 2012). Oxidative stress is propagated by radical molecules that exist in different forms, including superoxide ion ($O_2^{\cdot}$), hydroxyl ($HO^{\cdot}$), hydroperoxyl radical ($H_2O_2^{\cdot}$), peroxyl radical ($ROO^{\cdot}$), singlet oxygen species ($^1O_2$), alkoxyl radical ($RO^{\cdot}$) and peroxynitrite ($ONOO^{-}$) (Teow et al. 2007; Ou et al. 2002). Dietary antioxidants, enzymes and large molecules in healthy individuals stimulate cellular defenses and neutralize free radicals (Prior et al. 2005). Among the isolated antioxidants, phenolic compounds are an especially interesting group of molecules due to their ubiquity and pronounced protective effects (Orzechowski et al. 2002). Overall, epidemiological studies have demonstrated an inverse relationship between antioxidant rich diets and occurrence of chronic diseases (Hertog et al. 1993; Kaur & Kapoor 2008; Sánchez-Moreno et al. 1999).

Antioxidant compounds are also used in the food industry to prolong shelf life (Guilbert et al. 1996). A collective agreement entails that synthetic antioxidants, such as butylated hydroxyl-anisole (BHA) and butylated hydroxyl-toluene (BHT) pose higher toxicity and potential health risks relative to natural antioxidants (e.g. tocophenol and rosmarinic acid) (Kahl & Kappus 1993). In addition, natural antioxidants can be incorporated in our diet as nutraceuticals with health-promoting effects (Dillard & German 2000). Therefore, the search for antioxidants from natural sources has received attention.

In a previous investigation, I accumulated a list of 48 promising northern Ontario medicinal plants with claimed antioxidant, antibacterial, anticancer, anti-inflammatory and antiviral activity, among others. Through a literature survey, I found an absence of

ethnopharmacological research in this region despite an abundance of plant resources utilized in aboriginal communities for traditional medicinal practices. Hence, I selected twenty-one medicinal plants (from the original list of 48) from eleven plant families for the assessment of antioxidant capacities based on their ability to alleviate oxidative stress related diseases (i.e. cancer, inflammation) or claimed antioxidant activity (**Table 1**). In this study, I report on the total phenolic contents and *in vitro* antioxidant activity of the crude extract of these plants through DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt), ORAC (Oxygen radical absorption capacity), and EC_{50} (half maximal effective concentration) assays.

2. Results and Discussion

In this study, 21 northern Ontario medicinal plants were separated into leaf, flower, stem and root parts and extracted with ethanol. In total, 43 extracts from 21 medicinal plants were analyzed for their total phenolic contents and *in vitro* antioxidant activity to provide phytochemical evidence in support of their medicinal uses.

Antioxidant assays can be generally divided into two major groups: Hydrogen Atom Transfer (HAT) or single Electron Transfer (ET). The ET-based methods (i.e. DPPH, total phenolic content, and ABTS assay) use the end-point of redox reaction to quantify antioxidant activity; whereas, the HAT-based methods (i.e. ORAC assay) utilize a competition based kinetic scheme between antioxidants, radical initiator (AAPH) and oxidizable probe (fluorescein) to quantify the antioxidant capacity (Huang et al. 2005). As such, the reaction rate differences between antioxidants and radical species are not reflected in ET-based methods. Overall, no

single assay can provide a comprehensive analysis of antioxidant potential, thus, both ET and HAT-based methods were employed in this study (**Table 2)**.

2.1. DPPH Assay

The rate-determining step in DPPH assay involves a rapid electron transfer from the antioxidant to a $DPPH^*$ radical molecules (Foti et al. 2004). Upon reduction of the $DPPH^*$ radicals, the solution loses its deep violet color and this transition can be monitored at 517 nm. The antioxidant activity of 43 plant extracts in the DPPH assay range from 3.72 - 177.95 µM TE/ gdw, representing a variation of 48-fold. The leaf extract of *C. canadensis* displays the highest antioxidant activity, followed by *P. vulgaris* leaf, *L. palustre* leaf, *A. uva-ursi* leaf, *C. canadensis* stem, and *Apoc. androsaemifolium* L. leaf extracts. The lowest activity was observed for *S. purpurea* L. leaf followed by *T. ceratophorum* stem. According to a literature survey, high antioxidant capacity in the DPPH assay typically falls in the range of 90 - 100 µM TE/ gdw (Cai et al. 2005; Jastrzebski et al. 2007; Qin et al. 2011; Yao et al. 2010). Hence, antioxidant capacity above 80 µM TE/ gdw was considered significant in the present investigation. Among 43 extracts, 31 (72%), 9 (21%) and 3 extracts (7%) exhibit antioxidant activity between 0 - 80, 80 - 120 and 120 - 180 µM TE/ gdw, respectively. From the 12 extracts exhibiting antioxidant activity over 80 µM TE/ gdw, 75% belonged to the *Apocynaceae, Sarraceniaceae*, *Ericaceae, Cornaceae* and *Lamiaceae* plant families. Plants from *Poaceae, Cupressaceae* and *Asclepiadaceae* family exhibit moderate activity (between 60 - 80 µM TE/ gdw). The plant species from *Onagraceae, Asteraceae* and *Lycopodiaceae* families display low activity (mostly below 65 µM TE/ gdw). It is interesting to note that most extracts from the *Asteraceae* family display low antioxidant activity. Essential oils from plants of the *Asteraceae* family are known to contain antioxidant metabolites (Colombia 2010), however, many species from this family are reported to be weak scavengers of

the DPPH radical (Borneo et al. 2009; Miliauskas et al. 2004). A study by Borneo et al. (2009) on fifteen Argentinian species of *Asteraceae* family reported high EC_{50} values in the range of 198.0 - 2009.7 µg/ ml.

EC_{50} value is defined as the extract concentration necessary to reduce 50% of the DPPH radical. The EC_{50} estimates of 43 extracts range from 14.00 - 235.42 µg/ml, representing a variation of 17-fold. The lowest EC_{50} value was observed for *C. canadensis* leaf, followed by *L. palustre* leaf, *P. vulgaris* leaf, flower and stem, *A. uva-ursi* leaf and *C. canadensis* stem. The highest EC_{50} value was observed for *T. ceratophorum* stem. In accordance with prior studies (Cai et al. 2005; Mensor et al. 2001), plant extracts exhibiting EC_{50} below 30 µg/ ml were considered highly effective in neutralizing the DPPH radicals. In this study, only 9 extracts (21%) display EC_{50} values below 30 µg/ ml, with 77% of them belonging to the *Ericaceae, Cornaceae* and *Lamiaceae* families. It is exciting to note that the EC_{50} of *C. canadensis* (14.00 µg/ ml) was comparable to rutin (14.16 µg/ml) (Mensor et al. 2001), a dietary polyphenolic compound, which has reported protective effects against cancer (Alía et al. 2006), rheumatoid arthritis (Ostrakhovitch & Afanas'ev 2001) and other oxidative stress related diseases.

2.2. ABTS Assay

$ABTS^{*+}$ radical is generated by the persulfate oxidation of the $ABTS^{2-}$ molecule in aqueous solution (Huang et al. 2005). The $ABTS^{*+}$ radical is then reduced by antioxidants in mixture, resulting in a loss of green color. The antioxidant capacity of 43 plant extracts in the ABTS assay range from 7.00 - 172.67 µM TE/gdw, representing a variation of approximately 25-fold. Highest antioxidant capacity was observed for *C. canadensis* leaf extract, followed by the extracts of *P. vulgaris* leaf, *L. palustre* leaf, *C. canadensis* stem, *Arct. uva-ursi* leaf and *Apoc.*

androsaemifolium L. leaf. The lowest activity was found for *Arctium lappa* stem followed by *T. ceratophorum* stem. Results from the ABTS assay are especially interesting since absorption at 734 nm exempts color interferences from sample (Dudonne et al. 2009). According to previous studies using similar ABTS method parameters, antioxidant activity in the range of 80 - 100 µM TE/ gdw is considered significant (Jastrzebski et al. 2007; Thaipong et al. 2006; Wojdyło et al. 2009). Hence, 15 extracts (35%) display a significant antioxidant activity (greater than 80 µM TE/ gdw), with five of them exhibiting a capacity greater than 120 µM TE/gdw. Surprisingly, 80% of the 15 promising extracts belong to *Apocynaceae, Asclepiadaceae, Cupressaceae, Poaceae, Ericaceae, Cornaceae* and *Lamiaceae* plant families. *Onagraceae, Asteraceae* and *Lycopodiaceae* families display low to medium activity (majority below 70 µM TE/ gdw), which is in agreement with DPPH assay. It is interesting to note that the leaf extracts from *C. canadensis (Cornaceae), P. vulgaris (Lamiaceae)* and *L. palustre* (*Ericaceae*) display an exceptional activity in both DPPH and ABTS assays.

2.3. ORAC Assay

ORAC assay utilizes a radical initiator to generate peroxy radical (ROO^*), which favorably abstracts hydrogen from antioxidants to form hydroperoxide (ROOH). This preference retards the oxidation of the fluorescent probe, generating an extract specific fluorescent decay curve. The ORAC assay value for 43 plants extracts range from 6.72 - 466.99 µM TE/gdw, representing a variation of approximately 69-fold. The highest activity in ORAC assay is observed for *P. vulgaris* leaf, followed by *P. vulgaris* stem, *Anap. margaritacea* flower, *Arct. uva-ursi* leaf, *Achi. millefolium* L. flower, *L. palustre* leaf, *Anap. margaritacea* leaf, *Achi. millefolium* L. leaf and *Arct. uva-ursi* stem. The lowest value was observed for *P. frigidus* stem, followed by *R. hirta* L. stem. Although, the data from different studies vary depending on

experimental parameters, an antioxidant capacity around 150 µM TE/ gdw can be considered significant (Oomah et al. 2008). From 43 extracts, 20 (46.5%), 12 (28%) and 11 extracts (25.5%) exhibit activity between 0 - 150, 150 - 300 and 300 - 470 µM TE/ gdw, respectively. Among the 11 plant extracts exhibiting high antioxidant capacity (between 300 - 470 µM TE/ gdw), 64% of them belonged to *Ericaceae, Lamiaceae* and *Cupressaceae* plant families. Most plant extracts from *Apocynaceae, Cornaceae, Sarraceniaceae,* and *Asclepiadaceae* families display moderately high activity between 200 - 300 µM TE/ gdw. Plants from *Lycopodiaceae* and *Onagraceae* family exhibit low activity, which is consistent with ET-based assays. Interestingly, a 60-fold variation (6.72 - 404 µM TE/gdw) in activity was observed in plant species from *Asteraceae* family. In contrast to ET-based assays, extracts from *Achi. millefolium* and *Anap. margaritacea* plants of the *Asteraceae* family display an exceptionally high activity in the ORAC assay (between 210 - 404 µM TE/gdw). Furthermore, the *C. canadensis* leaf extract, which exhibited the highest activity in ET-based methods, displays only moderate activity in the ORAC assay. This discrepancy between ET and HAT-based methods has been reported in prior studies (Dudonne et al. 2009; Ou et al. 2002; Prior et al. 2005). According to Wright et al. (2001) and Ou et al. (2002), although HAT occurs simultaneously with ET mechanism, it plays a more dominant role in biological redox reactions. The ORAC assay also incorporates a biologically relevant radical source (ROO^{*}), reaction scheme (competitive and Kinetic), temperature (37 ^{o}C), pH (7.4) and salinity system (Dudonne et al. 2009; Prior et al. 2005). Hence, it is both mechanistically and physiologically relevant. Overall, it is very exciting to report that extracts from *P. vulgaris* and *L. palustre* display among the highest antioxidant activity in both ET and HAT-based methods.

2.4. Total Phenolic Contents

In this assay, the folin-ciocalteu phenol reagent reacts with phenolic compounds under basic conditions to form chromogens that can be detected at 750 nm. The total phenolic contents of 43 plant extracts range from 8.99 - 359.82 mg GAE/ 10 gdw, which represents a variation of 40-fold. The leaf extract of *C. canadensis* exhibits the highest phenolic content, followed by extracts of *P. vulgaris* leaf, *L. palustre* leaf, *C. canadensis* stem, *Arct. uva-ursi* leaf and *Apoc. androsaemifolium* L. leaf. The lowest phenolic content was found for *Achi. millefolium* L. stem, followed by *Ascl. incarnata* L. stem and *X. strumarium* L. stem. Through a survey of literature, it was determined that phenolic contents around 100 mg GAE/ 10 gdw can be considered significant (Kähkönen et al. 1999; Javanmardi et al. 2003; Li et al. 2008; Turkmen et al. 2005), although higher quantities have been reported in literature (Ghasemi et al. 2009; Guo et al. 2011). From 43 extracts, 26 (60%), 9 (21%) and 8 extracts (19%) exhibit total phenolic contents between 0 - 100, 100 - 200, and 200 - 350 mg GAE/ 10 gdw. Furthermore, the difference in total phenolic contents between varying families was quite distinct. Seven out of eight extracts exhibiting high phenolic content (200 - 350 mg GAE/ 10 gdw) belong to *Apocynaceae, Ericaceae, Cornaceae* and *Lamiaceae* plant families. Most extracts from *Asclepiadaceae, Sarraceniaceae*, *Poaceae* and *Cupressaceae* families exhibit phenolic contents between 90 - 200 mg GAE/ 10 gdw. Majority of species (81%) from *Onagraceae, Asteraceae* and *Lycopodiaceae* plant family exhibit low phenolic content (most below 80 mg GAE/ 10 gdw). These findings show great resemblance with ET-based methods. Surprisingly, *X. strumarium* leaf extract, which displays low activity in DPPH and ABTS assay, exhibits moderately level of phenolic content and antioxidant capacity in ORAC assay.

Among the different parts of plants, the following hierarchy in total phenolic contents and antioxidant capacities was found, with a few exceptions: leaf > flower > root > stem. Leaf and flower tissues in plants generally incur greater exposure to UV-B radiation from sunlight due to their higher surface areas for photosynthetic and mating purposes, respectively. As defense mechanisms, plants produce large quantities of phenolic metabolites (flavonoids, anthocyanin's, quercetin glycoside) in these tissues to cope with oxidative stress (Cen & Bornman 2006; Price et al. 1995). A study by Price et al. (1995) demonstrated that concentration of the quercetin glycoside (a natural flavonol) in grapes tissue increased 7.5-fold (4.5 mg/L to 33.7 mg/L) as a direct outcome of increased exposure to sunlight. However, some plants, i.e. *L. palustre, Apoc. androsaemifolium* and *C. canadensis*, display high antioxidant and total phenolic contents despite flourishing in forest understory. As such, resistance to herbivore or competition against other plants in the habitat may be alternative hypothesis to explain the high antioxidant capacity and phenolic contents in these extracts.

2.5. Correlation Analysis

Relationships between data from different assays were also analyzed in this study (**Table 3).** High correlation between the ABTS and DPPH assays ($r^2 = 0.92$, **Figure 1 a**) relates well with an inter-laboratory finding that ABTS and DPPH are easy to implement, show high correlation and give reproducible results (Buenger et al. 2006). However, relatively weak correlation was observed between EC_{50}, DPPH and ABTS assays ($r^2 = 0.69$ and $r^2 = 0.63$ with ABTS and DPPH assays, respectively, **Figure 1 b**). Insufficient incubation period in the EC_{50} assay (30 minutes) may have inhibited attainment of the steady state kinetics for some plant species (Sánchez-Moreno et al. 1999). This may have implications in obscuring the EC_{50} estimates, especially for plants with weak antioxidant capacity,

In this study, results from the ORAC assay display poor relationship with other radical scavenging assays and total phenolic contents ($r^2 = 0.32$, $r^2 = 0.32$, $r^2 = 0.40$, and $r^2 = 0.45$ with DPPH, ABTS, EC_{50} and total phenolic content assays, respectively, **Figure 1 c, 1 d and 2 b**). This discrepancy may be due to incorporation of a competition based kinetic reaction scheme or an alternate reaction mechanism inherent to HAT-based methods (Dudonne et al. 2009). Also, lack of solubilizing agents applied in the ORAC method could have confiscated activity of lipophilic phenolic groups in the aqueous medium (Cheng et al. 2006).

Relatively high correlations between the ET based antioxidant activity and total phenolic contents ($r^2 = 0.84$, $r^2 = 0.85$, $r^2 = 0.63$ with ABTS, DPPH and EC_{50} assay, respectively, **Figure 2 a and 2 c**) in plant extracts of our study conform to previous investigations (De-Oliveira et al. 2009; Kontogianni & Gerothanassis 2012; Maisuthisakul et al. 2008). Indeed, phenolic compounds are excellent nucleophiles composed of one or more aromatic rings bearing hydroxyl groups that quench free radicals by forming resonance and steric stabilized phenoxyl radicals, inhibiting lipid peroxidation and acting as breakers of oxidation reaction (Bors & Michel 2002; Rice-Evans et al. 1996; Zou et al. 2011).

3. Materials and Methods

3.1. Instruments and Chemicals

The absorbance was measured using Bio-Rad Smart Spec Plus spectrophotometer. Synergy HT Biotek using GEN 5 software was utilized for measurement of fluorescence. The chemicals 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,2'-Azobis(2-methylpropionamidine) dihydrochloride (AAPH), potassium persulfate, 6-hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid (Trolox), gallic acid, Folin-Ciocalteu phenol reagent and anhydrous sodium carbonate were purchased from Sigma-Aldrich (USA). ACS grade methanol was purchased from Fisher Scientific (USA).

3.2. Collection of Plant Material

Plant materials in this study were whole plant samples, including root and flower for some plants. They were collected during the late flowering season (June-August) in the Thunder Bay region, Ontario. The specimens were identified by Erika North, herbarium curator and contract lecturer at Lakehead University, Thunder Bay, ON, Canada. The scientific names were based on those proposed by Morton and Venn (1990). All voucher specimens were deposited in -80 ^{o}C fridge at the Bio-Refining Institute, Lakehead University.

3.3. Extraction

The collected plant samples were separated into root, stem, flower and leaf parts. The plant parts were dried in dark over 24 - 48 hours at 32 ^{o}C. The dried plant samples (2 g) were ground up, milled through *no. 40* mesh and extracted twice with 50ml of ethanol (95 % v/v) over

a period of 48 hours at room temperature. All extracts were filtered through *Whatman No.1* filter paper, evaporated to dryness in a rotary evaporator at 34 °C and stored at -80 °C in glass vials.

3.4. ABTS antioxidant Assay

For ABTS assay, the procedure by Thaipong et al. (2006) was used with modifications. Stock solutions of 7.4 mM ABTS and 2.6 mM potassium thiosulfate were mixed in equal quantities and allowed to react in darkness at room temperature for 12 - 16 hours. Then, 1.0 ml of this working solution was mixed with 20 ml methanol to obtain a reading of 1.16 ± 0.05 units at 734 nm. Plant extracts (150 µl) were allowed to react with 2850 µl of the diluted $ABTS^{*+}$ for 2 hours in dark. Then the absorbance was taken at 734 nM. This was done in triplicates and the results expressed as µM Trolox Equivalent/gram dry weight (µM TE/gdw). The Trolox standard curve was linear between 25 µM and 625 µM. Negative control consisted of 150 µl of methanol in 2850 µl of the diluted $ABTS^{*+}$ solution.

3.5. DPPH Antioxidant Assay

The DPPH assay was performed according to the method established by Brand-Williams et al. (1995). The stock solution was prepared by dissolving 24 mg DPPH in 100 ml methanol. The working solution was prepared by mixing 10 ml of stock solution in 45 ml methanol to obtain a reading of 1.13 ± 0.05 at 515 nm. Plant extracts (150 µl) were allowed to react with 2850 µl of DPPH working solution at room temperature for 24 hours in dark. The absorbance was read in triplicates at 515 nm and the results expressed as µM Trolox Equivalent/ gram dry weight (µM TE/gdw). The Trolox standard curve was linear between 25 µM and 825 µM. Negative control consisted of 150 µl of methanol in 2850 µl of the diluted DPPH solution.

3.6. Determination of EC_{50}

This assay was performed according to Mensor et al. (2001). Briefly, 1 ml of freshly prepared 0.3 mM DPPH methanol solution was added to 2.5 ml of sample solution in triplicate, and allowed to react at room temperature for 30 minutes. The absorbance was measured at 518 nm using methanol as blank. The EC_{50} value, extract concentration that can reduce 50 % of the initial DPPH radical concentration, was calculated through a dose-response curve expressed either as a linear or logarithmic relationship. DPPH solution (1.0 ml at 0.3 mM) plus methanol (2.5 ml) was used as a negative control and Trolox as positive control.

3.7. Oxygen Radical Absorption Capacity Assay

The ORAC procedure was performed according to Teow et al. (2007). Briefly, 100 µl of 12 nm fluorescein, 20 µl of methanol plant extract, and 80 µl of 24 mM AAPH (2,2'-Azobis(2-methylpropionamidine) dihydrochloride) were loaded and mixed in flat bottom, polystyrene 96-well microplate. The plate was agitated at slow speed for 15 seconds prior to first reading (excitation/ emission 485 nm/ 520 nm), in 1 min interval for 120 minutes at ambient conditions (pH 7.4, 37 ^{o}C). The area under the kinetic curve (AUC) was calculated using the PRISM 5 software (Macintosh system) and the ORAC value was expressed as µM TE/ gdw. AAPH and fluorescein was used as blank, AAPH in exclusion was used as negative control and fluorescein in absence of AAPH and extract was the positive control.

3.8. Total Phenolic Content (TPC) Assay

The total phenolic content assay was performed according to a procedure established by Ou et al. (2012). Briefly, at 0 min, 200 µl Folin-Ciocalteu phenol reagent was added to a reaction mixture of 200 µl of plant extract and 2.6 ml of ddH_20 and mixed vigorously at room

temperature. After 6 minutes, 2 ml of 7 % anhydrous sodium carbonate solution were added and mixed vigorously. The mixture was incubated in dark for 90 minutes and absorbance was read at 750 nm. The total phenolic content was expressed as mg Gallic Acid Equivalent/ 10 gdw (mg GAE/ 10 gdw). The negative control consisted of 2.6 of ml ddH_20, 200 µl Folin-Ciocalteu reagents and 200 µl of methanol.

3.9. Statistical Analysis

The results were expressed as means ± standard deviation (SD) of two or three replicate measurements. The standard errors and correlation coefficients were calculated in MS Excel and PRISM 5 software, respectively.

4. Conclusion

My current study establishes northern Ontario medicinal plants as promising candidates for ethnopharmacological research. In this study, 43 extracts from twenty-one northern Ontario medicinal plants were assayed for antioxidant activity through DPPH, ABTS and ORAC assays. The total phenolic content of medicinal plants was also determined. All extracts exhibit some level of antioxidant activity and phenolic contents; however, a few were exceptional. In all assays, extracts from *Ericaceae, Cornaceae* and *Lamiaceae* family display exceptional antioxidant capacities and phenolic contents. Whereas, plant extracts from *Onagraceae* and *Lycopodiaceae* family consistently performed poorly. Plant extracts from the *Asteraceae* family typically ranked poorly, however, some extracts (especially *A. margaritacea*) exhibit high phenolic content and remarkable activity in HAT and ET-based methods. Furthermore, phenolic content in medicinal plants correlated well with their antioxidant profile in ET-based assays,

promoting phenolics as excellent agents in negating oxidative stress. Also, with a few exceptions, phenolic contents and antioxidant capacities in different parts of medicinal plants was observed in the following hierarchy: leaf > flower > root > stem. Overall, in this study, *C. canadensis*, *P. vulgaris* and *L. palustre* display the highest antioxidant capacities and phenolic contents. The present study provides strong evidences in supporting the medicinal properties and traditional uses of these plants. Current investigation also promotes plant species from *Ericaceae, Cornaceae* and *Lamiaceae* families as model candidates in future phytochemical studies.

Table 1. Twenty-one northern Ontario medicinal plants and their medicinal properties.

Plant Family	Botanical Name	Conventional Name	Medicinal Property
Asteraceae	*Achillea millefolium* L.	Common Yarrow	Antimicrobial
	Anaphalis margaritacea	Pearly Everlasting	
	Arctium lappa	Burdock	Anti-inflammatory
	Grindelia squarrosa	Gumweed	Antibacterial,
	Petasites frigidus	Sweet Coltsfoot	Antibacterial
	Rudbeckia hirta L.	Goldenglow	Anti-inflammatory
	Solidago Canadensis	Goldenrod	Antiseptic
	Taraxacum ceratophorum	Dandelion	Antimicrobial
	Xanthium strumarium L.	Cocklebur	Antibacterial
Ericaceae	*Arctostaphylos uva-ursi*	Common Bearberry	Antibacterial
	Ledum palustre	Labrador Tea	Antibacterial, Anti-inflammatory
Asclepiadaceae	*Asclepias incarnata* L.	Milkweed	Antibacterial
Apocynaceae	*Apocynum androsaemifolium* L.	Spreading Dog Bane	Antibacterial
Cornaceae	*Cornus Canadensis* L.	Dogwood	Anti-inflammatory, anti-diarrheal,
Onagraceae	*Epilobium angustifolium* L.	Fireweed	Antibacterial
Poaceae	*Hierochloe odorata*	Sweet Grass	Antibacterial
Cupressaceae	*Juniperus communis* L.	Juniper	Antibacterial
Lycopodiaceae	*Lycopodium annotinum, L. clavatum*	Clubmoss	Estrogenic, antibacterial, antiviral,
Lamiaceae	*Prunella vulgaris*	Heal-all	Antibacterial, anti-inflammatory,
Sarraceniaceae	*Sarracenia purpurea* L.	Pitcher Plant	Antibacterial

Table 2. Antioxidant capacities and total phenolic content of 21 northern Ontario medicinal plants

Medicinal Plant	Plant Part	ABTS [a] (μM TE/ gdw)	DPPH [b] (μM TE/ gdw)	EC_{50} [c] (μg/ ml)	ORAC [d] (μM TE/ gdw)	Total Phenolic Content (mg GAE/ 10 gdw)
Achi. millefolium L.	Leaf	52.33 ± 0.55	61.15 ± 1.04	147.68 ± 1.06	355.85 ± 8.80	85.79 ± 0.55
	Flower	43.11 ± 0.25	49.74 ± 1.01	114.86 ± 0.61	385.16 ± 0.57	92.74 ± 1.07
	Stem	22.67 ± 0.31	32.05 ± 0.43	172.29 ± 1.76	230.80 ± 5.49	8.99 ± 0.03
Anap. margaritacea	Leaf	80.02 ± 0.66	80.77 ± 1.61	86.14 ± 0.67	370.77 ± 4.35	120.10 ± 2.34
	Flower	66.69 ± 0.83	66.79 ± 0.93	129.22 ± 1.88	404.39 ± 2.40	165.38 ± 2.10
	Stem	23.36 ± 0.14	22.31 ± 0.21	150.00 ± 8.89	118.38 ± 6.67	25.24 ± 0.11
Apoc. androsaemifolium L.	Leaf	104.44 ± 0.96	110.13 ± 3.11	34.46 ± 0.46	210.14 ± 5.64	239.96 ± 4.58
	Stem	37.44 ± 0.22	40.90 ± 0.11	197.54 ± 0.25	11.43 ± 0.62	25.79 ± 0.33
Arct. uva-ursi	Leaf	120.69 ± 1.05	113.08 ± 1.32	25.00 ± 0.75	397.94 ± 7.42	251.76 ± 0.64
	Stem	90.24 ± 0.55	84.36 ± 0.09	30.15 ± 1.16	344.47 ± 3.93	206.63 ± 3.40
Arctium. lappa	Leaf	30.78 ± 0.16	20.38 ± 0.34	151.68 ± 2.34	133.63 ± 18.63	62.18 ± 0.31
	Flower	22.00 ± 0.26	21.28 ± 0.23	150.00 ± 1.90	70.66 ± 5.68	57.04 ± 0.62
	Stem	7.00 ± 0.15	20.51 ± 0.42	187.50 ± 8.19	21.27 ± 0.51	18.71 ± 0.11
Ascl. incarnata L.	Leaf	88.22 ± 0.70	80.51 ± 2.33	33.91 ± 1.38	267.59 ± 3.54	95.93 ± 0.57
	Root	52.56 ± 0.92	60.51 ± 0.26	155.00 ± 2.45	26.28 ± 3.97	27.46 ± 0.27
	Stem	50.56 ± 0.41	26.67 ± 0.55	169.47 ± 0.56	11.12 ± 0.09	9.68 ± 0.02

C. canadensis L.	Leaf	172.67 ± 2.80	177.95 ± 3.95	14.00 ± 0.45	261.67 ± 4.85	359.82 ± 0.70
	Stem	127.11 ± 0.48	112.69 ± 0.95	27.50 ± 0.96	220.64 ± 5.54	256.35 ± 0.13
E. angustifolium L.	Leaf	49.24 ± 0.26	46.03 ± 0.43	51.69 ± 0.75	199.24 ± 4.49	61.63 ± 0.42
G. squarrosa	Leaf	35.33 ± 0.13	64.87 ± 0.32	172.29 ± 1.87	40.03 ± 0.90	78.01 ± 0.62
	Flower	18.67 ± 0.22	17.69 ± 0.59	217.12 ± 0.98	68.20 ± 0.50	49.68 ± 0.01
	Stem	51.67 ± 0.47	47.56 ± 1.57	166.67 ± 3.28	101.72 ± 1.87	35.93 ± 0.30
H. odorata	Leaf	95.00 ± 0.51	74.36 ± 2.69	109.00 ± 2.31	44.72 ± 0.85	119.82 ± 0.88
J. communis L.	Leaf	91.89 ± 0.23	72.18 ± 0.77	37.50 ± 0.68	393.07 ± 4.96	179.40 ± 2.30
L. palustre	Leaf	157.33 ± 2.13	161.54 ± 3.05	15.00 ± 0.06	376.76 ± 8.92	349.81 ± 1.90
	Stem	96.44 ± 0.57	97.18 ± 0.60	38.29 ± 0.17	259.35 ± 8.70	179.82 ± 1.37
L. annotinum	Leaf	36.89 ± 0.22	41.41 ± 0.56	166.67±0.89	121.39 ± 5.58	74.26 ± 1.85
L. clavatum	Leaf	69.89 ± 1.61	63.46 ± 1.50	111.23 ± 0.12	38.15 ± 3.40	44.26 ± 0.10
	Stem	57.78 ± 0.24	62.44 ± 1.01	101.32±0.46	58.50 ± 3.14	47.32 ± 0.15
P. frigidus	Leaf	57.56 ± 0.37	44.62 ± 0.80	35.00 ± 1.80	226.26 ± 3.02	128.85 ± 1.84
	Stem	84.22 ± 0.73	66.03 ± 0.95	51.68 ± 1.80	6.72 ± 2.80	99.95 ± 2.60
P. vulgaris	Leaf	160.00 ± 1.57	169.23 ± 4.70	17.23 ± 0.97	466.99 ± 5.80	350.65 ± 3.27
	Flower	94.33 ± 1.26	93.46 ± 0.61	20.00 ± 1.83	429.15 ± 0.19	206.63 ± 2.18
	Stem	98.89 ± 5.91	67.95 ± 0 .30	21.99 ± 0.68	305.66 ± 7.51	149.13 ± 3.25
R. hirta L.	Leaf	46.11 ±1.21	40.64 ± 0.54	189.99 ± 0.15	11.27 ± 0.51	29.96± 0.04
	Stem	68.67 ± 0.16	65.51 ± 1.41	127.98 ± 3.98	9.17 ± 0.051	44.96 ± 0.04
S. purpurea L.	Leaf	25.11 ± 0.57	3.72 ± 0.08	179.52 ± 0.30	99.64 ± 5.96	26.90 ± 0.31
	Root	78.22 ± 0.39	87.56 ± 2.69	37.29 ± 0.26	266.47 ± 4.54	161.35 ± 0.89
S. canadensis	Leaf	35.36 ± 0.61	35.90 ± 0.33	144.11 ± 5.60	264.03 ± 9.00	42.60 ± 0.16
	Stem	26.02 ± 0.04	27.82 ± 0.32	182.03 ± 1.62	51.07 ± 0.56	16.63 ± 0.02
T. ceratophorum	Stem	8.44 ± 0.07	7.44 ± 0.04	235.42 ± 5.47	60.36 ± 7.26	33.85 ± 0.23
X. strumarium L.	Leaf	67.91 ± 0.61	53.72 ± 0.11	35.65 ± 0.90	195.93 ± 5.76	149.40 ± 0.42
	Stem	16.02 ± 0.12	19.69 ± 0.01	203.56 ± 1.23	51.30 ± 0.82	13.29 ± 0.02

Note: all results are expressed as mean ± SD of three measurements

[a] ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt; [b] DPPH: 2,2-diphenyl-1-picrylhydrazyl; [c] EC_{50}: concentration necessary to neutralize 50% of DPPH radical at concentration of 82µM; [d] ORAC: oxygen radical absorption capacity assay

µM TE/ gdw: µM Trolox Equivalence/ gram dry weight

mg GAE/ 10 gdw: mg Gallic Acid Equivalence/ 10 gram dry weight.

Table 3. Correlation between antioxidant capacities and total phenolic contents

	Correlation Coefficient
DPPH : ABTS	0.92
DPPH : EC_{50}	0.63
DPPH : ORAC	0.32
DPPH : TPC[a]	0.85
ABTS : EC_{50}	0.69
ABTS : ORAC	0.32
ABTS : TPC[a]	0.84
ORAC : TPC[a]	0.45
ORAC : EC_{50}	0.40
EC_{50} : TPC[a]	0.63

[a] TPC: total phenolic contents

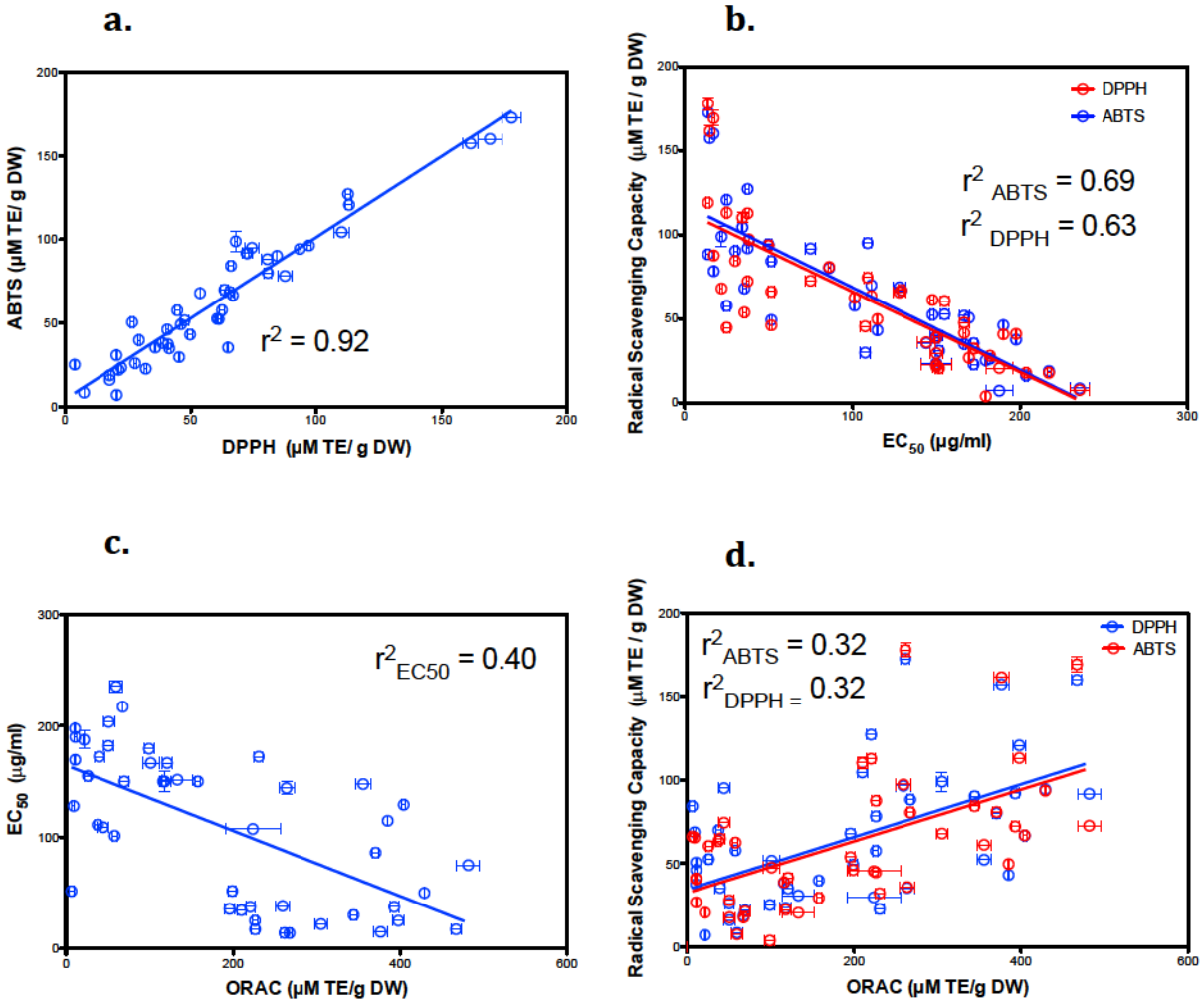

Figure 1: Correlations between DPPH, ABTS, EC_{50} and ORAC assays in 43 extracts from 21 northern Ontario medicinal plants. **a**. A correlation coefficient of $r^2 = 0.92$ was obtained between DPPH and ABTS assay data sets. **b.** The correlation coefficient between ABTS and DPPH assays versus EC_{50} values was $r^2 = 0.69$ and $r^2 = 0.63$, respectively. **c.** A correlation coefficient of $r^2 = 0.40$ was obtained between EC_{50} values and ORAC assay data sets. **d.** Correlation coefficients of $r^2 = 0.32$ was observed between DPPH, ABTS and ORAC assays.

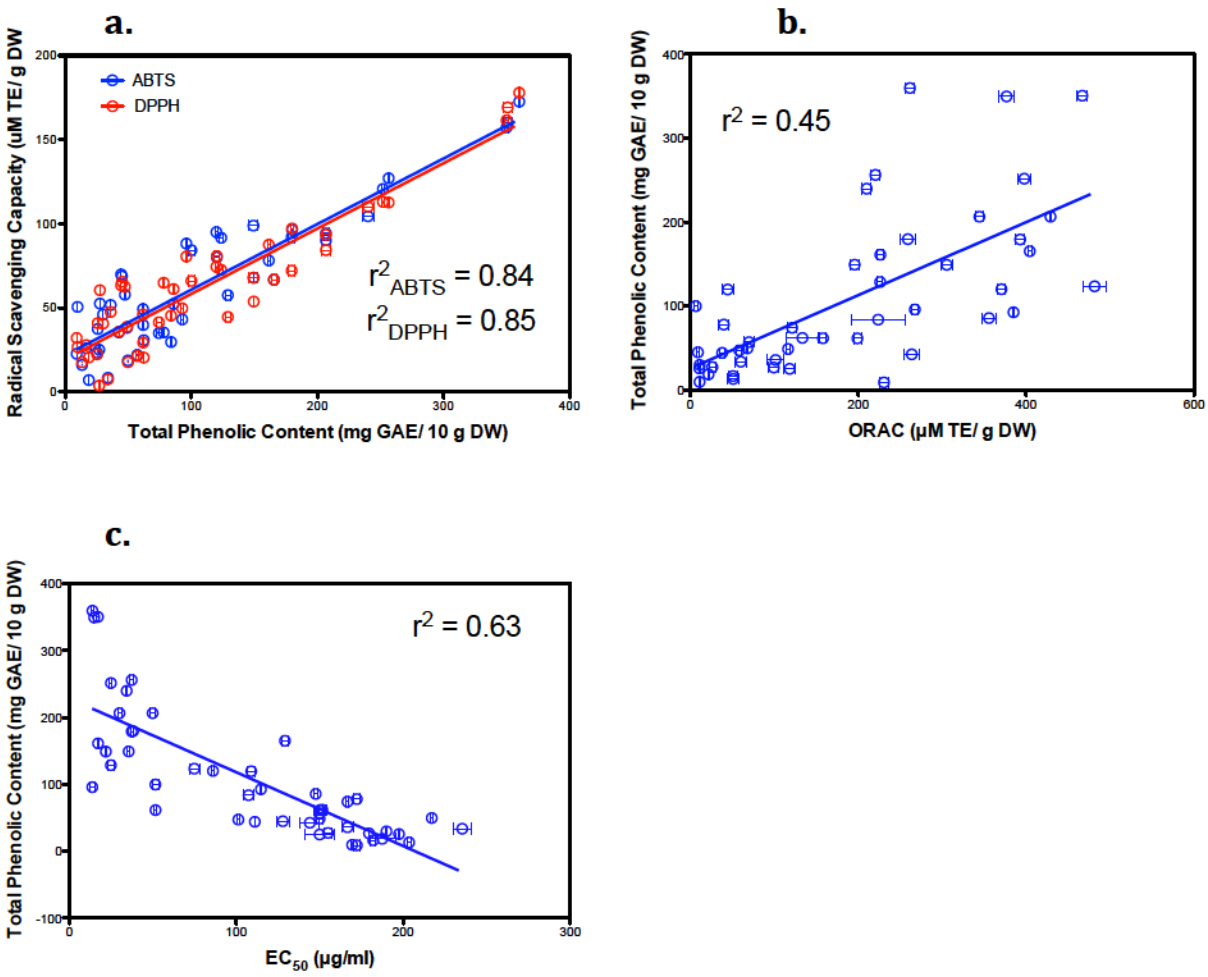

Figure 2: Correlation between total phenolic content and radical scavenging assays (DPPH, ABTS, EC_{50} and ORAC) of 43 extracts from 21 northern Ontario medicinal plants. **a.** The correlation coefficient for DPPH and ABTS assay versus total phenolic content was r^2 = 0.84 and r^2 = 0.85, respectively. **b.** Correlation coefficient of r^2 = 0.45 was obtained between ORAC assay and total phenolic content. **c.** The correlation values between total phenolic content and EC_{50} assay was r^2 = 0.63.

CHAPTER 3

In vitro Antimicrobial Bioactivities of Twenty-One Northern Ontario Medicinal Plants

Haider M. Hassan

ABSTRACT

Despite the abundance of medicinal plants in the northern Ontario region, the phytochemical and ethnobotanical investigations are scarce. In the present study, twenty-one northern Ontario medicinal plants were selected based on their uses in the treatment of inflammation, diarrhea and infectious diseases in First Nation communities. The crude extracts of these plants were analyzed for *in vitro* antibacterial activity against six different bacterium through the hole-plate diffusion and minimum inhibitory concentration (MIC) assays. Eight extracts were further shortlisted and analyzed for minimum bactericidal concentration (MBC) and time kill analysis. The crude extracts were also fractionated by polarity and analyzed in the MIC assay. Leaf and/ or flower extracts of *Xanthium strumarium*, *Anaphalis margaritacea, Arctostaphylos uva-ursi*, *Apocynum androsaemifolium* L., *Cornus canadensis, Solidago canadensis* and *Grindelia squarrosa* display the most promising activity. In the hole-plate diffusion assay, the *Xanthium strumariumi* leaf extract displays a larger zone of inhibition for *Bacillus cereus* than the ampicillin (100 µg/ml) positive control. In the MIC assay, majority of the extracts (62%) exhibit increased antibacterial activity in the medium polarity fraction. Particularly, the medium polarity fraction (Hex/ EtOAc/ MeOH 0:1:0) of *Anaphalis margaritacea* flower extract displays exceptional activity with bacterial inhibition at concentrations ranging from 0.08 - 1.25 mg/ml. Also, crude extract of *A. margaritacea* displays MBC values ranging from 0.16 - 5 mg/ml against *Aeromonas caviae, Micrococcus luteus, Bacillus cereus* and *Paenibacillus alvei.* The extract was lethal to these bacteria within 1 - 8 hrs at MBC. Overall, the observed bioactivities of medicinal plants correlate with their uses in traditional medicinal practices.

1. Introduction

Infectious diseases are a major concern in our society. The impending problem is traceable to the evolution of multiple drug resistant strains of pathogenic bacteria and the concentrated use of existing antibacterial drugs (Ahmad et al. 1998). The agricultural industry also suffers from microbial infestations on crops, with plant pathogens causing an approximate 20% reduction in crop yield per year worldwide (Oerke et al. 1994). The situation is especially dire since the discovery of new chemical entities has been declining steadily and hit a 20 year low in 2001 (Newman et al. 2003). This may be attributed to the retraction of pharmaceutical research on natural products due to the advent of high throughput screening (HTS) technology and combinatorial chemistry (Butler 2008; Newman & Cragg 2007; Harvey 2008; Lam 2007).

In the areas of infectious diseases, 70% of all drugs originated from plant sources between 1981-2002 (Lam 2007; Newman et al. 2003). Medicinal plants are currently the focus of advanced contemporary research on their myriad bioactivities. However, phytochemical research was found to be vastly lacking in the northern Ontario region despite an established practice of traditional medicine by several native aboriginal communities.

In a previous investigation, I accumulated a list of 48 northern Ontario medicinal plants that exhibit anti-inflammatory, antibacterial, antioxidant, antiviral, anticancer and antidiarrheal activity, among others. However, ethnopharmacological research on the antibacterial activity of medicinal plants in this region is limited. Hence, I selected twenty-one medicinal plants (**Table 1**), from the original list of forty-eight, based on their uses in the treatment of burns, cuts, inflammation, infections, diarrhea and mouth conditions. In accordance to North American First

Nations Ethnobotanical medicinal plants trend (Jones et al. 2000), plants utilized in aboriginal communities towards the treatment of these illnesses may be the source of antibacterial phytochemicals. Here in, for the first time, I present a rigorous analysis of the *in vitro* antibacterial activity of these medicinal plants through the hole-plate diffusion and minimum inhibitory concentration (MIC) assays. Crude extracts of these plants were also separated into fractions of varying polarity through manual liquid chromatography, and these fractions were assayed for their inhibitory activity in the MIC assay. The minimum bactericidal concentration (MBC) and time-kill analysis of a few short listed plants was also determined.

2. Results and Discussion

In this study, twenty-one northern Ontario medicinal plants were selected based on the North American First Nations Ethnobotanical Medicinal Plant trend (**Table 1)**. These plants were separated into leaf, flower, stem and root tissues and assayed for *in vitro* antibacterial activity through hole-plate diffusion and MIC assays. A few short-listed medicinal plants were further assayed for MBC value and time-kill analysis.

2.1. Hole-Plate Diffusion Assays

In hole-plate diffusion method, the active principle must diffuse through the LB agar medium in a homogenous fashion to give optimal results (Rios et al. 1988). In order to lower the detection limit, inoculated system was kept at low temperature (4 ^{o}C) prior to incubation, which favors diffusion in culture medium (Rios et al. 1988). The zone of inhibition (ZOI) was defined as the inhibition diameter around the hole (6 mm) in seeded agar plates. In this assay, 23 (53%)

out of 43 plant extracts from eight plant families (out of eleven) exhibit ZOI diameters in the range of 6.0 mm - 35.3 mm, representing a variation of approximately 6-fold (**Table 2**). The extracts of *S. canadensis* leaf, *Arct. uva-ursi* leaf and stem, *Anap. margaritacea* flower, stem and leaf, *C. canadensis* L. leaf and stem, *G. squarrosa* leaf and flower, *Apoc. androsaemifolium* L. leaf, *E. angustifolium* L. and *P. frigidus* leaf display the highest ZOI diameters. It is exciting to note that ZOI diameter from *X. strumarium* leaf extract for *B. cereus* was greater than the ampicillin positive control (100 µg/ml). The ZOI diameter for *M. luteus, M. avium* subsp. *avium* and *P. alvei* were comparable to ampicillin (100 µg/ml). The smallest inhibition zone of 6.0 ± 1.5 mm against *M. avium* subsp. *avium* was observed for the *Achi. millefolium* L. stem extract. Overall, only the extracts from *C. canadensis* L. leaf and *Arct. uva-ursi* stem and leaf, belonging to the *Cornaceae* and *Ericaceae* plant families, respectively, successfully inhibit the growth of all six bacteria tested.

In previous investigations using similar methods, ZOI diameters in excess of 12 mm can be considered significant (Donaldson et al. 2005; Kudi et al. 1999; Palombo & Semple 2001). Plant extracts from *Cornaceae* and *Ericaceae* family display moderate to high antibacterial activity, with the majority of ZOI diameters around 14 mm. The leaf extracts of *C. canadensis* (*Cornaceae*) and *Arct. uva-ursi* (*Ericaceae*) exhibit the highest inhibition diameters for *A. caviae*, a Gram-negative bacterium. This finding is especially intriguing since *A. caviae* demonstrates resistance to both chloramphenicol and ampicillin positive controls, a finding that conforms to prior studies on this bacteria (Vila et al. 2002). Plant extracts from *Cupressaceae, Onagraceae, Lycopodiaceae* and *Asclepiadaceae* families exhibit moderate to low antibacterial activity, with majority of ZOI diameters below 12 mm. However, plants from *Asteraceae* family exhibit the highest antibacterial activity, with majority of ZOI in the range of 15 - 35 mm

(**Figure 1a**). The *Asteraceae* family is composed of approximately 30,000 species belonging to over 1000 genera. This family is a rich source of sesquiterpenoids phytochemicals with documented antibacterial bioactivity (Wu et al. 2006). Particularly, the *Xanthium* genus is renowned for its use in alternative medicine for the treatment of infectious diseases (Anjoo & Ajay 2010). The antibacterial activity in *Xanthium* species has been attributed to the presence of xanthanolide sesquiterpenoids (i.e. xanthol and xanthanin) (Jawad et al. 1988; Sato et al. 1997).

2.2. Minimum Inhibitory Concentration (MIC) Assay

Many clinical studies have demonstrated a dose-dependent correlation between antibiotic concentration and bacterial inhibition in liquid cultures (Schentag 1990). Hence, the MIC value suffices as a measure of efficacy of bioactive metabolites in their inhibitory action against specific bacteria. In the MIC assay, resazurin, a nontoxic and non-fluorescent dye, was used as an indicator of cell growth and viability. Resazurin, initially dark blue, becomes pink and fluorescent when reduced to resorufin by oxidoreductases in living cells (Sarker et al. 2007; Mann & Markham 1998). It also generates a more distinct end-point color change than formazan based dyes and is relatively low cost (Griffin et al. 2000; Mann & Markham 1998).

In the MIC assay, thirty-seven (86%) out of 43 plant extracts inhibit the growth of one or more bacteria tested (**Table 3)**. Plants from all families exhibit some level of activity. The extracts of *S. canadensis* leaf, *X. strumarium* leaf, *Anap. margaritacea* leaf and flower, *Arct. uva-ursi* leaf, *C. canadensis* leaf, *G. squarrosa* leaf, stem and flower display the highest activity. Also, the extracts of *C. canadensis* L. leaf, *Anap. margaritacea* flower, *Achi. millefolium* L. leaf, *S. canadensis* leaf, *X. strumarium* leaf, *P. vulgaris* leaf, *Ascl. incarnata* L. leaf, *Arct. uva-ursi* stem and leaf successfully inhibit the growth of all six bacteria tested. In prior studies, two-fold

serial dilution ranges of 0.38 - 12.6 mg/ml (Buwa & van Staden 2006; Eldeen et al. 2005), 0.64 - 1.24 mg/ml (Yasunaka et al. 2005), 0.039 - 0.625 mg/ml (Mathabe et al. 2006) and 0.015 - 0.5 mg/ml (Newton et al. 2002) have been reported for the MIC assay. However, at higher concentration (i.e. 12 mg/ml), solubility of the extract in liquid broth may be reduced, unless solubilizing agents are applied. Hence, a concentration range of 0.04 - 5.00 mg/ml was utilized in this study. Fractions exhibiting MIC values below 1.25 mg/ml were considered significant inhibitors of bacterial growth in liquid broth. From 37 plant extracts, six extracts (16%) from *Asteraceae* and *Ericaceae* plant families exhibit high antibacterial activity, with the majority of MIC values between 0.08 - 1.25 mg/ml (**Figure 1b**). Plant extracts from *Poaceae, Cornaceae, Lamiaceae, Apocynaceae, Sarraceniaceae* and *Asclepiadaceae* family show moderate antibacterial activity, with the majority of MIC values around 1.25 - 2.50 mg/ml. In concert with hole-plate diffusion assay, plant extracts from *Cupressaceae, Lycopodiaceae* and *Onagraceae* plant families exhibit low antibacterial activity (majority of MIC values above 2.50 mg/ ml). Also, in agreement with hole-plate diffusion assay, extracts from the *Asteraceae* plant family generally display the highest antibacterial activity among all plant families.

In the MIC assay, a total of 4, 23, 2, and 1 extracts display increased antibacterial activity (i.e. lowest MIC values) in Hex/ EtOAc/ MeOH 5:1:0 (medium-low polarity), 0:1:0 (medium polarity), 0:9:1 (medium-high polarity) and 0:3:2 (high polarity) fractions, respectively. Hence, in the majority of extracts (62%), inhibitory activity was increased in the medium polarity fraction. This finding correlates with previous studies (Pekić et al. 1998; Swamy & Tan 2000; Syu et al. 1998), indicating that the ethyl acetate solvent shows a high selectivity towards natural products. This can be attributed to the accumulation of bioactive metabolites or the removal of potential antagonists. However, crude extracts of *Achi. millefolium* L. flower, *C. canadensis* leaf,

G. squarrosa flower, *P. frigidus* stem, *Apoc. androsaemifolium* leaf, *Arct. uva-ursi* and *P. vulgaris* stem demonstrate higher activities than individual fractions. The synergistic action of secondary metabolites in the crude extract has been reported in prior studies (Kohl et al. 1959; Si et al. 2006). A study by Kohl et al. (1959) demonstrated that the *Piper methysticum* crude extract was more effective in protection of the nervous system from strychnine (a toxic alkaloid) than its individual crystalline components. Also, Si et al. (2006) demonstrated that the Chinese green tea crude extract, which contains a mixture of compounds, displayed a higher degree of antibacterial activity than its individual components. Overall, none of the extracts display a significant antibacterial activity (MIC < 2.50 mg/ml) in the low polarity fraction (Hex/ EtOAc 9:1).

2.3. Minimum Bactericidal Concentration (MBC) Assay and Time-Kill Analysis

MBC is the concentration of plant extract that completely exterminates bacterial growth. The MBC and time-kill assay was performed on the crude extracts of most promising medicinal plants belonging to *Asteraceae, Apocynaceae, Ericaceae* and *Cornaceae* plant families. These extracts were selected due to their high tier performance in the hole-plate diffusion and MIC assays (refer to **Figure 1**).

In the MBC assay, none of the extracts achieved extermination of *E. coli* and *M. avium* subsp. *avium* bacteria (**Table 4)**. Among the bacteria that were complete annihilated by the shortlisted plants, *A. caviae* requires the highest bactericidal concentration, which may be attributed to a difference in cell wall barriers characteristic of all Gram-negative bacteria. In the time-kill analysis, most of the plant extracts proved lethal to *A. caviae, M. luteus* and *P. alvei* at MBC between 1 - 12 hours (**Figure 2**). In most cases, extracts of *Arct. uva-ursi* leaf, *G. squarrosa* flower and leaf and *A. margaritacea* flower were able to exterminate the bacteria

tested between 1 - 4 hrs. Particularly, the extract of *A. margaritacea* flower displays complete kill within 2 - 8 hours at bactericidal concentration of 0.16 mg/ml and 1.25 mg/ ml for *P. alvei* and *M. luteus*, respectively. Extract of *A. margaritacea* flower exterminated *A. caviae* within 1 hr at 5 mg/ml. However, none of the shortlisted extracts proved lethal to *B. cereus* at their respective MBC values; instead, a bacteriostatic effect was observed. Similar finding has been reported in prior studies (Ahmed et al. 2004; Viljoen et al. 2005), suggesting that *B. cereus* may develop resistance when grown in favorable conditions.

Among the shortlisted medicinal plants, a detailed time-kill analysis was performed on *Anaphalis margaritacea* flower extract due to its high bioactivity (**Figure 3**). *Anaphalis margaritacea* flower extract achieved maximum kill for *B. cereus* at 3 X MBC, whereas, at MIC, MBC and 2 X MBC a bacteriostatic effect was observed (**Figure 3b)**. The time kill analysis for *P. alvei* was of particular significance, since the extract was able to achieve maximum kill at a concentration of 80 µg/ml (MIC) within 2 hrs (**Figure 3c**). As expected, at higher concentrations of the extract (i.e. 3 X MBC and 2 X MBC) complete kill for *P. alvei* was observed within 1 hr or less. Findings in this study illustrate the high potency of this extract, especially against Gram-positive bacteria. It is exciting to note that *A. margaritacea* flower extract from *Asteraceae* family also exhibits considerably low MIC values (between 0.08 - 1.25 mg/ml) in the medium polarity fraction (Hex/ EtOAc/ MeOH 0:1:0). The antibacterial activity in this plant may be attributed to the presence of hydroxylactones (Ahmed et al. 2004). However, future studies need to be geared towards elucidating the exact source of its bioactivity.

2.4. Relationship Between Hole-plate Diffusion and MIC Assay

In the antimicrobial assays, fourteen plants that display no ZOI, exhibit activity in MIC assays. This discrepancy may be attributed to the diffusibility of the secondary metabolites in hole-plate diffusion assay, which is influenced by their size, polarity, conformation and ionic charge (Griffin et al. 2000). The ability of bioactive metabolites to diffuse through the agar medium may have also construed correlation between the two assays. For instance, *X. strumarium*, which displays the largest ZOI diameter in hole-plate diffusion assay, exhibits significantly higher MIC values relative to *Anap. margaritacea* and *S. canadensis*. Due to these challenges, many studies primarily consider the data from agar-diffusion method as preliminary and a qualitative evidence of antibacterial activity (Fernández-Pan et al. 2012). In broth dilution assays, the presence of organic solvents aid in the solubilization of non-polar metabolites and provide a significantly more accurate analysis of antimicrobial activity (Griffin et al. 2000). Broth dilution methods are also more quantitatively relevant since inhibitory action of bioactive metabolites is not determined by its diffusibility, but rather solubility in media and specific interaction with the target microorganism. However, a general correlation between high ZOI diameter and low MIC values, and vice versa, was observed. Majority of plants from *Asteraceae, Ericaceae* and *Cornaceae* family that display relatively high ZOI diameter also exhibit low MIC values. Further, plants from *Cupressaceae, Lycopodiaceae* and *Onagraceae* family that display relatively low ZOI diameters also exhibit high MIC values.

2.5. Bacterial Susceptibility

In the hole plate diffusion method, a total of 7, 23, 16, 17, 12 and 12 plant extracts inhibited the growth of *E. coli, B. cereus, M. avium* subsp. *avium, M. luteus, A. caviae* and *P.*

alvei, respectively. In the MIC assay, *E. coli* and *A. caviae* display the least sensitivity to extracts with the lowest observed MIC of 1.25 mg/ml. The highest sensitivity was displayed by *P. alvei* and *M. luteus* with the lowest observed MIC of 0.08 mg/ml and 0.31 mg/ml, respectively. This finding correlates well with previous studies (Kudi et al. 1999; Olano et al. 1996; Palombo & Semple 2001; Vlietinck et al. 1995) indicating that the Gram-negative bacteria are more resistant. This innate resistance can be attributed to a difference in cell wall structure, with the outer membrane of Gram-negative bacteria providing a protective barrier against antibiotics, detergents and many other environmental substances (Palombo & Semple 2001). Interestingly, *P. alvei* presented superior resistance on solid agar media, in contrast to liquid media. This may be attributed to the production of a lipopeptide derivative biosurfactant front on solid media by *P. alvei* that confers higher resistance to bactericides (Najafi et al. 2011). Overall, in my data there was no uniform response within or between bacterial strains in terms of susceptibility to the antibacterial substances in methanolic extracts of the medicinal plants. This may be attributed to differences in cell wall composition, inheritance genes, or plasmids that can be transmitted between bacterial species.

Species of *E. coli, M. avium* and *A. caviae* are potentially pathogenic to humans causing fevers, gastro-intestinal and extra-intestinal infections, mal-absorption, diarrhea, food poisoning, wound infections and bacteremia (Awan et al. 2009; Vila et al. 2003; von Reyn et al. 1994; Welch et al. 1981). Interestingly, *A. caviae* displays high resistance to the β lactam and cephalosporin antibiotics (i.e. ampicillin, cefazolin), though clinical isolates are more of a concern than environmental isolates (Awan et al. 2009; Ko et al. 1996; Vila et al. 2002; Vila et al. 2003). Further, *B. cereus* and *P. alvei* are food-borne pathogen and causative agent of foulbrood in honeybees, respectively (Antúnez et al. 2012; Ultee et al. 1998). Though, *M. luteus*

is a commercial strain, it can also be an opportunistic pathogen in patients with AIDS or immune-deficiency (Peces et al. 1997). Current study identifies several medicinal plants that could be a source of potential antibiotics or new chemical entities for agricultural crop protection programs or treatment of infectious diseases caused by these bacterium.

3. Materials and Methods

3.1. Instruments and Chemicals

Resazurin was purchased from Sigma-Aldrich (USA). Sodium chloride, potassium chloride, sodium bisphosphate, potassium phosphate monobasic, methanol, yeast extract, phytone and peptone were purchased from fisher scientific (USA).

3.2. Test Microorganisms

The microorganisms tested were all environmental isolates. They were representative of Gram negative, Gram positive, aerobic and facultative anaerobic categories. They comprised of *Bacillus cereus*, *Escherichia coli*, *Micrococcus luteus, Mycobacterium avium* subsp. *avium*, *Paenibacillus alvei* and *Aeromonas caviae*. They were streak plated on LB agar and maintained at 4 ^{o}C in dark. Stock freezer cultures were kept at -80 ^{o}C.

3.3. Collection of Plant Material

The plant materials in this study were whole plant samples, including root and flower for some plants. They were collected during the late flowering season (June-August) in Thunder Bay region, Ontario. The specimens were identified by Erika North, herbarium curator and

contract lecturer at Lakehead University, Thunder Bay, Ontario. The scientific names were based on those proposed by Morton and Venn (1990). All voucher specimens were deposited in -80 °C fridge at the Bio-Refining Institute, Lakehead University.

3.4. Extraction and Separation

The collected plant samples were separated into root, stem, flower and leaf parts. The plant parts were dried in dark over 24 - 48 hours at 30 °C. The dried plant samples (2 g) were ground up, milled through *no. 40* mesh and extracted twice with 50 ml of ethanol (85% v/v) over a period of 48 hours at room temperature. All extracts were filtered through *Whatman No. 1* filter paper, evaporated to dryness under rotary evaporator at 34 °C and stored at -80 °C in glass vials.

The extracts were subjected to manual LC on silica gel (40-60µm; 1 x 14cm) using Hex/ EtOAc/ MeOH 9:1:0, 5:1:0, 0:1:0, 0:9:1, 0:3:2, 5ml/min, in 100 ml aliquots. All fractions were dried in a rotary evaporator and high vacuum for 24 hours, and stored at -80 °C.

3.5. Hole-Plate Diffusion Assay

The procedure was followed according to Gallo and Riera (2005) with modifications. In this assay, 100 µl of $2x10^6$ CFU/mL bacterial suspensions were homogenously seeded into 25 ml of liquid broth agar and poured into 100 mm petri dishes. Then, 6 mm holes were aseptically bored into agar and 40 µl of 10 mg/ml methanolic extracts were pipetted into the wells. The plates were kept at 4 °C for 3 hours, then, incubated at 32 °C for 18 - 20 hours in dark. Chloramphenicol (100 µg/ml) and ampicillin (100 µg/ml) were used as positive controls and methanol was used as negative control. All results were recorded as mean of the triplicate and the zones of inhibition were determined as the diameter of inhibition zones around the holes.

3.6. MIC and MBC Assay

The minimum inhibitory concentration (MIC) assay was performed according to Sarkar et al. (2007) with modifications. First and second fractions from each extract were dissolved in EtOAc/ MeOH 6:1, whereas, the rest of fractions were dissolved in methanol (99.85 % v/v). The extracts were re-suspended to a concentration of 20 mg/ml. Then, 50µl of 20 mg/ml extract was aseptically added to 230 µl of LB broth in the first well of a sterile 96 well micro-plate. To each subsequent well, 180 µl of LB broth was added. Two fold serial dilutions, in 100 µl aliquots, were performed using a multichannel pipette to achieve a concentration gradient of 5.00 - 0.04 mg/ml. Prior to each subsequent dilution, precaution was taken to maximize homogeneity of the solution. Then, 20 µl of 2 x 10^6 CFU/ml bacterial suspensions in 1X PBS buffer were inoculated in their respective wells (to a final volume of 200 µl), the plate was wrapped with parafilm and placed in a plastic bag with mildly wet paper towel to prevent dehydration of bacteria. The plates were incubated at 32 °C for 18 hours in dark. After incubation, 10 µl of 0.04% resazurin in 1 X PBS buffer was added and the plate was further incubated in dark at 32 °C for 90 - 180 minutes. Micro-plates were viewed under short wavelength UV lamp for fluorescence and color change. The lowest concentration in which color changed from blue to pink, and displayed fluorescence, was taken as MIC. The MBC value was determined by plating 15 µl aliquots from wells without visible growth. Wells exhibiting complete annihilation of bacteria after incubation of LB agar plates at 32 °C for 18- 24 hrs were taken as MBC. A two-fold serial dilution of chloramphenicol (100 µg/ ml) and ampicillin (100 µg/ml) were used as positive controls, and EtOAc/ MeOH 6:1, 0:1 as negative control. The tests were performed in duplicates.

3.7. Time-Kill Analysis

Time-kill analysis was performed according to Imazatol et al. (1999). Bacterial suspensions prepared in 1 X PBS buffer were added in 2 ml of LB broth to a final concentration of $2x10^6$ CFU/ ml. The LB broth was prepared with a certain concentration of plant extracts and incubated at 30 °C with shaking at 200 rpm. After 30 min, 1 hr, 2 hr, 4 hr, 8 hr, 12 hr and 24 hr of incubation, 40 µl aliquot was acquired, serially diluted and plated on LB agar. The plates were incubated at 32 °C for 18 - 24 hrs and the numbers of viable cells were determined by counting the colonies. The test was performed in duplicates.

3.8. Statistical Analysis

The results were expressed as means ± standard deviation (SD) of two or three replicate measurements. The standard error was calculated in MS Excel v. 2011.

4. Conclusion

In conclusion, this study promotes northern Ontario region as a promising candidate for future ethnopharmacological research. In this study, 43 extracts from twenty-one northern Ontario medicinal plants were subjected to hole-plate diffusion and MIC assays to determine their antibacterial activity. Plants from eight families (out of 11) display activity in the hole-plate diffusion assay; however, extracts from all families exhibit activity in the MIC assay. Also, all extracts that actively inhibit bacteria in hole-plate diffusion assay show activity in the MIC assay. Plants from the *Asteraceae, Ericaceae* and *Apocynaceae* families display the highest antibacterial activity; whereas, plants from the *Cupressaceae, Lycopodiaceae* and *Onagraceae* families

display relatively low ZOI diameters and high MIC values. In the hole-plate diffusion and MIC assay, only the extracts from *C. canadensis* leaf and *A. uva-ursi* stem and leaf successfully inhibit the growth of all six bacteria tested. In the MIC assay, a majority (62%) of the extracts exhibit increased activity in their medium polarity fraction (Hex: EtOAc: MeOH 0:1:0). Further, in both MIC and hole-plate diffusion assays, plant extracts from *G. squarrosa, X. strumarium, A. margaritacea* and *A. uva-ursi* display among the highest antibacterial activity. In the MBC and time-kill analyses, the flower extract of *Anap. margaritacea* exterminated *B. cereus, A. caviae, P. alvei* and *M. luteus* within 2-8 hours at a concentration range of 0.08 - 2.50 mg/ml. Overall, the observed bioactivities of medicinal plants provide evidence for their traditional medicinal uses in native Aboriginal communities.

Table 1. Twenty-one northern Ontario medicinal plants and their medicinal properties.

Plant Family	Botanical Name	Conventional Name	Medicinal Property
Asteraceae	*Achillea millefolium* L.	Common Yarrow	Antimicrobial
	Anaphalis margaritacea	Pearly Everlasting	
	Arctium lappa	Burdock	Anti-inflammatory
	Grindelia squarrosa	Gumweed	Antibacterial,
	Petasites frigidus	Sweet Coltsfoot	Antibacterial
	Rudbeckia hirta L.	Goldenglow	Anti-inflammatory
	Solidago Canadensis	Goldenrod	Antiseptic
	Taraxacum ceratophorum	Dandelion	Antimicrobial
	Xanthium strumarium L.	Cocklebur	Antibacterial
Ericaceae	*Arctostaphylos uva-ursi*	Common Bearberry	Antibacterial
	Ledum palustre	Labrador Tea	Antibacterial, Anti-inflammatory
Asclepiadaceae	*Asclepias incarnata* L.	Milkweed	Antibacterial
Apocynaceae	*Apocynum androsaemifolium* L.	Spreading Dog Bane	Antibacterial
Cornaceae	*Cornus Canadensis* L.	Dogwood	Anti-inflammatory, anti-diarrheal,
Onagraceae	*Epilobium angustifolium* L.	Fireweed	Antibacterial
Poaceae	*Hierochloe odorata*	Sweet Grass	Antibacterial
Cupressaceae	*Juniperus communis* L.	Juniper	Antibacterial
Lycopodiaceae	*Lycopodium annotinum, L. clavatum*	Clubmoss	Estrogenic, antibacterial, antiviral,
Lamiaceae	*Prunella vulgaris*	Heal-all	Antibacterial, anti-inflammatory,
Sarraceniaceae	*Sarracenia purpurea* L.	Pitcher Plant	Antibacterial

Table 2. Hole-plate diffusion assay of 21 northern Ontario Medicinal Plants.

Medicinal Plant	Plant Part	Zone of Inhibition Diameter** (mm)					
		Gram Negative		Gram Positive			
		E. coli [a]	*A. caviae* [b]	*B. cereus* [c]	*M. luteus* [d]	*M. avium* [e]	*P. alvei* [f]
Achi. millefolium L.	Leaf	(-)	14.0 ± 0.0	(-)	(-)	12.0 ± 2.0	(-)
	Flower	(-)	16.0 ± 0.0	(-)	(-)	9.3 ± 1.2	8.0 ± 0.0
	Stem	(-)	(-)	(-)	(-)	6.0 ± 1.5	(-)
Anap. margaritacea	Leaf	(-)	(-)	22.7 ± 3.1	19.3 ± 1.2	18.6 ± 1.2	(-)
	Flower	(-)	22.0 ± 1.2	21.3 ± 3.1	19.3 ± 1.2	22.7 ± 1.2	23.0 ± 1.2
	Stem	(-)	20.0 ± 0.0	14.7 ± 3.1	13.3 ± 2.3	16.0 ± 2.0	(-)
Apoc. androsaemifolium L.	Leaf	15.3 ± 1.2	(-)	9.3 ± 1.2	(-)	16.0 ± 2.0	(-)
	Stem	(-)	(-)	(-)	(-)	(-)	(-)
Arct. uva-ursi	Leaf	16.6 ± 1.2	30.0 ± 0.0	16.7 ± 2.3	17.3 ± 1.2	18.0 ± 2.0	15.0 ± 0.0
	Stem	9.3 ± 1.2	22.0 ± 0.0	10.7 ± 2.3	10.0 ± 0.0	12.7 ± 4.6	12.0 ± 0.0
Arctium lappa	Leaf	(-)	(-)	(-)	(-)	(-)	(-)
	Flower	(-)	(-)	(-)	(-)	(-)	(-)
	Stem	(-)	(-)	(-)	(-)	(-)	(-)
Ascl. incarnata L.	Leaf	11.3 ± 1.2	(-)	10.0 ± 0.0	(-)	11.3 ± 1.2	(-)
	Root	(-)	(-)	(-)	(-)	(-)	(-)
	Stem	(-)	(-)	(-)	(-)	(-)	(-)
C. canadensis L.	Leaf	11.3 ± 1.2	26.7 ± 1.2	11.3 ± 1.2	14.7 ± 3.1	11.3 ± 1.2	12.0 ± 0.0
	Stem	10.0 ± 0.0	(-)	12.0 ± 5.3	9.7 ± 0.6	12.7 ± 1.2	20.0 ± 0.0

E. angustifolium L.	Leaf	9.0 ± 0.0	22.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	(-)
G. squarrosa	Leaf	(-)	18.0 ± 0.0	15.0 ± 1.0	19.3 ± 1.2	13.3 ± 1.2	15.3 ± 0.6
	Flower	(-)	21.3 ± 1.2	19.3 ± 2.3	23.3 ± 1.2	15.0 ± 1.0	16.0 ± 0.0
	Stem	(-)	(-)	(-)	(-)	(-)	(-)
H. odorata	Leaf	(-)	(-)	(-)	(-)	(-)	(-)
J. communis L.	Leaf	(-)	(-)	8.7 ± 1.2	11.3 ± 1.2	10.7 ± 1.2	20.0 ± 0.0
L. palustre	Leaf	(-)	(-)	(-)	(-)	(-)	(-)
	Stem	(-)	(-)	(-)	(-)	(-)	(-)
L. annotinum	Leaf	(-)	(-)	(-)	12.7 ± 1.2	12.0 ± 0.0	(-)
L. clavatum	Leaf	(-)	(-)	(-)	8.0 ± 0.0	7.3 ± 1.2	(-)
	Stem	(-)	(-)	(-)	(-)	8.7 ± 1.2	(-)
P. frigidus	Leaf	(-)	14.0 ± 0.0	12.0 ± 0.0	8.0 ± 0.0	8.7 ± 1.2	(-)
	Stem	(-)	(-)	(-)	(-)	(-)	(-)
P. vulgaris	Leaf	(-)	(-)	(-)	(-)	(-)	(-)
	Flower	(-)	(-)	(-)	(-)	(-)	(-)
	Stem	(-)	(-)	(-)	(-)	(-)	(-)
R. hirta L.	Leaf	(-)	(-)	(-)	(-)	(-)	(-)
	Stem	(-)	(-)	(-)	(-)	(-)	(-)
S. purpurea L.	Leaf	(-)	(-)	(-)	(-)	(-)	(-)
	Root	(-)	(-)	(-)	(-)	(-)	(-)
S. canadensis	Leaf	(-)	(-)	12.0 ± 2.0	13.3 ± 4.2	14.0 ± 1.2	12.0 ± 0.0
	Stem	(-)	16.0 ± 0.0	(-)	14.0 ± 0.0	(-)	9.0 ± 0.0
T. ceratophorum	Stem	(-)	(-)	(-)	(-)	(-)	(-)
X. strumarium L.	Leaf	(-)	(-)	26.7 ± 1.2	35.3 ± 1.2	30.0 ± 1.2	25.0 ± 1.2
	Stem	(-)	(-)	(-)	(-)	(-)	(-)
Chloramphenicol (100µg/ml)		32.0 ± 0.0	(-)	42.0 ± 0.0	52.0 ± 0.0	50.0 ± 0.0	50.0 ± 0.0
Ampicillin(100µg/ml)		30.0 ± 0.0	(-)	22.0 ± 0.0	44.0 ± 0.0	36.0 ± 0.0	36.0 ± 0.0
99.99% MeOH (40µl)		(-)	(-)	(-)	(-)	(-)	(-)

Note: all results are expressed as mean ± SD of three measurements.
(-): No zone of inhibition was observed.
[a] *Escherichia coli*; [b] *Aeromonas caviae*; [c] *Bacillus cereus*; [d] *Micrococcus luteus*; [e] *Mycobacterium avium* subsp. *avium*; [f] *Paenibacillus alvei*
[**] The Zone of Inhibition is defined as the inhibition diameter around the 6 mm holes bored in the agar

Table 3. Minimum inhibitory concentration of 21 northern Ontario medicinal plants

Medicinal Plant	Plant Part	Fraction	Minimum Inhibitory Concentration (mg/ml)					
			Gram Negative		Gram Positive			
			E. coli [f]	*A. caviae* [g]	*B. cereus* [h]	*M. luteus* [i]	*M. avium* [j]	*P. alvei* [k]
Anap. margaritacea	Leaf [C]	*a, b*	A >5					
		c	>5	1.25	1.25	0.31	>5	0.16
		d	>5	5.00	5.00	0.63	>5	0.31
		e	>5	5.00	1.25	2.50	>5	1.25
		Cr	>5	5.00	2.50	1.25	>5	0.16
	Flower [C]	*a*	A >5					
		b	2.50	5.00	2.50	2.50	2.50	2.50
		c	1.25	1.25	0.16	0.08	1.25	0.08
		d	1.25	1.25	1.25	1.25	1.25	1.25
		e	1.25	1.25	1.25	1.25	0.63	1.25
		Cr	>5	5.00	0.31	0.31	5.00	0.08

	Stem	*All fractions*	A >5					
Achi. millefolium L.	Leaf [C]	*a*	>5	>5	>5	>5	>5	2.5
		b	>5	>5	>5	>5	>5	2.5
		c	>5	5.00	5.00	>5	>5	0.63
		d	>5	>5	>5	>5	>5	2.50
		e	>5	>5	>5	>5	>5	5.00
		Cr	A >5					
	Flower [Cr]	*a, b*	A >5					
		c	>5	5.00	2.50	2.50	>5	1.25
		d	>5	5.00	5.00	5.00	>5	2.50
		e	>5	>5	2.50	2.50	>5	1.25
		Cr	>5	5.00	5.00	2.50	>5	0.63
	Stem	*All fractions*	A >5					
Ascl. incarnata L.	Leaf [C]	*a, b*	A >5					
		c	5.00	2.50	2.50	2.50	5.00	2.50
		d	A >5					
		e	5.00	>5	>5	>5	2.50	5.00
		Cr	2.50	>5	5.00	5.00	2.50	2.50
	Root [C]	*a, b*	A >5					
		c	>5	>5	5.00	>5	>5	2.50
		d	>5	>5	5.00	>5	>5	>5
		e	>5	5	5.00	>5	>5	2.50
		Cr	>5	>5	5.00	>5	>5	2.50
	Stem [C]	*a, b*	A >5					
		c	>5	>5	5.00	>5	>5	0.31
		d	>5	>5	>5	>5	>5	1.25
		e	>5	>5	>5	>5	>5	2.50
		Cr	>5	>5	>5	>5	>5	2.50
Arctium lappa	Leaf [E]	*a, b, c, d*	A >5					
		e	>5	5.00	>5	>5	>5	5.00
		Cr	>5	5.00	5.00	5.00	>5	5.00
	Flower [D]	*a*	>5	>5	>5	>5	>5	5.00
		b	>5	>5	>5	>5	>5	2.50
		c	>5	5.00	>5	2.50	>5	2.50
		d	>5	5.00	>5	2.50	>5	0.63
		e	>5	5.00	>5	5.00	>5	1.25
		Cr	>5	5.00	5.00	2.50	>5	2.50
	Stem	*All fractions*	A >5					
Arct. uva-ursi	Leaf [C]	*a*	A >5					
		b	>5	>5	>5	5	>5	2.5
		c	5.00	1.25	0.63	0.63	5.00	0.63
		d	5.00	2.50	2.50	2.50	2.50	2.50
		e	>5	5.00	5.00	>5	>5	5.00
		Cr	>5	5.00	2.50	2.50	>5	1.25
	Stem [Cr]	*a, b*	A >5					
		c	5.00	2.50	2.50	2.50	2.50	2.50
		d	5.00	5.00	2.50	5.00	2.50	2.50
		e	>5	5.00	>5	>5	>5	5.00
		Cr	5.00	1.25	1.25	1.25	2.50	1.25
	Leaf [Cr]	*a, b*	A >5					
		c	>5	2.50	5.00	2.50	>5	2.50
		d	>5	5.00	5.00	2.50	>5	2.50
		e	>5	>5	5.00	5.00	>5	>5

Apoc. androsaemifolium L.		*Cr*	>5	5.00	1.25	1.25	2.50	1.25
	Stem [C]	*a*	A >5					
		b	>5	>5	5.00	5.00	>5	5.00
		c	>5	2.50	5.00	2.50	>5	2.50
		d	>5	>5	>5	>5	>5	5.00
		e	A >5					
		Cr	>5	>5	5.00	5.00	>5	>5
C. canadensis L.	Leaf [Cr]	*a*	A >5					
		b	>5	5.00	1.25	2.50	5.00	2.50
		c	5.00	5.00	2.50	5.00	5.00	2.50
		d	5.00	5.00	5.00	5.00	5.00	2.50
		e	>5	5.00	5.00	>5	>5	>5
		Cr	>5	1.25	1.25	1.25	1.25	1.25
	Stem [C]	*a, b*	A >5					
		c	>5	>5	5.00	5.00	>5	2.50
		d	>5	5.00	5.00	>5	>5	5.00
		e	>5	2.50	2.50	>5	>5	5.00
		Cr	>5	2.50	2.50	>5	>5	2.50
E. angustifolium L.	Leaf [B]	*a*	A >5					
		b	5.00	5.00	5.00	5.00	5.00	2.50
		c	>5	5.00	5.00	>5	>5	5.00
		d	>5	>5	5.00	>5	>5	5.00
		e	>5	>5	>5	>5	>5	5.00
		Cr	5.00	5.00	5.00	5.00	5.00	5.00
G. squarrosa	Leaf [C]	*a*	>5	>5	>5	>5	>5	2.50
		b	>5	>5	>5	>5	>5	2.50
		c	>5	2.50	0.63	0.63	>5	0.63
		d	>5	5.00	2.50	2.50	>5	1.25
		e	>5	5.00	5.00	>5	>5	5.00
		Cr	>5	5.00	1.25	2.50	>5	1.25
	Flower [Cr]	*a, b*	A >5					
		c	>5	2.50	2.50	0.63	>5	2.50
		d	>5	>5	5.00	>5	5.00	1.25
		e	>5	>5	>5	>5	>5	5.00
		Cr	>5	5.00	0.63	1.25	>5	0.31
	Stem [C]	*a*	A >5					
		b	>5	5.00	5.00	5.00	5.00	2.50
		c	2.50	5.00	0.63	5.00	0.63	0.31
		d	>5	5.00	1.25	5.00	1.25	1.25
		e	>5	5.00	5.00	>5	>5	5.00
		Cr	>5	5.00	5.00	5.00	5.00	5.00
H. odorata	Leaf [C]	*a, b*	A >5					
		c	>5	2.50	5.00	1.25	5.00	0.63
		d	>5	2.50	5.00	1.25	5.00	1.25
		e	>5	2.50	>5	2.50	>5	2.50
		Cr	>5	5.00	5.00	2.50	>5	1.25
J. communis L.	Leaf [C]	*a, b*	A >5					
		c	>5	>5	5.00	5.00	>5	5.00
		d	>5	>5	>5	>5	>5	5.00
		e	A >5					
		Cr	>5	>5	5.00	>5	>5	>5
	Leaf [C]	*a*	A >5					
		b	>5	2.50	>5	5.00	>5	>5

L. palustre		*c*	>5	2.50	2.50	2.50	>5	1.25
		d	>5	5.00	5.00	2.50	>5	5.00
		e	>5	>5	5.00	2.50	>5	2.50
		Cr	>5	5.00	5.00	2.50	>5	1.25
	Stem [C]	*a, b*	A >5					
		c	>5	5.00	5.00	5.00	>5	2.50
		d	>5	>5	5.00	5.00	>5	2.50
		e	>5	>5	5.00	>5	>5	5.00
		Cr	A >5					
L. annotinum	Leaf [B]	*a*	A >5					
		b	>5	5.00	>5	2.50	>5	1.25
		c	>5	>5	>5	>5	>5	5.00
		d, e						
		Cr	2.50	5.00	2.50	2.50	2.50	5.00
L. clavatum	Leaf [C]	*a, b*	A >5					
		c	>5	>5	5.00	5.00	>5	1.25
		d	>5	>5	2.50	5.00	>5	1.25
		e	>5	>5	>5	>5	>5	5.00
		Cr	>5	>5	>5	>5	>5	5.00
	Stem [C]	*a, b*	A >5					
		c	>5	5.00	5.00	2.50	>5	5.00
		d	>5	5.00	5.00	5.00	>5	2.50
		e	>5	>5	5.00	5.00	>5	5.00
		Cr	>5	>5	>5	5.00	>5	5.00
P. frigidus	Leaf [B]	*a*	A >5					
		b	>5	1.25	2.50	2.50	1.25	0.63
		c	>5	5.00	5.00	5.00	>5	1.25
		d	>5	>5	>5	>5	>5	5.00
		e	>5	>5	>5	>5	>5	5.00
		Cr	>5	5.00	2.50	>5	>5	1.25
	Stem [Cr]	*a*	A >5					
		b	>5	>5	>5	>5	>5	5.00
		c	>5	>5	>5	>5	>5	5.00
		d	>5	>5	>5	>5	>5	5.00
		e	A >5					
		Cr	>5	>5	>5	>5	>5	2.50
P. vulgaris	Leaf [B]	*a*	A >5					
		b	>5	5.00	5.00	1.25	5.00	0.31
		c	>5	5.00	2.50	2.50	>5	1.25
		d	>5	>5	>5	2.50	>5	2.50
		e	>5	>5	>5	>5	>5	5.00
		Cr	5.00	5.00	5.00	5.00	5.00	2.50
	Flower [C]	*a, b*	A >5					
		c	>5	5.00	>5	5.00	>5	2.50
		d	>5	>5	>5	5.00	>5	5.00
		e	>5	5.00	>5	>5	>5	>5
		Cr	A >5					
	Stem [Cr]	*a, b, c*	A >5					
		d	>5	>5	>5	>5	>5	2.50
		e	>5	>5	5.00	5.00	>5	>5
		Cr	>5	>5	5.00	>5	>5	2.50
	Leaf [C]	*a, b*	A >5					
		c	>5	>5	>5	>5	>5	0.31

R. hirta L.		*d*	>5	>5	>5	>5	>5	1.25
		e	>5	>5	>5	>5	>5	2.50
		Cr	>5	>5	>5	>5	>5	1.25
	Stem [D]	*a*	A >5					
		b	>5	>5	>5	>5	>5	2.50
		c	A >5					
		d	>5	>5	>5	>5	>5	1.25
		e	>5	>5	5.00	>5	>5	5.00
		Cr	>5	>5	5.00	>5	>5	2.50
S. purpurea L.	Leaf [C]	*a, b*	A >5					
		c	>5	5.00	2.50	2.50	5.00	1.25
		d	>5	>5	5.00	>5	>5	2.50
		e	>5	5.00	>5	2.50	>5	5.00
		Cr	>5	5.00	>5	5.00	>5	2.50
	Root	*All fractions*	A >5					
S. canadensis	Leaf [C]	*a*	>5	>5	>5	>5	>5	5.00
		b	>5	5.00	5.00	1.25	2.50	1.25
		c	>5	5.00	>5	5.00	>5	0.16
		d	5.00	5.00	5.00	5.00	2.50	1.25
		e	5.00	>5	>5	>5	>5	5.00
		Cr	>5	>5	5.00	2.50	>5	0.31
	Stem	*All fractions*	A >5					
T. ceratophorum	Stem	*All fractions*	A >5					
X. strumarium L.	Leaf [C]	*a*	5	5.00	1.25	2.50	2.50	1.25
		b	>5	2.50	>5	2.50	>5	1.25
		c	>5	1.25	2.50	2.50	>5	0.63
		d	>5	>5	5.00	5.00	>5	2.50
		e	>5	5.00	2.50	2.50	5.00	5.00
		Cr	>5	5.00	2.50	2.50	>5	2.50
	Stem [C]	*a*	>5	>5	>5	>5	>5	2.50
		b	>5	>5	>5	>5	>5	2.50
		c	>5	2.50	5.00	5.00	>5	2.50
		d	>5	>5	>5	>5	>5	2.50
		e	>5	>5	>5	>5	>5	2.50
		Cr	>5	>5	>5	>5	>5	5.00

Note: All results are expressed as mean of duplicate measurements

[f] *Escherichia coli*; [g] *Aeromonas caviae*; [h] *Bacillus cereus*; [i] *Micrococcus luteus*; [j] *Mycobacterium avium* subsp. *avium*; [k] *Paenibacillus alvei*

a: Hex/ EtOAc 9:1; *b*: Hex/EtOAc 5:1; *c*: Hex/ EtOAc/ MeOH 0:1:0; *d*: EtOAc/MeOH 9:1; *e*: EtOAc/ MeOH 3:2; *Cr*: crude extract

A, B, C, D, E, Cr denotations on plant part indicate the fraction with the lowest observed MIC values for bacteria tested

A >5 denotes minimum inhibitory concentration beyond the 5 mg/ml range

Table 4. MIC and MBC values for the crude extract of shortlisted medicinal plants

Medicinal Plant	Plant Part	MIC (MBC) [g] (mg/ml)					
		Gram Negative		Gram Positive			
		E. coli [a]	*A. caviae* [b]	*B. cereus* [c]	*M. luteus* [d]	*M. avium* [e]	*P. alvei* [f]
Anap. margaritacea	Leaf	>5 (>5)	2.50 (2.50)	0.63 (1.25)	1.25 (1.25)	>5 (>5)	0.16 (0.63)
	Flower	>5 (>5	5.00 (5.00)	0.31 (0.63)	0.31 (1.25)	5.00 (>5)	0.08 (0.16)
Apoc. androsaemifolium	Leaf	>5 (>5)	>5 (>5)	1.25 (2.50)	1.25 (1.25)	2.50 (>5)	1.25 (1.25)
Arct. uva-ursi	Leaf	>5 (>5)	>5 (>5)	>5 (>5)	2.50 (2.50)	>5 (>5)	1.25 (1.25)
C. canadensis	Leaf	>5 (>5)	1.25 (1.25)	1.25 (2.50)	1.25 (1.25)	2.50 (>5)	1.25 (1.25)
G. squarrosa	Leaf	>5 (>5)	5.00 (5.00)	0.63 (1.25)	1.25 (1.25)	>5 (>5)	0.31 (0.63)
	Flower	>5 (>5)	5.00 (5.00)	0.63 (0.63)	2.50 (2.50)	>5 (>5)	1.25 (1.25)
X strumarium	Leaf	>5 (>5)	>5 (>50	>5 (>5)	>5 (>5)	>5 (>5)	2.50 (2.50)

[a] *Escherichia coli*; [b] *Aeromonas caviae*; [c] *Bacillus cereus*; [d] *Micrococcus luteus*; [e] *Mycobacterium avium* subsp. *avium*; [f] *Paenibacillus alvei*

[g] The value enclosed in the brackets corresponds to the MBC value

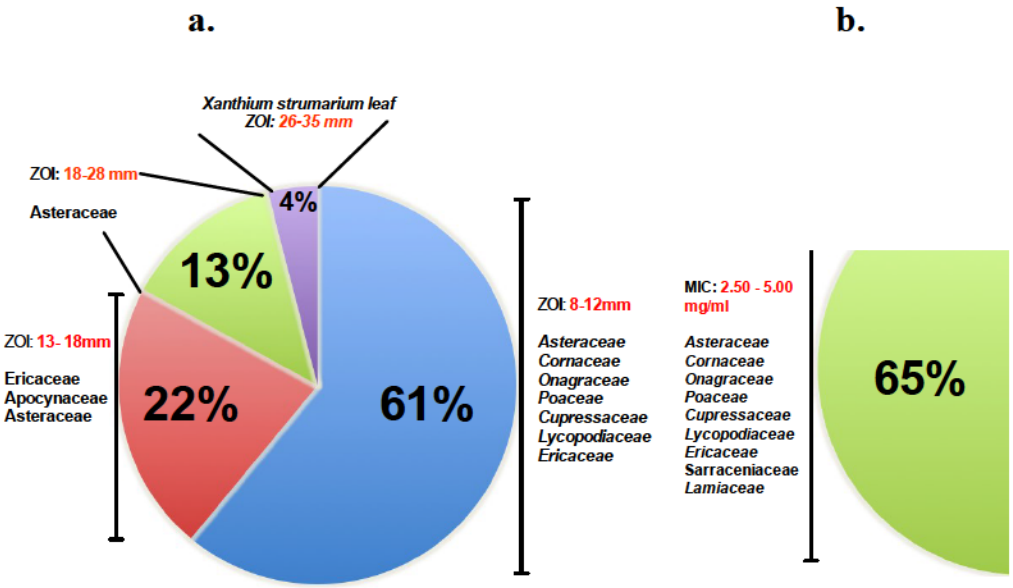

Figure 1. Distribution of antibacterial activity in hole-plate diffusion and MIC assays. **a.** Statistical distribution of antibacterial activity by plant family in hole-plate diffusion assay. **b.** Statistical distribution of antibacterial activity by plant family in MIC assay.

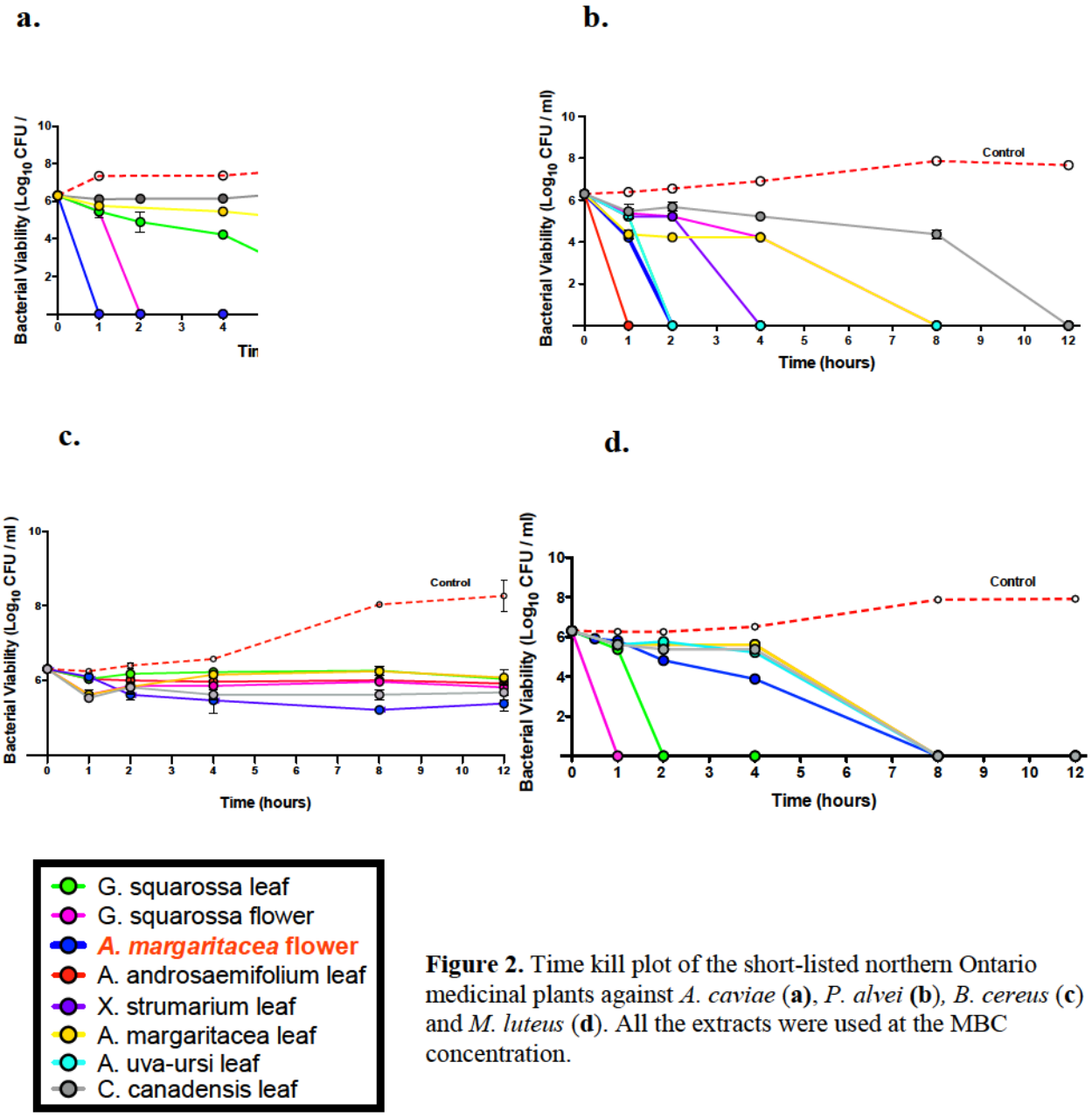

Figure 2. Time kill plot of the short-listed northern Ontario medicinal plants against *A. caviae* (**a**), *P. alvei* (**b**), *B. cereus* (**c**) and *M. luteus* (**d**). All the extracts were used at the MBC concentration.

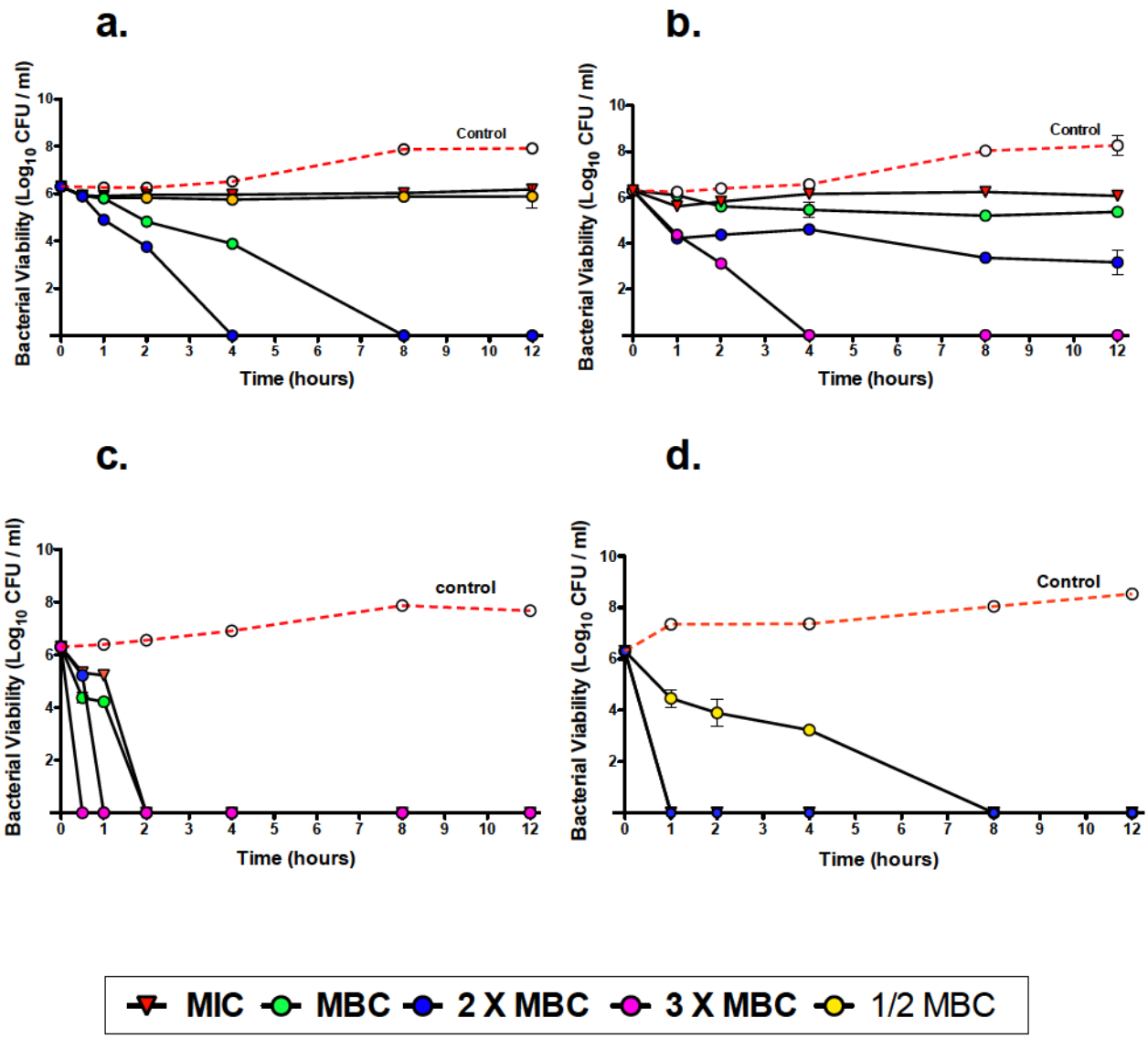

Figure 3. Time kill plot of *Anap. margaritacea* flower against *M. luteus* (**a**), *B. cereus* (**b**), *P. alvei* (**c**) and ***A. caviae*** (**d**). The extract was used at concentrations equivalent to MIC, MBC, 2 X MBC, 3 X MBC and/ or ½ MBC values.

CHAPTER #4

General Discussion and Future Directions

Haider M. Hassan

1. General Discussion

In this study, twenty-one northern Ontario medicinal plants were selected based on their traditional medicinal uses towards the treatment of oxidative stress related and infectious diseases. These plants were separated into leaf, flower, stem and root samples and extracted with ethanol. In total, forty-three plant extracts from twenty-one medicinal plants were analyzed for the assessment of their antioxidant and antibacterial activity. In the antioxidant assay, both ET (DPPH and ABTS assay) and HAT-based methods (ORAC assay) were utilized. The total phenolic content of medicinal plants was also determined. In the antibacterial assays, agar-diffusion (Hole-plate diffusion) and broth dilution (MIC assay) methods were employed. Then, a few plants were shortlisted and analyzed for the determination of MBC value and time kill analyses.

In this investigation, plant extracts from *Lycopodiaceae* and *Onagraceae* family consistently display moderate to low antioxidant and antibacterial activity. The low antioxidant and antibacterial profiles of *L. clavatum* and *L. annotinum* from the *Lycopodiaceae* family has been reported in prior studies (Calderón et al. 2012; Konrath et al. 2012). The low antioxidant activity may be attributed to presence of a thicker cuticle lined with waxy particles in these plants (Sun et al. 2005). This characteristic tends to reduce exposure to harmful near-infrared radiation (NIR) from sunlight via increased reflection at leaf surface (Orhan et al. 2007). A study by Slaton et al. (2001) demonstrated a high correlation ($r^2 = 0.93$) between a thicker cuticle and increased NIR reflection. However, contrary to my data on *E. angustifolium* (*Onagraceae*), previous studies have reported moderately high antioxidant activity for this plant (Li et al. 2012; Shikov et al. 2006). Though low antibacterial activity for *E. angustifolium* is consistent with prior studies

(Borchardt et al. 2008 a; Borchardt et al. 2008 b). For instance, Borchardt et al. (2008) demonstrated inhibition diameters for *E. angustifolium* in a similar range (7- 20 mm) to those observed in present study (9 - 22 mm). Interestingly, consistent with a study by Miceli et al. (2009) on *J. communis* from Turkey, the *J. communis* leaf extract (*Cupressaceae*) displayed antibacterial activity, albeit low, only against Gram-positive bacteria. This activity has been attributed to the presence of flavonoids, particularly apigenen (Miceli et al. 2009). The leaf extract of *J. communis* also displayed moderate radical scavenging capability, which has been attributed to the presence of quercetin-hexoside and isoscutellarein-8-O-hexoside flavonoids (Miceli et al. 2009).

Plant extracts from the *Asteraceae* family display moderate to high antibacterial activity, but low antioxidant capacities. Similar observations have been reported in prior studies. A study by Juteau et al. (2002) on the essential oil of *Artemisia annua* L. (*Asteraceae*) reported a MIC value of 0.05 mg/ml against *E. coli*, however, a relatively weak antioxidant activity. A study by Mothana et al. (2011) on Yemeni medicinal plants (*Carthamus tinctorius* L., *Centaurea pseudosinaica* Czerep.) of *Asteraceae* family, reported low antioxidant activity (EC_{50}: 100 - 540 µg/ ml) and a moderately high antibacterial activity (ZOI: 9-14 mm) against five Gram-positive and Gram-negative bacteria. However, there were exceptions to this trend. In the antibacterial studies, ten and four plant extracts from this family exhibited no antibacterial activity in the hole-plate diffusion and MIC assays, respectively. Extracts from *A. margaritacea* leaf and flower display exceptional antibacterial and moderately high antioxidant activity. Also, contrary to my data, a study by Candan et al. (2003) on *A. millefolium* (*Asteraceae*) from Turkey reported a relatively high antioxidant activity for this plant. In the present investigation, *A. millefolium* displays a low phenolic content (below 100

mg GAE/ 10 gdw) and performs poorly in ABTS and DPPH assay (activity below 60 µM TE/ gdw). However, it exhibits an unexpectedly high activity in ORAC assay in the range of 230 - 355 µM TE/ gdw for stem, leaf and flower extracts. Overall, in agreement with Candan et al. (2003), *A. millefolium* displays moderately low ZOI diameters (between 6 - 16 mm) and MIC values (majority over 1.25 mg/ml).

1.1. Most Promising Medicinal Plants

The leaf, stem and/ or flower extracts of *Xanthium strumarium*, *Anaphalis margaritacea, Solidago canadensis* and *Grindelia squarrosa* from the *Asteraceae* family exhibit the largest ZOI and lowest MIC values. Decoctions of these plants are used in the aboriginal community to alleviate infectious diseases. For instance, a paste extracted from the leaf tissues of *Anaphalis margaritacea* is routinely applied on wounds, rashes or cuts to prevent infections. Previous studies on this plant have isolated multiple hydroxylactones responsible for antimicrobial activity (Ahmed et al. 2004). *Xanthium strumarium,* a common weed, has also been used for the treatment of tuberculosis, diarrhea, malaria, constipation, bacterial and fungal infections (Anjoo & Ajay 2010). Xanthol, xanthinin and xanthanolide sesquiterpenes have been identified as the active metabolites (Jawad et al. 1988; Sato et al. 1997). *Solidago canadensis* is also a well-researched plant and the antimicrobial activity has been attributed to the presence of several clerodane-type diterpenes (Kruszewska et al. 2004; Lu et al. 1993). However, research on *Grindelia squarrosa* was found to be vastly lacking. Previous study on a separate species, *Grindelia tarapacana,* has isolated manoyloxide type diterpenoids exhibiting pronounced antibacterial and anti-tuberculosis activity (Zhou et al. 1995). Some of the diterpenoids isolated displayed a more potent anti-tuberculosis activity (MIC = 32 µg/ml) than the anti-tuberculosis agent pyrazinamide (40 µg/ml). Therefore, a biochemical

analysis on *Grindelia squarrosa*, a North American species of gumweed, could yield promising new antibacterial compounds and future studies should be tailored towards this aspect.

The extracts from *Cornaceae, Lamiaceae* and *Ericaceae* family display highest antioxidant activity. From these plant families, the leaf extracts of *Arctostaphylos uva-ursi, P. vulgaris*, *L. palustre*, and *C. canadensis* display the most promising activity. *P. vulgaris* (also known as heal all) is well known for its antioxidant, anti-inflammatory, antibacterial and antidiarrheal activity (Lee et al. 2008; Liu & Ng 2000; Psotova et al. 2006; Skottová et al. 2004). The crude extract of heal-all consists of rosmarinic acid (Psotová et al. 2003), triterpenoids, flavonoids, anthocyanins, oleanoic acid, betulinic acid, caffeic acid, tannins and anionic polysaccharides (Cai et al. 2004; Psotová et al. 2005). Rosmarinic acid is a known antibacterial, antiviral, antioxidant and anti-inflammatory agent (Petersen & Simmonds 2003). Oleanoic, caffeic, and betulinic acid are also well known antioxidant agents. Previous research on *L. palustre* (Labrador tea) has shown antioxidant, anti-inflammatory, antimicrobial, and anticancer activity (Dufour et al. 2007). HPLC and GC-MS analysis of the Labrador tea aerial parts has isolated monoterpenes, sesquiterpenes, esculin, quercetin glycosides, acetylated flavonoids, glucoside fraxin and dihydric alcohols (Chosson et al. 1998; Fylaktakidou et al. 2004; Nam 2006). *Arctostaphylos uva-ursi* is also well-researched plants and its bioactivity has been attributed to presence of arbutin (Jahodár et al. 1985). Overall, *C. canadensis* (Dogwood) exhibits the highest antioxidant activity in SET-assays and the total phenolic content; however, it ranked relatively poorly in the ORAC assay. This activity could be attributed to a poor competition for peroxy radical, or a lack of solubilizing agents applied in ORAC method that could have confiscated the activity of lipophilic

phenolic groups (Cheng et al. 2006). Overall, through a research survey, no isolation or *in vivo* study on the extracts of *C. canadensis* was identified in literature. This presents a unique and promising incentive for future research on *C. canadensis*.

2. Future Directions

An antioxidant compound is a substance that significantly reduces the oxidation of an oxidizable substrate at low concentrations. The current study identifies the antioxidant activity of medicinal plants through DPPH, ABTS, ORAC and total phenolic content assays. However many other *in vitro* and *in vivo* methods exist in literature that can elucidate the important characteristics of compounds from crude plant extracts.

Oxidative stress can cause lipid peroxidation, especially in polyunsaturated fatty acids (PUFA), since presence of a double bond weakens the C-H bond on the adjacent carbon atoms, facilitating H^+ removal (Niki 2010). The oxidation of PUFA or other lipids can have detrimental effects in cells as the membrane characteristics and function of associated receptors or proteins may be impaired. The oxidized lipids can further degrade to unsaturated aldehydes (Repine et al. 1997), which can also alter cell function by inhibiting antiproteases and cyclooxygenases, activating latent procollagenese, and reacting with arachidonic acid (Niki 2010). Measurement of the products of lipid peroxidation (e.g. Malondialdehyde and F2- isoprostanes) are routinely used to determine antioxidant activity *in vitro* and *in vivo*. Thiobarbituric acid-reactive substance (TBARS) assay is used to quantify antioxidant capacity of extracts based on their ability to reduce the formation of malondialdehyde - thiobarbituric acid (MDA-TBA) adduct. However, this assay lacks sensitivity and specificity

due to side reactions of TBA with hemoglobin and rapid metabolism of MDA, which represents only 1% of lipid peroxides (Pincemail et al. 1996). Templar et al. (1999) reported higher sensitivity in TBARS assay by incorporating HPLC to separate MDA-TBA from interfering compounds, and subsequent detection at 532 nm. However, the most reliable method to quantify oxidative stress by lipid peroxidation is through measurement of F2-isoprostanes (Liu et al. 2009). F2-isoprostane biomarkers are structurally stable, produced *in vivo*, and are present in relatively high concentrations (Montuschi et al. 2007). Increased plasma concentration of F2-isoprostane levels has been reported for smokers (Dietrich et al. 2002), patients suffering from oxidative stress during acute hyperglycemia type 2 diabetes, (Sampson et al. 2002) and breast cancer (Stoll 2002). Through support from the National Institute of health (NIH), Milne et al. (2007) published an excellent method in *Nature Protocols* to quantify F2-isoprostanes as the most reliable biomarker of oxidative stress *in vitro* or *in vivo*. Their protocol utilizes GC-MS to determine accurate levels of F2-isoprostanes in 12 - 16 urine or blood samples in 6 - 8 hours.

Studies also measure oxidative damage on DNA by quantifying urinary excretion of the DNA-repair product, 8-hydroxydeoxyguanosine (8-ohdG), through HPLC (Svoboda & Kasai 2004). However, this method is rarely implemented due to higher cost and time allotment. In my perspective, future antioxidant studies should be tailored towards determination of TBARS *in vitro* for the most promising medicinal plants and isolation of the potentially bioactive metabolites. However, HPLC-MS, GC-MS or LC-MS can be performed directly on the crude extracts to determine the major constituents, in reference to standard phenolic compounds. At the most advanced stage, the bioactive components can be assayed for their

therapeutic and lethal concentration (mice studies) and effect on F2-isoprostane concentration *in vivo* as a measure of antioxidant action in a biological system.

The antimicrobial activity of medicinal plants was evaluated through hole-plate diffusion and minimum inhibitory concentration (MIC) assays. The future steps could be to assess the antibacterial activity of medicinal plants on clinical isolates, particularly *Pseudomonas* species, methicillin resistant *Staphylococcus aureus* (MRSA), *mycobacterium tuberculosis*, *Aeromonas caviae* (Clinical isolate) and *Aeromonas hydrophilia* due to their high pathogenicity and/ or multiple drug resistance. Also, the bacteriostatic and/ or bactericidal antibacterial action of bioactive metabolites in crude extract must be determined. Further, the *in vivo* studies on treatment of bacteremia in mice or pigs, prevention of vegetable or food decay (e.g. Butternut squash) or mouth borne diseases in mice are also important to conduct. Overall, isolation strategies should be performed on the most promising medicinal plants to determine the architectures of bioactive metabolites responsible for the antibacterial activity.

APPENDIX A

Calibration Curves for Antioxidant Assays

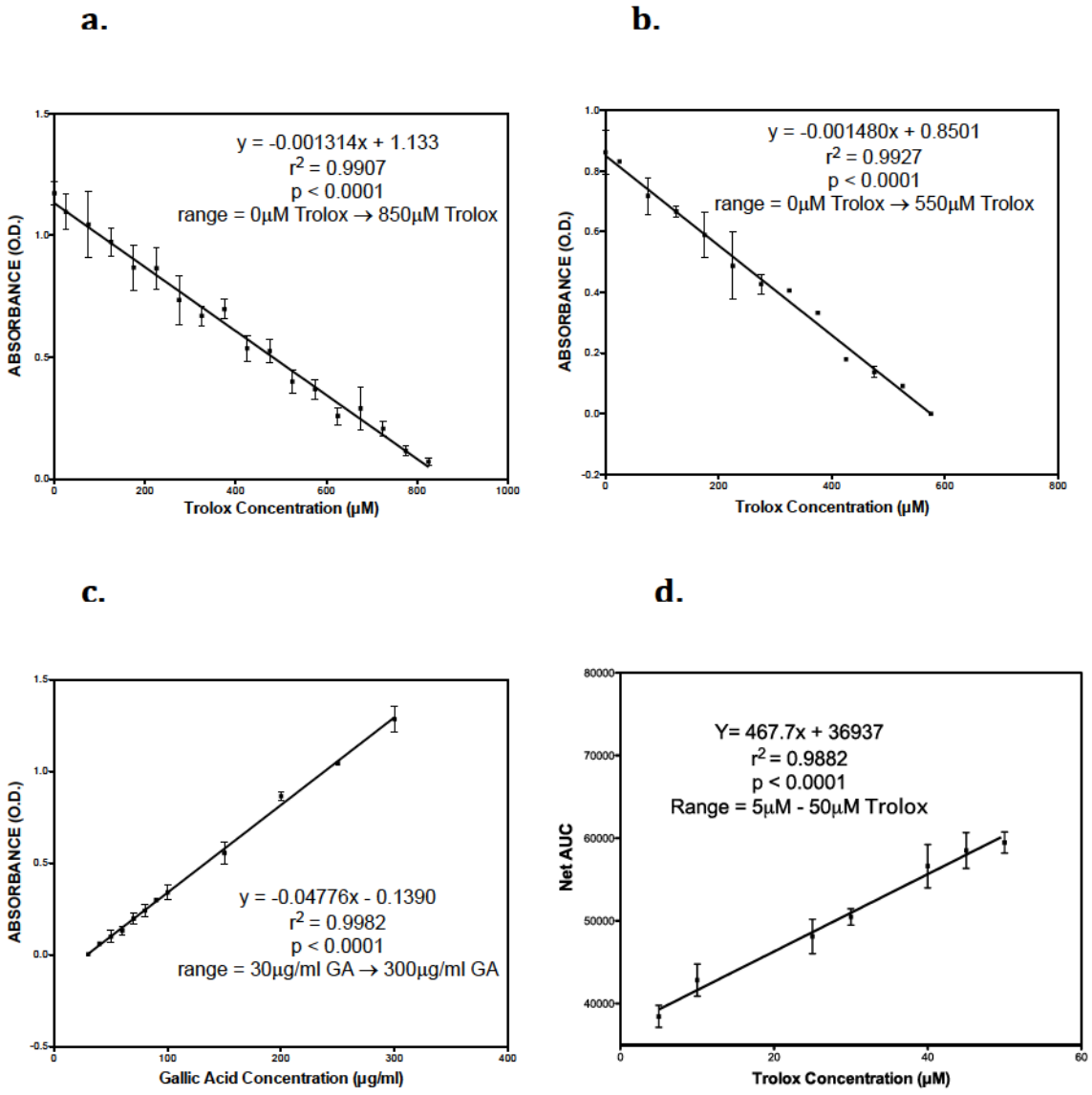

Figure 1. Calibration curves for ORAC, DPPH, ABTS, and total phenolic content assays. **a.** DPPH calibration curve. **b.** ABTS Calibration curve. **c.** Calibration curve for total phenolic content assay. **d.** Calibration curve for ORAC assay.

Appendix B

Characteristics of Crude and LC Fractions

Table 1. Mass of 43 crude extracts from 21 northern Ontario medicinal plants

Medicinal Plant	Plant Part	Crude Extract mass (mg / gdw) [a]
Achi. millefolium L.	Leaf	82.8
	Flower	64.0
	Stem	45.4
Anap. margaritacea	Leaf	194.4
	Flower	162.3
	Stem	62.6
Apoc. androsaemifolium L.	Leaf	197.9
	Stem	61.1
Arct. uva-ursi	Leaf	152.7
	Stem	105.8
Arctium lappa	Leaf	167.9
	Flower	135.6
	Stem	103.6
Ascl. incarnate L.	Leaf	166.5
	Root	177.8
	Stem	219.5
C. canadensis L.	Leaf	115.7
	Stem	79.4
E. angustifolium L.	Leaf	162.0
G. squarrosa	Leaf	157.5
	Flower	144.5
	Stem	88.0
H. odorata	Leaf	86.5
J. communis L	Leaf	108.9
L. palustre	Leaf	327.6
	Stem	145.2
L. annotinum	Leaf	100.0
L. clavatum	Leaf	172.8
	Stem	98.3
P. frigidus	Leaf	185.2
	Stem	115.3
P. vulgaris	Leaf	131.6
	Flower	115.2
	Stem	80.0
R. hirta L	Leaf	118.9
	Stem	68.6
S. purpurea L.	Leaf	348.6
	Root	112.4
S. canadensis	Leaf	130.8
	Stem	119.7
T. ceratophorum	Stem	118.6
X. strumarium L.	Leaf	110.0
	Stem	64.7

[a] The extract mass entails mg of crude extract weight / gram of dried plant weight

Table 2. Physical characteristics of the LC fractions and their respective recovery

Medicinal Plant	Plant Part	Fraction mass and Physical Characteristics**					% Yield [f]
		F1[a]	*F2*[b]	*F3*[c]	*F4*[d]	*F5*[e]	
Achi. millefolium L.	Leaf	Orange-yellowish oil (13.4 mg)	Light green oil (13.7 mg)	Dark green oil (20.5 mg)	Light green precipitate (15.4 mg)	Brownish colored precipitate (25.7 mg)	80.2
	Flower	Clear oil (13.8 mg)	Light green oil (14.7 mg)	Dark green oil (21.7 mg)	Greenish orange precipitate/ solid (19.7 mg)	Orange-brownish solid/ precipitate (37.8 mg)	72.4
	Stem	Yellowish paste (14.3 mg)	Yellowish paste (15.1 mg)	Dark green paste (20.0 mg)	Yellowish green solid (13.1 mg)	Yellowish green precipitate (69.0 mg)	94.6
Anap. margaritacea	Leaf	Yellowish oil (36.7 mg)	Yellowish oil (26 mg)	Greenish solid (77.2 mg)	Light green solid (31.5 mg)	Dark reddish orange solid (177.8 mg)	89
	Flower	Yellowish oil (26.7 mg)	Yellowish oil (49.8 mg)	Greenish solid (52.5 mg)	Yellowish-green solid (59.8 mg)	Dark reddish orange solid (103.4 mg)	83.5
	Stem	Yellow oil (16.4 mg)	Yellow green oil (19.2 mg)	Green paste (18.1 mg)	Yellowish green precipitate (27.3 mg)	Greenish precipitate (30.0 mg)	84.7
Apoc. androsaemifolium L.	Leaf	Yellowish oil (15.8 mg)	Greenish yellow paste (14.6 mg)	Dark green oil/ paste (19.9 mg)	Dark green precipitate (21.0 mg)	Dark green precipitate (33.8 mg)	86.8
	Stem	Off white oil paste (14.3 mg)	Off white oil paste (19.6 mg)	Dark green oil (22.3 mg)	Light green solid (14.9 mg)	Off whitish-green precipitate or crystal--its kind of fluffy (84.2 mg)	80.5
Arct. uva-ursi	Leaf	Greenish yellow (17.5 mg)	Light green noil (19.5 mg)	Off white greenish paste (24.9 mg)	Yellowish but slight green solid (29.2 mg)	Yellowish green precipitate (37.95 mg)	79.1
	Stem	Off white oil/ paste (20.4 mg)	Clear (18.7 mg)	Light green paste (31.8 mg)	Light green solid (52.8 mg)	Yellowish precipitate (59.0 mg)	76.6
Arctium lappa	Leaf	Yellowish dark oil (16.2 mg)	Light yellow oil (14.4 mg)	Dark green-brownish oil (19.5 mg)	Light green oil/ paste (15.5 mg)	Dark brownish oil/ paste (45.8 mg)	68
	Flower	Colorless oil (16.9 mg)	Colorless oil/ paste (19.7 mg)	Dark greenish precipitate/ paste (19.6 mg)	Light green paste (14.1 mg)	Dark green-brownish crystal/ precipitate (42.9 mg)	61

	Stem	Off white wax (12 mg)	Off white waxy oil (13.4 mg)	Dark green paste (25.7 mg)	Light green solid (20.5 mg)	Light greenish precipitate (43.0 mg)	97
Ascl. incarnate L.	Leaf	Yellowish oil (14.3 mg)	Yellowish oil (14.4 mg)	Green oil (19.9 mg)	Green oil (13.4 mg)	Green precipitate (57.3 mg)	90
	Root	Off white paste (13.0 mg)	Off white paste (13.8 mg)	Light green paste (16.9 mg)	Light green paste (17.4 mg)	Very fluffy, light green/ white and seems to have crystalized (144.6 mg)	93
	Stem	Off white paste (12.3 mg)	Off whitish paste (20.6 g)	Dark green precipitate (22.5 mg)	Light green solid (17.6 mg)	Yellowish green crystal foaming (263.7 mg)	95
C. canadensis L.	Leaf	Colorless oil (15.6 mg)	Yellowish oil (32.0 mg)	Dark green paste (40.0 mg)	Light green precipitate (30.0 mg)	Yellowish green solid (105.0 mg)	85.6
	Stem	Yellowish oil (22.0 mg)	Light green oil (29.0 mg)	Green yellowish paste (33.0 mg)	Off white greenish precipitate (53.0 mg)	Yellowish orange solid (157.8 mg)	93.2
E. angustifolium L.	Leaf	Yellowish oil (13.0 mg)	Greenish oil (21.7 mg)	Yellowish oil (25.0 mg)	Yellowish oil (17.4 mg)	Orange precipitate (25.0 mg)	79
G. squarrosa	Leaf	Yellowish oil (12.0 mg)	Yellowish oil (17.6 mg)	Dark green paste (18.9 mg)	Yellowish green paste (23.1 mg)	Reddish precipitate (29.1 mg)	80
	Flower	Yellowish oil (12.4 mg)	Yellowish oil (17.7 mg)	Dark green paste (17.5 mg)	Yellowish green paste (19.5 mg)	Reddish precipitate (27.0 mg)	89.2
	Stem	Off white oil (12.3 mg)	Light yellowish oil (17.6 mg)	Light green precipitate (23.6 mg)	Light green paste (14.4 mg)	Dark green-brownish crystal (84.0 mg)	90
H. odorata	Leaf	Dark yellowish oil (15.0 mg)	Yellowish oil (14.9 mg)	Dark green oil/ paste (24.4 mg)	Dark green precipitate (15.1 mg)	Dark brownish-orange red crystal formed (97.2 mg)	76
J. communis L	Leaf	Nothing eluted	Off white paste (12.7 mg)	Dark green solid (18.6 mg)	Dark green solid (14.6 mg)	Brownish precipitate (40.1 mg)	97
L. palustre	Leaf	Yellowish oil (15.2 mg)	Light green oil/ paste (17.8 mg)	Dark green oil (78.9 mg)	Light green solid (76.6 mg)	Brownish dark crystals formed (143.9 mg)	82
	Stem	Colorless oil (12.3 mg)	Light yellowish-green oil (17.6 mg)	Light green precipitate (23.6 mg)	Light green paste (14.4 mg)	Dark green-brownish crystal (84.0 mg)	78
L. annotinum	Leaf	Yellowish	Greenish	Greenish	Yellowish-	Reddish	82.2

		oil (22.6 mg)	yellow oil (27.5 mg)	solid (32.0 mg)	green solid (14.3 mg)	orange solid (125.6 mg)	
L. clavatum	Leaf	Whitish paste (12.9 mg)	Yellowish paste (16.8 mg)	Green paste (13.3 mg)	Light green precipitate/ solid (17.2 mg)	Orange-greenish solid (37.1 mg)	62.3
	Stem	White oil/ paste (14.1 mg)	Off white oil/ paste (17.1 mg)	Dark green precipitate (24.0 mg)	Off white-greenish precipitate (17.8 mg)	Orange oily paste (46.5 mg)	82.6
P. frigidus	Leaf	Colorless oil/ paste (14.7 mg)	Yellowish green paste (17.2 mg)	Green oil/ paste (17.6 mg)	Light greenish yellow precipitate (23.8 mg)	Colorless precipitate (22.2 mg)	81.6
	Stem	Yellowish green paste (16.4 mg)	Yellowish green paste (13.0 mg)	Light green paste (16.7 mg)	Off whitish solid (15.0 mg)	Light green solid (86.0 mg)	58
P. vulgaris	Leaf	Yellowish oil (25.9 mg)	Greenish oil (26.3 mg)	Green very oily (31.2 mg)	Green precipitate forming (132.2 mg)	Orange crystal (137.2 mg)	82.1
	Flower	Colorless oil (12.4 mg)	Yellowish oil/ paste (20.0 mg)	Dark green precipitate (29.0 mg)	Light green solid / powder (17.8 mg)	Brownish crystal (80.4 mg)	76
	Stem	Off-white paste (13.7 mg)	Yellowish-green paste (12.0 mg)	Light greenish white powdery solid (20.4 mg)	Whitish Green precipitate-powdery material (30.0 mg)	Dark reddish brown solid/ paste (47.3 mg)	97
R. hirta L	Leaf	Off white wax (7 mg)	Yellowish oil (15.3 mg)	Dark green paste (24.7 mg)	Light green precipitate (13.4 mg)	Light green solid (30.7 mg)	93
	Stem	Yellowish green paste (16.7 mg)	Colorless paste (16.6 mg)	Dark Green precipitate (24.3 mg)	Light green solid (14.3 mg)	Light greenish / yellow solid (37.1 mg)	88
S. purpurea L.	Leaf	Yellowish oil (22.0 mg)	Yellowish oil (24.0 mg)	Dark green paste (32.5 mg)	Light green solid (27.0 mg)	Yellowish crystallization (102.0 mg)	92.6
	Root	Colorless paste (20.0 mg)	Colorless paste (20.0 mg)	Yellowish green solid (44.1 mg)	Whitish solid at sides but green in middle (48.5 mg)	Orange solid, but also seems like a paste (97 mg)	90
S. canadensis	Leaf	Yellowish oil (15.0 mg)	Whitish yellow oil (13.4 mg)	A green paste (87.0 mg)	Light green solid (30.0 mg)	Orangish red precipitate (91.2 mg)	84.6
	Stem	Off whitish paste/ oil (15.2 mg)	Off whitish paste/ oil (14.4 mg)	Green paste (17.3 mg)	Light green precipitate (13.4 mg)	Dark green waxy material (32.5 mg)	83
T. ceratophorum	Stem	Off-white paste (12.3	Yellowish-green paste	Light greenish	Whitish Green	Dark reddish brown solid/	97

		mg)	(12.0 mg)	white powdery solid (20.4 mg)	precipitate-powdery material (15 mg)	paste (27.3 mg)	
X. strumarium L.	Leaf	Yellowish oil (15.8 mg)	Whitish yellow oil (15 mg)	A green paste (19.7 mg)	Light green solid (24.0 mg)	Greenish precipitate (82.0 mg)	77
	Stem	Waxy substance, off white (14.0 mg)	Waxy substance, off white (14.4 mg)	Light green solid (15.4 mg)	Light green solid (12.9 mg)	Light green precipitate (31.6 mg)	88.9

[a] Hex/ EtOAc 9:1; [b] Hex/EtOAc 5:1; [c] Hex/ EtOAc/ MeOH 0:1:0; [d] EtOAc/MeOH 9:1; [e] EtOAc/ MeOH 3:2
[f] % yield = [(mass $_{F1}$ + mass $_{F2}$ + mass $_{F3}$ + mass $_{F4}$ + mass $_{F5}$) / (Mass crude extract added in LC)] * 100%
**The characteristics and mass of fractions was determined after they were dried under high vacuum for 24 hours. The fractions were sealed with parafilm and stored at -80 °C until required for analysis.

Bibliography

Abascal, K., Yarnell, E. 2008. Botanical Medicine for Cystitis. Alt. Comp. Ther. **14:** 69-77.

Ahmad, I., Mehmood, Z., Mohammad, F. 1998. Screening of some Indian medicinal plants for their antimicrobial properties. J. Ethnopharmacol. **62:** 183-193.

Ainge, G.D., Lorimer, S.D., Gerard, P.J., Ruf, L.D. 2002. Insecticidal activity of huperzine A from the New Zealand clubmoss, *Lycopodium varium*. J. Agric. Food Chem. **50:** 491-494.

Albert, D., Zündorf, I., Dingermann, T., Müller, W.E., Steinhilber, D., Werz, O. 2002. Hyperforin is a dual inhibitor of cyclooxygenase-1 and 5-lipoxygenase. Biochem. Pharmacol. **64:** 1767-1775.

Amarowicz, R., Pegg, R.B., Rahimi-Moghaddam, P., Barl, B., Weil, J.A. 2004. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chem. **84:** 551-562.

Ames, B.N., Gold, L.S., Willett, W.C. 1995. The causes and prevention of cancer. Proc. Natl. Acad. Sci. **92:** 5258.

An, M., Pratley, J.E., Haig, T., Liu, D.L. 2005. Whole-range assessment: a simple method for analysing allelopathic dose-response data. Nonlinearity Biol. Toxicol. Med. **3:** 245-259.

Argus, G.W., Coupe, R., MacKinnon, A., Pojar, J.1999. Plants of Northern British Columbia. Lone Pine Pub., Vancouver, AB. 352pp.

Arnason, T., Hebda, R.J., Johns, T. 1981. Use of plants for food and medicine by native peoples of eastern Canada. Can. J. Bot. **59:** 2189-2325.

Alía, M., Mateos, R., Ramos, S., Lecumberri, E., Bravo, L., Goya, L. 2006. Influence of quercetin and rutin on growth and antioxidant defense system of a human hepatoma cell line (HepG2). EU. J. Nurt. **45:** 19-28.

Ahmed, A.A., Hussein, T.A., Mahmoud, A.A., Farag, M.A., Paré, P.W., Wojcińska, M., Karchesy, J., Mabry, T.J. 2004. Nor-ent-kaurane diterpenes and hydroxylactones from *Antennaria geyeri* and *Anaphalis margaritacea*. Phytochem. **65:** 2539-43.

Anjoo, K., Ajay, S. 2010. Phytopharmacological review of *Xanthium strumarium* L. (Cocklebur). Int. J. Green Pharm. **4:** 129-139.

Antúnez, K., Anido, M., Branchiccela, B., Harriet, J., Campá, J., Zunino, P. 2012. American Foulbrood in Uruguay: twelve years from its first report. J. Invertebr. Pathol. **110:** 129-31.

Awan, M.B., Maqbool, A., Bari, A., Krovacek, K. 2009. Antibiotic susceptibility profile of Aeromonas spp. isolates from food in Abu Dhabi, United Arab Emirates. New Microbiol. **32:** 17-23.

Bai, D.L. 1993. Traditional Chinese medicines and new drug development. Pure Appl. Chem. **65:** 1103-1112.

Barile, E., Bonanomi, G., Antignani, V., Zolfaghari, B., Sajjadi, S.E., Scala, F., Lanzotti, V. 2007. Saponins from Allium minutiflorum with antifungal activity. Phytochem. **68:** 596-603.

Barnes, J., Anderson, L.A., Phillipson, J.D. 2001. St John's wort (*Hypericum perforatum* L.): a review of its chemistry, pharmacology and clinical properties. J. Pharm. Pharmacol. **53:** 583-600.

Bekesiova, I., Nap, J.P., Mlynarova, L. 1999. Isolation of high quality DNA and RNA from leaves of the carnivorous plant *Drosera rotundifolia*. Plant Mol. Biol. Rep. **17:** 269-277.

Benkeblia, N. 2004. Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). Lebensm. Wiss. Technol. **37:** 263-268.

Benthin, B., Danz, H., Hamburger, M. 1999. Pressurized liquid extraction of medicinal plants. J. Chromatogr. **837:** 211-219.

Betoni, J.E., Mantovani, R.P., Barbosa, L.N., Di Stasi, L.C., Fernandes Junior, A. 2006. Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases. Mem. Inst. Oswaldo Cruz. **101:** 387-390.

Borchardt, J.R., Wyse, D.L., Sheaffer, C.C., Kauppi, K.L., Fulcher, R.G., Ehlke, N.J., Biesboer, D.D., Bey, R.F. 2008. Antimicrobial activity of native and naturalized plants of Minnesota and Wisconsin. J. Med. Plant Res. **2:** 98-110.

Brock, A., Herzfeld, T., Paschke, R., Koch, M., Dräger, B. 2006. *Brassicaceae* contain nortropane alkaloids. Phytochem. **67:** 2050-2057.

Bryan, M, Bryan, S, McClymont, T, North, E1993. Checklist of Vascular plants of Thunder Bay District. 1 ed. Thunder Bay Field Naturalist, Thunder Bay, ON. 58pp.

Bryant, J.P., Reichardt, P.B., Clausen, T.P. 1992. Chemically mediated interactions between woody plants and browsing mammals. J. Range Manage. 18-24.

Buchsbaum, R., Valiela, I., Swain, T. 1984. The role of phenolic compounds and other plant constituents in feeding by Canada geese in a coastal marsh. Oecologia. **63:** 343-349.

Burden, R.S., Kemp, M.S. 1984. Sesquiterpene phytoalexins from *Ulmus glabra*. Phytochem. **23:** 383-385.

Bussmann, R.W., Sharon, D., Perez, F., Díaz, D., Ford, T., Rasheed, T., Silva, Y.B.R. 2008. Antibacterial activity of northern-peruvian medicinal plants. Arnaldoa. **15:** 127-148.

Borneo, R., León, A.E., Aguirre, A., Ribotta, P., Cantero, J.J. 2009. Antioxidant capacity of medicinal plants from the Province of Córdoba (Argentina) and their *in vitro* testing in a model food system. Food. Chem. **112:** 664-670.

Borchardt, J.R., Wyse, D.L., Sheaffer, C.C., Kauppi, K.L., Fulcher, R.G., Ehlke, N.J., Biesboer, D.D., Bey, R.F. 2008b. Antioxidant and antimicrobial activity of seed from plants of the Mississippi river basin. J. Med. Plant Res. **2:** 81-93.

Bors, W., Michel, C. 2002. Chemistry of the antioxidant effect of polyphenols. Ann. NY. Acad. Sci. **957:** 57-69.

Brand-Williams, W., Cuvelier, M., Berset, C. 1995. Use of free radical method to evaluate antioxidant activity. Lebensmittel Wissenschaft und Technologie. **28:** 25-30.

Buenger, J., Ackermann, H., Jentzsch, A., Mehling, A., Pfitzner, I., Reiffen, K.A., Schroeder, K.R., Wollenweber, U. 2006. An interlaboratory comparison of methods used to assess antioxidant potentials. Int. J. Cosmet Sci. **28:** 135-46.

Butler, M.S. 2008. Natural products to drugs: natural product-derived compounds in clinical trials. Nat. Prod. Rep. **25:** 475-516.

Buwa, L.V., van Staden, J. 2006. Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa. J. Ethnopharmacol. **103:** 139-42.

Cantos, E., Espín, J.C., López-Bote, C., de la Hoz, L., Ordóñez, J.A., Tomás-Barberán, F.A. 2003. Phenolic compounds and fatty acids from acorns (*Quercus* spp.), the main dietary constituent of free-ranged Iberian pigs. J. Agric. Food Chem. **51:** 6248-6255.

Carlson, H.J., Douglas, H.G., Robertson, J. 1948. Antibacterial Substances Separated from Plants. J. Bacteriol. **55:** 241-248.

Carpenter, D.J. 2011. St. John's wort and S-adenosyl methionine as "natural" alternatives to conventional antidepressants in the era of the suicidality boxed warning: what is the evidence for clinically relevant benefit? Altern. Med. Rev. **16:** 17-39.

Cai, Y.Z., Xing, J., Sun, M., Zhan, Z.Q., Corke, H. 2005. Phenolic antioxidants (hydrolyzable tannins, flavonols, and anthocyanins) identified by LC-ESI-MS and MALDI-QIT-TOF MS from Rosa chinensis flowers. J. Agric. Food Chem. **53:** 9940-8.

Calderón, A.I., Simithy-Williams, J., Sanchez, R., Espinosa, A., Valdespino, I., Gupta, M.P. 2012. Lycopodiaceae from Panama: A new source of acetylcholinesterase inhibitors. Nat. Prod. Res. **27**: 500-505

Candan, F., Unlu, M., Tepe, B., Daferera, D., Polissiou, M., Sökmen, A., Akpulat, H.A. 2003. Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. *millefolium* Afan. (Asteraceae). J. Ethnopharmacol. **87:** 215-20.

Cerdeiras, M.P., Albores, S., Etcheverry, S., Lucian, V., Soubes, M., Vazquez, A. 2007. Antimicrobial Activity of Xanthium cavanillesii Extracts. Pharm. Biol. **45:** 251-254.

Cha, Y.Y., Lee, E.O., Lee, H.J., Park, Y.D., Ko, S.G., Kim, D.H., Kim, H.M., Kang, I.C., Kim, S.H. 2004. Methylene chloride fraction of Scutellaria barbata induces apoptosis in human U937 leukemia cells via the mitochondrial signaling pathway. Clin. Chim. Acta. **348:** 41-48.

Chan, K. 2003. Some aspects of toxic contaminants in herbal medicines. Chemosphere. **52:** 1361-1371.

Chevallier, A. 1996. The encyclopedia of Medicinal Plants. 1 ed. Dorling Kindersley, Bedford, NS. 336pp.

Choo, C.Y., Hirasawa, Y., Karimata, C., Koyama, K., Sekiguchi, M., Kobayashi, J., Morita, H. 2007. Carinatumins A-C, new alkaloids from *Lycopodium carinatum* inhibiting acetylcholinesterase. Bioorg. Med. Chem. **15:** 1703-1707.

Coe, F.G., Anderson, G.J. 1996. Screening of medicinal plants used by the Garífuna of eastern Nicaragua for bioactive compounds. J. Ethnopharmacol. **53:** 29-50.

Copp, B.R. 2003. Antimycobacterial natural products. Nat. Product Rep. **20:** 535-557.

Cutler, H.G. 1985. Secondary metabolites from plants and their allelochemic effects Bioregulators for Pest Control. American Chemical Society, Washington, DC. 455-468.

Çırak, C., Raduvsiene, J., Janulis, V., Ivanauskas, L. 2007. Secondary metabolites in *Hypericum perfoliatum*: variation among plant parts and phenological stages. Bot. Helv. **117:** 29-36.

Cen, Y.P., Bornman, J.F. 2006. The effect of exposure to enhanced UV-B radiation on the penetration of monochromatic and polychromatic UV-B radiation in leaves of *Brassica napus*. Physiologia. Plantarum. **87:** 249-255.

Cheng, Z., Moore, J., Yu, L. 2006. High-throughput relative DPPH radical scavenging capacity assay. J. Agric. Food Chem. **54:** 7429-36.

Chosson, E., Chaboud, A., Chulia, A.J., Raynaud, J. 1998. Dihydroflavonol glycosides from *Rhododendron ferrugineum*. Phytochem. **49:** 1431-1433.

Colombia, D.C. 2010. Composition and antibacterial activity of essential oils obtained from plants of the Lamiaceae family against pathogenic and beneficial bacteria. Rev. Colom. Cienc. Pecua. **23:** 451-461

Dall'Agnol, R., Ferraz, A., Bernardi, A.P., Albring, D., Nör, C., Sarmento, L., Lamb, L., Hass, M., von Poser, G., Schapoval, E.E.S. 2003. Antimicrobial activity of some *Hypericum* species. Phytomed. **10:** 511-516.

Daniel, M. 2006. Medicinal plants: chemistry and properties. Science Pub Inc, Enfield, NH. 266pp.

Datta, B.K., Datta, S.K., Rashid, M.A., Nash, R.J., Sarker, S.D. 2000. A sesquiterpene acid and flavonoids from Polygonum viscosum. Phytochem. **54:** 201-205.

Dorman, H.J.D., Deans, S.G. 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J. Appl. Microbiol. **88:** 308-316.

Dreikorn, K. 2000. Phytotherapeutic agents in the treatment of benign prostatic hyperplasia. Curr. Urol. Rep. **1:** 103-109.

Duinker, P., Matakala, P., Zhang 1991. Community forestry and its implications for Northern Ontario. Forestry Chron. **67:** 131-135.

Daud, A., Gallo, A., Riera, A.S. 2005. Antimicrobial properties of *Phrygilanthus acutifolius*. J. Ethnopharmacol. **99:** 193-7.

Dietrich, M., Block, G., Hudes, M., Morrow, J.D., Norkus, E.P., Traber, M.G., Cross, C.E., Packer, L. 2002. Antioxidant supplementation decreases lipid peroxidation biomarker F(2)-isoprostanes in plasma of smokers. Cancer Epidemiol. Biomarkers Prev. **11:** 7-13.

Donaldson, J.R., Warner, S.L., Cates, R.G., Gary Young, D. 2005. Assessment of antimicrobial activity of fourteen essential oils when using dilution and diffusion methods Pharm. Bio. **43:** 687-695.

Dillard, C.J., German, J.B. 2000. Phytochemicals: nutraceuticals and human health J. Sci. Food Agric. **80:** 1744-1756.

Dudonne, S., Vitrac, X., Coutie re, P., Woillez, M., Me rillon, J.M. 2009. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. J. Agric. Food Chem. **57:** 1768-1774.

Dufour, D., Pichette, A., Mshvildadze, V., Bradette-Hébert, M.E., Lavoie, S., Longtin, A., Laprise, C., Legault, J. 2007. Antioxidant, anti-inflammatory and anticancer activities of methanolic extracts from *Ledum groenlandicum* Retzius. J. Ethnopharmacol. **111:** 22-8.

Edilmesi, F.B.T. 2002. Induction of phytoalexin accumulation in broad bean (*Vicia faba* L.) cotyledons following treatments with biotic and abiotic elicitors. Turk. J. Agric. For. **26:** 343-348.

Etkin, N.L. 2008. An ethnobotany of Darwin's gardens. Ethnobotany Res. Appl. **6:** 325-334.

Eldeen, I.M., Elgorashi, E.E., van Staden, J. 2005. Antibacterial, anti-inflammatory, anti-cholinesterase and mutagenic effects of extracts obtained from some trees used in South African traditional medicine. J. Ethnopharmacol. **102:** 457-64.

Fajer, E.D., Bowers, M.D., Bazzaz, F.A. 1992. The effect of nutrients and enriched CO_2 environments on production of carbon-based allelochemicals in Plantago: a test of the carbon/nutrient balance hypothesis. Am. Nat. **140:** 707-723.

Fant, F., Vranken, W.F., Borremans, F.A. 1999. The three-dimensional solution structure of Aesculus hippocastanum antimicrobial protein 1 determined by 1H nuclear magnetic resonance. Proteins. **37:** 388-403.

Farnsworth, NR, Morris, RW 1976. Higher plants- the sleeping giant of drug discovery. Am. J. Pharmacy. **148:** 46-52.

Feresin, G.E., Tapia, A., Sortino, M., Zacchino, S., de Arias, A.R., Inchausti, A., Yaluff, G., Rodriguez, J., Theoduloz, C., Schmeda-Hirschmann, G. 2003. Bioactive alkyl phenols and embelin from *Oxalis erythrorhiza*. J. Ethnopharmacol. **88:** 241-247.

Foti, M.C., Daquino, C., Geraci, C. 2004. Electron-transfer reaction of cinnamic acids and their methyl esters with the DPPH* radical in alcoholic solutions. J. Org. Chem. **69:** 2309-14.

Fernández-Pan, I., Royo, M., Ignacio Maté, J. 2012. Antimicrobial activity of whey protein isolate edible films with essential oils against food spoilers and foodborne pathogens. J. Food Sci. **77:** M383-90.

Fylaktakidou, K.C., Hadjipavlou-Litina, D.J., Litinas, K.E., Nicolaides, D.N. 2004. Natural and synthetic coumarin derivatives with anti-inflammatory/ antioxidant activities. Curr. Pharm. Des. **10:** 3813-33.

Fraser, M.H., Cuerrier, A., Haddad, P.S., Arnason, J.T., Owen, P.L., Johns, T. 2007. Medicinal plants of Cree communities (Quebec, Canada): antioxidant activity of plants used to treat type 2 diabetes symptoms. Can. J. Physiol. Pharmacol. **85:** 1200-1214.

Galván, I.J., Mir-Rashed, N., Jessulat, M., Atanya, M., Golshani, A., Durst, T., Petit, P., Amiguet, V.T., Boekhout, T., Summerbell, R., Cruz, I., Arnason, J.T., Smith, M.L. 2008. Antifungal and antioxidant activities of the phytomedicine pipsissewa, *Chimaphila umbellata*. Phytochem. **69:** 738-746.

Gomiero, T., Pimentel, D., Paoletti, M.G. 2011. Is There a Need for a More Sustainable Agriculture? Crit. Rev. Plant Sci. **30:** 6-23.

Gottshall, R.Y., Lucas, E.H. 1949. The occurrence of antibacterial substances active against Mycobacterium tuberculosis in seed plants. J. Clin. Invest. **28:** 920-923.

Gupta, R.K., Möller, H.J. 2003. St. John's Wort. An option for the primary care treatment of depressive patients? Eur. Arch. Psychiatry Clin. Neurosci. **253:** 140-148.

Gurevitch, J., Scheiner, S.M., Fox, G.A. 2002. The ecology of plants. Sinauer Associates, Sunderland, MA. 523pp.

Güllüce, M., Adigüzel, A., Öugütçü, H., Sengül, M., Karaman, I., Sahin, F. 2004. Antimicrobial effects of Quercus ilex L. extract. Phytother. Res. **18:** 208-211.

Ghasemi, K., Ghasemi, Y., Ebrahimzadeh, M.A. 2009. Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. Pak. J. Pharm. Sci. **22:** 277-81.

Griffin, S.G., Markham, J.L., Leach, D.N. 2000. An agar dilution method for the determination of the minimum inhibitory concentration of essential oils. J. Essential Oil Res. **12:** 249-255.

Guilbert, S., Gontard, N., Gorris, L.G.M. 1996. Prolongation of the shelf-life of perishable food products using biodegradable films and coatings. Food Sci. Tech. **29:** 10-17.

Guo, X.D., Ma, Y.J., Parry, J., Gao, J.M., Yu, L.L., Wang, M. 2011. Phenolics content and antioxidant activity of tartary buckwheat from different locations. Molecules. **16:** 9850-67

Hadacek, F., Greger, H. 2000. Testing of antifungal natural products: methodologies, comparability of results and assay choice. Phytochem. Anal. **11:** 137-147.

Hagen, R.D. 1989. Isolation and identification of 5-hydroxy-indore-3-acetic acid and 5-hydroxy tryp- tophan, major allelopathic aglycons in quackgrass (Agropyronrepens). J. Agri. food Chem. **37:** 1143-1149.

Hazlett, D.L., Sawyer, N.W. 1998. Distribution of Alkaloid-Rich Plant Species in Shortgrass Steppe Vegetation. Conserv. Biol. **12:** 1260-1268.

Hälvä, S., Craker, L.E. 1996. Manual for Northern herb growers. HSMP Press, Lexington, MA. 110pp.

Heatherly, A.N. 1998. Healing plants: a medicinal guide to native North American plants and herbs. 1 ed. Lyons Press, Toronto, ON. 288pp.

Hemmati, S., Schneider, B., Schmidt, T.J., Federolf, K., Alfermann, A.W., Fuss, E. 2007. Justicidin B 7-hydroxylase, a cytochrome P450 monooxygenase from cell cultures of *Linum perenne* Himmelszelt involved in the biosynthesis of diphyllin. Phytochem. **68:** 2736-2743.

Hoffmann, J.J., Timmermann, B.N., McLaughlin, S.P., Punnapayak, H. 1993. Potential antimicrobial activity of plants from the southwestern United States. Pharm. Biol. **31:** 101-115.

Hostanska, K., Reichling, J., Bommer, S., Weber, M., Saller, R. 2003. Hyperforin a constituent of St John's wort (*Hypericum perforatum* L.) extract induces apoptosis by

triggering activation of caspases and with hypericin synergistically exerts cytotoxicity towards human malignant cell lines. Eur. J. Pharm. Biopharm. **56:** 121-132.

H**uang, W.H., Zhang, Q.W., Meng, L.Z., Yuan, C.S., Wang, C.Z., Li, S.P. 2011.** Oplopanphesides A-C, three new phenolic glycosides from the root barks of *Oplopanax horridus*. Chem. Pharm. Bull. (Tokyo). **59:** 676-679.

H**udec, J., Burdová, M., Kobida, L., Komora, L., Macho, V., Kogan, G., Turianica, I., Kochanová, R., Lozek, O., Habán, M., Chlebo, P. 2007.** Antioxidant capacity changes and phenolic profile of Echinacea purpurea, nettle (Urtica dioica L.), and dandelion (Taraxacum officinale) after application of polyamine and phenolic biosynthesis regulators. J. Agric. Food Chem. **55:** 5689-5696.

H**arvey, A.L. 2008.** Natural products in drug discovery. Drug Disc. today. **13:** 894-901.

H**ertog, M.G., Feskens, E.J., Hollman, P.C., Katan, M.B., Kromhout, D. 1993.** Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet. **342:** 1007-1011.

H**uang, D., Ou, B., Prior, R.L. 2005.** The chemistry behind antioxidant capacity assays. J. Agric. Food Chem. **53:** 1841-56.

I**brahim, M.A., Kainulainen, P., Aflatuni, A., Tiilikkala, K., Holopainen, J.K. 2001.** Insecticidal, repellent, antimicrobial activity and phytotoxicity of essential oils: With special reference to limonene and its suitability for control of insect pests (Review). Agric. Food Sci. Finland. **10:** 243-260.

I**saeva, E.V., Lozhkina, G.A., Ryazanova, T.V. 2010.** A study of the alcohol extract from balsam poplar buds. Russ. J. Bioorganic Chem. **36:** 929-933.

I**shimoto, H., Fukushi, Y., Yoshida, T., Tahara, S. 2000.** Rhizopus and Fusarium are selected as dominant fungal genera in rhizospheres of *Brassicaceae*. J. Chem. Ecol. **26:** 2387-2399.

I**washina, T., Kitajima, J. 2000.** Chalcone and flavonol glycosides from *Asarum canadense* (*Aristolochiaceae*). Phytochemistry. **55:** 971-974.

I**mazato, S., Ebi, N., Tarumi, H., Russell, R.R., Kaneko, T., Ebisu, S. 1999.** Bactericidal activity and cytotoxicity of antibacterial monomer MDPB. Biomaterials. **20:** 899-903.

Jastrzebski, Z., Medina, O.J., Marlen Moreno, L., Gorinstein, S. 2007. In vitro studies of polyphenol compounds, total antioxidant capacity and other dietary indices in a mixture of plants (Prolipid). Int. J. Food Sci. Nutri. **58:** 531-541.

Javanmardi, J., Stushnoff, C., Locke, E., Vivanco, J.M. 2003. Antioxidant activity and total phenolic content of Iranian *Ocimum accessions*. Food chem. **83:** 547-550.

Jahodár, L., Jílek, P., Páktová, M., Dvoráková, V. 1985. Antimicrobial effect of arbutin and an extract of the leaves of *Arctostaphylos uva-ursi* in vitro. Cesk. Farm. **34:** 174-8.

Jawad, A.L., Mahmoud, M.J., Al-Naib, A. 1988. Antimicrobial activity of *xanthium strumarium* extract. Fitoterapia. **59:** 220-221.

Jelager, L., Gurib-Fakim, A., Adsersen, A. 1998. Antibacterial and antifungal activity of medicinal plants of Mauritius. Pharm. Biol. **36:** 153-161.

Jemal, A., Bray, F., Center, M.M., Ferlay, J., Ward, E., Forman, D. 2011. Global cancer statistics. CA. Cancer J. Clin. **61:** 69-90.

Jing-Yan, W., Zhao-Pu, L.I.U. 2010. Alkaloid Accumulation in Catharanthus roseus Increases with Addition of Seawater Salts to the Nutrient Solution. Pedosphere. **20:** 718-724.

Jirovský, D., Kosina, P., Myslínová, M., Stýskala, J., Ulrichová, J., Simánek, V. 2007. HPLC analysis of rosmarinic acid in feed enriched with aerial parts of *Prunella vulgaris* and its metabolites in pig plasma using dual-channel coulometric detection. J. Agric. Food. Chem. **55:** 7631-7637.

Jones, N.P., Arnason, J.T., Abou-Zaid, M., Akpagana, K., Sanchez-Vindas, P., Smith, M.L. 2000. Antifungal activity of extracts from medicinal plants used by First Nations Peoples of eastern Canada. J. Ethnopharmacol. **73:** 191-198.

Jøhnk, N., Hietala, A.M., Fossdal, C.G., Collinge, D.B., Newman, M.A. 2005. Defense-related genes expressed in Norway spruce roots after infection with the root rot pathogen Ceratobasidium bicorne (anamorph: Rhizoctonia sp.). Tree Physiol. **25:** 1533-1543.

Juteau, F., Masotti, V., Bessière, J.M., Dherbomez, M., Viano, J. 2002. Antibacterial and antioxidant activities of *Artemisia annua* essential oil. Fitoterapia. **73:** 532-5.

Kapusta, I., Janda, B., Szajwaj, B., Stochmal, A., Piacente, S., Pizza, C., Franceschi, F., Franz, C., Oleszek, W. 2007. Flavonoids in horse chestnut (*Aesculus hippocastanum*) seeds and powdered waste water byproducts. J. Agric. Food. Chem. **55:** 8485-8490.

Keen, R.W., Deacon, A.C., Delves, H.T., Moreton, J.A., Frost, P.G. 1994. Indian herbal remedies for diabetes as a cause of lead poisoning. Postgrad. Med. J. **70:** 113-114.

De Keukeleire, J., Ooms, G., Heyerick, A., Roldan-Ruiz, I., Van Bockstaele, E., De Keukeleire, D. 2003. Formation and accumulation of alpha-acids, beta-acids, desmethylxanthohumol, and xanthohumol during flowering of hops (*Humulus lupulus* L.). J. Agric. Food Chem. **51:** 4436-4441.

Kobaisy, M., Abramowski, Z., Lermer, L., Saxena, G., Hancock, R.E., Towers, G.H., Doxsee, D., Stokes, R.W. 1997. Antimycobacterial polyynes of Devil's Club (*Oplopanax horridus*), a North American native medicinal plant. J. Nat. Prod. **60:** 1210-1213.

Krishnaiah, D., Sarbatly, R., Bono, A. 2007. Phytochemical antioxidants for health and medicine--A move towards nature Biotechnol. Mol. Biol. Rev. **1:** 97-104.

Kubota T., Yahata H., Yamamoto S., Hayashi S., Shibata T., Kobayashi J. 2009. Serratezomines D and E, new Lycopodium alkaloids from *Lycopodiumserratum* var. *serratum*. Bioorg. Med. Chem. Lett. **19:** 3577-3580.

Kumagai, T., Müller, C.I., Desmond, J.C., Imai, Y., Heber, D., Koeffler, H.P. 2007. Scutellaria baicalensis, a herbal medicine: anti-proliferative and apoptotic activity against acute lymphocytic leukemia, lymphoma and myeloma cell lines. Leuk. Res. **31:** 523-530.

Kumar, P., Bhatt, R.P., Singh, L. 2010. Identification of phytochemical content and antibacterial activity of *Juniperus communis* leaves. Int. J. Biotechnol. Biochem. **6:** 87-91.

Kumari, R., Agrawal, S.B., Singh, S., Dubey, N.K. 2009. Supplemental ultraviolet-B induced changes in essential oil composition and total phenolics of *Acorus calamus* L. (sweet flag). Ecotoxicol. Environ. Saf. **72:** 2013-2019.

Kahl, R., Kappus, H. 1993. Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E. Z Lebensm Unters Forsch. **196:** 329-38.

Kaur, C., Kapoor, H.C. 2008. Antioxidants in fruits and vegetables--the millennium's health. Int. J. Food Sci. Tech. **36:** 703-725.

Kähkönen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.P., Pihlaja, K., Kujala, T.S., Heinonen, M. 1999. Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem. **47:** 3954-3962.

Kohl, M.W., Keller, F., Williams, R.E., Toekes, M.I., Cronheim, G.E. 1959. A chemical and pharmacological investigation of *Piper methysticum* Forst. J. Med. Pharm. Chem. **1:** 95-103.

Kontogianni, V.G., Gerothanassis, I.P. 2012. Phenolic compounds and antioxidant activity of olive leaf extracts. Nat. Prod. Res. **26:** 186-9.

Ko, W.C., Yu, K.W., Liu, C.Y., Huang, C.T., Leu, H.S., Chuang, Y.C. 1996. Increasing antibiotic resistance in clinical isolates of *Aeromonas* strains in Taiwan. Antimicrob. Agents Chemother. **40:** 1260-2.

Konrath, E.L., Neves, B.M., Lunardi, P.S., Passos, C.d.o.s. .S., Simões-Pires, A., Ortega, M.G., Gonçalves, C.A., Cabrera, J.L., Moreira, J.C., Henriques, A.T. 2012. Investigation of the in vitro and ex vivo acetylcholinesterase and antioxidant activities of traditionally used Lycopodium species from South America on alkaloid extracts. J. Ethnopharmacol. **139:** 58-67.

Kruszewska, H., Zareba, T., Tyski, S. 2004. Examination of antimicrobial activity of selected non-antibiotic drugs. Acta. Pol. Pharm. **61 Suppl:** 18-21.

Kudi, A.C., Umoh, J.U., Eduvie, L.O., Gefu, J. 1999. Screening of some Nigerian medicinal plants for antibacterial activity. J. Ethnopharmacol. **67:** 225-8.

Lam, K.S. 2007. New aspects of natural products in drug discovery. Trends Microbiol. **15:** 279-289.

Large, R., Brocklesby, M., Brocklesby, H. 1938. A hypoglycemic substance from the roots of Devil's Club (*Fatsia horrida*). Can. Med. Assoc. J. **39:** 32-35.

De La Rosa, T.M., Julkunen-Tiitto, R., Lehto, T., Aphalo, P.J. 2001. Secondary metabolites and nutrient concentrations in silver birch seedlings under five levels of daily UV-B exposure and two relative nutrient addition rates. New Phytologist. **150:** 121-131.

Lee, H.Y., Mun, T.K., Lim, C.J., Kim, C.K., Sung, T.K. 1992. Antibacterial activity of *Ulmus pumila* L. extract. Kor. J. Appl. Microbiol. Biotechnol. **20:** 1-5

Li, T.S.C. 2000. Medicinal plants. Technomic Pub. Co., Lancaster, Pa. 517pp.

Loew, D., Kaszkin, M. 2002. Approaching the problem of bioequivalence of herbal medicinal products. Phytother. Res. **16:** 705-711.

Lopez, A.D. 2006. Global burden of disease and risk factors. Oxford University Press, New York, NY. 475pp.

Lou, H., Yuan, H., Ma, B., Ren, D., Ji, M., Oka, S. 2004. Polyphenols from peanut skins and their free radical-scavenging effects. Phytochem. **65:** 2391-2399.

Luczkiewcz, M., Cisowski, W. 2001. Optimisation of the second phase of a two phase growth system for anthocyanin accumulation in callus cultures of *Rudbeckia hirta*. Plant cell Tiss. Org. Cult. **65:** 57-68.

Luczkiewicz, M., Zárate, R., Dembińska-Migas, W., Migas, P., Verpoorte, R. 2002. Production of pulchelin E in hairy roots, callus and suspension cultures of *Rudbeckia hirta* L. Plant Sci. **163:** 91-100.

Lee, S.J., Sung, N.J., Jeong, H.G., Shin, J.H., Chung, Y.C., Seo, J.K. 2008. Antioxidant activities of methanol extracts from Prunella vulgaris. J. Kor. Soc. Food Sci. Nutr. **37:** 1535-1541

Li, C., Tang, Z., Huang, M., Tao, N., Feng, B., Huang, S. 2012. Antioxidant efficacy of extracts produced from pickled and dried mustard in rapeseed and peanut oils. J. Food Sci. **77:** 394-400

Liu, F., Ng, T.B. 2000. Antioxidative and free radical scavenging activities of selected medicinal herbs. Life Sci. **66:** 725-35.

Liu, W., Morrow, J.D., Yin, H. 2009. Quantification of F2-isoprostanes as a reliable index of oxidative stress in vivo using gas chromatography-mass spectrometry (GC-MS) method. Free Radica. Biol. Med. **47:** 1101.

Li, H.B., Wong, C.C., Cheng, K.W., Chen, F. 2008. Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. Food Sci. Tech. **41:** 385-390.

Lu, T., Menelaou, M.A., Vargas, D., Fronczek, F.R., Fischer, N.H. 1993. Polyacetylenes and diterpenes from *Solidago canadensis*. Phytochem. **32:** 1483-1488.

MacKinnon, A. 2009. Edible and Medicinal Plants of Canada. Lone Pine Pub, Edmonton, AB. 448p.

Mathes, M.C. 1963. Antimicrobial substances from aspen tissue grown in vitro. Science. **140:** 1101-1102.

Matovic, M., Mihajlov, M., Comic, L., Curcic, S. 1996. Antimicrobe activity of certain plant species with a special emphasis on the effects of biochemical products of juniper (*Juniperus communis* L.) on micro-organisms. Zbornik. Radova. **45:** 11-15.

Mattes, B.R., Clausen, T.P., Reichardt, P.B. 1987. Volatile constituents of balsam poplar: the phenol glycoside connection. Phytochem. **26:** 1361-1366.

McCutcheon, A.R., Roberts, T.E., Gibbons, E., Ellis, S.M., Babiuk, L.A., Hancock, R.E., Towers, G.H. 1995. Antiviral screening of British Columbian medicinal plants. J. Ethnopharmacol. **49:** 101-110.

Merhi, F., Tang, R., Piedfer, M., Mathieu, J., Bombarda, I., Zaher, M., Kolb, J.P., Billard, C., Bauvois, B. 2011. Hyperforin inhibits akt1 kinase activity and promotes caspase-mediated apoptosis involving bad and noxa activation in human myeloid tumor cells. PLoS One. **6:** e25963.

Michalska, K., Kisiel, W. 2003. Sesquiterpene lactones from *Taraxacum obovatum*. Planta Med. **69:** 181-183.

Mishra, S., Tyagi, A., Singh, I.V., Sangwan, R.S. 2006. Changes in lipid profile during growth and senescence of Catharanthus roseus leaf Brazilian. J. Plant Physiol. **18:** 447-454.

Mohagheghzadeh, A., Faridi, P., Shams-Ardakani, M., Ghasemi, Y. 2006. Medicinal smokes. J. Ethnopharmacol. **108:** 161-184.

Moore, P. 1980. The advantages of being evergreen Nature. Trends Ecol. Evol. **285:** 535-535

Morita, H, Ishiuchi K, Haganuma A, Hoshino T, Obara Y, Nakahata N 2005. Complanadine B, obscurumines A and B, new alkaloids from two species of *Lycopodium*. Tetrahedron. **61:** 1955-1960.

Mothana, R.A., Kriegisch, S., Harms, M., Wende, K., Lindequist, U. 2011. Assessment of selected Yemeni medicinal plants for their in vitro antimicrobial, anticancer, and antioxidant activities. Pharm. Biol. **49:** 200-10

Mowrey, D.B. 1990. Next generation herbal medicine: guaranteed potency herbs. Keats Pub. Inc., New Canaan, Conn. 157pp.

Mulabagal, V., Tsay, H.S. 2004. Plant cell cultures-an alternative and efficient source for the production of biologically important secondary metabolites. Int. J. Appl. Sci. Eng. **2:** 29-48.

Murch, S.J., Haq, K., Rupasinghe, H.P., Saxena, P.K. 2003. Nickel contamination affects growth and secondary metabolite composition of St. John's wort (*Hypericum perforatum L.).* Environ. Exp. Bot. **49:** 251-257.

Murthy, P.G., Mamtharani, D.R., Tejas, T.S and Niranjan M. Surlikerimath 2011. Phytochemical analysis, in vitro anti-bacterial and antioxidant activities of wild onions sps. Int. J. Pharm. B. Sci. **2:** 230-237.

Müller, W.E., Rolli, M., Schäfer, C., Hafner, U. 1997. Effects of hypericum extract (LI 160) in biochemical models of antidepressant activity. Pharmacopsychiatry. **30 (2):** 102-107.

Mann, C.M., Markham, J.L. 1998. A new method for determining the minimum inhibitory concentration of essential oils. J. Appl. Microbiol. **84:** 538-44.

Mathabe, M.C., Nikolova, R.V., Lall, N., Nyazema, N.Z. 2006. Antibacterial activities of medicinal plants used for the treatment of diarrhoea in Limpopo Province, South Africa. J. Ethnopharmacol. **105:** 286-93.

Maisuthisakul, P., Pasuk, S., Ritthiruangdej, P. 2008. Relationship between antioxidant properties and chemical composition of some Thai plants. J. Food Comp. Anal. **21:** 229-240.

Markesbery, W.R. 1997. Oxidative stress hypothesis in Alzheimer's disease. Free radical Biol. Med. **23:** 134.

Mensor, L.L., Menezes, F.S., Leitão, G.G., Reis, A.S., Santos, T.C., Coube, C.S., Leitão, S.G. 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res. **15:** 127-130.

Miliauskas, G., Venskutonis, P.R., Van Beek, T.A. 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food chem. **85:** 231-237.

Miceli, N., Trovato, A., Dugo, P., Cacciola, F., Donato, P., Marino, A., Bellinghieri, V., La Barbera, T.M., Güvenç, A., Taviano, M.F. 2009. Comparative analysis of flavonoid profile, antioxidant and antimicrobial activity of the berries of Juniperus communis L. var. communis and *Juniperus communis* L. var. saxatilis Pall. from Turkey. J. Agric. Food Chem. **57:** 6570-7.

Milne, G.L., Sanchez, S.C., Musiek, E.S., Morrow, J.D. 2007. Quantification of F2-isoprostanes as a biomarker of oxidative stress. Nature Protocols. **2:** 221-226.

Montuschi, P., Barnes, P., Roberts, L.J. 2007. Insights into oxidative stress: the isoprostanes. Curr. Med. Chem. **14:** 703-17.

Nagai T., Myoda T., Nagashima T. 2005. Antioxidative activities of water extract and ethanol extract from field horsetail (tsukushi) *Equisetum arvense* L. Food Chem. **91:** 389-394.

Newman, D.J., Cragg, G.M., Snader, K.M. 2003. Natural products as sources of new drugs over the period 1981-2002. J. Nat. Prod. **66:** 1022-1037.

Newman, D.J., Cragg, G.M. 2007. Natural products as sources of new drugs over the last 25 years. J. Nat. Prod. **70:** 461-477.

Nonaka, G.I., Miwa, N., Nishioka, I. 1982. Stilbene glycoside gallates and proanthocyanidins from *Polygonum multiflorum*. Phytochem. **21:** 429-432.

Nam, B. 2006. Extracts and Essential Oil of Ledum palustre L. Leaves and Their Antioxidant and Antimicrobial Activities. J. Food Sci. Nutr. **11:** 100-104.

Newton, S.M., Lau, C., Gurcha, S.S., Besra, G.S., Wright, C.W. 2002. The evaluation of forty-three plant species for in vitro antimycobacterial activities; isolation of active constituents from *Psoralea corylifolia* and *Sanguinaria canadensis*. J. Ethnopharmacol. **79:** 57-67.

Niki, E. 2010. Assessment of antioxidant capacity in vitro and in vivo. Free Radical Biol. Med. **49:** 503-15.

Oerke, E.C., Dehne, H.W., Schonbeck, F., Weber, A. 1994. Crop Protection and Crop Production. 3rd ed. Amsterdam, NH. 808pp.

Orhan, Ozcelik, B., Aslan, S., Kartal, M., Karaoglu, T., Sener, B., Terzioglu, S., Choudhary, M. 2007. Antioxidant and antimicrobial actions of the clubmoss *Lycopodium clavatum* L. Phytochem. Rev. **6:** 189-196.

De Oliveira, A.C., Valentim, I.B., Silva, C.A., Bechara, E.J.H., de Barros, M.P., Mano, C.M., Goulart, M.O.F. 2009. Total phenolic content and free radical scavenging activities of methanolic extract powders of tropical fruit residues. Food Chem. **115:** 469-475.

Oomah, B.D., Blanchard, C., Balasubramanian, P. 2008. Phytic acid, phytase, minerals, and antioxidant activity in Canadian dry bean (*Phaseolus vulgaris* L.) cultivars. J. Agric. Food Chem. **56:** 11312-9.

Orzechowski, A., Ostaszewski, P., Jank, M., Berwid, S.J. 2002. Bioactive substances of plant origin in food--impact on genomics. Reprod. Nutr. Dev. **42:** 461-77.

Olano, I., Paz, E.A., Cerdeíras, M.P., Fernández, J., Ferreira, F., Moyna, P., Soubes, M., Vazquez, A., Vero, S., Bassagoda, M.J. 1996. Screening of Uruguayan medicinal plants for antimicrobial activity. Part II. J. Ethnopharmacol. **53:** 111-5.

Ostrakhovitch, E.A., Afanas'ev, I.B. 2001. Oxidative stress in rheumatoid arthritis leukocytes: suppression by rutin and other antioxidants and chelators. Biochem. Pharmacol. **62:** 743-6.

Ou, B., Huang, D., Hampsch-Woodill, M., Flanagan, J.A., Deemer, E.K. 2002. Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: a comparative study. J. Agric. Food. Chem. **50:** 3122-3128.

Ou, B., Bosak, K.N., Brickner, P.R., Iezzoni, D.G., Seymour, E.M. 2012. Processed Tart Cherry Products-Comparative Phytochemical Content, in vitro Antioxidant Capacity and in vitro Anti-inflammatory Activity. J. Food Sci. **77:** H105-12.

Parajuli, P., Joshee, N., Rimando, A.M., Mittal, S., Yadav, A.K. 2009. In vitro antitumor mechanisms of various Scutellaria extracts and constituent flavonoids. Planta Med. **75:** 41-48.

Pareek, S.K., Gupta, V., Bhatt, K.C., Negi, K.S., Sharma, N. 2005. Plant genetic resources: horticultural crops. Pages 279-289 *in* B.S. Dhillon, R.K. Tayagi, S. Saxena, and G.J. Randhawa, eds. Medicinal and Aromatic Plants. Narosa Publishing House Pvt. Ltd, New Delhi, India.

Park, J.S., Yi, G.H., Nam, M.H., Park, S.H. 2001. Production of 8-epi-tomentosin by plant cell culture of Xanthium strumarium. Biotechnol. Bioprocess. Eng. **6:** 51-55.

Pastirova, A., Repak, M., Eliaova, A. 2004. Salicylic acid induces changes of coumarin metabolites in Matricaria chamomilla L. Plant Sci. **167:** 819-824.

Post, D.M., Urban, J.E. 1995. Antimicrobial activity of dogwood fruits (*Cornus drummondii*) from winter food caches of eastern woodrats (*Neotoma floridana*). J. Chem. Ecol. **21:** 419-425.

Puupponen-Pimiä, R., Nohynek, L., Meier, C., Kähkönen, M., Heinonen, M., Hopia, A., Oksman-Caldentey, K.M. 2001. Antimicrobial properties of phenolic compounds from berries. J. Appl. Microbiol. **90:** 494-507.

Palombo, E.A., Semple, S.J. 2001. Antibacterial activity of traditional Australian medicinal plants. J. Ethnopharmacol. **77:** 151-157.

Peces, R., Gago, E., Tejada, F., Laures, A.S., Alvarez-Grande, J. 1997. Relapsing bacteraemia due to *Micrococcus luteus* in a haemodialysis patient with a Perm-Cath catheter. Nephrology Dialysis Transplantation. **12:** 2428-2429.

Pekić, B., Kovač, V., Alonso, E., Revilla, E. 1998. Study of the extraction of proanthocyanidins from grape seeds. Food chem. **61:** 201-206.

Petersen, M., Simmonds, M.S.J. 2003. Rosmarinic acid Phytochemistry. **62:** 121-125.

Pincemail, J., Defraigne, J.O., Limet, R. 1996. Oxidative stress in clinical situations--fact or fiction? EU. J. Anasth. **13:** 219-234.

Price, S.F., Breen, P.J., Valladao, M., Watson, B.T. 1995. Cluster sun exposure and quercetin in Pinot noir grapes and wine. Am. J. Enc. Vitic. **46:** 187-194.

Prior, R.L., Wu, X., Schaich, K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J. Agric. Food Chem. **53:** 4290-302.

Psotova, J., Svobodova, A., Kolarova, H., Walterova, D. 2006. Photoprotective properties of Prunella vulgaris and rosmarinic acid on human keratinocytes. J. Photochem. Photobiol. B. **84:** 167-74.

Psotová, J., Kolár, M., Sousek, J., Svagera, Z., Vicar, J., Ulrichová, J. 2003. Biological activities of Prunella vulgaris extract. Phytother. Res. **17:** 1082-7.

Psotová, J., Chlopcíková, S., Miketová, P., Simánek, V. 2005. Cytoprotectivity of Prunella vulgaris on doxorubicin-treated rat cardiomyocytes. Fitoterapia. **76:** 556-61.

Qin, P., Wu, L., Yao, Y., Ren, G. 2011. Changes in phytochemical compositions, antioxidant and α-glucosidase inhibitory activities during the processing of tartary buckwheat tea. Food. Res. Int. *in press.*

Rafii, M.S., Walsh, S., Little, J.T., Behan, K., Reynolds, B., Ward, C., Jin, S., Thomas, R., Aisen, P.S., Alzheimer's Disease Cooperative Study 2011. A phase II trial of huperzine A in mild to moderate Alzheimer disease. Neurology. **76:** 1389-1394.

Ralph, S.G., Yueh, H., Friedmann, M., Aeschliman, D., Zeznik, J.A., Nelson, C.C., Butterfield, Y.S., Kirkpatrick, R., Liu, J., Jones, S.J., Marra, M.A., Douglas, C.J., Ritland, K., Bohlmann, J. 2006. Conifer defence against insects: microarray gene

expression profiling of Sitka spruce (*Picea sitchensis*) induced by mechanical wounding or feeding by spruce budworms (*Choristoneura occidentalis*) or white pine weevils (Pissodes strobi) reveals large-scale changes of the host transcriptome. Plant Cell Environ. **29:** 1545-1570.

R**apaport, M.H., Nierenberg, A.A., Howland, R., Dording, C., Schettler, P.J., Mischoulon, D. 2011.** The treatment of minor depression with St. John's Wort or citalopram: failure to show benefit over placebo. J. Psychiatr. Res. **45:** 931-941.

R**ichards, W.C., Takai, S. 1988.** Production of cerato-ulmin in white elm following artificial inoculation with *Ceratocystis ulmi*. Physiol. Mol. Plant Pathol. **33:** 279-285.

R**omani, A., Pinelli, P., Galardi, C., Mulinacci, N., Tattini, M. 2002.** Identification and quantification of galloyl derivatives, flavonoid glycosides and anthocyanins in leaves of *Pistacia lentiscus* L. Phytochem. Anal. **13:** 79-86.

Romo, J.T. 2011. Clubmoss, precipitation, and microsite effects on emergence of graminoid and forb seedlings in the semiarid Northern Mixed Prairie of North America. J. Arid. Environ. **75:** 98-105

Rowe, D.J., Baker, A.C. 2009. Perioperative risks and benefits of herbal supplements in aesthetic surgery. J. Aesthet. Surg. **29:** 150-157

R**oy, J., Bergeron, J.M. 1990.** Branch-cutting behavior by the vole (*Microtus pennsylvanicus*). J. Chem. Ecol. **16:** 735-741.

R**epine, J.E., Bast, A., Lankhorst, I.D.A. 1997.** Oxidative stress in chronic obstructive pulmonary disease. Am. J. Resp. Crit. Care Med. **156:** 341-357.

R**ios, J.L., Recio, M.C., Villar, A. 1988.** Screening methods for natural products with antimicrobial activity: a review of the literature. J. Ethnopharmacol. **23:** 127-149.

R**ice-Evans, C.A., Miller, N.J., Paganga, G. 1996.** Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free radical Biol. Med. **20:** 933-956.

S**ato, Y., Suzaki, S., Nishikawa, T., Kihara, M., Shibata, H., Higuti, T. 2000.** Phytochemical flavones isolated from Scutellaria barbata and antibacterial activity against methicillin-resistant *Staphylococcus aureus*. J. Ethnopharmacol. **72:** 483-488.

S**calbert, A., Haslam, E. 1987.** Polyphenols and chemical defence of the leaves of *Quercus robur*. Phytochem. **26:** 3191-3195.

Scheck, A.C., Perry, K., Hank, N.C., Clark, W.D. 2006. Anticancer activity of extracts derived from the mature roots of Scutellaria baicalensis on human malignant brain tumor cells. BMC Complement. Altern. Med. **6:** 27.

Schimel, J.P., Van Cleve, K., Cates, R.G., Clausen, T.P., Reichardt, P.B. 1996. Effects of balsam poplar (*Populus balsamifera*) tannins and low molecular weight phenolics on microbial activity in taiga floodplain soil: implications for changes in N cycling during succession. Can. J. Bot. **74:** 84-90.

Shale, T.L., Stirk, W.A., van Staden, J. 1999. Screening of medicinal plants used in Lesotho for anti-bacterial and anti-inflammatory activity. J. Ethnopharmacol. **67:** 347-354.

Shang, X., He, X., He, X., Li, M., Zhang, R., Fan, P., Zhang, Q., Jia, Z. 2010. The genus *Scutellaria* an ethnopharmacological and phytochemical review. J. Ethnopharmacol. **128:** 279-313.

Shoemaker, M., Hamilton, B., Dairkee, S.H., Cohen, I., Campbell, M.J. 2005. In vitro anticancer activity of twelve Chinese medicinal herbs. Phytother. Res. **19:** 649-651.

Schumacker, P.T. 2006. Reactive oxygen species in cancer cells: Live by the sword, die by the sword. Cancer Cell. **10** (3): 175-176

Singh, S.R., Levine, M.A. 2007. Potential interactions between pharmaceuticals and natural health products in Canada. J. Clin. Pharmacol. **47:** 249-258.

Small, E., Catling, P.M. 1999. Canadian medicinal crops. 1st ed. Nrc Research Press, Ottawa, ON. 240pp.

Smith, L.W., Culvenor, C.C.J. 1981. Plant sources of hepatotoxic pyrrolizidine alkaloids. J. Nat. Prod. **44:** 129-152.

Smith, M., Boon, H.S. 1999. Counseling cancer patients about herbal medicine. Patient Educ. Couns. **38:** 109-120.

Sun, S., Du, G.J., Qi, L.W., Williams, S., Wang, C.Z., Yuan, C.S. 2010. Hydrophobic constituents and their potential anticancer activities from Devil's Club (*Oplopanax horridus Miq.*). J. Ethnopharmacol. **132:** 280-285.

Sampson, M.J., Gopaul, N., Davies, I.R., Hughes, D.A., Carrier, M.J. 2002. Plasma F2 Isoprostanes Direct evidence of increased free radical damage during acute hyperglycemia in type 2 diabetes. Diabetes care. **25:** 537-541.

Sarker, S.D., Nahar, L., Kumarasamy, Y. 2007. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods. **42:** 321-4.

Sato, Y., Oketani, H., Yamada, T., Singyouchi, K., Ohtsubo, T., Kihara, M., Shibata, H., Higuti, T. 1997. A xanthanolide with potent antibacterial activity against methicillin-resistant *Staphylococcus aureus*. J. Pharm. Pharmacol. **49:** 1042-4.

Sánchez-Moreno, C., Larrauri, J.A., Saura-Calixto, F. 1999. A procedure to measure the antiradical efficiency of polyphenols. J. Sci. Food Agric. **76:** 270-276.

Schentag, J.J. 1990. Correlation of pharmacokinetic parameters to efficacy of antibiotics: relationships between serum concentrations, MIC values, and bacterial eradication in patients with gram-negative pneumonia. Scand. J. Infect. Dis. Suppl. **74:** 218-34.

Shikov, A.N., Poltanov, E.A., Dorman, H.J., Makarov, V.G., Tikhonov, V.P., Hiltunen, R. 2006. Chemical composition and in vitro antioxidant evaluation of commercial water-soluble willow herb (*Epilobium angustifolium* L.) extracts. J. Agric. Food Chem. **54:** 3617-3624.

Si, W., Gong, J., Tsao, R., Kalab, M., Yang, R., Yin, Y. 2006. Bioassay-guided purification and identification of antimicrobial components in Chinese green tea extract. J Chromatogr. A. **1125:** 204-10.

Skottová, N., Kazdová, L., Oliyarnyk, O., Vecera, R., Sobolová, L., Ulrichová, J. 2004. Phenolics-rich extracts from *Silybum marianum* and *Prunella vulgaris* reduce a high-sucrose diet induced oxidative stress in hereditary hypertriglyceridemic rats. Pharmacol. Res. **50:** 123-30.

Slaton, M.R., Raymond Hunt, E., Smith, W.K. 2001. Estimating near-infrared leaf reflectance from leaf structural characteristics. Am. J. Bot. **88:** 278-84.

Sohal, R.S., Weindruch, R. 1996. Oxidative stress, caloric restriction, and aging Science. New York, NY. **273:** 59.

Stoll, B.A. 2002. N-3 fatty acids and lipid peroxidation in breast cancer inhibition. Br. J. Nutr. **87:** 193-8.

Sun, T.-X., Edwards, D., LI, C.-S. 2005. The stomatal apparatus of *Lycopodium japonicum* and its bearing on the stomata of the Devonian lycophyte *Drepanophycus spinaeformis*. Bot. J. Linnean Soc. **149:** 209-216.

Svoboda, P., Kasai, H. 2004. Simultaneous HPLC analysis of 8-hydroxydeoxyguanosine and 7-methylguanine in urine from humans and rodents. Anal. Biochem. **334:** 239-50.

Swamy, S.M., Tan, B.K. 2000. Cytotoxic and immunopotentiating effects of ethanolic extract of *Nigella sativa* L. seeds. J. Ethnopharmacol. **70:** 1-7.

Syu, W.J., Shen, C.C., Don, M.J., Ou, J.C., Lee, G.H., Sun, C.M. 1998. Cytotoxicity of curcuminoids and some novel compounds from *Curcuma zedoaria*. J. Nat. Prod. **61:** 1531-1534.

Tai, J., Cheung, S., Cheah, S., Chan, E., Hasman, D. 2006. In vitro anti-proliferative and antioxidant studies on Devil's Club *Oplopanax horridus*. J. Ethnopharmacol. **108:** 228-235.

Tanaka, N., Nishikawa, K., Ishimaru, K. 2003. Antioxidative capacity of extracts and constituents in *Cornus capitata* adventitious roots. J. Agric. Food Chem. **51:** 5906-5910.

Thommasen, H.V., Wilson, R.A., McIlwain, R.G. 1990. Effect of Devil's Club Tea on Blood Glucose Levels in Diabetes Mellitus. Can. Fam. Physician. **36:** 62-65.

Templar, J., Kon, S.P., Milligan, T.P., Newman, D.J., Raftery, M.J. 1999. Increased plasma malondialdehyde levels in glomerular disease as determined by a fully validated HPLC method. Nephrol. Dial. Transpl. **14:** 946-951.

Teow, C.C., Truong, V.D., McFeeters, R.F., Thompson, R.L., Pecota, K.V., Yencho, G.C. 2007. Antioxidant activities, phenolic and β-carotene contents of sweet potato genotypes with varying flesh colors. Food Chem. **103:** 829-838.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., Hawkins Byrne, D. 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J. Food Comp. Anal. **19:** 669-675.

Turkmen, N., Sari, F., Velioglu, Y.S. 2005. The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. Food chem. **93:** 713-718.

Varma, J., Dubey, N.K. 1999. Prospectives of botanical and microbial products as pesticides of tomorrow. Curr. Sci (Banglore). **76:** 172-178.

Vasinauskiene, M., Radusiene, J., Zitikaite, I., Surviliene, E. 2006. Antibacterial activities of essential oils from aromatic and medicinal plants against growth of phytopathogenic bacteria. Agron. Res. **4:** 437-440.

Vila, J., Marco, F., Soler, L., Chacon, M., Figueras, M.J. 2002. In vitro antimicrobial susceptibility of clinical isolates of *Aeromonas caviae*, *Aeromonas hydrophila* and *Aeromonas veronii* biotype sobria. J. Antimicrobial Chemother. **49:** 701-702.

Vila, J., Ruiz, J., Gallardo, F., Vargas, M., Soler, L., Figueras, M.J., Gascon, J. 2003. Aeromonas spp. and traveler's diarrhea: clinical features and antimicrobial resistance. Emerg. Infect Dis. **9:** 552-555.

Vlietinck, A.J., Van Hoof, L., Totté, J., Lasure, A., Vanden Berghe, D., Rwangabo, P.C., Mvukiyumwami, J. 1995. Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. J. Ethnopharmacol. **46:** 31-47.

Viljoen, A.M., Subramoney, S., van Vuuren, S.F., Başer, K.H., Demirci, B. 2005. The composition, geographical variation and antimicrobial activity of Lippia javanica (Verbenaceae) leaf essential oils. J Ethnopharmacol. **96:** 271-277.

Von Reyn, C.F., Maslow, J.N., Barber, T.W., Falkinham, J.O., Arbeit, R.D. 1994. Persistent colonisation of potable water as a source of *Mycobacterium avium* infection in AIDS. Lancet. **343:** 1137-1141.

Ultee, A., Gorris, L.G., Smid, E.J. 1998. Bactericidal activity of carvacrol towards the food-borne pathogen *Bacillus cereus*. J. Appl. Microbiol. **85:** 211-218.

Westfall, R.E., Glickman, B.W.2004. Conservation of Indigenous medicinal plants in Canada. Proceedings of the Species at Risk 2004 Pathways to Recovery Conference, BC. Victoria, BC.

Willard, T., McCormick, J. 1992. Edible and medicinal plants of the Rocky Mountains and neighboring territories. Wild Rose College of Natural Healing, Victoria, BC. 278pp.

Wink, M. 1987. Quinolizidine alkaloids: biochemistry, metabolism, and function in plants and cell suspension cultures. Planta Med. **53:** 509-514.

Wink, M., Schneider, D. 1990. Fate of plant-derived secondary metabolites in three moth species (*Syntomis mogadorensis*, *Syntomeida epilais*, and *Creatonotos transiens*). J. Comp. Physiol. B. **160:** 389-400.

Wittstock, U., Gershenzon, J. 2002. Constitutive plant toxins and their role in defense against herbivores and pathogens. Curr. Opin. Plant Biol. **5:** 300-307.

Witzell, J.W.J., Martín, J.A.M.J.A. 2008. Phenolic metabolites in the resistance of northern forest trees to pathogens-past experiences and future prospects. Can. J. For. Res. **38:** 2711-2727.

Woods-Panzaru, S., Nelson, D., McCollum, G., Ballard, L.M., Millar, B.C., Maeda, Y., Goldsmith, C.E., Rooney, P.J., Loughrey, A., Rao, J.R., Moore, J.E. 2009. An examination of antibacterial and antifungal properties of constituents described in traditional Ulster cures and remedies. Ulster. Med. J. **78:** 13-15.

Wu, J., Hu, D., Wang, K.X. 2008. Study of *Scutellaria baicalensis* and Baicalin against antimicrobial susceptibility of *Helicobacter pylori* strains in vitro. Zhong Yao Cai. **31:** 707-710.

Welch, R.A., Dellinger, E.P., Minshew, B., Falkow, S. 1981. Haemolysin contributes to virulence of extra-intestinal E. coli infections. Nature. **294:** 665-667.

Wojdyło, A., Figiel, A., Oszmiański, J. 2009. Effect of drying methods with the application of vacuum microwaves on the bioactive compounds, color, and antioxidant activity of strawberry fruits. J. Agric. Food Chem. **57:** 1337-1343.

Wright, J.S., Johnson, E.R., DiLabio, G.A. 2001. Predicting the activity of phenolic antioxidants: theoretical method, analysis of substituent effects, and application to major families of antioxidants. J. Am. Chem. Soc. **123:** 1173-83.

Wu, Q.X., Shi, Y.P., Jia, Z.J. 2006. Eudesmane sesquiterpenoids from the *Asteraceae* family. Nat. Prod. Rep. **23:** 699-734.

Yarnell, E., Abascal, K. 2008. Antiadhesion herbs. Alternat. Complement. Ther. **14:** 139-144.

Yao, Y., Sang, W., Zhou, M., Ren, G. 2010. Antioxidant and alpha-glucosidase inhibitory activity of colored grains in China. J. Agric. Food Chem. **58:** 770-774.

Yasunaka, K., Abe, F., Nagayama, A., Okabe, H., Lozada-Pérez, L., López-Villafranco, E., Muñiz, E.E., Aguilar, A., Reyes-Chilpa, R. 2005. Antibacterial activity of crude extracts from Mexican medicinal plants and purified coumarins and xanthones. J. Ethnopharmacol. **97:** 293-299.

Yazaki, K., Sasaki, K., Tsurumaru, Y. 2009. Prenylation of aromatic compounds, a key diversification of plant secondary metabolites. Phytochem. **70:** 1739-1745.

Ye, F., Xui, L., Yi, J., Zhang, W., Zhang, D.Y. 2002. Anticancer activity of *Scutellaria baicalensis* and its potential mechanism. J. Altern. Complement. Med. **8:** 567-572.

Yildirim, A., Mavi, A., Kara, A.A. 2003. Antioxidant and antimicrobial activities of *Polygonum cognatum* Meissn extracts. J. Sci. Food Agric. **83:** 64-69.

Yin, S., Fan, C.Q., Wang, X.N., Yue, J.M. 2006. Lycodine-Type Alkaloids from *Lycopodium casuarinoides*. Helv. Chim. Acta. **89:** 138-143.

Yin, X., Zhou, J., Jie, C., Xing, D., Zhang, Y. 2004. Anticancer activity and mechanism of *Scutellaria barbata* extract on human lung cancer cell line A549. Life Sci. **75:** 2233-2244.

Yu, J., Liu, H., Lei, J., Tan, W., Hu, X., Zou, G. 2007. Antitumor activity of chloroform fraction of *Scutellaria barbata* and its active constituents. Phytother. Res. **21:** 817-822.

Yu, C., Zhou, Y., Han, Y., Wu, F., Qi, B. 1986. The structures of huperzine A and B,' two new alkaloids exhibiting marked anticholinesteraseactivity. Can. J. Chem. **64:** 837-839.

Zhou, L., Fuentes, E.R., Hoffmann, J.J., Timmermann, B.N. 1995. Diterpenoids from *Grindelia tarapacana*. Phytochem. **40:** 1201-1207.

Zou, Y., Chang, S.K., Gu, Y., Qian, S.Y. 2011. Antioxidant activity and phenolic compositions of lentil (*Lens culinaris* var. Morton) extract and its fractions. J. Agric. Food Chem. **59:** 2268-2276.