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**Exploration and characterization of bioactive phytochemicals in native Canadian plants for human health**

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Tsao, R. and Liu, Z. 2007. **Exploration and characterization of bioactive phytochemicals in native Canadian plants for** **human health.** Can. J. Plant Sci. **87**: 1045–1053. The boundary between medicine and food is not always clear in many ancientcultures. Many plants have traditionally been used in both culinary and healing practices. Herbs, in particular, have shown this dual functionality. Scientific information on herbal medicines has been limited to exotic plants, and only a few herbal plants native to, or grown in, Canada, such as American ginseng, Echinacea, St. John’s wort and feverfew, have been studied. Thorough inves-tigations have not been carried out, and there is a lack of information about native Canadian plants and their potential as medici-nal plants, particularly in terms of their chemical composition, biological activity and potential use for disease prevention. Also, from the marketing point of view, many of the existing herbs have only a small niche in the marketplace, so over production and consequent price depression can easily happen, as seen in the ginseng industry. There is obviously a need for multidisciplinary collaboration among herbalists, botanists, chemists and other scientists, since introducing native plants into mass production requires knowledge of environmental impact, genetic variability and the effects of other factors on the bioactive components. This review is intended to introduce the needs, techniques and challenges of such an approach with an emphasis on chemical and bio-chemical characterizations.

**Key words**: Phytochemicals, native plants, medicinal plants, aboriginal plants

Tsao, R. et. Liu, Z. 2007. **Examen et caractérisation des substances chimiques bioactives dans les plantes indigènes du** **Canada pour la santé humaine**. Can. J. Plant Sci. **87**: 1045–1053. La frontière entre médecine et alimentation n’est pas toujoursclaire dans maintes cultures antiques. De nombreuses plantes ont toujours été employées pour leurs vertus culinaires ou curatives. Cette dualité se remarque particulièrement chez les fines herbes. En homéopathie, les données scientifiques se bornent aux espèces exotiques et seules quelques herbacées parmi les plantes indigènes du Canada ont été étudiées, notamment le ginseng américain, l’échinacée, le millepertuis et le chrysanthème-matricaire. Aucune étude exhaustive n’a été entreprise et on manque d’informations sur les plantes indigènes du Canada ainsi que leur potentiel en tant que plantes médicinales. On ne sait notamment pas grand-chose sur leur composition chimique, leur activité biologique ni leur utilité éventuelle pour prévenir la maladie. Du point de vue com-mercial, bon nombre des fines herbes existantes occupent une très petite niche sur le marché, de sorte qu’il peut y avoir aisément surproduction et chute des prix, ainsi qu’on a pu le voir dans l’industrie du ginseng. Manifestement, les homéopathes, les botanistes, les chimistes et d’autres scientifiques doivent collaborer, car le passage des plantes indigènes à la production de masse exige une solide connaissance des répercussions sur l’environnement, de la variabilité génétique et des effets d’autres paramètres sur les composés bioactifs. Cet article expose les besoins, les techniques et les enjeux d’une telle approche, en insistant sur la caractérisation chimique et biochimique.

**Mots clés**: Substances phytochimiques, plantes indigènes, plantes médicinales, plantes autochtones

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The father of modern medicine, Hippocrates, in 460 BC, said “Let your food be your medicine and your medicine be your food”. The idea of "\_…≈Õ¨‘¥" or “medicines and foods are of the same origin” can be traced back to as early as the Official Records (ZhouLi ÷‹¿Ò) of the Zhou Dynasty, 1046-771 BC, of China. The first systemic medicinal book,ª∆µ ƒ⁄æ≠ (Huang Di Nei Jing 476-221 BC), interest-ingly, published during the same era as Hippoctates,



describes the theories and applications of traditional medi-cines, many of which clearly stated the importance of inte-grating foods and medicines. Most of these traditional medicines are of plant origin. Most other cultures have been using plants as a source of medicines; some of them may be food plants, others may not. In fact, some 80% of the world's population still rely upon plants for primary health care; even today, in Western medicine, and despite progress in

**Abbreviations**: FRAP, ferric reducing/antioxidant power; HAT, hydrogen atom transfer; LC-DAD-MS, liquid chromatog-raphy combined with diode array and MS detectors; LDL, low-density lipoprotein; ORAC, oxygen radical absorbance capac-ity; PCL, photochemiluminescence assay; ROS, reactive oxygen species

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synthetic chemistry, a quarter of prescription medicines are still derived either directly or indirectly from plants (Fowler 2006).

In recent years, research and development of nutraceuti-cals and functional foods have become a hot topic. Increased interest in plants as a source of novel bioactives for the prevention of the world’s top killing diseases, i.e., cancer and heart diseases, has led to a totally new way of promoting human health and wellness. This is not only from a therapeutic point of view, but more from a preventive point of view, that is, risk reduction by daily doses of func-tional foods or food supplements (nutraceuticals).

Among the diseases suffered by Canadians, cardiovascu-lar and heart diseases remain the top killers, followed by the various cancers. Other industrialized countries, such as the United States of America, have a similar picture of the lead-ing cause of death (Table 1). While there are many risk fac-tors for these chronic human diseases that we cannot change, such as age, sex and genetic inheritance, many oth-ers, such as lifestyle-related factors can help us to signifi-cantly reduce the risks. One-third of all cancers, for example, are avoidable by changing dietary habits (Milner 1994; World Cancer Research Fund 1997). Cardiovascular and heart disease are also found to be influenced by diet (Duthie and Brown 1994). These chronic degenerative dis-eases have been linked to oxidative stresses caused by excessive free radicals and reactive oxygen species (ROS), such as the superoxide anion (•O2–), hydroxyl radical (OH•) and the peroxy radical (ROO•), which react with vital bio-molecules such as lipids, proteins and nucleic acids (e.g., DNA), causing the aforementioned major health problems. Free radicals and ROS are neutralized by antioxidant defence mechanisms.

Fruits and vegetables are certainly the richest dietary sources of phytochemicals; however, other non-food plants, particularly those that have been used as traditional medi-cines, can be even more diverse sources of bioactives. Bioactive phytochemicals in many of these plants, particu-larly native Canadian plants, have not been systematically explored as potential candidates for the prevention, risk reduction or even cure of chronic human diseases. The prin-cipal bioactives in many Canadian plants used by Aboriginal people have not been chemically and biological-ly characterized. This paper is, therefore, to briefly review native plants of Canada for their traditional uses and current research status. Our intention is to bring this subject to the attention of Canadian researchers so an assorted effort is made in exploring the potential of these plants.

**CANADIAN PLANTS USED AS HERBAL MEDICINES**

Ethnobotanical research is a proven method for the discov-ery of plant-based medicines. Of the more than 120 pure drugs derived from plants in current commercial use, three-quarters were discovered through scientific investigations of traditional uses (Soejarto and Farnsworth 1989). However, it is important that this type of research be conducted with the full cooperation of Aboriginal communities (Marles et al. 2000; Marles, 2001). While relatively exotic regions are

richer in such resources, and scientific investigations tend to be heavily biased to those plants, many botanicals can be found in our own backyards. Hundreds of plant species have been found to be native to Canada and many of them have been traditionally used by Aboriginal people as herbal med-icines (http://www.evergreen.ca/nativeplants/search/index. php; Marles et al. 2000). Marles (2001), with the help of over 100 Aboriginal elders, has summarized information on the use of more than 200 species of plants as foods and med-icines. A publication by the Department of Indian Affairs and Northern Development of Canada listed 16 medicinal plants commonly used by the First Nations people of Canada (Table 2, DIAND 1997).

Most of the traditional medicinal plants in these lists have not been scientifically examined. Even for those we know relatively well, there is still a lack of information on culti-vation, chemical profiles, active components, toxicity and pharmacology. To screen bioactives from the vast collection of native Canadian plants for the promotion of human health is certainly a more systematic approach. It is a huge task that requires collaboration among scientists from all disciplines, and most importantly, the participation of native Canadians.

One such approach is taken by McCune and Johns (2002). They have selected 34 plant species from the published lit-erature as traditionally used by the Indigenous Peoples of the boreal forest in Canada for three or more symptoms of diabetes, or its complications, and measured the antioxidant activities of the methanolic extracts. They found that the antioxidant activities supported the contribution of these tra-ditional medicines in a lifestyle historically low in the inci-dence of diabetes. The fruit of staghorn sumac (*Rhus hirta*), a native woody shrub found in Ontario and most of the east-ern Canada, topped the list in its antioxidant activities (McCune and Johns 2002). Unfortunately, the active ingre-dient of the staghorn sumac fruit is not known, although gal-lotannins and ellagitannins have been found in the leaf of the plant (Niemetz and Gross 2005).

Another approach that might speed up the process is, per-haps, from well-studied traditional medicines from other cultures, such as traditional Chinese medicine. Exploring native Canadian plants that belong to the same species or families that have already been scientifically studied and used by other cultures could offer a shortcut to the under-standing and utilization of Canadian plants. Distinction between the two approaches may not always be clear; for example, Native Indians had used wild North American gin-seng for its healing properties long before French coloniza-tion (Pritts 1995); however, until it was exported to China in 1721, when the uses of Oriental ginseng for various reme-dies were already well documented and highly popular, wild North American ginseng (*Panax quinquefolius*) was not known outside Canada. It is said that after the introduction of North American ginseng to China, Chinese manuscripts and traditional medical writing documented the benefits of its consumption. The Chinese have since prized this herb for its healing and health-promoting properties (Duke 1989). The high demand and the claimed traditional use have brought it to the attention of the scientific community, lead-ing to investigations of its active ingredients, toxicology,

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**Table 1. Top causes of death in the United States of America and Canada**



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| Disease category | Common name of disease | USA**z** | Canada**y** |
| Diseases of the heart | Heart attack (mainly) | 28.5% | 26.6% |
| Malignant neoplasms | Cancer | 22.8% | 27.2% |
| Cerebrovascular disease | Stroke | 6.7% | 7.4% |
| Chronic lower respiratory disease | Emphysema, chronic bronchitis | 5.1% | 4.5% |
| Unintentional injuries | Accidents | 4.4% | 4.0% |
| Diabetes mellitus | Diabetes | 3.0% | 2.6% |



**z**2002 data, National Vital Statistics Report, Volume 53, Number 5 (October 2004), http://www.cdc.gov/nchs/data/nvsr/nvsr53/nvsr53\_05acc.pdf.

**y**1997 data, http://www40.statcan.ca/l01/cst01/health36.htm.



**Table 2. Selected medicinal plants used by the First Nations people of Canadaz**



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| Plant names | Traditional medicinal uses |
|  |  |
| Balsam fir | The gum, needles, bark or buds are used for several purposes, such as treating colds and indigestion |
| Common juniper | The gum, roots, berries and bark are used to treat wounds and sprains or as a diuretic |
| Common yarrow | Used alone or in association with other plants, it helps relieve colds and fevers as well as diarrhoea and vomiting |
| False Solomon's seal | Backaches and skin afflictions, as well as itching, are treated using a leaf-based preparation |
| Golden Alexanders | The root tea soothes fever and induces sleep |
| Jack-in-the-pulpit | A purgative using the roots of this plant soothes stomach aches |
| Paper birch | Different preparations made from the internal bark of paper birch are used to treat skin afflictions, wounds and stomach aches |
| Red baneberry | The roots of the red baneberry are used to treat colds and flu. It is also used to control menstrual irregularity |
| Red osier dogwood | Various bark-based preparations are used to treat eye diseases as well as colds, and to heal wounds |
| Staghorn sumac | Several parts are used, alone or with other plants, as a tonic, and to treat rheumatism or to heal wounds |
| Tobacco | Tobacco smoke blown into the ears helps relieve earaches. This plant is also used in many sacred rituals |
| Water avens | The roots prepared in the form of a decoction are used to treat diarrhoea |
| White cedar | The leaves, bark gum and branches are used for many purposes, such as treating colds, skin irritation and rheumatism. The wood |
|  | is also used in the sweat lodge |
| Wild ginger | The rhizome, alone or in association with other plants, is used as a tonic to treat colds, fevers or stomach cramps |
| Wintergreen | Depending on how it is prepared, it can regulate blood circulation, fight colds or act as an all-purpose medication |
| Yellow clintonia | The leaves are used to soothe infections, wounds and burns, and also to ward off mosquitoes |
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**z**DIAND publications # QS-6101-011-BB-A1 (1997).

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mode of actions and efficacies in humans. Scientific research has also led to the development of commercial products; Cold-fx®, a well-known herbal medicine with ginseng extract as the major active ingredient, is a good example.

In addition to North American ginseng, native plants that have been used widely and shown medicinal properties are Echinacea, St. John’s wort and feverfew. The origins, chem-istry, pharmacology, physiological functions and clinical uses of these herbs have been well documented (Hobbs 1994; Ernst and Pittler 2000; Ernst 2002; Garg and Hershey 2003; Muller 2003).

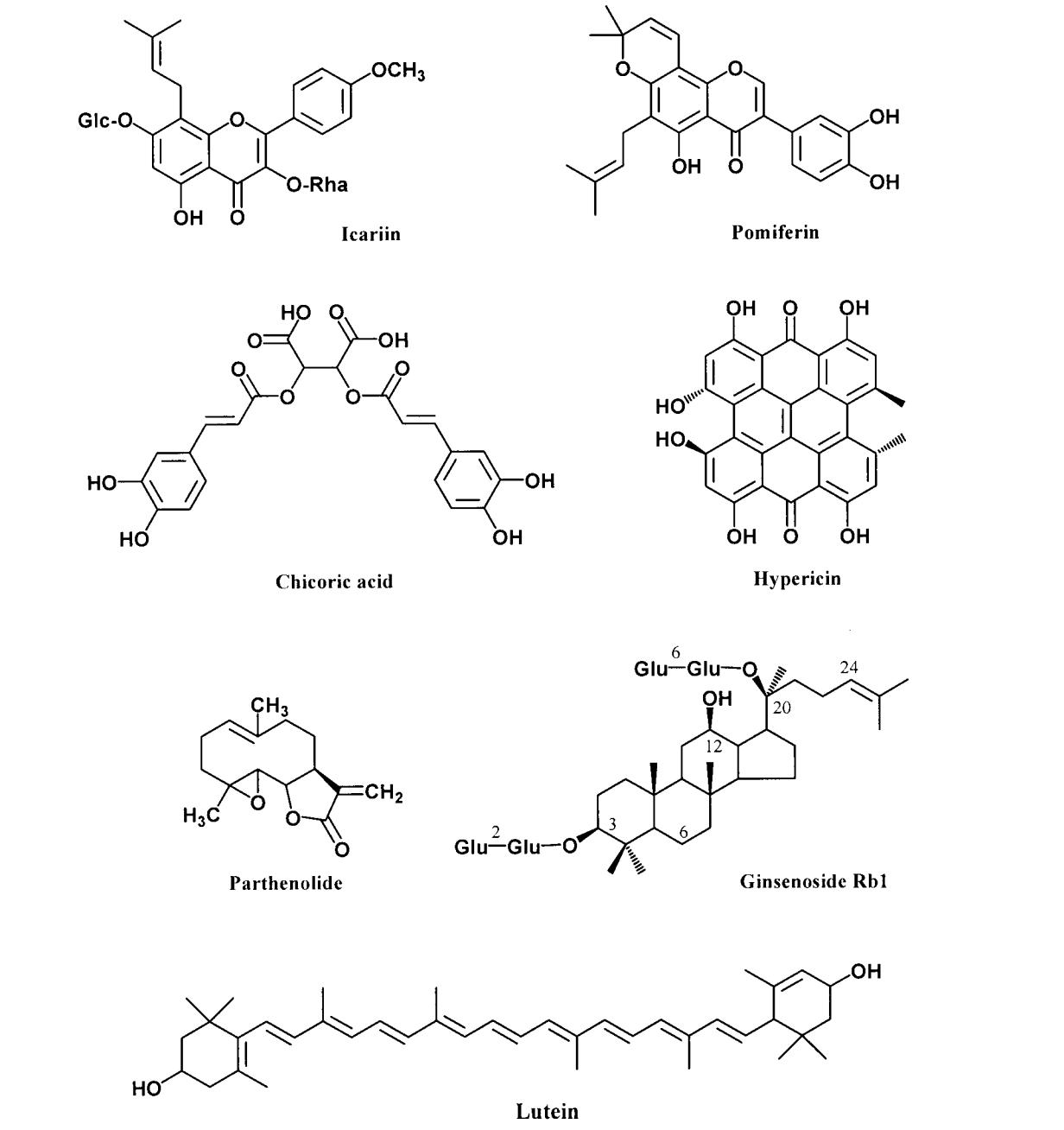
The Aboriginal people of Canada had used wild *P. quin-quefolius* for its healing properties, such as for the treatmentfor coughs, headaches, rheumatism and fevers, long before French colonization (Pritts 1995). Currently, this species and its extract are mainly used as a tonic, performance and mood enhancer (Table 3); however, recent studies have shown that it may have other attributes, such as antioxidant and angiogenesis regulation (Sengupta et al. 2004; Kim et al. 2007). The active compounds of *P. quinquefolius* are mostly ginsenosides (Schlag and McIntosh 2006) (Fig. 1).

Although ginseng has been reported for its positive effects, such as general improvement in energy and mental abilities, many clinical trials have failed to show any signif-icant improvement over the control (Garg and Hershey 2003). Different Echinacea species have traditionally been

used to treat colds, flues and infections, especially of the respiratory tract (Barnes et al. 2002). *Echinacea purpurea* is a native species, and oral and topical applications have been used to boost the immune system and relieve inflammatory conditions (Bauer and Wagner 1991; Speroni et al. 2002). Extract of Echinacea was also found to be a strong antioxi-dant (Hu and Kitts 2000). Echinacosides, particularly caffe-ic acid derivatives, are considered to be the major active components in Echinacea plants (Table 3, Fig. 1). A recent review of clinical data suggests that when taken early in the course of a common cold, Echinacea may be safe and effec-tive in reducing its severity and duration (Garg and Hershey 2003).

St. John’s wort (*Hypericum perforatum*) has traditionally been used as sedative for the relief of restlessness or ner-vousness (Barnes et al. 2002). A meta-analysis of 23 ran-domized controlled trials of 1757 outpatients concluded that St. John's wort extract was a more effective antidepressant than a placebo, and is comparable to conventional antide-pressants, particularly in patients with mild to moderate depression (Linde et al. 1996). The active components are known to be hypericin and hyperforin-related compounds and flavonoids (Williams et al. 2006) (Fig. 1). Murch and Saxena (2006) have used this special plant as a model for large-scale production of bioactives with defined qualities including high yield, concentration and predictable phyto-chemical profiles.

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**Fig. 1.** Active components in selected native plants. Icariin (Epimedium); pomiferin (Osage orange); chicoric acid (Echinacea); hypericin(St John's wort); parthenolide (feverfew); ginsenolide Rb1 (north American ginseng); lutein (marigold).

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Another commonly accepted native herb is feverfew (*Tanacetum parthenium* L.), which has been used to treat migraine headache and other inflammation-related symp-toms. It has been shown to cause vasodilatation and to reduce inflammation by inhibiting secretion of arachidonic acid and serotonin (Garg and Hershey, 2003). The active ingredient is considered to be parthenolide; however, other components may also contribute to its activities, such as antioxidant activity (Wu et al. 2006) (Fig. 1).

**EXPLORING BIOACTIVES FROM NATIVE PLANTS** To develop plant-based natural health products or nutraceu-ticals, all links in the value-chain need to be considered. No commercial products can be developed without sustainable supplies of the feedstock, the raw material. Knowledge from Aboriginal people is of the greatest importance, and consul-tation with and acceptance by Aboriginal Councils and Elders have been recommended in ethnobotanical research activities (Marles 2001). Most native plants are only found

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in the wild; therefore, cultivation in large acreage is neces-sary once a plant is found to have a high potential. Certainly, every step along the value-chain is a challenge; however, cultivation, production and postharvest processing are beyond the scope of this review. It is the authors’ intention to focus mainly on the chemical and biological characteri-zation of these native plants.

**Chemical Characterization and Profiling**

*Extraction, Separation, Structure Identification*

The lack of information on the phytochemical composition is perhaps the first challenge faced by scientists. In addition, normally only certain parts (leaf, flower, bark or root) or conditions of the part (fresh vs. dry) are used in traditional treatments. This requires a well-planned extraction and frac-tionation strategy. Solvent extraction is a conventional approach. Fresh samples are often first extracted with water-miscible organic solvents such as methanol, ethanol, ace-tonitrile or acetone, alone or in mixture with different percentages of water. Dry materials are often ground and then extracted similarly, except that water or a highly polar solvent is often necessary in order for the solvent to pene-trate plant cells. The solvent is evaporated, and the aqueous crude extract is often further partitioned with other organic solvents, such as ethyl ether, ethyl acetate or hexane, depending on the solubility of the active ingredient. The organic layer is then subjected to various chromatographic fractionation and purification procedures. The remaining water layer can be further partitioned with solvents such as *n*-butanol for highly water-soluble bioactives. Identity of thepurified compounds is elucidated based on the collective information from ultraviolet/visible (UV/Vis) and nuclear magnetic resonance (NMR) spectrum, infrared (IR) and mass spectrometric (MS) data, and other instrumental analy-sis. Liquid chromatography combined with diode array and MS detectors (LC-DAD-MS) is considered the most valu-able tool for the detection and analysis of known and unknown compounds. Various new extraction, separation and detection technologies are now available, and readers are referred to recent reviews (Tsao and Deng 2004).

It must be pointed out that chemical characterization, including the extraction, fractionation, purification and structural identification, can only be meaningful when the purified and identified compounds or fractions containing such have the same or similar biological activity as the plant or its extract. This is often done by the so-called bioassay-guided fractionation or purification technique. Proper in vitro and in vivo assay methods are used at each step of the fractionation and purification process (also see below in Biological Characterization). Only those with activity are followed for further purification.

*Fingerprinting and Quality Control*

The lack of scientific data on the phytochemical composi-tion of native plants and the fact that the active principals of such plants are not always known make quality control of these plant materials being used as medicines or food sup-plements difficult.

Even for well-known herbs, the chemical markers known to be present in the specific plant may not necessarily be the

cause of the physiological effect (Schaneberg et al. 2003). Another difficulty by measuring only the active components or chemical markers of an herbal product, is from the fact that the same compound can be found in different plants, leaving room for adulteration. It would be an even greater challenge for herbal products such as ginseng, as the active ingredients are a group of similar compounds (ginseno-sides); however the slight difference in the ratios of differ-ent ginsenosides can make a big difference in the bioactivity or side effect, as it is well known that the physiological effect of North American ginseng is different (or even oppo-site) from that of Chinese ginseng (*Panax ginseng*). The dif-ferences have been attributed to the ratio of two major ginsenosides (Sengupta et al. 2004). For these reasons, chemical fingerprinting, particularly chromatographic fin-gerprinting, has been used as an additional method of qual-ity control (Schaneberg et al. 2003; Sengupta et al. 2004).

Chromatographic fingerprinting is equally important for new native plants, as many factors can affect the phyto-chemical profiles of a particular species (Tsao et al. 2006). Phytochemical profiles, or fingerprints, are a less critical issue for single active ingredients purified from a plant. Phytochemical profiling is certainly a critical marker in the standardization of plant-based natural health products such as St. John’s wort (Murch and Saxena 2006).

**Biological Characterization**

Methods for the evaluation of biological activity or physio-logical function depend on what the plant was originally used for, and there is no set assay protocol for all plants or their extracts. Many native plants and traditional uses of these plants are related to the general enhancement of health, such as improving the immune system and reducing inflammatory symptoms. Most chronic diseases that modern humans face, including cancer, coronary and cardiovascular heart diseases and diabetes, are considered to be caused by excess reactive oxygen species in the body. Phytochemicals that can neutralize these free radicals (antioxidants) there-fore play an important role in alleviating these chronic dis-eases. For this reason, various assay methods have been developed to evaluate the antioxidant activities of phyto-chemicals.

*In Vitro Chemical Models for Antioxidant Activities*

The oxidative and antioxidant processes in the human body involve systems far more complicated than any single chem-ical or biochemical model. Although many in vitro models and methods have been developed for the evaluation of antioxidant activities under laboratory conditions, one must understand that there is no perfect system from which we can learn about the “true” antioxidant capacity of a single antioxidant or a complex medium of antioxidant phyto-chemicals (Tsao and Deng 2004). Nevertheless, at the First International Congress on Antioxidant Methods in 2004, it was proposed by the world’s leading scientists, that proce-dures and applications for three assays be considered for standardization: the oxygen radical absorbance capacity (ORAC) assay, the Folin-Ciocalteu method, and the Trolox equivalent antioxidant capacity (TEAC) assay (Finley 2005;

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**Table 3. Selected native plants, active compounds and treatments**



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| Plant names | Treatments | Active compounds |
|  |  |  |
| St John's wort | Anxiety, depression, insomnia | Hypericin, hyperforin |
| (*Hypericum perforatum*) |  |  |
| Echinacea | Prevention and treatment of common cold, wound healing | Echinacosides, caffeic acid derivatives, |
| (*Echinacea purpurea*) |  | polysaccharides, glycoproteins |
| Feverfew | Migraine prophylaxis | Parthenolide |
| (*Tanacetum parthenium* L.) |  |  |
| North American ginseng | Tonic, performance enhancer, mood enhancer, adaptogen | Ginsenosides Rb1, Re, Rg1, Rc and Rd |
| (*Panax quinquefolius*) |  |  |
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Data extracted from Garg and Hershey (2003).

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Prior et al. 2005). The photochemiluminescence assay (PCL) was not included in the White Papers, however; it has a great potential to be a standard method because of its high degree of automation, and more importantly, its high sensi-tivity (nanomolar range) compared with other assays (Schlesier et al. 2002; Tsao et al. 2005).

Although each method is based on a single mechanism, the overall antioxidant capacity from assays of different mechanisms may bring us one step closer to the true picture in the biological system. ORAC and PCL are based on the hydrogen atom transfer (HAT) reaction, and thus are con-sidered the most relevant to human biology (Prior et al. 2005; Tsao et al. 2006). The Folin-Ciocalteu method (FC) and the Ferric reducing/antioxidant power (FRAP) assays are single electron transfer (SET) based assays that measure the reducing power of an antioxidant. FC is normally expressed as total phenolic contents. SET and HAT mecha-nisms almost always occur together in all samples, with the balance determined by antioxidant structure and pH (Prior et al. 2005). It is strongly recommended to use at least two methods due to the differences between the test systems investigated (Schlesier et al. 2002).

*Ex Vivo Biochemical Models and Cell Culture Technique* Chemical models are easy to use and can be wonderful screening tools for the evaluation of antioxidant capacities of phytochemicals; however, these model systems lack bio-logical relevance. Oxidative damage by reactive oxygen species (ROS) to vital biomolecules, such as DNA and low-density lipoprotein (LDL), have been implicated in chronic diseases such as cancer and atherosclerosis. For this reason, many ex vivo biochemical models based on biomarkers such as DNA and LDL have frequently been used for the assessment of the protective roles of antioxidants. The cop-per-induced LDL peroxidation method uses copper (II) to initiate the oxidation reaction, and antioxidant activity is measured by spectrophotometric monitoring of the peroxi-dation product, the conjugated dienes, over time (Katsube et al. 2006). The Comet assay, or single cell gel electrophore-sis (SCGE) assay, has been used to assess oxidative damage to DNA, and has only recently been adapted to measure the antioxidative role of phytochemicals (Singh et al. 1988; Russo et al. 2003; O’Brien et al. 2006).

In addition to assays based on the protective roles of antioxidants, cell culture techniques have recently been developed and used in the assessment of antioxidant activi-

ties of phytochemicals and their potential role in preventing chronic human diseases (Liu and Finley 2005). Furthermore, biomarkers such as antioxidative phase II enzymes including superoxide dismutase (SOD), catalase, reduced glutathione (GSH), GSH peroxidase (GPx), glu-tathione reductase (GR), GSH S-transferase (GST), and NAD(P)H:quinone oxidoreductase 1 (NQO1) have also been measured during or after animal or human clinical tri-als on antioxidants. Many antioxidants have been found to promote activities of these enzymes (Kilanczyk and Bryszewska 2003; Cao and Li 2004).

**Case Studies**

*Lutein from Marigold*

Marigold (*Tagetes erecta* L.) is a plant found mainly in the Americas, particularly Mexico. Its bright yellow and orange colour and good adaptability to different environmental con-ditions make marigold a popular ornamental plant. The whole plant, or part of the plant, has been used in tradition-al medicinal practices for the treatment of different ailments including as analgesics, antiseptics, carminatives, diuretics, expellants, stimulants, vermifuges, and vermin repellents (Tsao et al. 2007). Marigold has received more attention in recent years due mainly to its high lutein content and the increasing evidence of the various physiological roles of lutein in the maintenance of human health and disease pre-vention (Fig. 1). Lutein and zeaxanthin have been found to inhibit the auto-oxidation of cellular lipids, to protect against oxidant-induced cell damage, cancer and cardiovas-cular diseases, but most importantly to prevent age-related macular degeneration (AMD) (Tsao et al. 2007).

In southern Ontario, Canada, *T. erecta* has been grown as a rotation crop for nematode control in tobacco fields (Reynold et al. 2000). Planting *T. erecta* as an inter-crop between tomato plants has also been found useful in reduc-ing nematode populations, although the efficiency was not as good as that of synthetic nematicide (Vasudevan et al. 1997). The nematicidal activity of *T. erecta* has been identi-fied from the root exudates. This has prompted us to inves-tigate the possibility of complete utilization of this plant.

The carotenoid profile of the marigold flower was first investigated by Tsao et al. (2004), and the identities of all possible lutein esters and their isomers were revealed using LC-MS. Different from the free lutein in popular vegetables, lutein in the marigold flower was found to be in ester forms with different long-chain fatty acids (Tsao et al. 2004; Tsao

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and Yang 2006). Extraction and hydrolysis conditions were further studied in our laboratory, and a method using high-speed counter-current chromatography was developed for gram level purification of high-quality lutein (Tsao and Yang 2006). This provided us with enough pure material for further studies on the antioxidant and anti-cancer activities of lutein (Wang et al. 2006). Using PCL assay and the β–carotene-linoleic acid model system (β-CLAMS) method, we found that lutein showed a greater antioxidant activity than the other two common carotenoids, β–carotene and lycopene. Lutein was found to be not only non-muta-genic, but it showed an anti-mutagenic effect (Wang et al. 2006). Chromosome aberration test using Chinese hamster ovary cells also indicated that lutein had a strong anti-clas-togenicity effect (Wang et al. 2006). Our findings provide scientific evidence for the safe use and beneficial health effects of lutein from this plant.

*Prenylated Flavonoids/isoflavones in Native Plants*

The surge in interest in the health benefits of isoflavones has prompted our effort in the search for isoflavones in other botanical sources. One such plant is the Osage orange [*Maclura pomifera* (Raf.) Schneid], a tree of the Moraceae or Mulberry family. It is a tree that is native to North America, from a small region in the United States known as the home of the Osage Indians (hence the common name “Osage orange”) through to Southern Ontario, Canada. The tree was widely planted as hedge trees throughout the US Midwest and southern Ontario, Canada (hence its other name “hedge apple”), and it played an important role in con-verting the prairies into productive agricultural land (Smith and Perino 1981; Barnett and Burton 1997; Tsao et al. 2003). Although the tree of Osage orange has been used as hedge trees and hardwood, the inedible fruit has been used as an enduring pest management home remedy in the US Midwest (Peterson et al. 2000; Tsao et al. 2003).

Chemical profiling of the phytochemicals in Osage orange fruit showed two main compounds, osajin and pomiferin, both of which belong to prenylated isoflavones (Peterson et al. 2000; Tsao et al. 2003) (Fig. 1). Using two in vitro chemical model systems, FRAP and β-CLAMS, we found that only pomiferin showed strong antioxidant activi-ties, which were comparable with those of vitamins C and E and the synthetic antioxidant BHT. Further biological eval-uations of anti-cancer and other activities are underway in our laboratory.

Our interest in prenylated isoflavones from Osage orange has also led us to other prenylated flavonoids, such as the widely used traditional Chinese medicinal plant, Epimedium. Prenylated flavonoids were found to have stronger antioxidant activity against microsomal lipid per-oxidation, and greater antiproliferative and cytotoxic effects against several human cancer cell lines than non-prenylated flavonoids (Ye and Lou 2005). Prenylated flavonoids are found to be responsible for risk reduction of cardiovascular diseases (Guan et al. 1996) and to enhance the immune function (Li et al. 1994). *Epimedium* plants can survive in a cold climate, and are currently grown in Canada as orna-mental plants for their beautiful flowers. We are currently

investigating the phytochemical profiles of Epimedium plants grown in Ontario, and more interestingly, a close Canadian cousin of Epimedium, *Vancouveria hexandra*.

**CONCLUSIONS**

Despite the lack of in-depth studies on their chemical and biological properties, the native plants of Canada present a great source of bioactives that may contribute significantly to human health and wellness. Exploring the use of these plants requires concerted effort from all links of the value-chain of these plants, although certain links might be weak-er than others for some plants. The scientific community also needs to work closely with Aboriginal people in order to develop a sustainable strategy for utilizing traditional medicines. Once plants are identified as a subject of research, the most important step is perhaps the characteri-zation of bioactive compositions. This is often done by bioassay-guided fractionation and purification processes; thus, methods used for extraction, detection and bioassays will play important roles. Arguably, most plants have tradi-tionally been used to treat multiple illnesses or for general wellness. Antioxidant activities, albeit limited, are perhaps among the first general approaches to the full understanding of the specific physiological functions of such plants or the bioactive components in the plants.

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