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Inter and Intra-Specific Differences in Medicinal Plant Use for the Treatment of Type II Diabetes Symptoms by the Cree Elders of Eeyou Istchee (QC)

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Inter and Intra-Specific Differences in Medicinal Plant Use for the Treatment of Type II Diabetes Symptoms by the Cree Elders of Eeyou Istchee (QC)

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Résumé

Au Canada, nous remarquons une prédominance du diabète de type 2 au sein des communautés autochtones. Une approche ethnobotanique est utilisée en collaboration avec la Nation Crie de Eeyou Istchee afin de déterminer quels traitements à base de plantes peuvent être utilisés pour contrer les différentes conditions qui, collectivement, forment le diabète. Les pharmacopées de deux communautés cries, soit celles de Waskaganish et de Nemaska, ont été établies puis comparées à celles de étudiées antérieurement : communautés Whapmagoostui et Mistissini. Malgré les différences géographiques de ces groupes, leurs utilisations sont majoritairement semblables, avec pour seule exception le contraste entre les communautés de Nemaska et de Whapmagoostui.

De plus, nous avons complété l'évaluation du taux cytoprotecteur des aiguilles, de l'écorce et des cônes de l'épinette noire (*Picea mariana*). Les extraits provenant de tous les organes des plantes démontrent une protection qui dépend de la concentration. La réponse spécifique d'organes peut varier selon l'habitat; ainsi, les plantes poussant dans les tourbières ou dans les forêts, sur le littoral ou à des terres l'intérieur démontrent des différences quant à leur efficacité. Bref, l'écorce démontre une relation dose-effet plus forte dans la forêt littorale, tandis que les aiguilles n'indiquent pas de changements significatifs selon leur environnement de croissance. La bioactivité observée démontre une corrélation avec le contenu phénolique et non avec l'activité de l'agent antioxydant. Ces résultats contribuent à péciser les activités antidiabétiques des plantes de la forêt boréale canadienne, telles qu'identifiées au niveau cellulaire par les guérisseurs Cries.

Mots-clés

Plantes médicinales, Diabète de Type II, Neuropathie, *Picea mariana*, ethnobotanique, agent antioxydant, phénol, cytoprotection, Autochtones, Premières Nations, cellules PC12-AC

Abstract

In Canada there is an overwhelming prevalence of type II diabetes in First Nations communities. Here an ethnobotanical approach has been used in cooperation with the Cree Nation of Eeyou Istchee to focus on finding plant based treatments for the conditions which collectively make up the symptoms of diabetes. The pharmacopoeias of two Cree communities (Waskaganish and Nemaska) are elucidated then compared with previously studied populations (Whapmagoostui and Mistissini). Despite differences in north-south east-west geography, plant ranking and use matrices were similar with the exception of Nemaska/Whapmagoostui.

We have also completed the evaluation of Black spruce (*Picea mariana*) needle, bark and cone cytoprotectivity. Extracts from all organs exhibited concentration-dependent protection. Organ-specific response was habitat and growth environment dependent with plants grown either in bog or forest habitats in coastal or inland environments exhibiting differences in efficacy. Observed bioactivity correlated with total phenolic content but not with antioxidant activity. Together, these results contributed to the understanding of antidiabetic activity of Canadian boreal forest plants identified by the Cree of Eeyou Istchee healers at the cellular level.

Keywords

Medicinal plants, Type II Diabetes, Neuropathy, *Picea mariana*, Ethnobotany, Antioxidant, Phenolics, Cytoprotection, aboriginal, PC12-AC cells

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List of Abbreviations

PC12-AC: a clonal derivate of the rat adrenal pheochromocytoma cell line
TAAM: CIHR Team in Aboriginal Antidiabetic Medicines
UV: ultraviolet
RPMI: complete media (10% horse serum, 5% new born calf serum)
PBS: phosphate buffer solution
LD_{50}: lethal dosage to achieve 50% viability
DMSO: Dimethyl Sulphoxide
BSA: bovine serum albumin
WST: soluble formazan dye
DPPH: 1,1-diphenyl-2-picryl-hydrazyle radical
AA.: Ascorbic acid
EC_{50}: efficiency concentration at 50%
IC_{50}:inhibitory concentration at 50%
HPLC-DAD: high performance liquid chromatography – photodiode array detector
MANOVA: Multivariate Analysis of Variance
SIV: syndromic importance value
FC: frequency of citation value
ROS: reactive oxygen species
TNFα: tumor necrosis factor
GLUT-4: glucose transporter type 4
S-GLU-1: surface glucose transporter 1
H1: hypothesis 1
H2: hypothesis 2
APS: aboriginal peoples survey
FNIHS: First Nations and Inuit regional health survey
G319S: mutation of the HNF1A (encoding hepatocyte nuclear factor) gene

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Chapter 1: Introduction

An Introduction to Diabetes in Aboriginal Populations

1.1 Introduction to Diabetes

Diabetes is a group of metabolic disorders that affect glucose uptake (Health Canada, 2002). Although the disease manifests itself in many forms, three main categories exist: type I, type II, and gestational.

Type I diabetes is mainly a genetic disorder and is normally diagnosed during childhood. In this case, the beta cells of the pancreas are attacked by the autoimmune system, causing irreversible damage and ceasing the production of insulin. Without insulin, glucose cannot be readily transported from the blood into the insulin sensing cells, which leaves the afflicted person hyperglycemic. Left untreated, the disease quickly becomes fatal (Health Canada, 2002). Patients with type I diabetes require insulin injections in order to control their blood sugar. Complications such as neuropathy, nephropathy and retinopathy arise from increased oxidative stress. It is estimated that only 10% of all diabetes cases are of this category. This is true for the overall Canadian population and specifically for aboriginal communities, so when we refer to a diabetes epidemic we are referring to type II which represents 90% of all diabetes cases (Health Canada, 2000). Gestational diabetes which only affects pregnant women is also a major problem in first nation communities; however, the condition is considered temporary and is not part of our focus.

Type II diabetes results from the development of insulin resistance in target tissues, mainly the muscle and liver cells (Health Canada, 2002). As a result, insulin levels in afflicted individuals can be either abnormally high if beta cells are compensating for lowered sensitivity, or if at a later stage of disease progression, abnormally low since the

beta cells become exhausted. High blood sugar above 7mmol/L is characteristic of type II diabetics (Meltzer et al., 1998). Symptoms of type II diabetes include arthritis/rheumatism, frequent headaches, back and/or kidney pain, diarrhea, swelling and/or inflammation, general weakness, increased appetite, heart and/or chest pain, increased thirst, abscesses and/or boils, blurred vision, increased urination, foot numbness and/or foot sores, slow healing infections, and sore and/or swollen limbs (Leduc et al., 2006). These symptoms and related complications are the parameters that will be used in order to identify medicinal plants that could be used for the treatment of diabetes in this study.

Symptoms and complications of type I and type II diabetes are caused by the stress placed on the microvascular and macrovascular systems from oxidative damage due to hyperglycemia (Schultz Johansen et al., 2005). Although it is possible to regulate blood glucose levels with conventional treatment options, it is difficult for most. Fluctuations can lead to damage of the kidneys, eyes, nerves, heart and blood vessels (Rahimi et al., 2005). The result can be as severe as blindness, renal failure, cardiovascular disease, loss of feeling or tingling in the extremities and/or the development of slow healing sores which can only be treated by amputation. Insulin injection therapy is sometimes employed in severe type II cases; however, for most this type of diabetes is controllable with the help of oral medication which increases insulin sensitivity and/or proper diet and an active lifestyle.

1.2 **Diabetes in Canadian Aboriginal Populations**

Aboriginal people in Canada suffer from higher rates of diabetes than the overall population and are considered one of the highest risk groups for diabetes in the world (Health Canada, 2000; Yu and Zinman, 2007). Waskaganish and Nemaska, are two of nine

Cree communities making up the Eeyou Istchee territory within Québec and have high rates of diabetes (18% and 17.5% respectively) (Kuzmina et al., 2008). Diabetes was virtually unknown in these communities before the 1940s (Legaré, 2004). Prevalence remained low through the 1980's when it reached 5.2%. By 1991, 7.1% of the population was diagnosed with diabetes, and now the age standardized prevalence is 25% (Kuzmina et al., 2008). High rates of complications are also characteristic of these populations. For example, in 2002 a study was conducted in which 58% of participants suffered from kidney problems, 11% from retinopathy, 12% from neuropathy and 13% had some form of vascular complication (Legaré, 2004). The rapid increase in prevalence among Canadian First nations has been attributed to a genetic predisposition towards obesity and diabetes, and a shift towards a Westernized lifestyle.

1.3 **Genetic Predisposition**

The trend towards a sedentary lifestyle and poor diet is becoming a global problem and certainly a Canadian one; however, the consequences are magnified in indigenous populations where there is a genetic predisposition for weight gain and type II diabetes. Scientists believe that aboriginals have what are termed "thrifty genes"(Neel, 1999; Yu and Zinman, 2007).

Theoretically, thrifty genes help to maintain fat reserves and are leftover from a time when our ancestors lived a life of feast and famine as hunter gatherers (Ritenbaugh and Goodby, 1989). In times when food was plentiful, a person with such a genetic makeup would easily store fat despite physical activity and this was viewed, and still is to some degree, as being healthy among many communities (Boston et al., 1997; Neel, 1999; Ritenbaugh and Goodby, 1989). When a period of famine came, often during the winter,

those with excess energy stores would survive while those without were at greater risk of starvation. With this lifestyle, there is a genetic selection over time towards a "thrifty" metabolism. Nowadays there is no threat of famine and activity levels are reduced since the acquisition of food involves a simple trip to the grocery store (Boston et al., 1997; Chakravarthy and Booth, 2004; Ritenbaugh and Goodby, 1989). For aboriginal populations, the ability to store fat efficiently is no longer a benefit but a burden.

Genetic predisposition to obesity is polygenic and therefore highly complex, making the attribution of specific genes or pathways difficult (Hegele et al., 2003). One gene specific to the Oji-Cree, called HNF1A, has been identified. It encodes for a transcription factor which acts on an insulin production gene. It is not the wild type HNF1A gene that has the thrifty characteristics however; it is when the gene possesses the G319S mutation found in the Cree population that the phenotype is created (Hegele et al., 2003).

The G319S mutation was found in Northwestern Ontario and Manitoba populations of Oji-Cree (Hegele et al., 2003). The presence of this mutation was strongly correlated to the development of diabetes and could be used quite accurately to predict whether an individual would develop diabetes by the age of 50. Although the identification of such a marker is interesting, 60% of diabetics in this population did not have the mutation, providing evidence that there are several genetic factors at play. Furthermore, without an environmental stress, individuals with the mutation could have been free of diabetes. This is possible because the mutation confers only a reduction in the activity of the transcription factor rather than eliminating all of its function. In agreement with others, it is concluded that the additional stress comes from poor diet and a lack of exercise, resulting in obesity (Hegele et al., 2003).

1.4 **Perception of Diabetes – The Roadblocks**

In general, First Nation communities consider diabetes to be a disease originating from the “Whiteman”, like tuberculosis and other disease epidemics that came with the arrival of Europeans (Young et al., 2000). They blame the increasing numbers of diabetes cases on the consumption of “Whiteman’s food” and the adoption of a sedentary, non-nomadic lifestyle, spent mostly on reserves (Boston et al., 1997). Scientists recognize that the traditional diet of bush food did not contain refined carbohydrates, unlike the fast and processed foods of a modern Westernized diet (Gittelsohn et al., 1996). Hence, if it is the incorporation of processed foods and the trend towards a sedentary lifestyle imposed by modern values that are to blame, it is counterproductive to cure the disease with what is perceived to be "Whiteman's" foods, lifestyle regimens and medicine. In this situation the development of recommendations that incorporate traditional food and medicinal practices would be beneficial.

Aboriginal communities are divided in their perception of traditional and modern medicine. Among the older generation there is a general distrust of Western foods and medicines (Gittelsohn et al., 1996). Younger generations, on the other hand, seem to be losing their trust in traditional medicine and are becoming increasingly interested in modern conveniences. There is a need for compromise and flexibility in medical/medicinal treatment for these populations (Letendre, 2002). Difficulty in understanding treatment, incompatibility with lifestyle, lack of medical treatment and medical facilities can all contribute to what has been seen as low compliance to modern medicine. This all translates to a very high incidence of resulting microvascular and macrovascular complications (Legaré, 2004).

According to the Aboriginal Peoples Survey (APS), in 1998 over 90% of diabetic aboriginals had seen a health care provider within the year. This demonstrates interest in and utilization of modern medicine in these communities. However, a First Nations and Inuit Regional Health Survey (FNIHS) found that more than 80% of diabetic aboriginals felt that the health care system was in need of improvement. Main concerns were the lack of staff, chronic care facilities, home care, education about medication, disease prevention and mental health counseling (Young et al., 2000).

A 1998 study in Milwaukee, Wisc, surveyed Native Americans using urban health care centers and the largest groups represented were Ojibway (20.7%), Oneida (20.0%), Chippewa (11.3%), and Menominee (8.0%) (Marbella et al., 1998). Of the surveyed patients, 38% were seeing a healer in addition to a physician. Most of the patients went to the healer for spiritual reasons, highlighting a need for a more holistic approach to medicine. This is reiterated when 86% of patients not seeing a healer responded that they would consider it in the future. The types of healers sought out were spiritual (50.1%), herbalist (42.1%) and medicine men (28.1%) (total exceeds 100% since some patients sought out more than one kind of healer). Of those who saw both a physician and a healer only 14.8% told their physician, and of those who did not tell their physician it was because they felt physicians would not understand, would want too many details or would not believe in the healer's abilities. Patients who received different recommendations from their healer than their physician had a difficult time deciding what medicines to take and how, but most often valued their healer's advice the most (Marbella et al., 1998). Studies such as these clarify the shortcomings of urban medical treatment facilities, mainly highlighting a

need for holistic methods that can incorporate all aspects of aboriginal life in a culturally sensitive way.

1.5 **Benefits of Plant Based Medicine**

For Canadian aboriginals, traditional medicine refers to the medical practices established before European colonization, which brought new diseases and Western medical influences (Johnston, 2002). Religious conversion has also brought changes in the utilization and delivery of traditional medicine (Niezen, 1997). This is because, unlike Western medical practices, traditional medicine is rooted in spiritual belief. For this reason, the increasing disinterest in traditional practices among aboriginal youth will undoubtedly continue to influence changes in the spiritual aspect of traditional treatment methods. Although much change is expected, an ongoing interest in plant and animal based treatment methods will likely remain as this type of treatment has been resilient even throughout the conversion of the Hudson's Bay Cree (Niezen, 1997).

Arnason et al. (1981) summarize plants used for food and medicine by aboriginals of eastern Canada. More than 400 plants were listed as being used medicinally, and of those 105 were identified as having known biochemical constituents with medicinal potential. Monoterpenes, polyacetylenes, alkaloids, tannins and salicylates are among the most frequently identified as medicinal constituents, many of which are found in conifers that are characteristic of the boreal landscape where our study area is found (Arnason et al., 1981).

In recent ethnobotanical surveys of northern Quebec, James Bay Cree and Inuit populations, many of the plants identified by Arnason et al. (1981) were reported to have anti-diabetic potential (Fraser et al., 2007; Leduc et al., 2006). Sixteen plants from Mistissini and 20 plants from Whapmagoostui were used for the treatment of diabetes

symptoms. All of these plants have been tested for their antioxidant potential and many have notable activity. Interviews by Leduc et al. (2006) and Fraser et al. (2007) revealed that plant part, preparation and selection are all important in order to ensure usefulness of plant medicines. An *in vitro* study on *Picea glauca* (Moench) Voss, a species closely related to *Picea mariana* (Mill.) Britton, Sterns & Poggenb. (a major focus of this thesis), has shown that symptom specific responses of plant extracts (neuropathy) are possible in the treatment of diabetes (Harris et al., 2008). In this case, differences in the plant part used were proven to affect activity in agreement with elder recommendations.

Despite having a multitude of conventional treatment options, diabetes complications remain common (Health Canada, 2002). This is due, in part, to the fact that conventional treatments target a specific metabolic aberration while the disease is characterized by having several (Tiwari and Rao, 2002). Crude plant extracts could be more beneficial due to the synergistic effects of phytochemicals found in these preparations. Plants that could reduce oxidative damage, restore the health of beta cells, increase insulin secretion, increase sensitivity to insulin, mimic insulin and control blood glucose levels or combinations of these would be interesting. Some examples of plants with anti-diabetic properties exist and can be found as part of traditional pharmacopeias around the world (Marles and Farnsworth, 1995; Tiwari and Rao, 2002).

One way in which plants can be beneficial is by providing antioxidants (Ames et al., 1993; Soumyanath, 2006). It is generally accepted that antioxidants provide overall protection against complications in diabetes and are beneficial for health in general. This is because the normal functioning of our metabolism produces reactive oxygen species (ROS) that damage lipids, proteins and carbohydrates. In a healthy individual the body produces

scavenger enzymes, that neutralize the harmful ROS (Ames et al., 1993). However, in diabetics the production of scavengers is insufficient and/or the amount of ROS produced is much higher than normal leaving the system overrun (Ames et al., 1993; Schultz Johansen et al., 2005). Many phenolic compounds like tannins have antioxidant activity and are often quantified to determine the potential level of protection a plant may have against ROS (Fraser et al., 2007; Spoor et al., 2006).

High antioxidant activity appears to be a common characteristic of Canadian aboriginal treatments for diabetes symptoms (McCune and Johns, 2002). As evidence for this, McCune and Johns compared plants used for three or more symptoms of diabetes to commonly consumed Western foods in Native communities. The majority (89%) scored higher than the Western food items in antioxidant studies, while 14% and 23% were on par with ascorbic acid and green tea/Trolox respectively (McCune and Johns, 2002).

Interestingly, it seems that plants with high levels of antioxidants can also be capable of increasing glucose uptake at the cellular level (Spoor et al., 2006; Tiwari and Rao, 2002). This may be due to the influence of ROS on specific regulatory molecules in the insulin dependent glucose transport pathway (Tiwari and Rao, 2002). ROS increases the expression of TNF-α, a cytokine capable of inhibiting tyrosine phosphorylation of the insulin receptor and post receptor signaling intermediates. A lack of phosphorylization renders insulin incapable of stimulating glucose uptake via GLUT-4 (glucose transporter) causing hyperglycemia. In response to what is perceived as a lack of insulin, GLUT-4 is down regulated, decreasing its presence and further hindering glucose uptake (Tiwari and Rao, 2002). This is one way in which insulin resistance could be increasing over time in diabetics. In this case, increased hyperglycemia further increases ROS production, thereby

restarting the cycle (Tiwari and Rao, 2002). Some antioxidants, including polyphenols, have shown an ability to down regulate TNF-α by reducing ROS. The synergistic properties seen in the James Bay Cree plants (antioxidant and glucose uptake) could be due to this type of protection (Spoor et al., 2006; Tiwari and Rao, 2002).

Besides increasing glucose uptake in insulin sensitive cells, antioxidants protect and may even help to restore beta cells in the pancreas (Chakravarthy et al., 1980). The health of alloxan-induced diabetic rat pancreases have shown improvement after treatment with *Pterocarpus marsupium* Roxb. (Fabaceae), an Ayurvedic medicinal plant (Chakravarthy et al., 1980). Similar results have been found for *Gymnema sylvestre* (Retz.) Schult. (Apocynaceae) in streptozotocin-induced diabetic rats (Tiwari and Rao, 2002).

Plants are also able to reduce the availability of glucose (Tiwari and Rao, 2002). Many reduce its presence by inhibiting carbohydrate hydrolyzing enzymes such as *a*-amylase and *a*-glucosidase (Kim et al., 2000). Others reduce glucose uptake from the intestine by inhibiting S-GLUT-1 (Kobayashi et al., 2000). Furthermore, plants like *Trigonella foecum-graecum* L. (Fabaceae) can affect glucose availability by modifying gastric emptying (Srinivasan, 2006). In *T. foecum-graecum*, the fiber reduces the total amount of glucose taken up and slows its release, which prevents spikes in blood glucose levels (Chandalia et al., 2000).

Myrcia multiflora (Lam.) DC. (Myrtaceae) is a Brazilian medicinal plant that is commonly known as plant insulin (Tiwari and Rao, 2002). Contrary to its name, the plant does not act like insulin; instead, it limits the diabetic's glucose absorption and therefore can prevent complications. Specifically, it is capable of inhibiting aldose reductase and *a*-glucosidase. Inhibition of aldose reductase prevents damage from sugar accumulation like

what is typical in the diabetic lens (Tiwari and Rao, 2002; Yoshikawa et al., 1998). Like S-GLUT-1, α-glucosidase inhibition occurs in the intestine which prevents glucose absorption before it can cause complications (Yoshikawa et al. 1998; Tiwari and Rao 2002). This is yet another example of how medicinal plants could have multiple benefits.

1.6 **Environmental Influences**

Crude plant extracts that offer multiple levels of protection, alone or in combination, would be of more benefit than a single target drug; however, fluctuations in the composition and concentration of beneficial compounds can be problematic. There are many factors associated with growth environment that may influence the phytochemistry of medicinal plants. Fluctuating compounds are ones involved in protecting against UV damage, herbivory, plant competition (allelopathy) or may be associated with different types of stress like waterlogging or drought (McCune and Johns, 2007). Other abiotic factors linked to climate or nutrient availability could also have an impact (Connor et al., 2002; Estiarte et al., 1994; Wang and Zheng, 2001).

Although specific examples of environmental influence on antioxidant phytochemistry exist, most have been conducted on commercially available cultivars. In general, plants from Mistissini and Whapmagoostui have not been the focus of such studies with the exception of *Vaccinium* spp. (Leduc et al., 2006). In a two year study (1998, 1999, (Connor et al., 2002)) it was found that phenolic content in blueberries of the same cultivar varied significantly from location to location and year to year. From this, they concluded that differences in chemistry must be due to environmental influence. In this case antioxidant activity fluctuated in unison with phenolic content. This is a trend that has also been observed for several of the Cree antidiabetic plants (Spoor et al., 2006; Wang and

Zheng, 2001). One purpose of this study is to determine whether antidiabetic activities of *Picea mariana* could be influenced by its growing environment.

1.7 **Study Goal**

The overall goal of this study is to aid in recommending plants for use as traditional medicine in the modern facilities of First Nations communities. In order to succeed in making broad generalizations, the traditional medicine of four James Bay Cree communities will be compared. Similar histories of experience and the sharing of knowledge between communities increase the likelihood that their pharmacopoeias will be similar. Furthermore, ethnobotanical and phytochemical evidence support this (Arnason et al., 1981; Fraser et al., 2007; Holmes, 1884; Leduc et al., 2006; Marles et al., 2000; Marles and Farnsworth, 1995; Marshall and Chiskamish, 1996; Marshall et al., 1989; Seigfried, 1994; Strath, 1903). Specifically, regression analysis of plant ranking from literature sources and Mistissini interviews showed positive correlation and Mantel and Podani algorithm results confirmed similarities (Leduc et al., 2006). Therefore hypothesis 1(**H1):** Although diversity in flora will vary from one community to the next, if locally available, plant use will remain consistent for the treatment of diabetes symptoms in all four pharmacopoieas. Differential activity for same species comparisons has, however been demonstrated by Fraser et al. (2007) for plants harvested from different communities. Namely antioxidant capacity of *Larix laricina*, *Rhododendron groenlandicum* and *Pinus banksiana* varied depending on whether they were grown in Whapmagoostui (north-coastal) or Mistissini (south-inland).

To ensure reliability of recommendations, environmental influence on antidiabetic activity will be further evaluated using *P. mariana* as a model plant. *P. mariana* has been

chosen for several reasons: 1. It grows in drastically different environments and is very plastic, having several growth forms, and it is often stunted in stressful environments like bogs (Begin and Filion, 1999; Kershaw, 2001; Legasy et al., 1995; Newmaster et al., 1997) 2. It already has shown promise as an antioxidant (Fraser et al. 2007, Spoor et al. 2006); 3. A closely related species, *P. glauca* (Wang et al., 2000; Weng and Jackson, 2000) has shown promise as a protector against diabetic neuropathy *in vitro* (Harris et al., 2008).

Hypothesis 2 (**H2):** In the nutrient-poor, waterlogged conditions of bogs (Zoltai and Vitt, 1995), where plants tend to be stunted (Kershaw, 2001), *P. mariana* will have more stress related compounds and therefore have a greater ability to protect PC12 cells from high glucose conditions.

Although the tandem use of traditional and conventional medicine is nothing new in developing countries (Bodeker, 2001), it is a novel approach to solving the problem of diabetes in Canadian aboriginal communities. We hope at the completion of this study to provide evidence that more culturally specific treatment recommendations are possible.

The results of this study are divided into two main chapters. Chapter 2: examines the findings of our ethnobotanical study and chapter 3 focuses on *P. mariana* bioactivity. In Chapter 2, we present the plants that were mentioned for the treatment of diabetes symptoms in Waskaganish and Nemaska. Plants are ranked using a syndromic importance value and then all TAAM interviewed communities are compared and contrasted using the Spearman rank analysis, Mantel analysis and Podani algorithm. Relationships between plants and specific symptoms are explored using correspondence analysis.

In chapter 3 we evaluate *P. mariana*'s ability to prevent glucose toxicity in PC12-AC cells *in vitro* (a diabetic neurophathy model). Protective activities are detailed for

needle, bark and cone taking the influence of growth environment into account. Regression analysis is used to determine if there are correlations between phenolic content, antioxidant activity and bioactivity. Conclusions based on these findings are drawn in the fourth and final chapter where we explore *P. mariana*'s prospective use as a sustainable resource for diabetes treatment.

Chapter 2: Ethnobotany

An Ethnobotanical Approach to Finding Culturally Appropriate Treatment Options for Canadian Aboriginals Suffering From Diabetes: Inter and Intra-specific differences in Medicinal Plant Use

Ashleigh D. Downing, Pierre S. Haddad, Timothy Johns, János Podani and Alain Cuerrier

Abstract

In order to identify plants with potential to treat the multiple symptoms of diabetes we surveyed local knowledge of two Eeyou Istchee Cree communities located in northern Quebec and made comparisons of plant use with two communities interviewed previously. Forty elders and healers from Waskaganish and Nemaska were surveyed using a semi-structured approach. Two plants previously unrecorded were identified: *Heracleum maximum* Bartram and *Thuja occidentalis* L.. Thirteen plants were confirmed between the two surveys and were ranked based on their potential to treat diabetes using the syndromic importance value (SIV) and the frequency of citation (FC) method for each community. The SIV ranking was then calculated compiling data across communities including the previously surveyed populations of Mistissini and Whapmagoostui. The degree of correlation between ranking of common plants was then measured using the Spearman rank test. Nemaska/Mistissini, Waskaganis/Whapmagoostui, and Mistissini/Whapmagoostui had correlation values >0.5 and were deemed similar. In the Mantel analysis, used to compare plant use matrices, there was a lack of correlation between communities with the exception of the Waskaganish/Nemaska comparison. Conversely, the Podani algorithm showed similarities between matrices in all cases except the Nemaska/Whapmagoostui comparison which agreed with the Spearman test results. Symptom species associations were also evaluated for the two new communities and for all communities grouped as one population. In the overall evaluation, among several associations, it was only *Abies balsamea* (L.) Mill (that was associated with the vision symptom) that was mentioned in more than one community. Other associations in the overall correspondence analysis were *Juniperus communis* L. (Whapmagoostui) and *Lycopodium clavatum* L. (Mistissini) for urination problems and *Vaccinium vitis-idae* L. (Whapmagoostui) for vision problems. The plants mentioned therein should be tested for their ability to treat associated symptoms *in vitro/vivo* bioassays to further TAAM's goal of finding traditional plant based remedies for diabetes treatment.

2.1 **Introduction**

Diabetes is a chronic disease capable of afflicting people from all walks of life and is expected to affect 366 million individuals by 2030 (Wild et al., 2004). Although, the problem is global, it impacts indigenous groups more dramatically (Yu and Zinman, 2007); Canadian aboriginal populations have a threefold higher prevalence of diabetes as compared to the national average (Health Canada, 2000; Young et al., 2000; Yu and Zinman, 2007). This represents a dramatic increase in the disease as it was not present in First Nation populations less than 100 years ago. The diabetes epidemic has been attributed to the acculturation that took place during European colonization and therefore to a changing lifestyle (Boston et al., 1997; Chakravarthy and Booth, 2004; Health Canada, 2000; Robinson et al., 1995). The Québec Cree of Eeyou Istchee are particularly affected by this, leaving 1 in 4 with type II diabetes (Kuzmina et al., 2008). Researchers and health care professionals agree that the problem of diabetes must be dealt with immediately.

Participatory research in Cree communities has revealed that lifestyle recommendations and treatments in modern facilities are often culturally inappropriate (Boston et al., 1997; Johnston, 2002). This leads to low levels of compliance resulting in higher levels of more severe complications including neuropathy, nephropathy, retinopathy and morbidity (Young et al., 2000). The CIHR Team in Aboriginal Antidiabetic medicine (TAAM) is a multidisciplinary group of researchers and community members working on this problem. The team is focused on incorporating plant based medicine from local traditional practices into modern health care facilities in Eeyou Istchee communities.

Plants have been used for millennia to treat diabetes in places like China, India and Egypt (Oubré et al., 1997). The incorporation of traditional and alternative medicine into the modern framework is a natural progression in developing countries; however, in

Canada it is a novel approach (Bodeker, 2001). Based on phytochemical and ethnobotanical evidence we believe that boreal plants are capable of treating diabetes, but that a short history of the disease has not allowed for the manipulation of plants for this purpose. Therefore, boreal antidiabetic medicine is essentially in its infancy and needs to be promoted.

The use of an ethnobotanical approach ensures that treatments are not simply herbal but also culturally appropriate (Oubré et al., 1997). Surveys for the TAAM project were initially conducted in Mistissini and Whapmagoostui (Fraser et al., 2007; Leduc et al., 2006). Although many plants were mentioned and tested from the first two locations it was important to invite other communities to join the project. The two newly participating communities, which are the topic of this paper, are Nemaska and Waskaganish. Allowing them to share their knowledge offers an opportunity to identify new plants and to compare pharmacopeia use between all interviewed communities. Comparisons can serve as a tool in determining which plants are appropriate for use in a particular community. Since the availability of local plants is relatively consistent in Nemaska and Waskaganish, we predict that their use will be similar. Furthermore, we expect that similar histories of experience and knowledge sharing have led to comparable plant use between all of the Eeyou Istchee communities surveyed. Therefore, Spearman rank tests, Mantel tests and Podani algorithm analysis comparing plant importance and use should agree, showing that SIV values and matrices are similar. We surmise that in many cases symptom specific links between plants found through correspondence analysis will also be reflected in the literature. This consultation of the literature will serve as a cross reference in determining the importance of plants for the treatment of diabetes symptoms.

2.2 **Materials and Methods**

2.2.1 Ethnobotanical Survey

Waskaganish and Nemaska are two of nine Eeyou Istchee Cree communities in the north of Quebec (Beaulieu, 1998). Waskaganish is a small community of approximately 2,045 occupants located at the mouth of the Rupert River and has access to James Bay. Nemaska is inland on Lake Champion and is the second smallest of the communities having around 680 inhabitants (MSSS, 2009). Both communities are at similar latitudes within the 51^{rst} parallel. Previously similar studies have been done in Mistissini (South) and Whapmagoostui (North) located at the 50° and 55° N parallels respectively (Beaulieu, 1998).

Interviewees were identified by community members as being particularly knowledgeable in herbal medicine. Interviews began in June and took place in the home of willing participants. The average age of participants was 74 yrs in Waskaganish and 68 yrs in Nemaska, although some elders could not remember their age or were not willing to share this information. Two younger knowledgeable participants, namely two Cree culture teachers, took part as well (one from each community). Couples in general preferred to be interviewed together and were therefore counted as n=1.

The survey was the same semi-structured questionnaire that had previously been used by Leduc et al. (2006) and Fraser et al. (unpublished) in the communities of Mistissini and Whapmagoostui. The survey is structured around 15 symptoms of diabetes and its complications: (1) arthritis/rheumatism, (2) frequent headaches, (3) back and/or kidney pain, (4) diarrhea, (5) swelling and/or inflammation, (6) general weakness, (7) increased appetite, (8) heart and/or chest pain, (9) increased thirst, (10) abscesses and/or boils, (11)

blurred vision, (12) increased urination, (13) foot numbness and/or foot sores, (14) slow healing infections, and (15) sore and/or swollen limbs. Surveys and methods were approved by the Université de Montréal ethics comity, "Comité d'éthique de la recherché de la faculté des arts et des sciences (CÉRFAS) (see appendix I). During the interview process we obtained prior informed consent; Cree Elders and Healers were aware that they could decline or stop the interview at any time and that they did not have to answer all questions. Interviews were recorded following the agreement of the Elder or Healer. Three voucher specimens of each plant cited in the interviews were collected and used to confirm the identity of the plants mentioned. Specimens were deposited in the Marie-Victorin herbarium (MT)(Appendix VIII and VIIII).

2.2.2. Ranking

Two methods of ranking were employed: the syndromic importance value (SIV, (Leduc et al., 2006)) and the frequency of citation calculation (FC, (Ladio and Lozada, 2004)). The syndromic importance value was calculated for each plant in order to assign a ranking order for the comparison of the plants relative importance as a potential diabetes treatment. It is also used to compare pharmacopoeias among communities and to rank all species from TAAM surveys as one overall population. Sphagnum moss and lichen species were not included as their identification was not clear. The SIV was calculated using the equation below,

$$SIV = \frac{\left[\frac{\sum ws}{S}\right] + \left[\frac{\sum wf}{SF}\right]}{2} = \frac{\sum ws + \left[\frac{\sum wf}{F}\right]}{2s}$$

where "∑ ws" is the sum of the weight of all the symptoms for which the plant was mentioned (for example, if it was mentioned for 15 symptoms, then ∑ws=1). "∑wf" is the sum of the symptom weight multiplied by the number of times it was mentioned for that use, "F" is the total number of interviews, while "S" is the number of symptoms in the survey (*i.e.* 15)(Leduc et al., 2006).

The FC was used as a second method of ranking for comparison. Ranks were calculated for each plant by dividing the "# of interviews in which the plant was mentioned" by "the total # of interviews" (Ladio and Lozada, 2004).

2.2.3 Statistics – Comparing Communities

A Spearman rank correlation test was used (Biostats program) to measure how similarly plants ranked between communities (ie. Waskaganish *vs* Nemaska, Waksaganish *vs* Whapmagoostui, Nemaska *vs* Whapmagoostui, Nemaska *vs* Mistissini, Waskaganish *vs* Mistissini and Mistissini *vs* Whapmagoostui). This analysis was only performed on SIV results since the FC calculation gave equal ranking to many plants. To perform this analysis, plants that were not common to both communities were removed from the list. Since gaps in SIV ranking results from the removal of plants the list was re-ranked so that in both communities species would be ranked 1 – n (n = number of species in common) without skipping any number. To further expand comparisons, the Mantel test and Podani algorithm were used to evaluate matrix correlations. In the case of the Mantel test, Euclidian distance was measured taking simple presence/absence of species mention (1 or

0) into account for the 15 symptoms (10,000 permutations, R program). With the Podani algorithm, we have compared community plant use matrices which specify the number of mentions for each plant and symptom. Podani correlations were calculated using Manhattan differences via 10,000 permutations of rows, columns, or both (Leduc et al., 2006).

In order to understand the association between species and symptoms, we used correspondence analysis (R program). This analysis was represented visually by scatter plots for each community.

2.3 **Results**

2.3.1. Ranking

In Waskaganish, 19 interviews were completed with a total of 25 participants. Twelve plants were listed, two of which, *Heracleum maximum* and *Thuja occidentalis* (see Table 2.1.), were new to the project. *Picea glauca, Abies balsamea, Larix laricina* (Du Roi) K. Koch*, Picea mariana* and *Rhododendron groenlandicum* (Oeder) Kron & Judd were the top five plants listed using the SIV ranking method. Ranking using the FC method of Ladio & Lozada (2004) placed *Abies balsamea, Alnus incana* ssp. *rugosa* (L.) Moench ssp. rugosa (Du Roi) R.T. Clausen*, Larix laricina, Picea mariana* and *Populus balsamifera* L. in the top five positions (see Table 2.2). *Abies balsamea, Larix laricina* and *Picea mariana* were in the top five for both methods whereas *Picea glauca, Rhododendron goenlandicum, Alnus incana* ssp. *rugosa* and *Populus balsamifera* were not common to both.

Table 2.1 Species mentioned by informants in Waskaganish in decreasing order of SIV ranking. Common, Latin and Cree names are provided as well as the number of symptoms (S) each species was mentioned for, the number of informants (I) who have mentioned that species and the plant organs (O) that were utilized. L = leaf, Br = branch, G = gum, W = wood, B = bark, C = cone, Wa = wood ash, R = root

SIV	Rank	Common	Latin	Cree	S	I	O
0.02034	1	White Spruce	*Picea glauca*	Minhikw	9	4	L, Br, G, W,
0.01749	2	Balsam Fir	*Abies balsamea*	Inaasht	6	15	G, L, Br,
0.01201	3	Tamarack	*Larix laricina*	Watnagan	4	10	B
0.01018	4	Black Spruce	*Picea mariana*	Inaahtikw	4	8	G,B,Br
0.00764	5	Labrador Tea	*Rhododendron groenlandicum*	Kachichepukw	4	5	L
0.00716	6	Jack Pine	*Pinus banksiana*	Ushchishk	3	3	B, L, C
0.00597	7	White-Cedar	*Thuja occidentalis*	Maastchiisk	3	7	Br,L
0.00511	8	Cow Parsnip	*Heracleum maximum*	Wiipashtk	2	4	R
0.00470	9	Gray Alder	*Alnus incana* ssp. *rugosa*	Atushpi	1	13	B, Br
0.00382	10	Balsam Poplar	*Populus balsamifera*	Mitus	1	7	B, Wa
0.00331	11	Pitcher Plant	*Sarracenia purpurea*	Ayigadash	1	1	L
0.00312	12	Mountain Ash	*Sorbus* spp.	Mushkuminanatikw	1	1	B

Table 2.2 Species mentioned by informants in Waskaganish in order of rank based on the FC method (Ladio & Lozada 2004).

Species	% Frequency	Rank
Abies balsamea	78.9	1
Alnus incana ssp. *rugosa*	68.4	2
Larix laricina	52.6	3
Picea mariana	42.1	4
Populus balsamifera	36.8	5
Thuja occidentalis	36.8	5
Rhododendron groenlandicum	26.3	6
Heracleum maximum	21.1	7
Picea glauca	21.1	7
Pinus banksiana	15.8	8
Sorbus spp.	5.3	9
Saracenia purpurea	5.3	9

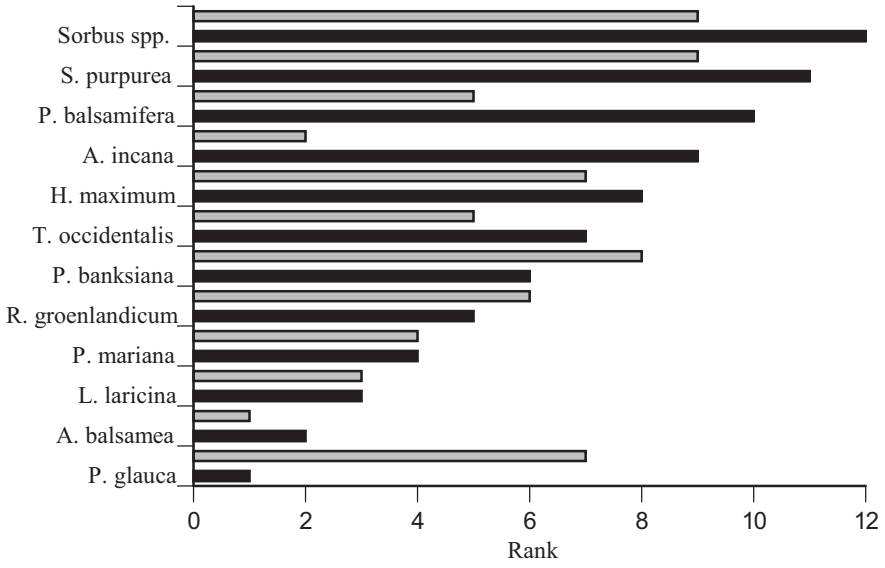

Figure 2.2 Bar graph depicting the differences in SIV (black) and FC ranking (gray) of the 12 plants mentioned in Waskaganish.

In the smaller community of Nemaska 15 interviews with 15 participants led to the identification of 10 plants, none of which were new from previous studies. Ranking was similar using both the SIV and FC method (see Tables 2.3. & 2.4 and Figure 2.3) for this

community. Interesting, however, is the position of *Picea glauca* as it falls from the first position in Waskaganish to the last in Nemaska.

Table 2.3 Species mentioned by informants in Nemaska in decreasing order of SIV ranking. Common, Latin and Cree names are provided as well as the number of symptoms (S) each species was mentioned for, the number of informants (I) who have mentioned that species and the plant organs (O) that were utilized. L = leaf, Br = branch, G = gum, W = wood, B = bark, C = cone, Wa = wood ash.

SIV	Rank	Common	Latin	Cree	S	I	O
0.01525	1	Black spruce	*Picea mariana*	Inaahtikw	5	7	G, L, C
0.01154	2	Gray alder	*Alnus incana* ssp. *rugosa*	Atushpi	4	14	B, Br,
0.00756	3	Tamarack	*Larix laricina*	Watnagan	2	7	B
0.00749	4	Balsam Fir	*Abies balsamea*	Inaasht	2	6	G, B,
0.00652	5	Balsam Poplar	*Populus balsamifera*	Mitus	2	4	Wa, B
0.00614	6	Labrador Tea	*Rhododendron groenlandicum*	Kachichepukw	3	4	L
0.00593	7	Jack Pine	*Pinus banksiana*	Ushchishk	2	3	C
0.00298	8	Mountain Ash	*Sorbus* spp.	Mushkuminanatikw	2	2	B, Br
0.00149	9	Sheep Laurel	*Kalmia angustifolia*	Uishichipukw	1	1	L
0.00138	10	White Spruce	*Picea glauca*	Minhikw	1	2	Br

Table 2.4 Species mentioned by informants in Nemaska in order of rank based on the FC method.

Species	% Frequency	Rank
Alnus incana ssp. *rugosa*	93.3	1
Picea mariana	46.7	2
Larix laricina	46.7	2
Abies balsamea	40.0	4
Populus balsamifera	26.7	5
Rhododendron groenlandicum	26.7	5
Pinus banksiana	20.0	6
Sorbus spp.	13.3	7
Picea glauca	13.3	7
Kalmia angustifolia	6.7	8

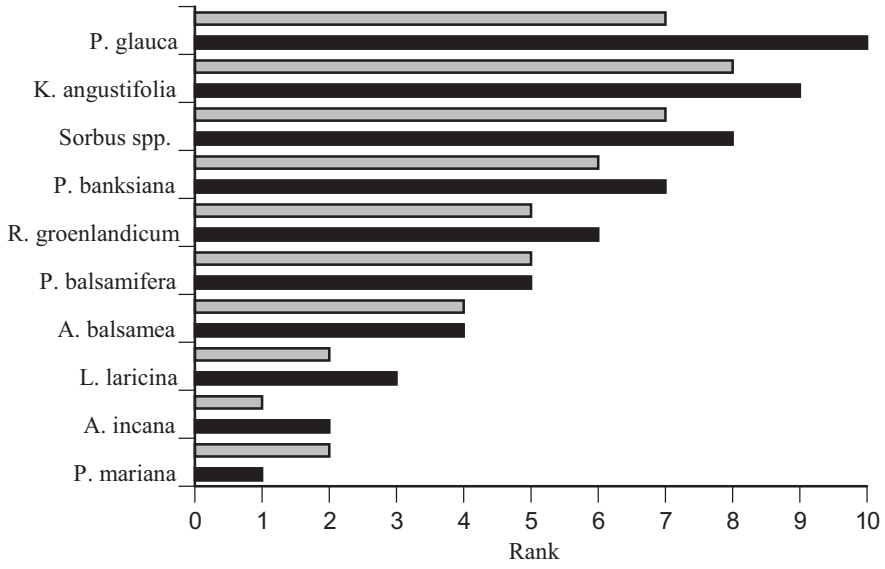

Figure 2.3 Bar graph depicting the similarity in SIV (black) and the FC ranking (gray) of the 10 plants mentioned in Nemaska.

Thuja occidentalis, Heracleum maximum and *Sarracenia purpurea* L. were all uniquely mentioned in Waskaganish. *Kalmia angustifolia* L. was the only plant mentioned in Nemaska that was not mentioned in Waskaganish; however, this plant had been mentioned before in Mistissini (Leduc et al., 2006).

When the interview data for all four communities are lumped together there are a total of 23 plants (table 2.5). After ranking the species of all four communities as a single sample, the top five plants are *Rhododendron groenlandicum, Rhododendron tomentosum* Harmaja ssp. subarticum*, Larix laricina, Picea mariana* and *Picea glauca.*

Table 2.5 List of 23 plant species mentioned by informants in all surveyed communities and their SIV ranks. Zero represents plants that were not mentioned in that community.

	Plant	Ranks			
		Whapmagoostui	Waskaganish	Mistissini	Nemaska
1	*Rhododendron groenlandicum*	1	5	1	6
2	*Larix laricina*	2	3	2	3
3	*Rhododendron tomentosum*	3	0	0	0
4	*Picea glauca*	4	1	14	10
5	*Picea mariana*	5	4	4	1
6	*Kalmia angustifolia*	6	0	12	9
7	*Juniperus communis*	7	0	0	0
8	*Sorbus* spp.	8	12	5	8
9	*Salix planifolia*	10	0	7	0
10	*Vaccinium vitis-idaea*	11	0	13	0
11	*Leymus mollis*	12	0	0	0
12	*Pinus banksiana*	13	6	9	7
13	*Empetrum nigrum*	14	0	0	0
14	*Cladonia rangiferina*	15	0	0	0
15	*Alnus incana* spp. *rugosa*	0	9	6	2
16	*Abies balsamea*	0	2	3	4
17	*Vaccinium angustifolium*	0	0	11	0
18	*Lycopodium clavatum*	0	0	10	0
19	*Gaultheria hispidula*	0	0	15	0
20	*Sarracenia purpurea*	0	11	8	0
21	*Populus balsamifera*	0	10	0	5
22	*Thuja occidentalis*	0	7	0	0
23	*Heracleum maximum*	0	8	0	0

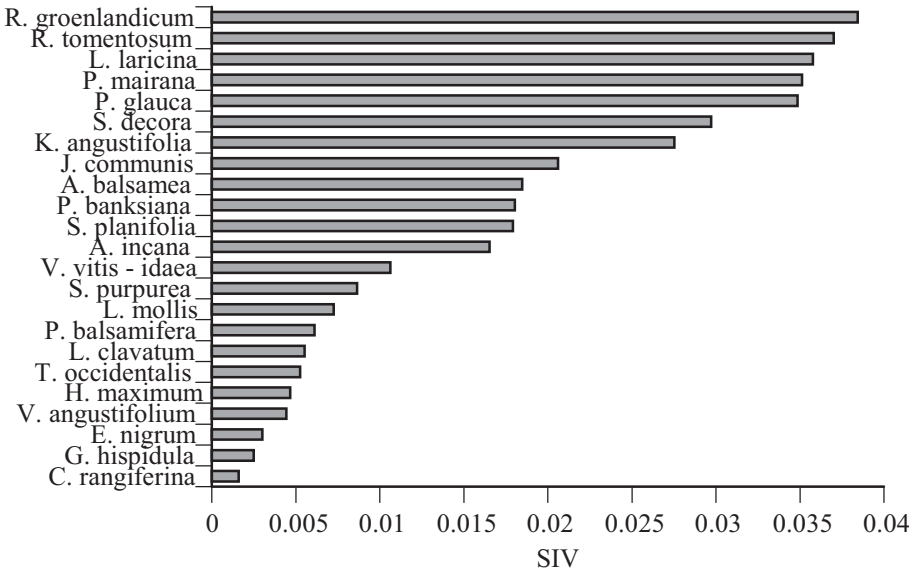

Figure 2.4 Bar graph representation of SIV ranking for all 23 plants. SIV's were calculated after merging data from Waskaganish, Nemaska, Whapmagoostui and Mistissini, and treating the master dataset as information from one population. Plants are listed from the highest SIV to the lowest.

2.3.2 Comparing Communities

In order to determine whether plant use within the pharmacopoeias of the four communities were similar, several methods were employed.

Spearman rank correlation test. Starting with the Spearman rank correlation test (see table 2.6), we find that Nemaska and Mistissini ($r = 0.60$) pharmacopoeias are the most similarly ranked, followed closely by Waskaganish and Whapmagoostui ($r = 0.54$) and Mistissini and Whapmagoostui ($r = 0.52$). Nemaska and Whapmagoostui have some similarity in ranking ($r = 0.29$) while Waskaganish and Mistissini are not well correlated ($r = 0.13$). Unexpectedly, Waskaganish and Nemaska are not similarly ranked at all ($r = 0.05$). However, for Wakaganish/Nemaska and Waskaganish/Mistissini, a lack of correlation may in part be due to the position of *Picea glauca*. In Nemaska and Mistissini this plant is ranked 10th and 14th respectively and in Waskaganish it is number one. If you remove this

species from the analyses the correlation coefficient increases to r = 0.4286 (Waskaganish/Nemaska) and r = 0.6190 (Waskaganish/Mistissini), which means they are very similar. In the case of the Spearman rank correlation test we are using the plant's importance as determined by the SIV ranking method. The SIV takes the weight of the symptoms into account distinguishing it from the other two methods of comparison (ie. Mantel test and Podani algorithm).

Mantel test. When analyzing matrices which have been simplified into mention (value = 1) or lack of mention (value = 0) for the Mantel test, we find that only Waskaganish and Nemaska have similar plant use (r=0.5773, p=0.0002). All other matrix comparisons gave *p* values well over 0.05 meaning that they are no more similar than two matrices randomly generated.

Podani algorithm. This changes, however, when we look at the Podani analysis which takes the frequency of mentions into account. For all but one community comparisons, *p* values were smaller than 0.05 meaning that the matrices were significantly similar when compared to matrices randomly generated. The only comparison that was not found similar was the Nemaska/Whapmagoostui relationship and this agrees with Spearman results.

Table 2.6 Community pharmacopoeia comparisons as evaluated by three statistical methods, Spearman rank correlation test, Mantel analysis and Podani algorithm. Spearman rank correlation test was a measurement of how similarly plants were ranked between communities (r >0.5 considered similar). The Mantel analysis evaluated whether the same plants were used for the same symptoms (p<0.05, were considered similar) while the Podani algorithm compared the number of times each plants was mentioned ($p \leq 0.05$, were considered similar).

	Spearman	Mantel		Podani		
Comparison	r	r	p	Row	Column	Both
Waskaganish /Nemaska	0.0500	0.5773	0.0002	0.0001	0.0008	0.0000
Waskaganish /Whapmagoostui	0.5429	0.0580	0.3337	0.0408	0.1128	0.0138
Nemaska /Whapmagoostui	0.2857	-0.1717	0.1825	0.2771	0.4485	0.2214
Nemaska /Mistissini	0.6000	0.0902	0.2643	0.0051	0.0020	0.0042
Waskaganish /Mistissini	0.1333	0.1303	0.1919	0.0021	0.0382	0.0007
Mistissini /Whapmagoostui	0.5167	0.2021	0.1347	N/A	N/A	0.0223

2.3.3 Symptom-Species Associations

For the sake of clarity, all outliers and clusters obtained from the correspondence analysis were included in tables 2.7 and 2.8. However, for the discussion of relationships, we will limit our definition to plants being mentioned more than one time for a given symptom. In Waskaganish, *Populus balsamifera* and *Alnus incana* ssp. *rugosa* stood out as treatments for abscesses and/or boils. *Abies balsamea*, *Picea mariana*, *Heracleum maximum* and *Larix laricina* all come out as being associated with the treatment of infection. *Abies balsamea* was also closely linked in this community to problems of blurred vision. With only two mentions, *Picea glauca* was connected to the treatment of heart and/or chest pain. *Thuja occidentalis* was also coupled with this symptom and is considered strongly linked since it had six mentions. Finally, *Rhododendron groenlandicum* was associated with urination problems, one of the most heavily weighted symptoms by physicians in our survey with two mentions in Waskaganish.

Table 2.7 Symptom-species associations revealed as a result of analyzing two dimensional perceptual maps (see figure 2.5 and 2.6, appendix II & III) stemming from the correspondence analyses of Waskaganish and Nemaska contingency tables.

Plants	Symptoms	# mentions
Waskaganish		
P. balsamifera	abscesses/boils	7
A. incana ssp. *rugosa*	abscesses/boils	13
P. banksiana	inflammation	1
A. balsamea	infections	8
P. mariana	infections	7
H. maximum	infections	3
L. laricina	infections	7
A. balsamea	vision problems	9
P. glauca	heart/chest pain	2
T. occidentalis	heart/chest pain	6
T. occidentalis	headache	1
R. groenlandicum	urination problems	2
Nemaska		
P. glauca	headache	2
A. incana ssp. *rugosa*	arthritis/rheumatism	1
A. incana ssp. *rugosa*	inflammation	1
A. balsamea	vision problems	5
L. laricina	infections	6
P. mariana	foot sores	1
Sorbus spp.	diarrhea	1
R. groenlandicum	urination problems	1
P. balsamifera	urination problems	1
P. banksiana	heart problems	1

In Nemaska, corespondance analysis was less valuable because there were fewer plants which were also mentioned less frequently. Many symptom-species associations occurred when plants were uniquely mentioned for that symptom (see table 2.7). *Picea glauca*, however, was linked to the treatment of headaches with two mentions, while *Larix laricina* was connected to the healing of infections. Like in Waskaganish, *Abies balsamea* was repeatedly mentioned for the treatment of blurred vision and correspondence analysis confirmed that relationship (see table 2.8).

In the overall analysis of all four communities *Abies balsamea*; *Lycopodium clavatuem*, *Juniperus communis* and *Vaccinium vitis-idaea* stood out but in this case as plants associated with urination problems. With the exception of *Abies balsamea* all associations were unique to a given community; that is, when an association was observed it occurred only in that community and not in any of the other three.

Table 2.8 Symptom-species associations revealed as a result of analyzing two dimensional perceptual maps (figure 2.7, see appendix IV) stemming from the correspondence analyses of the merged contingency tables of all four communities (Whapmagoostui, Waskaganish, Mistissini and Nemaska). Species outliers and the symptom they were associated with are mentioned here with specific identification of the communities in which the species were actually mentioned.

Species	Symptom	Communities(#mentions)
L. clavatum	urination problems	Mist(2)
J. communis	urination problems	Whap(34)
A. balsamea	vision problems	Mist(10), Wask(9) and Nem(5)
V.vitis - idaea	vision problems	Whap(15)

2.4 **Discussion**

With the exception of *Heracleum maximum*, *Thuja occidentalis and Saracenia purpurea* in Waskaganish and *Kalmia angustifolia* in Nemaska, all plants were common to both communities surveyed in this study. This represents shared knowledge which is likely the result of a similar history of experience. Differences in pharmacopoeia, are highlighted by the Spearman rank, Mantel test and Podani algorithm and represent underlying distinctions between compared communities. Here we suggest that discrepancies between the Podani algorithm and the Spearman rank correlation results is due to the position of *Picea glauca*. *Picea glauca*'s relative importance as determined by ranking was also not agreed upon using the SIV (first place) and FC (seventh place) methods.

During interviews in Waskaganish, *Picea glauca* was described as being close to the coast or river edges. Since Waskaganish is a coastal community, it is likely that this community is surrounded by a higher density of this tree species as compared to Nemaska, an inland population. This ranking trend is mirrored when looking at *Picea glauca* in Whapmagoostui, a coastal community (rank = 4) and Mistissini, an inland community (rank = 14). In both Nemaska and Mistissini, inland communities, this plant is present but ranked last. Since the ranking of *P. glauca* was affected by a difference in species distribution and abundance we re-calculated the Spearman rank coefficient for Waskaganish/Nemaska and Waskaganish/Mistissini comparisons. Removing *P. glauca* resulted in a strong correlation for these comparisons ($r = 0.4286$ and $r = 0.6190$).

If we look at the geography and the history of these communities this level of correlation makes sense. These communities are not only in proximity to one another but there was also a period of time when Nemaska residents set up camp in neighbouring communities including Waskaganish and Mistissini. Their relocation was prompted by the James Bay Hydro-Electric Project and the closing of the Hudson's Bay Company fur trading outpost (Nemaska, 2009). The sharing of knowledge during this period represents a horizontal transmission of information and may also account for the similarity of plant use highlighted by the Spearman and Podani analyses (Heinrich et al., 1998). In this comparison the Mantel test did not show a significant amount of correlation ($p = 0.2643$) but this may be due to the fact that, given the number of zeros in our matrices, the analysis was not a good fit. The number of zeros in the matrices is in part due to the fact that many plants are not used for multiple symptoms. For example if a plant is used by both communities but only for one symptom that leaves 14 zeros in each matrix. A small number

of participants particularly like what we had in Nemaska can have the same effect. Unfortunately another compounding factor could be a loss of knowledge with respect to plant based medicine for these symptoms. It was also apparent that some symptoms used in the interview process such as loss of appetite and general weakness were considered the result of a hard days work and were "cured" with the consumption of food. Therefore these conditions were not thought of as symptoms to any disease and in their case would represent an area in the matrices where a lack of citation would be represented as a zero result. We see similar results for Whapmagoostui/Waskaganish and Whapmagoostui/Mistissini comparisons where it is only the Mantel test that does not show similarities. It is possible that in cases where there are a large number of zeros that it is not possible to determine if the two matrices are similar with simple presence/absence data. In comparing Nemaska and Whapmagoostui we see no similarities in plant use using any of the statistical manipulations.

It is important to take into account that we have only considered plants that are common to both communities being compared using these statistical methods. Plants that were not mentioned by both communities under comparison represent unique aspects of their pharmacopoeias relative to one another. In some cases like with *P. glauca* it could be a lack of presence or abundance that results in no citation. In order to elucidate unique attributes within their pharmacopoeias floristic surveys would have to be conducted for all four communities. In the case of *Saracenia purpurea*, absence of the species is the main reason for its lack of citation in Whapmagoostui due to its distribution (Forest and Legault, 1977). The same is true for *Thuja occidentalis,* which is reaching its northern limit in the Waskaganish area. Conversely, *Rhododendron tomentosum* ssp. *subarticum* likely only

grows in Whapmagoostui and not in the more southern communities (Cuerrier, personal communication). On the other hand, where distribution is not a factor, it is simply that these groups do not use the same plants in the same way (Johns et al., 1990).

When considering all four communities a total of 23 plants have been identified and ranked, two of which were newly mentioned, *Heracleum maximum* and *Thuja occidentalis*. In general, the importance of top ranking plants remained after pooling all four communities. One unexpected result came when looking at *R. tomentosum* ssp. s*ubarticum*'s rank before pooling in the Whapmagoostui dataset (3rd position) and after pooling (2nd position). When this plant was ranked taking all datasets into account it actually placed higher overall despite its lack of mention in the other communities. This is likely due to the fact that this plant was mentioned for all symptoms at least once and in more than five out of fifteen cases mentioned by >10 participants. A large number of mentions for this plant shows that this information is common knowledge within Whapmagoostui and that it is considered a powerful plant and likely a cure all. *P.glauca* ranks 5th overall despite the fact that it ranked in last place for both Mistissini and Nemaska. Its strength is likely attributable to its tie with the treatment of slow healing infections and foot sores, which were heavily weighted symptoms by physicians in the development of the SIV calculation.

In comparison to the FC ranking we believe the SIV gives a richer picture. The FC method, which was used for the two communities in question but not for the overall ranking, is based only on the number of times the plant has been mentioned by informants. It does not take into the account the relation of the symptoms to the disease. In general the SIV is likely a better method for identifying plants which have the most potential in treating

this specific disease. However, the usefulness of this calculation seems to be dependent upon the population size and amount of information acquired from it. In Waskaganish the SIV rank gave us a slightly different picture than the FC whereas in Nemaska the results of the two methods are comparable.

Since, for the most part the four communities have similar uses of plants, we will now compare and contrast what is already known about the use of plants by the Cree for the treatment of diabetes symptoms (see table 2.9). If we consider the results from our correspondence analysis, starting with *Sorbus* species which was linked to the diarrhea symptom (Nemaska), we find that it is also mentioned in the literature. This reinforces the link made in the survey. It also corroborates the use of *Thuja occidentalis* and *Picea* spp. (*Picea glauca* in our study) for heart and/or chest ailments. *Populus balsamifera* was used for treating abscesses and/or boils just as was found in Waskaganish. The strong correlation between vision problems and the use of *Abies balsamea* in the overall correspondence analysis was reiterated in the literature as well(Marshall et al., 1989). *Rhododendron groenlandicum* was cited for urination problems in both study communities as well as in Whapmagoostui and in the literature survey (Marles et al., 2000). In the overall correspondence analysis, *Juniperus communis* is likewise linked to urination problems.

Infection was a common ailment treated by the Cree, so it is not unexpected that we find a number of plants linked to this symptom (Arnason et al., 1981). *Abies balsamea*, *Picea mariana* and *Larix laricina* are common plants in the boreal forest and have known antibiotic properties, so it fits that they are associated both in our study and in the literature (Marles et al., 2000). In table 2.9, most of the species from our survey are represented and are highlighted in bold, although not always for the same symptoms. Other than differences

in the types of symptoms treated there are also many plants which are not mentioned in the four TAAM surveys. Many of these plants are not in our study area but are found in proximity to Cree communities in other parts of Canada.

Table 2.9 Literature review of plants used for the treatment of diabetes symptoms by Cree Nations (Holmes, 1884; Marles et al., 2000; Marshall and Chiskamish, 1996; Marshall et al., 1989; Seigfried, 1994; Strath, 1903). TAAM plants are represented in bold.

Diabetes Symptom	Species Represented in the literature
Arthritis/Rheumatism	***Abies balsamea,*** *Achillea millefolium, Acorus americanus, Betula papyrifera, Caulophyllum thalictroides, Gaultheria procumbens,* ***Heracleum maximum****,* ***Juniperus communis****,* ***Larix larcina****,* ***Rhododendron groenlandicum****, Medicago sativa, Nuphar lutea,* ***Picea glauca****, Rumex aquaticus, Shepherdia canadensis,* ***Sorbus*** **spp.***, Valeriana dioica*
Headaches	*Acorus americanus,* ***Sarracenia purpurea****, Sium suave, Helenium autumnale. var montanum, Hymenoxys richardsonii, Mentha arvensis, Valeriana dioica, Achillea millefolium, Monarda fistulosa, Nuphar lutea,* ***Rhododendron groenlandicum***
Back and/or kidney pain	***Abies balsamea****,* ***Juniperus communis****,* ***Rhododendron groenlandicum****,* ***Picea*** **spp.***, Achillea millefolium, Nuphar lutea, Symphiotrichum puniceum, Symphoricarpos albus, Equisetum arvensis, Matricaria matricarioides, Matteuccia struthiopteris, Betula papyrifera,* ***Sorbus*** **spp.***,* ***Sarracenia purpurea****, Acorus americanus, Chimaphila umbellate*
Diarrhea	***Rhododendron*** **sp.***,* ***Thuja occidentalis****,* ***Picea mariana****,* ***Alnus incana ssp. rugosa****, Achillea millefolium, Amelanchier alnifolia, Rubus idaeus, Populus tremuloides,* ***Salix*** *bebbiana, Cornus sericea, Mentha arvensis, Prunus virginiana, Rosa acicularis , Conyza canadensis, Acorus americanus, Cornus stolonifera, Fragaria virginiana, Heuchera richardsonii, Arctostaphylos uva-ursi,* ***Kalmia angustifolia,*** *Prunus virginiana*
Swelling and/or inflammation	*Achillea millefolium, Nuphar lutea,Maianthemum, Shepherdia canadensis, Sorbus* spp.*, Alnus viridis* ssp. *crispa*
General weakness	*Valeriana dioica, Mentha arvensis*
Heart and or chest pain/heart ailments	***Picea mariana*** *, Fragaria virginiana, Nuphar lutea, Populus tremuloides, hymenochaetaceae, Plantago major, Prunus virginiana,* ***Populus balsamifera****, Acorus americanus, Valeriana dioica, Sium suave,* ***Abies balsamea****,* ***Thuja occidentalis****,* ***Picea*** **spp.***,* ***Larix laricina****,* ***Rhododendron groenlandicum****,* ***Gaultheria hispidula****, Helianthus nuttallii, Lonicera dioica,* ***Sorbus*** *scopulina, Mentha arvensis, Rubus idaeus, Campanula rotundifolia, Amelanchier alnifolia, Chimaphila umbellata*
Abscesses and/or boils	***Larix laricina,*** *Nuphar lutea,* ***Abies balsamea****,* ***Rhododendron sp.****, Betula sp., Acorus americanus, Achillea millefolium, Symphyotrichum leave var laeves, ,Mentha arvensis, Monotropa uniflora,* ***Picea mariana****, Polygala senega, Geum aleppicum,* ***Populus balsamifera****, Populus tremuloides, Plantago* sp.*, Heracleum lanatum, Lilium philadelphicum,* ***Salix*** **sp**.
Blurred vision	***Salix*** **sp**.*,Oxalis montana, Cornus sericea,* ***Abies balsamea****,* ***Larix laricinia****, Rosa acicularis,* ***Vaccinium vitis-idaea***
Increased Urination and/or bladder problems	*Lycopodium* sp.*, Vaccinium vitis-idaea, Lonicera dioica,* ***Juniperus communis****,* ***Rhododendron groenlandicum****, Matricaria matricarioides,* ***Sarracenia purpurea****, Urtica dioica* ssp. *gracilis,* ***Thuja occidentalis***
Foot numbness and/or foot sores	*Calla palustris,* ***Heracleum maximum****, Chenopodium album, Nuphar lutea*

Infections	*Achillea millefolium,* ***Abies balsamea****, Aralia nudicaulis,* ***Rhododendron groenlandicum****, Nuphar lutea, Acorus americanus,* ***Picea mariana, Larix larcina****, Polygala senega,* ***Juniperus*** **spp**. *Petasites sagittatus,Plantago major, Picea glauca , Chamerion angustifolium,* ***Populus balsamifera***
Sore and/or swollen limbs	***Heracleum maximum****, Nuphar lutea,* ***Achillea millefolium****, Maianthemum* sp., *Shepherdia canadensis*
Diabetes	*Achillea millefolium, Acorus americanus, Oplopanax horridus,* ***Populus balsamifera****, Populus tremuloides* *Ribes americanum,* ***Sorbus*** *scopulina*

Other Canadian aboriginal groups have treatments for these symptoms as well and it is often possible to recognize similarities. For instance the Dene of Western Canada treat diarrhea with *Rhododendron groenlandicum,* as was found in our study and in the literature (Marles et al., 2000). *Larix laricina*, is also used by this group to treat boils and by the Metis to treat infection. First Nations of the Maritime Provinces treat diarrhea using *Prunus virginiana* L.*,* a plant not mentioned in our survey but which was recorded for the Cree (table 2.9) (Van Wart, 1948). *Larix laricina*, which is used by the Ojibway to treat headache, is also on record as a treatment for symptoms of diabetes by the Cree, specifically infection, blurred vision, abscesses and/or boils, arthritis/rheumatism and heart problems (Youngken, 1925). In this way the use of plants for other symptoms of diabetes by other groups serves to complement and help identify potential plants for further testing. Plants such as *Larix laricina*, capable of treating many of the problems in the diabetes spectrum at once, can be very interesting for future investigations.

The newly identified plants *Heracleum maximum* and *Thuja occidentalis* are also found in the literature; however, the uses cited in the Waskaganish survey have not yet specifically been recorded for the Cree (see table 2.10). Several of the symptoms cited in the literature are, however manifestations of diabetes (headache, abscesses/toothache, arthritis/rheumatism, chest pain and diarrhea), which affirms the anti-diabetic potential of these plants. Further disease specific *in vitro* investigation is necessary but should be done cautiously, as these two plants contain toxins. *Heraculeum maximum* contains furanocoumarins that are triggered by ultraviolet light and *Thuja occidentalis* contains thujone, a known neurotoxin (Marles et al., 2000). It is likely that the traditional method of

preparing these remedies eliminates toxicity. For example the decoction of cedar in water helps to eliminate the hydrophobic toxin thujone (Marles et al., 2000).

Table 2.10 Cree usage of *Heracleum maximum* and *Thuja occidentalis* in the literature and in TAAM surveys (Holmes, 1884; Marles et al., 2000; Marshall and Chiskamish, 1996; Marshall et al., 1989; Seigfried, 1994).

Plant	Literature		TAAM survey	
	symptom	organ	Symptom	Organ
Heracleum maximum	aches, headache, toothache, arthritis/rheumatism, colds, impetigo, scabies, painful limbs, worms in flesh	root	infections, urination problems	Root
Thuja occidentalis	chest pain, congested chest, cough, cuts, diarrhea, diuretic, impetigo scabies, stomach problems	leaves	heart/chest pain, headache, back/kidney pain	leaves

2.5. Conclusion

This ethnobotanical survey has furthered our understanding of plant use among the Cree of Eeyou Istchee and has allowed for more informed *in vitro* and *in vivo* investigation. Many related projects have been completed and are ongoing in tandem with the one represented here. One such caveat is an *in vitro* study of *Picea mariana*'s bioactivity in relation to high glucose stress in PC12-AC cells (a diabetic neuropathy model). This has emerged from the information found here that *P. mariana* is tied to symptoms related to diabetic nerve damage. These symptoms include slow healing infections and foot sores. Further phytochemical investigations involving *Heracleum maximum* and *Thuja occidentalis*, the newly identified plants, are also underway. The ethnobotanical data herein will help to determine the suitability of future bioassays.

The similarities in pharmacopoeia found here will likely ease the transition of traditional medicines into modern facilities. Recommendations that can be made general and standardized will be easier to validate and will likely breed more confidence among

health care practitioners. It is however, important not to forget what makes each communities' pharmacopoeia unique. Recommending plants that are not locally available or used are not necessarily culturally appropriate. For example we can now avoid recommending *Rhododendron tomentosum* ssp. *subarticum* despite its favourable rank in Waskaganish, Nemaska or Mistissini as this would not be in keeping with their respective pharmacopoeias. Recommendations involving a plant found in the given communities but for a purpose outside of the traditional use may also confer cultural inappropriateness.

In reality it is up to the community members to decide what is "culturally appropriate" for their treatment. It may be enough that all treatments come from the Cree of Eeyou Istchee for their trusted use, or perhaps individuals will prefer to only use plants from their own local pharmacopeia. Focus groups which address these questions have been completed and their analysis is underway (Tabib, unpublished). With the information from both of these studies TAAM should be able to make recommendations to communities when positive results are obtained.

The incorporation of traditional medicine should improve the health status of diabetes patients in Eeyou Istchee by having encouraged the community to participate in the development of their own personalized healthcare. It is anticipated that a better understanding and fulfilment of social and cultural needs will decrease the incidence of the disease and the severity of complications suffered. In discussion with community members, we are seeing that diabetes patients want to take charge of their health and that a new sense of confidence in old traditions is bourgeoning. We would like to acknowledge that this project was made possible due to the wisdom embodied by the elders and healers who have lead us throughout these investigations. As an elder once said "you (the scientists) are

paddling the canoe but we the elders are in the back steering and navigating the way". It is with this motto for our collaboration that we continue these studies.

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Chapter 3- *Picea mariana* Bioactivity

Growth Environment and Organ Specific Variation in Cytoprotective Antidiabetic Activities of *Picea mariana*: A Plant Used for Treatment of Diabetes Symptoms by the Cree of Eeyou Istchee (Quebec, Canada)

Ashleigh D. Downing, Andrew Tang, Fida Ahmed, Cory S. Harris, Ammar Saleem, Pierre S. Haddad, Timothy Johns, Alain Cuerrier, John T. Arnason and Steffany A.L. Bennett

Abstract

The Cree of Eeyou Istchee, suffer from a high rate of diabetes and its complications partly due to the incompatibility of the western lifestyle and diabetes regimens with Cree culture. As part of a search for alternative medicine based on traditional practice and pharmacopoeia this project has evaluated the biological activity of alcohol extracts of needle, bark and cone one plant *Picea mariana* (Mill.) Britton, Sterns & Poggenb., in preventing glucose toxicity to PC12-AC cells *in vitro* (a diabetic neurophathy model). All extracts were well-tolerated *in vitro* exhibiting an LD_{50} of 25 µg/ml or higher. Extracts were then tested for their ability to protect PC12 cells from hyperglycaemic challenge at physiologically relevant concentrations of 0.25, 0.5, 1.0 and 2.0 µg/ml. Extracts from all organs tested exhibited a cytoprotective concentration-dependent response. Furthermore, organ-specific protection was habitat and growth environment dependent with plants grown either in bog or forest habitats in coastal or inland environments exhibiting different cytoprotective efficacies. These differences in activity correlated with total phenolic content but not antioxidant activity. Together, these results provide further understanding of the antidiabetic activity of Canadian boreal forest plants identified by the Cree of Eeyou Istchee healers at the cellular level. Their activity is relevant to diabetic peripheral neuropathic complications and shows that their properties can be optimized by harvesting in optimal growth environments.

3.1 **Introduction**

In recent years, there has been increasing recognition of the deteriorating health status of Canadian First Nation peoples with respect to chronic diseases (Newbold, 1998; Tookenay, 1996). Among the chronic diseases afflicting these populations, diabetes has stood out as a literal epidemic (Health Canada, 1999; 2000; Young et al., 2000). Low levels of compliance due to treatment incompatibility has led to high levels of severe complications in these populations (Boston et al., 1997; Gittelsohn et al., 1995; Legaré, 2004; Marbella et al., 1998; Young et al., 2000). The proposed solution, as part of the CIHR Team in Aboriginal Antidiabetic Medicines (TAAM), is to make traditional treatment options readily available alongside conventional treatment (Letendre, 2002; Marbella et al., 1998). To accomplish this goal, semi-structured ethnobotanical surveys have been conducted in Misstisini, Whapmagoostui (Fraser et al., 2007; Leduc et al., 2006), Waskaganish and Nemaska (Chapter 2).

Picea mariana (Mill.) Britton, Sterns & Poggenb., commonly known as black spruce, has emerged in these surveys as a top ranking plant. Specifically, it is a treatment for symptoms related to diabetic neuropathy (Downing in preparation;Fraser et al.2007; Leduc et al, 2006). To date, only antidiabetic activities of a cone extract have been investigated by the TAAM team. The extract showed high antioxidant activity, insulin sensitizing and glitazone-like effects, as well as protection of PC12 cells, a model of peripheral neuronal precursors from high glucose insult (Fraser et al., 2007; Spoor et al., 2006). Given these data and that extracts from needles of *Picea glauca* (Moench) Voss (a closely related species) also protect PC12 cells from hyperglycemic challenge, we compared the cytoprotective efficacy of extracts prepared from three different organs

(needle, bark and cone) of *P. mariana* collected at different geographical locations and ecological conditions. Our primary goal was to establish whether cytoprotective activity was dependent upon habitat and growth environment and to elucidate what the ideal collection conditions might be for this plant. Here we describe how cytoprotective and mitogenic activities differ between extracts sampled from coastal and inland populations (Waskaganish to Mistissini) in areas where bog (low land) met forest (high land). Nine Eeyou Istchee communities across a varied northern Quebec landscape may incorporate the use of traditional pharmacopeia into clinics. The evaluation of local harvesting is essential to determine whether plant organs will yield similar benefits under different growing conditions. The development of this research question has come directly from the elders of Eeyou Istchee who emphasize the importance of plant selection and regional variation.

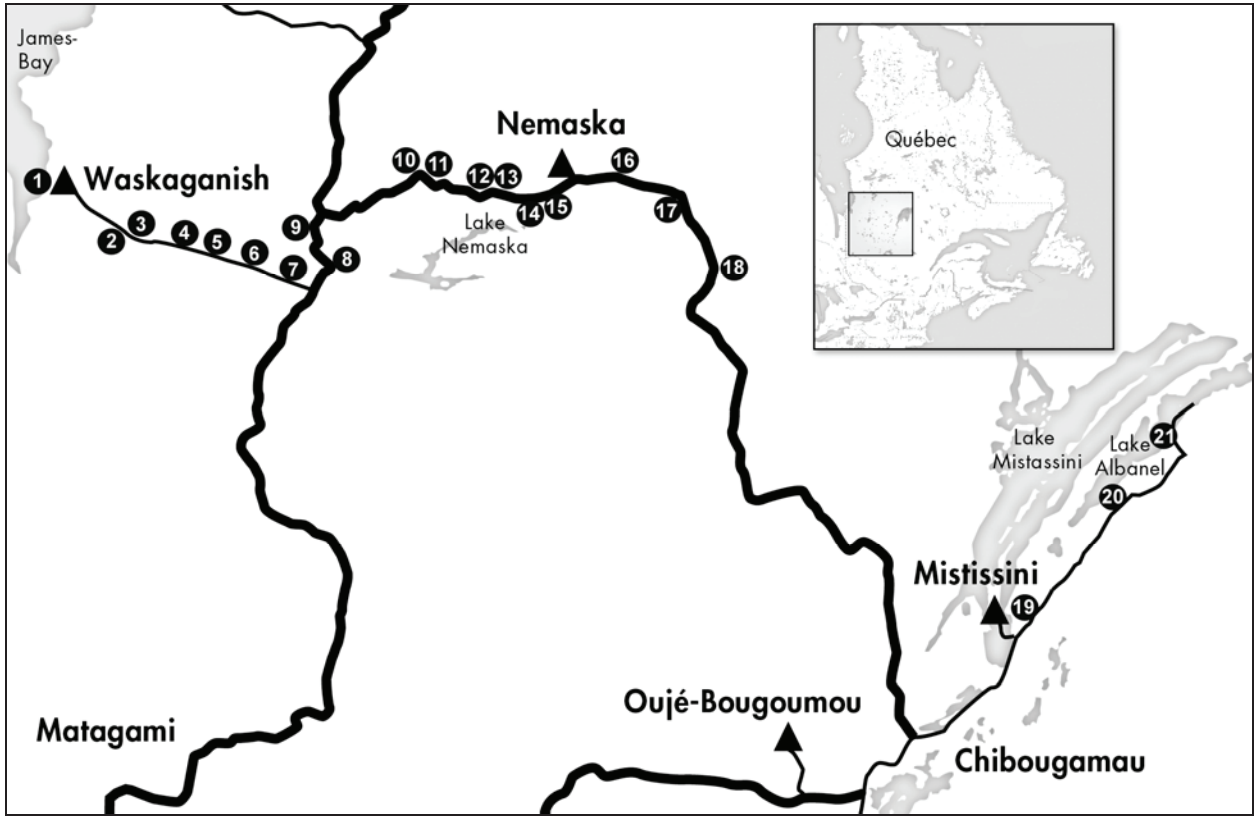

Figure 3.1 Map representing plant sampling locations (white number in black circle). The markers represent both the high and low land locations since they were next to each other. For example the circle labelled "3" represents bog and forest populations 3. At this location materials for B3N, B3B, B3C, F3N, F3B and F3C were collected. Cree communities are represented by a ▲ symbol. This map was simplified using photoshop, from "The Far North Nunavik and Baie-James" tourist map which was sourced from the Base de données géographiques et administratives du Québec (BDGA).

3.2 **Materials and Methods**

3.2.1 Plant Material

P. mariana organs (needle, bark and cone) were harvested in proximity to the Cree communities of Waskaganish, Nemaska and Mistissini located within the 50° and 51° N latitudes (Quebec, Canada). Twenty one populations were selected (Fig 3.1). Plants were harvested at the boundaries of high land (forest) and low land (bog) to enable collection from two distinct habitats (bog or forest) within the same geographical growth environment (measured as proximity to the James Bay coast). Growth environment was considered coastal if it was < 50km from the coast and inland if further. To ensure that habitats were distinct, bog stands were defined as trees growing in > 30 cm sphagnum moss. Forests were defined as having trees growing in organic soil with < 20 cm sphagnum moss ground cover. Separate populations had to be at least 5 km apart and in most cases were much further in order to sample across all three communities. Needles and bark were harvested from ten trees within a 50 m radius for each habitat. Cones were collected from the same trees when available. However they were often unreachable or not present. All bulk materials were air dried upon collection and dried to completion in a plant drier at 40ºC prior to shipment. The dried samples were extracted at the University of Ottawa. Voucher specimens were deposited at the Marie-Victorin, Université de Montréal, Herbarium (MT)(appendix VIIII).

3.2.2 Extract preparation

Twenty populations of needles were extracted. Pooled samples were made by weighing out 0.2 g of material from each tree (10x) resulting in a total of 2 g per location. The 2 g sample was placed in a magic bullet blender (Homeland Housewares) along with 20 ml of 80% ethanol (10 ml/g material) and collected in 50 ml falcon tubes (Fisher). After

blending, the sample was sonicated for 20 min and the first extraction mixture was stored at 4ºC for one week. The ethanol extract was centrifuged for 15 min at 1811 g and the liquid supernatant was removed by pipette and stored at 4ºC. Ten ml of fresh 80% ethanol was added to the pellet, sonicated and stored at 4°C. The second supernatant was then removed and added in the same fashion as the first. Alcohol was then removed from the pooled supernatant using a Labconco Centrivap at 40ºC for 72 h. The alcohol-free extract was lyophilized using a freeze drier (Edwards Pirani 501) for 72 h. The result was a dry stable extract that was homogenized and stored in a freezer at -20ºC. Nine of the twenty populations were included for the analysis of bark extracts. It was necessary in the case of bark and cone to grind samples in a Willey mill (2 mm mesh) tree by tree before evenly pooling the populations. As before 2 g of the pooled, sample was processed using the same protocol as for the needles. In the case of cones, however, only five extracts were prepared by pooling 4 trees per population due to a lack of material. A total of sixty four different extracts were prepared (20 needle bog, 20 needle forest, 9 bark bog, 9 bark forest and 6 cone bog = 64).

Extraction of needles for the isolation of compounds was completed using 1 kg mixed bog and forest black spruce needles. Needles were ground using a Willey mill and a 2 mm mesh. Five liters of 80 % ethanol was added to the needles and left to stir for 24 h. The alcohol was then collected using vacuum-filtration through Whitman paper no 1. An additional 5 L of 80% ethanol was added to the needle filtrate and left to stir for 24 h. Supernatant was collected as before and combined with the first aliquot. Alcohol was removed using a rotary evaporator and samples were then lyophilized. The dry extract was homogenized and stored at 4ºC.

3.2.3 Cell Culture and Bioactivity Assays

Cells of the clonal derivate of the PC12 rat pheochromocytoma cell line (American Type Culture Collection), PC12-AC (Brewer et al., 2002), were maintained in 10 cm dishes with 10 ml complete media (RPMI, 10% horse serum, 5% new born calf serum) at 37ºC with 5% atmospheric CO_2. Complete media provided a normoglucose environment (11 mM glucose). Bioactivity was assessed as previously described (Harris et al., 2008; Harris et al., 2007). To elicit glucose toxicity (hyperglycemia), complete medium was replaced with this same serum-free media supplemented with 150 mM glucose and 0.025% BSA. Following 96 h (hyperglycemia assays) of treatment, WST (Roche Diagnostics) was added to each well and incubated for 60 minutes before spectrophotometric analysis at 420 nm (formazan) and 620 nm (reference). Cultures were compared to cell-free treatment media incubated for the same period. Cell number per well was calculated from standard curves derived from wells containing known cell densities. Standardization allowed data to be compared across replicates. For LD_{50} assessments, each compound was tested at eight different concentrations in quintuplicate over two separate experiments (n=15 per concentration). For hyperglycemia assays, each compound was tested at four different concentrations in a minimum of triplicate measures (n=3-6). Data from control cultures were combined across plates (n=54). Percent viability was calculated as follows: % viability = $\text{cell number}_{(\text{treatment well})}$ / $\text{mean cell number}_{(\text{control})}$. Assigned bioactivities (cytotoxic, cytoprotective, or mitogenic) based on this screen were validated by direct assessment of viable cell number using Trypan Blue hemocytometer counts.

3.2.4 DPPH Antioxidant Assay

Extracts were tested for their radical scavenging activity using the 1,1-diphenyl-2-picryl-hydrazyle radical (DPPH) test as in Owen and Johns, 2002. Ascorbic acid (AA) was used as a control to generate a standard curve for the calculation of its IC_{50}:the concentration where 50% of the DPPH radical is quenched by AA. The following Least squares regression equation was used [$\hat{y}_i - y_0 = \frac{\sum_i \{(x_i - x_0)(y_i - y_0)\}}{\sum_i (x_i - x_0)^2}(x_i - x_0)$] in order to determine the line of best fit for the IC_{50} calculation where y=absorbance at 517nm and x= concentration in ppm. This method is employed over the traditional method of discerning the linear portion of the line visually and we believe it to be a more accurate method of extrapolation. Pure catechin and epicatechin compound was used as positive controls for comparison to crude plant extract samples. Extracts identified as having bioactivity in both screening paradigms and following direct validation were tested to determine whether antioxidant activity correlated with cytoprotection or mitogenicity in hyperglycemic media. Three independent assays were run with samples tested in duplicate.

3.2.5 Total Phenolics Test

The Folin-Ciocalteau method was used as described by Singleton and Rossi (1965), and later modified by Harris et al. (2008). A standard curve was constructed using dilutions of quercetin (Sigma Aldrich Co) at concentrations of 1.0, 0.75, 0.5, 0.25, 0.10, and 0.05 mg/ml. Eight hundred micro liters of Folin reagent was added to 160ul of sample with 540ul 7.5% $NaHCO_3$ and stored in the dark for 2 h. Absorbance was recorded at 725 nm using a spectrophotometer (Molecular Devices SpectraMax 5). Fifteen milligrams of crude extract was re-solubilized in 80% ethanol at 10 mg/ml inside 1.5 ml centrifuge tubes. The

samples were then vortexed until totally dissolved and sonicated for 15 min. These extracts were dyed using the same procedure and the concentration determined using the standard curve.

3.2.6 Fractionation and Isolation of Compounds

Pooled needle extracts (100 g) containing bog and forest needles were adsorbed on 200g of silica gel (200-300 nm) in the presence of 100% methanol. Absorbed extract was left to air dry for 2 days before being placed on the top of a column (60 cm x 10 cm). In order to complete the fractionation process, hexane, ethyl acetate and methanol were used as mobile phases. The first phase run was 2 L of 100% hexane and progressed to 2 L of 90:10 (hexane:ethyl acetate). Each subsequent addition reduced hexane by 10% until 100% ethyl acetate was attained. The same procedure was followed with 100% methanol. Fractions were collected in 200 ml aliquots. Solvent from each fraction was evaporated and remnants were separated using silicon plate thin layer chromatography.

3.2.7 HPLC

Samples were prepared by dissolving 40 mg of crude extract in 1 ml of methanol (Sigma Aldrich). A standard of 600 μl of resveratrol (1 mg/ml) (Sigma, St Louis, MO) was added to 500 μl of extract to make a total volume of 1100 μl. The mixture was filtered through a 0.2 μm PTFE nylon membrane filter (Chromatographic Specialities Inc., Brockville, ON, Canada) and sonicated for 5 min before injection.

Needle, bark and cone extracts were characterized by HPLC-DAD analysis using a novel method for the identification of phenolic metabolites. Analyses were performed on an Agilent 1100 HPLC-DAD (Palo Alto, CA, USA) comprising an autosampler, a quaternary pump, a column thermostat, and a photodiode array detector (DAD). Separations were

performed on an YMC ODS-AM column (100 ×2 mm i.d.; 3 μm particle size) (Waters, Mississauga, Canada) at 55ºC and a flow rate of 0.3 mL/min. The elution conditions consisted of HPLC grade water (Sigma) in solvent A and acetyl nitrile (Sigma) as solvent B with initial conditions starting at 85:15 (%A:%B) at the elapsed time of 0 min, 35:65 at 15 min, 0:100 at 15.1 min and held until 20 min. The injection volume was 1 μL of extract individually injected through a 100 μL loop of the autosampler. The chromatographic separation was monitored at 280 nm by DAD. The internal standard, resveratrol, was tested for comparison to ensure there was no interference between it and the eluting compounds. Pungenin's identity was confirmed in needles using this HPLC method and in comparing values to the literature. The isolated pungenin was used as an external standard for HPLC analysis of crude needle extract.

3.3 **Results**

3.3.1 Bioactivity

Upon extraction, 18%, 25% and 30% of dried needles, bark and cone were sequestered. For each milligram of that extract, 146.5$\pm$4.2 µg (needles), 270.3$\pm$13.9 µg (bark) and 398.7$\pm$48.8 l µg (cone) were phenolic compounds. Each extract was screened at 0.25-2 µg/ml for capacity to alter glucotoxicity *in vitro* (Appendix V, VI and VII, Tables 3.1-3.3). Glucotoxicity reduced the number of viable cells to 56.8% that of normoglucose controls (Appendix v, vi, vii, C vs. HG). Samples were attributed biologically active if at least two or more concentrations elicited a statistically significant change in viable cell number. Compounds that increased viable cell number to values significantly higher than the normoglucose controls (> 100%) were considered mitogenic. Compounds that significantly protected cells from high glucose toxicity (56.8%) without exceeding

normoglucose viability values were considered cytoprotective; compounds that enhanced glucotoxicity at any concentration were considered cytotoxic. Maximal test concentrations were at least one log concentration below their LD_{50} in normoglucose media (data not shown).

Table 3.1 Bioactivity of *Picea marianna* needle extracts in hyperglycemic media.

Extract ID[a]	**Bioactivity in hyperglycemic media**[b]
1FN	Cytoprotective
1BN	None
2FN	None
2BN	None
3FN	None
3BN	None
4FN	None
4BN	None
5FN	None
5BN	None
6FN	Cytoprotective
6BN	Cytoprotective
7FN	None
7BN	Cytoprotective
8FN	Mitogenic
8BN	Mitogenic
9FN	None
9BN	Cytoprotective
10FN	Cytoprotective
10BN	None
11FN	None
11BN	None
13FN	None
13BN	None
14FN	None
14BN	None
15FN	None
15BN	None
16FN	Cytoprotective
16BN	None
17FN	Cytoprotective
17BN	Cytoprotective
18FN	Cytoprotective
18BN	Cytoprotective
19FN	None
19BN	Cytoprotective
20FN	None
20BN	None
21FN	Cytoprotective
21BN	None

[a]The extract ID code defines the growth environment (Area#1-21, Fig 3.1.), habitat (B, Bog or F, forest) and organ (N, needle). For example, 1FN is an extract prepared from needles collected in a forest habitat at location #1.

[b]Bioactivity was classified comparing a 96 h treatment in hyperglycemic (150 mM) serum-free media. Viable cell number following extract treatment was established using the WST assay compared to standard curves of known cell number. Vehicle control was 0.1% DMSO. Compounds that at two or more concentrations (a) increased viable cell number to values significantly higher than the normoglucose controls (> 100%) were classified as mitogenic, (b) significantly protected cells from high glucose toxicity without apparent mitogenic activity were classified as cytoprotective, or (c) enhanced glucotoxicity were classified as cytotoxic. Statistics were ANOVA, *post-hoc* Tukey tested vs. vehicle-treated cultures in normo- or high glucose media. All concentration-response data and statistical analyses are presented in Appendices v, vi and vii.

Table 3.2 Bioactivity of *Picea marianna* bark extracts in hyperglycemic media

Extract ID[a]	Bioactivity in hyperglycemic media[b]
1FB	Mitogenic
1BB	Mitogenic
2FB	Cytoprotective
2BB	Cytoprotective
3FB	Cytoprotective
3BB	Cytoprotective
4FB	Cytoprotective
4BB	Cytoprotective
5FB	None
5BB	None
6FB	Cytoprotective/Mitogenic
6BB	Cytoprotective/Mitogenic
7FB	Cytoprotective
7BB	Cytoprotective
11FB	None
11BB	Cytoprotective
14FB	None
14BB	None

[a]The extract ID code defines the growth environment (Area#1-21, Fig 3.1.), habitat (B, Bog or F, forest) and organ (B, bark). For example, 1FB is an extract prepared from bark collected in a forest habitat at location #1.

[b]Bioactivity was classified using a 96 h treatment in hyperglycemic (150 mM) serum-free media. Viable cell number following extract treatment was established using the WST assay compared to standard curves of known cell number. Vehicle control was 0.1% DMSO. Compounds that at two or more concentrations (a) increased viable cell number to values significantly higher than the normoglucose controls (> 100%) were classified as mitogenic, (b) significantly protected cells from high glucose toxicity without apparent mitogenic activity were classified as cytoprotective, or (c) enhanced glucotoxicity were classified as cytotoxic. Samples classified with dual bioactivities (ie. Cytoprotective/Mitogenic) are possible when two concentrations satisfy our definition of one bioactivity (ie. Cytoprotective) and two other concentrations from the same sample are in another category (ie. Mitogenic). Statistics were ANOVA, *post-hoc* Tukey tested vs. vehicle-treated cultures in normo- or high glucose media. All concentration-response data and statistical analyses are presented in Appendices v, vi and vii.

Table 3.3 Bioactivity of *Picea marianna* cone extracts in hyperglycemic media.

Extract ID[a]	**Bioactivity in hyperglycemic media**[b]
3BC	Cytoprotective
7BC	Cytoprotective
11BC	Mitogenic
12BC	Mitogenic
14BC	None
15BC	None

[a]The extract ID code defines the growth environment (Area#1-21, Fig 3.1.), habitat (B, Bog or F, forest) and organ (C, cone). For example, 1BC is an extract prepared from cones collected in a bog habitat at location #1.

[b]Bioactivity was classified comparing a 96 h treatment in hyperglycemic (150 mM) serum-free media. Viable cell number following extract treatment was established using the WST assay compared to standard curves of known cell number. Vehicle control was 0.1% DMSO. Compounds that at two or more concentrations (a) increased viable cell number to values significantly higher than the normoglucose controls (> 100%) were classified as mitogenic, (b) significantly protected cells from high glucose toxicity without apparent mitogenic activity were classified as cytoprotective, or (c) enhanced glucotoxicity were classified as cytotoxic. Statistics were ANOVA, *post-hoc* Tukey tested vs. vehicle-treated cultures in normo- or high glucose media. All concentration-response data and statistical analyses are presented in Appendices v, vi and vii.

We found that 50% of all organ extracts improved viability of PC12 cultures under hyperglycemic conditions. Moreover, all extracts were well-tolerated in that none of them extracts enhanced glucose cytotoxicity. We identified twenty-four cytoprotective (38% of total) and seven mitogenic (9% of total) extracts. Two extracts (3% of total) exhibited both cytoprotective and mitogenic activities dependent upon the concentration tested (Table 3.1, 3.3, Appendix v, vi, vii). When comparing the overall bioactivity of extracts prepared from needle, bark and cone collected at all regions and habitats, we found that protective effects were maximal at the lowest concentrations tested in all cases and were not organ-specific

with the exception that bark and cone were more effective mitogens in high glucose media than needle extracts (Tables 3.1-3.3, Appendix v, vi, vii, Fig 3.2).

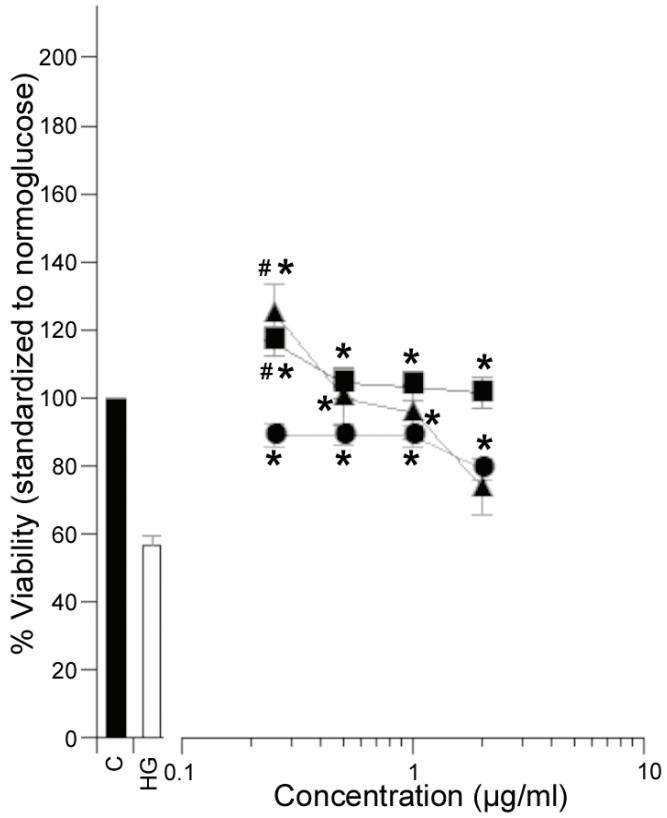

Figure 3.2 Comparison of cytoprotective and mitogenic activities of *P. mariana* needle (●), bark (■) and cone (▲) extracts. MANOVA analysis was used to average all extracts based on their organ type and concentration. Significant cytoprotection (*) or mitogenic activity (#) was afforded when Dunnett post hoc tests were $p \leq 0.05$ as compared to the normoglucose (**C**) and high glucose (**HG**) controls. All organs showed significant protection at all concentrations except cone at 2 ug/ml (needle n= 120, bark n= 60, cone n=18).

3.3.2 Regional Variation

A primary concern of the TAAM team is to provide laboratory data assessing the importance of plant selection and regional variation emphasized by the elders and healer of the Eeyou Istchee in their traditional preparations. Their insights are crucial to ensuring that plant usage will yield similar benefits in all communities. We found that fifty percent of *P. mariana* needle, bark, and cone extracts (32 extracts) did not exhibit significant biological

activity in our screening paradigm. To test whether extract efficacy depended upon the growth habitat where the plant had been harvested, we compared efficacy of needle and bark extracts collected in bog and forest habitats. Cone extracts were not included in this analysis as we were only able to collect cones from bog sites.

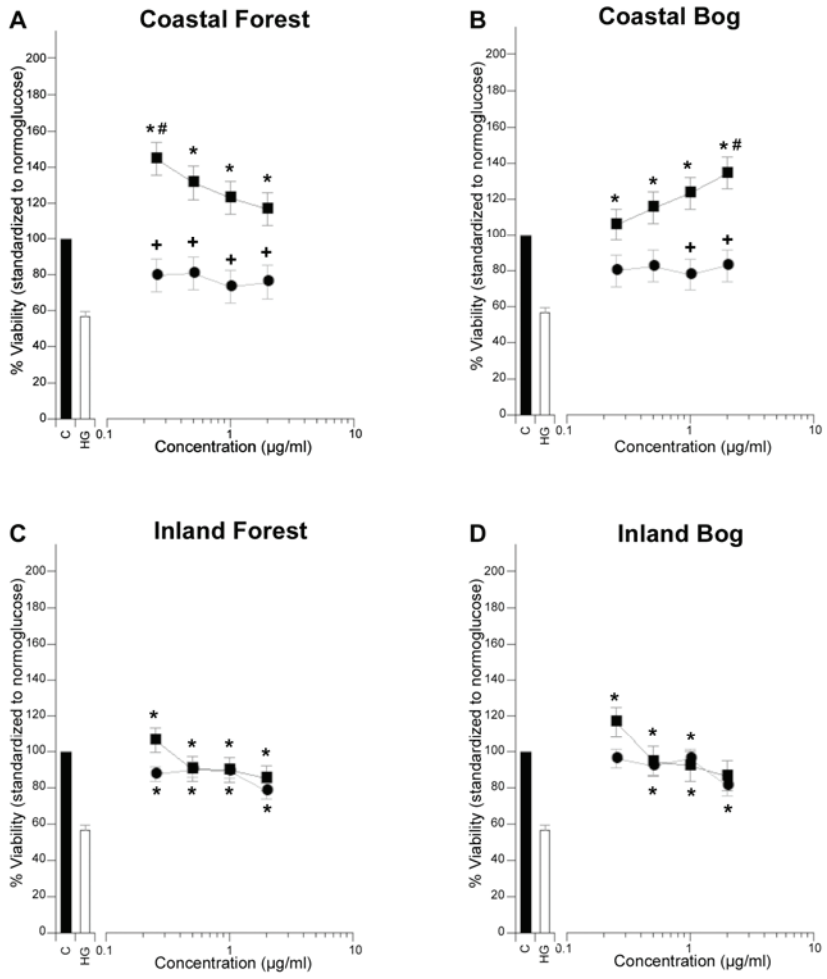

Figure 3.3 Comparison of cytoprotective and mitogenic activities by growth environment. MANOVA analysis was used to determine the mean viability with respect to habitat (forest/ bog), growth environment (coastal/inland), organ type (Needle = ●, Bark = ■) and concentration (0.25, 0.50, 1.00, 2.00ug/ml) interactions. Significant protection (*) or mitogenic activity (#) was identified by Dunnett post hoc tests ($p \leq 0.05$) by comparison to the (**C**) normoglucose or (**HG**) high glucose controls respectively. The same p value was used to denote a significant difference between needle and bark (+) at specified concentrations. (**A**) Comparison of bark and needle extract protection in coastal forest environments (bark n= 12 needle n= 12). (**B**) Comparison of bark and needle extract protection in coastal bog environments (bark n= 12, needle n= 12). (**C**) Comparison of bark and needle extract protection in inland forest environments (bark n= 18, needle n= 51). (**D**) Comparison of bark and needle extract protection in inland bog environments (bark n= 18, needle n= 45).

We did not detect any statistically significant differences between the bioactivites of needle and bark extracts grown in different habitats (data not shown). To test whether

growth environment and habitat interacted to produce organs with different bioactivities, samples collected in different growth habitats, were grouped into coastal or inland environments (Fig 3.1). This analysis revealed a statistically significant interaction between growth environment, habitat, and organ bioactivity (Fig 3.3). Extracts prepared from bark collected in coastal environments showed cytoprotection where needles extracts did not (Fig 3.3 A & C). Moreover, in coastal-derived extracts, activity was maximal (and concentration-dependent) at low concentrations in extracts prepared from bark collected in a forest habitat (Fig 3.3 A) and at high concentrations in extracts prepared from bark collected in a bog habitat (Fig 3.3 B). At their highest percent viability extracts were even mitogenic. This interaction was organ-specific. Neither growth environment nor habitat altered the cytoprotective efficacy of needle extracts (Fig 3.3 A-D).

3.3.3 Total Phenolics and Antioxidant Activity

These data suggest that the quantity of biologically active compounds are responsible for organ-specific, habitat dependant cytoprotective bioactivities of the extracts. It has previously been suggested by Spoor et al. (2006), that antioxidant activity is responsible for antidiabetic activities in *P. mariana* cone extracts. To further explore this hypothesis, we measured total phenolic content in the needle, bark and cone extracts (Fig 3.4) and correlated this content with the antioxidant activity of selected extracts using the Folin-Ciocalteau total phenolics method and DPPH antioxidant tests employed in previous TAAM studies (Spoor et al., 2006).

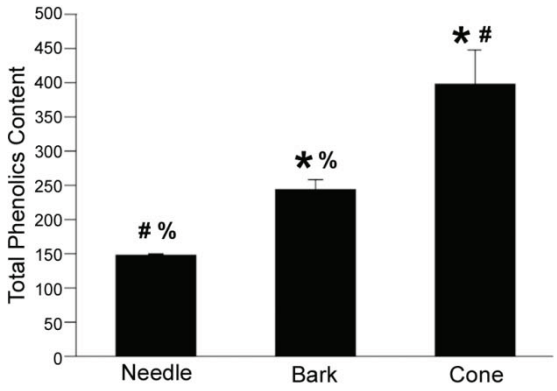

Figure 3.4 Total phenolic content in terms of quercetin equivalents, of *P. mariana* needle, bark and cone extracts. Needle, bark and cone phenolic content was found to be significantly different from each other at $p \leq 0.05$ ((*) = significantly different from needle; (#) = significantly different from bark; (**%**) = significantly different from cone). Cone had the highest phenolic content with 398.68 (SE = 48.77, n = 5), followed by bark at 244.08 (SE = 14.51, n = 18) and needle with 146.34µg/milligram extract (SE = 3.78, n = 40).

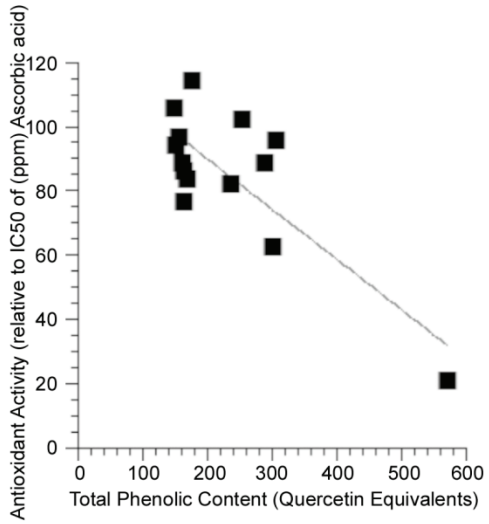

Figure 3.5 Regression analysis of total phenolic content with respect to antioxidant activity. Total phenolic content is measured in terms of quercetin equivalents and antioxidant activity is calculated with respect to the ascorbic acid standard curve. The trend shows that as phenolic content increases the amount of plant extract (EC_{50} in ppm) necessary to reach the IC_{50} of ascorbic acid decreases and therefore, antioxidant activity increases with increasing phenolic content ($r^2 = 0.63$, $p \leq 0.05$, n = 14). The data passed a normality test in order to ensure that it follows a normal distribution.

We found that the combined phenolic content was highest in cone extracts and lowest in needle (Fig 3.4). DPPH antioxidant assays were completed on fourteen selected extracts and plotted with respect to phenolic content (Fig 3.5). A significant correlation between antioxidant activity and total phenolic content was detected (r^2= 0.63, $p<0.05$, Fig 3.5) Total phenolic content positively correlated with bioactivity (r^2= 0.30, p=0.05, Fig 3.6, Tables 3.1-3.3). Antioxidant activity however, was not correlated with bioactivity (r^2=0.046, p=0.460, Fig 3.7). Taken together, these results suggest that, while the phenolic content of organ extracts prepared from plants harvested in different habitats and growth environments likely underlies bioactivity, the oxyradical scavenging capacity of these phenolics is not the primary mechanisms of action.

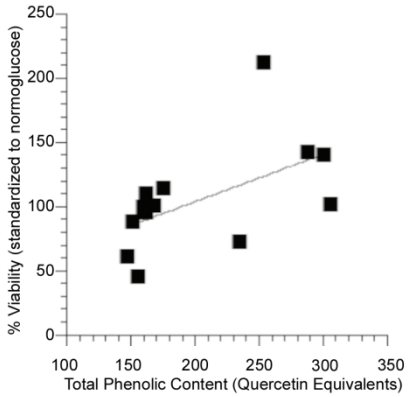

Figure 3.6 Regression analysis of total phenolic content with respect to PC12-AC bioactivity in high glucose media. The trend shows that as phenolic content increases the percent viability increases ($p\leq0.05$, n=13).

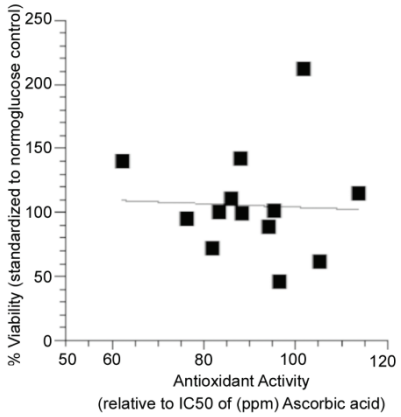

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Figure 3.7 Regression analysis of antioxidant activity with respect to PC12-AC bioactivity in high glucose media. Antioxidant activity is measured with respect to ascorbic acid and bioactivity is represented as percent viability with respect to normoglucose control. We do not see any kind of a trend here so antioxidant activity is not likely the main mechanism of protection ($p = 0.89$, $n=13$).

3.4 **Discussion**

There is a paucity of strategies effective in managing chronic pain, infection, paralysis, and loss of sensation association with peripheral diabetic neuropathy (Kuzmina et al., 2008; Legaré, 2004). Based on our ethnobotanical studies, we have observed that preparations from various organs of *P. mariana* are used to treat slow healing infections, sores and numbness (particularly of the extremities), all symptoms related to diabetic neuropathy. We demonstrate for the first time that *P. mariana* needles and bark are effective protectors of peripheral neuronal precursor survival when exposed to hyperglycemic conditions. We confirm the protection of cones as was first demonstrated by Spoor et al. (2006). We have also shown that harvest location can impact the antidiabetic bioactivities of *P. mariana*, particularly for bark extracts, which tended to be more effective when obtained from inland forest populations than from to coastal and inland bog populations. In general the strength of the observed activity of needle, bark and cone is correlated with the total phenolic concentration of the extracts. The one notable exception

was a cone extract that was suspected to have caused oxidation, a negative response, in PC12-AC cells. The presence of too many antioxidants can actually be harmful, resulting in oxidation and therefore a decrease in cell survival. This is something that was observed in preliminary DPPH tests at higher extract concentrations (Diouf et al., 2009).

These *in vitro* findings validate the importance of *P. mariana'*s role as part of the Cree pharmacopoeia and underline the importance of plant selection and extract strength. In a review of Canadian aboriginal plant use, Marles et al. (2000), has reported that this plant is used by the Cree to treat heart problems, high blood pressure, diarrhea, chicken pox, ear and skin infections, rashes, scabies, shortness of breath, sore eyes, sore mouth, sore stomach and sore breasts from breastfeeding. Here we have added, to TAAMs ethnobotanical and *in vitro* findings (Fraser et al., 2007; Leduc et al., 2006; Martineau et al., 2010; Spoor et al., 2006) by contributing positive evidence that boreal plants from the Cree pharmacopoeia are capable of treating diabetes symptoms. Specifically we have shown at the cellular level, that this traditional medicine may be effective in the treatment of a "new" disease afflicting First Nation peoples, namely the potential to manage diabetic peripheral symptoms by protecting peripheral neurons and their precursors from hyperglycemic insult.

These results are similar to those found in Harris et al., (2008) for *P. glauca,* except that all *P. mariana* organs showed protection. In contrast to Harris et al. (2008) findings, *P. mariana* needles were generally the lowest protector against diabetic insult. One factor that should be re-examined in the case of *P. glauca* is concentration range. It may be necessary to test bark and cone at much lower concentrations than 10 µg/ml. This is recommended because our graphs often showed a decreasing dose response from the lowest concentration

to the highest (figure 3.2). Conversely, given strong correlations in both studies with total phenolic content, these data could also suggest species differences in the phenolic constituents of the different organs that underlie the cytoprotective and mitogenic bioactivities. This is a good argument for the comprehensive fractionation based assays of organs collected at different locations and under different growth habitats in order to identify active components.

To this end, we have initiated preliminary HPLC fractionation and isolation of compounds to begin the required bioassay-guided fractionation necessary to test this hypothesis. We have found pungenin and several pungenin-like compounds to be the main constituents of needle extracts, yet absent from bark and cone extracts (unpublished data; see also Harris et al., (2008) for similar results with extracts from *P. glauca*). Pungenin also known as the glycoside of 3,4 dihydroxyacetophenone was first isolated and identified from the leafy branches of *Picea pungens* Engelm or Colorado spruce (Neish, 1957). It was later identified in *Picea glauca* as well and found to be seasonally variable, having higher quantities in the winter than in the summer (Neish, 1958). More recently pugenin has been identified in *Ficus septica* Burm f. a plant often employed in folk medicine of the Western Pacific, thought to have anticancer and anti-inflammatory potential (Lansky et al., 2008; Ueda et al., 2009). This is interesting because inflammation is a symptom associated with obesity related diabetes (Wellen and Hotamisligil, 2006). Anti-inflammatory activities have already been shown for *Picea mariana* by Diouf et al. (2009) so it would be interesting to see if pungenin is responsible for some of this activity. This has particular relevance to us as it is also highly soluble in hot water and is likely easily decocted, a method often employed by traditional practitioners (Neish, 1957).

Primary fractionation of *P. mariana* bark samples did not yield any pure compounds but close examination of the chromatographs indicated that these extracts are likely enriched in stilbenes. Stilbenes are a group of biologically active molecules previously identified in *Abies*, *Picea*, *Pinus*, *Juniperus*, *Rheum* and *Morus* species (Chrzascik, 2009). These compounds have been shown to, improve blood circulation, lower cholesterol, have estrogenic effects, stimulate protein synthesis, have fungicidal activities, stimulate bone and cartilage development, have anti-cancer activities and are strong antioxidants (Chrzascik, 2009). Chromatographs suggest that there are similarities between the stilbenes found in *Picea mariana* and that of the most widely studied stilbene resveratrol, a known cytoprotector found in red wine (Fremont, 2000). For cones, chromatograms suggest high concentrations of tannins and this will require further mass spectrometry analysis for isolation and identification. While still preliminary, this approach can identify the bioactive compounds that underlie species differences as well as reflect the observed growth environment and habitat impact upon extract efficiency.

Our data are also consistent with the commercial application of Pinaceae family members as sources of antidiabetic pharmacetucials. Pycnogenol® is made from the bark of *Pinus pinaster* Aiton and has been purported to exhibit antidiabetic activity. In clinical trials with diabetic patients, Pycnogenol® has shown an ability to reduce blood glucose levels, cardiovascular risk factors and, more interestingly for this study, to halt the progression of and even improve retinopathy (Liu et al., 2004; Spadea and Balestrazzi, 2001; Zibadi et al., 2008). As *P. mariana* and *P. pinaster* are in the same family we would expect their phytochemistry to be similar and perhaps have comparable bioactivities. Diouf et al., (2009) have indeed shown the phyochemistry of *P. pinaster* and *P. mariana* to be

comparable. Morevoer, it is the proanthocyanidin rich fractions that appear to be of interest, as these biomolecules make up the bulk of the Pycnogenol® supplement. Further, *P. mariana* extracts have been shown not only to be good antioxidants, as demonstrated here, but also confer anti-inflammatory protection (Diouf et al., 2009; Walshe-Roussel et al., 2009), which may provide further avenues for diabetes treatment. For example, TNF-α, an inflammatory cytokine, which is overproduced in the adipose tissues of obese individuals and in the presence of high levels of free fatty acids is associated with insulin resistance (Shoelson et al., 2006; Wellen and Hotamisligil, 2006). In this case TNF-α inhibits insulin action by blocking the downstream signaling from the insulin receptor. In chapter one we discussed how this same cytokine is simulated by the production of ROS (Tiwari and Rao, 2002). Plants which could prevent the chronic low level inflammatory responses that are associated with obesity, would make excellent candidates for diabetes treatment by preventing or alleviating insulin resistance and this may be the case for *Picea mariana* (Walshe-Roussel et al., 2009; Wellen and Hotamisligil, 2006).

We further show that extract efficacy depends upon careful selection of plant organs depending upon growth environment and habitat as indicated by the Cree elders and healers of Eeyou Istchee. This is an important finding as it supports the efforts of traditional practitioners in ensuring equivalent benefits across communities. Variation in activity by growth environment was observable mainly for bark. In this case, we recommend inland populations or, if harvesting in proximity to the coast, to choose forest sites instead of bog sites. Conveniently, this is easier ground to navigate and a less fragile habitat. However, if a bog is the only site available for harvesting, it may be possible to achieve the same level of protection in coastal sites, for example, by simply using more plant material per treatment

preparation. In the case of needles, neither growth environment nor habitat altered the cytoprotective efficacy of extracts, so no special recommendations are warranted. A lack of specificity should allow collectors to avoid over harvesting in one area and therefore, decreases the possibility of harmful environmental impacts.

3.5 **Conclusion**

P. mariana is an effective protector of peripheral neuronal precursor survival when cells are subjected to high glucose conditions. All organs tested in this diabetic neuropathy model, that is needles, bark and cone, proved to be efficacious. As all organ parts are protective and the boreal forest of Canada is highly populated by this tree species, we believe *P. mariana* is an excellent candidate for a renewable source of medicine. Further *in vitro* investigations are necessary to elucidate the underlying mechanisms of action and phenolic variation. In vivo, tests in diabetes specific animal models are also needed to confirm that bioactivity remains upon ingestion. Of course the successful application of this study's findings will also depend on the continuation of an already interdependent relationship developed by the TAAM team, health care professionals and the Eeyou Istchee communities. As the bonds we have built are strong, we are confident that not only *P. mariana* but many of the plants from the Cree pharmacopeia will become part of a new holistic form of diabetes treatment, culturally acceptable to the Cree of Eeyou Istchee and supported by traditional practice and evidence-based inquiry.

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Chapter 4 – Conclusion

Here we have contributed to the recording of medicinal plant uses for two Cree communities. We have identified two new plants, *Heraculeum maximum* and *Thuja occidentalis*, for the treatment of diabetes symptoms and have provided new avenues for *in vitro* analysis. The relationships between community pharmacopeias have been examined, which has increased our understanding of shared knowledge, while giving us an appreciation for what makes them unique. In Waskaganish, *Thuja occidentalis, Heracleum maximum* and *Sarracenia purpurea* were used exclusively in comparison to Nemaska, where the use of *Kalmia angustifolia* was distinct. However, what makes these two communities different from each other was what made them similar with reference to the other communities. For example, both *Sarracenia purpurea* and *Kalmia angustifolia* had been identified as medicinal plants in Mistissini (Leduc et al., 2006). With respect to plant importance as measured by SIV ranking and evaluated by the Spearman rank test, ranking of common plants was similar in all community comparisons when *Picea glauca* was not considered. This plant likely represents a difference of importance based on abundance. It would be interesting to go deeper in elucidating why plants are similarly ranked or otherwise using floristic and geographical data analysis. Plant use with respect to symptoms and citation number (i.e. Podani algorithm) was also similar for all community comparisons except Nemaska/Whapmagoostui. Differences here were likely magnified due to the small sample size and therefore low number of mentions of fewer plants in Nemaska. For this reason we conclude that the Spearman rank test and Podani algorithm has provided positive evidence for our H1 hypothesis. Mantel analysis provided contradictory results to the Podani analysis, only showing a correlation between Nemaska/Waskaganish. It is likely that this method was not well suited for this analysis but we cannot ignore these

results(Dutilleul et al., 2000). It would be useful to either try using squared Euclidean distance in the Mantel test as described by Dutilleul et al. (2000) or to find some other means of comparing presence or absence of use for these communities in order to confirm our suspicions. The development of a method which takes into account how many community members have been consulted and somehow weighs each mention accordingly may be helpful. In this respect, this study has served to highlight the utility of some statistical methods and the limitations of others for the field of ethnobotany.

Further to this, symptom-species correlations have been examined for the purpose of *in vitro* investigation. These correlations have given us the opportunity to examine relationships for the two new communities and for all four pooled pharmacopeias. Overall we can say that there are many plants which are associated with several symptoms of diabetes and this is demonstrated by the clumping of species and symptoms in the overall correspondence analysis (figure 2.7, appendix IV). Specifically strong relationships were observed in Waskaganish correspondence analysis for *Alnus incana* ssp *rugosa* (abscesses and boils), *Populus balsamifera* (abscesses and boils), *Thuja occidentalis* (heart/chest pain) and *Abies balsamea* (infections). Likewise in Nemaska *Larix laricina* had strong ties to the infection symptom. In both of these communities and in the pooled analysis *Abies balsamea* was well linked to vision problems.

Despite the value of these analyses, it is our position that all mentions of plant use be taken into account, as much information has been lost with the passing on of the previous generation in these communities. Therefore a lack of citation may not necessarily mean a lack of importance or efficacy. Although we have broached the topic of symptom-species relationships statistically, the specific mentions of preparation, animal uses and

their related symptoms have been left out. This information is considered either sacred or the types of uses mentioned were outside the scope of this report and have therefore been omitted. In the future we hope to not only put the aforementioned findings to use through the incorporation of traditional medicine but also to give all the knowledge, not just that represented here, back to the community in a comprehensive way. As a contribution to the field of ethnobotany this study is representative of work done entirely in cooperation with the community and should serve as an example for future ethnobotanical investigations.

In the second half of the report we have validated the utility of an ethnobotanical approach in identifying plants for the treatment of type II diabetes (Oubré et al., 1997). We demonstrate that *Picea mariana*'s high SIV ranking translated into cytoprotection of peripheral neuronal precursors from high glucose insult. All plant organs, needles, bark and cone, conferred this protection, but it was sometimes dependent upon growth environment and habitat. For instance organ specificity was observed in that protection in coastal environments was only significant for bark and not needles. Again in coastal conditions, cytoprotection of bark extracts were stronger when source material was harvested from forests rather than bogs. This disproves our H2 hypothesis that bog environments would have stronger activities than forest habitats. Likely the compounds that are protective in the PC12 bioassays are not triggered by stresses from low levels of nutrients or acidic conditions characteristic of bogs (Zoltai and Vitt, 1995). Further studies which quantify environmental fluctuations between the two habitats would be necessary for comparison to biologically active phytochemical fluctuations to determine specific cause and effect conclusions. The findings from chapter three therefore hold significance in the field of ethnobotany as well as chemical ecology.

The differences highlighted in the bioassays were predicted to exist by the Elders. This emphasizes the importance of following up on the guidance of community members in these types of study projects and holds relevance for any situation in which people are trying to incorporate traditional medicines into clinics. Further, variations highlighted here underline the importance of conducting similar experiments tailored to the biology of any plant being proposed for incorporation. Specifically for *Picea mariana,* the next step will be to further evaluate its active compounds and drug interaction biology *in vitro* as well as its overall bioactivity *in* vivo (Tam et al., 2009). Thus far we have identified pungenin in the needles and stillbene like compounds in the bark but we still need to examine their bioactivity. Total phenolic content and its associated antioxidant strength has also been evaluated but only the phenolic content held a direct correlation to bioactivity. One of the shortcomings of this study would be the lack of an in depth phytochemical analysis, which would be necessary for standardization and validation purposes. It should, however, be reiterated that it is not the goal of TAAM to develop alternative medicines or pharmaceuticals. However, it has been left open to the Cree of Eeyou Istchee to determine if steps will be taken towards the development of plant remedies for this purpose.

Several elders have expressed willingness and interest in the widespread use of resulting remedies. Emphasis has been that the development of any alternative medicine for the Cree or other peoples should be done similarly in respect of the traditional knowledge and related ideals. *Picea mariana* could accommodate distribution outside of Eeyou Istchee populations as there is a wealth of resources to draw from in the North American boreal forests. These forests cover six million square kilometres and are dominated primarily by black spruce (Begin and Filion, 1999; Karst, 2010). As needle, bark and cone all conferred

protection at small concentrations, this represents a large quantity of available plant material. Furthermore this abundance of material would make the development of sustainable harvesting possible and as we already have a model in Pycnogenol®, it should be easier to put into practice (Rohdewald, 2002).

For the time being observational studies that follow healer treatment of diabetic volunteers in Mistissini are taking place. *In vitro* results like the *Picea mariana* study serve to translate the efficacy of the plants chosen by the Cree healers. Thus far healer centered treatment in which solely healers administer traditional medicine is the goal, however it is important to realize this is the ideal situation. Without the training of new traditional practitioners within the participating communities healer centered treatment can only last as long as the existing healers are able to practice. Despite renewed interest in traditional medicine within the communities (personal communication), time is running out and there are only a few traditional practitioners and many patients. It is likely that the double burden of teaching new practitioners and treating patients is too much for already ageing healers. For this reason alternative forms of plant based medicine based on traditional practice become more important and it is necessary that through these studies we garner the trust of health care practitioners. In any case, whether it be in the form of healer consultation or delivery by a health care practitioner the hope is that these treatments improve the quality of life in these communities by providing culturally appropriate holistic diabetes management.

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Appendix I

Université de Montréal

COMITÉ D'ÉTHIQUE DE LA RECHERCHE DE LA FACULTÉ DES ARTS ET DES SCIENCES (CÉRFAS)

CERTIFICAT D'ÉTHIQUE

Le Comité d'éthique de la recherche de la Faculté des arts et des sciences, selon les procédures en vigueur, a examiné le projet de recherche intitulé :

« Inter and intra-specific differences of medicinal plant use among the First Nations Elders of Waskaganish and Nemaska »

et soumis par : ***Ashleigh Downing, étudiante à la maîtrise, Département de sciences biologiques***

Le Comité a conclu que la recherche proposée respecte les règles d'éthique énoncées à la « Politique sur la recherche avec des êtres humains » de l'Université de Montréal.

Tout changement anticipé au protocole de recherche doit être communiqué au CÉRFAS qui devra en évaluer l'impact au chapitre de l'éthique afin de déterminer si une nouvelle demande de certificat d'éthique est nécessaire.

Toute interruption prématurée du projet ou tout incident grave devra être immédiatement signalé au CÉRFAS.

[redacted]

Jean Decarie, président
Comité d'évaluation accélérée

[redacted]

Gilbert Renaud, président
CÉRFAS

Date de délivrance : 27 MAI 2008

Appendix II

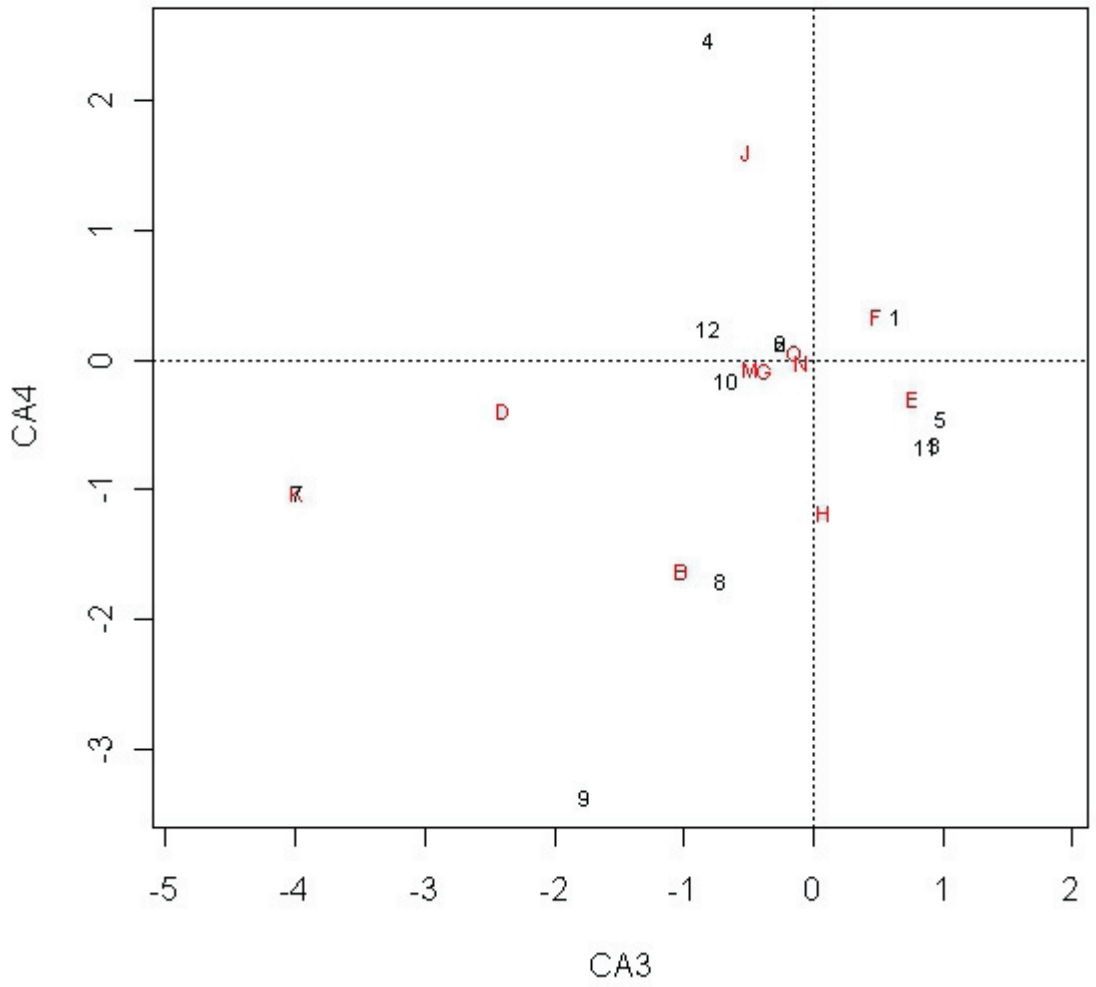

Figure 2.5 Two dimensional (3rd and 4th dimension) scatter plot resulting from the correspondence analysis of plant species mentioned in Waskaganish interviews (1-12) and diabetes symptoms (A-O). Symptoms and species which are close to one another are closely associated. Plant species; 1. *Abies balsamea*, 2. *Populus balsamifera*, 3. *Picea mariana*, 4. *Thuja occidentalis*, 5. *Heracluem maximum*, 6. *Alnus incana* spp. *rugosa*, 7. *Pinus banksiana*, 8. *Rhododendron groenlandicum*, 9. *Sorbus decora*, 10. *Sarracenia purpurea*, 11. *Larix laricina*, 12. *Picea glauca*. Symptoms; A. Increased Thirst, B. Urination, C.Increased Appetite, D.Weakness, E.Slow Healing Infections, F.Blurred Vision, G.Foot sores/numbness, H.Diarrhea, I.Rheumatism/Arthritis, J.Heart and/or Chest Pain, K.Inflammation, L.Sore/Swollen limbs, M.Headache, N.Back and/ or kidney Pain, O. Abscesses and/or Boils

AppendixIII

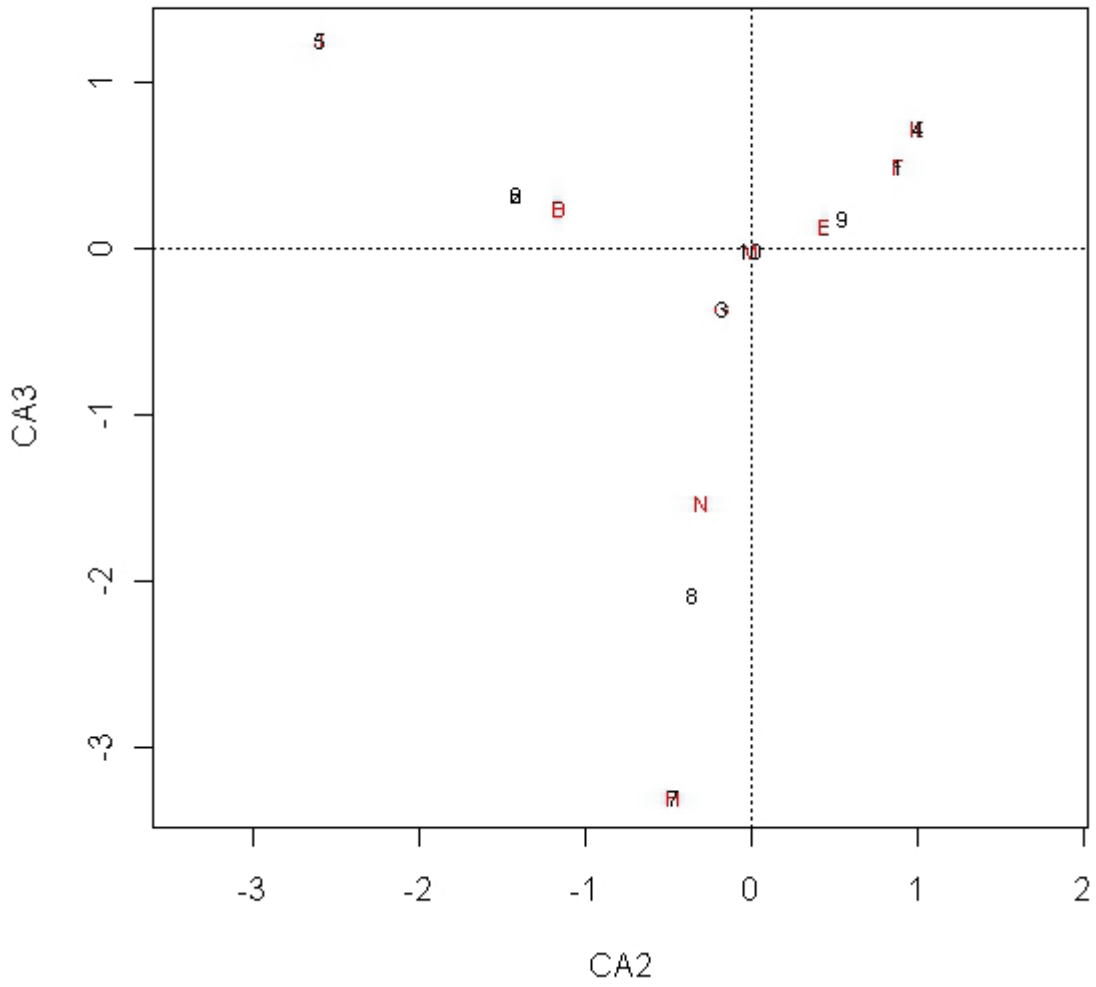

Figure 2.6 Two dimensional scatter plot (2nd and 3rd dimension) resulting from the correspondence analysis of plant species mentioned in Nemaska interviews(1-10) and diabetes symptoms (A-O). Symptoms and species which are close to one another are closely associated. Plant species;1. *Abies balsamea*, 2. *Populus balsamifera*, 3. *Picea mariana*, 4. *Alnus incana* ssp. *rugosa*, 5. *Pinus banksiana*, 6. *Rhododendron groenlandicum*, 7. *Sorbus decora*, 8. *Kalmia angustifolia*, 9. *Larix laricina*, 10. *Picea glauca*. Symptoms; A. Increased Thirst, B. Urination, C.Increased Appetite, D.Weakness, E.Slow Healing Infections, F.Blurred Vision, G.Foot sores/numbness, H.Diarrhea, I.Rheumatism/Arthritis, J.Heart and/or Chest Pain, K.Inflammation, L.Sore/Swollen limbs, M.Headache, N.Back and/ or kidney Pain, O. Abscesses and/or Boils

Appendix IV

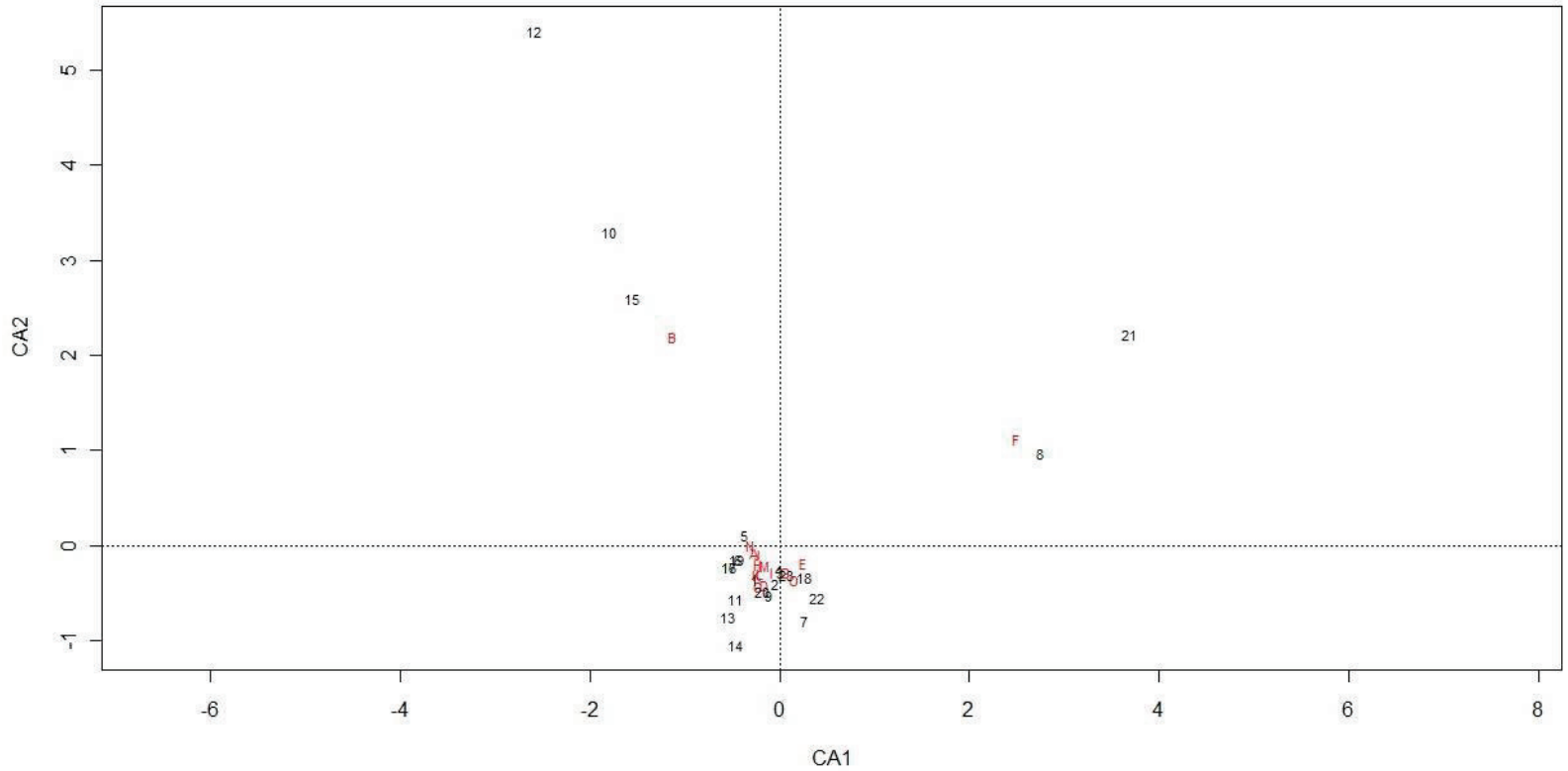

Figure 2.7 Two dimensional scatter plot resulting from the correspondence analysis of pooled plant species from Waskaganish, Nemaska, Mistissini and Whapmagoostui interviews(1-23) and diabetes symptoms (A-O). Symptoms and species which are close to one another are closely associated. Plant species; 1. *Rhododendron groenlandicum*, 2. *Larix laricina*, 3. *Picea glauca*, 4. *Picea mariana*, 5. *Sorbus decora*, 6. *Pinus banksiana*, 7. *Alnus incana* spp. *rugosa*, 8. *Abies balsamea*, 9. *Rhododendron tomentosum*, 10. *Juniperus communis*, 11. *Leymus mollis*, 12. *Empetrum nigrum*, 13. *Cladonia rangiferina*, 14. *Vaccinium angustifolium*, 15. *Lycopodium clavatum*, 16. *Gaultheria hispidula*, 17. *Thuja occidentalis*, 18. *Heracluem maximum*, 19. *Kalmia angustifolia*, 20. *Salix planifolia*, 21. *Vaccinium vitis-idaea*, 22. *Sarracenia purpurea*, 23. *Populus balsamifera*. Symptoms; A. Increased Thirst, B. Urination, C.Increased Appetite, D.Weakness, E.Slow Healing Infections, F.Blurred Vision, G.Foot sores/numbness, H.Diarrhea, I.Rheumatism/Arthritis, J.Heart and/or Chest Pain, K.Inflammation, L.Sore/Swollen limbs, M.Headache, N.Back and/ or kidney Pain, O. Abscesses and/or Boils

Appendix V

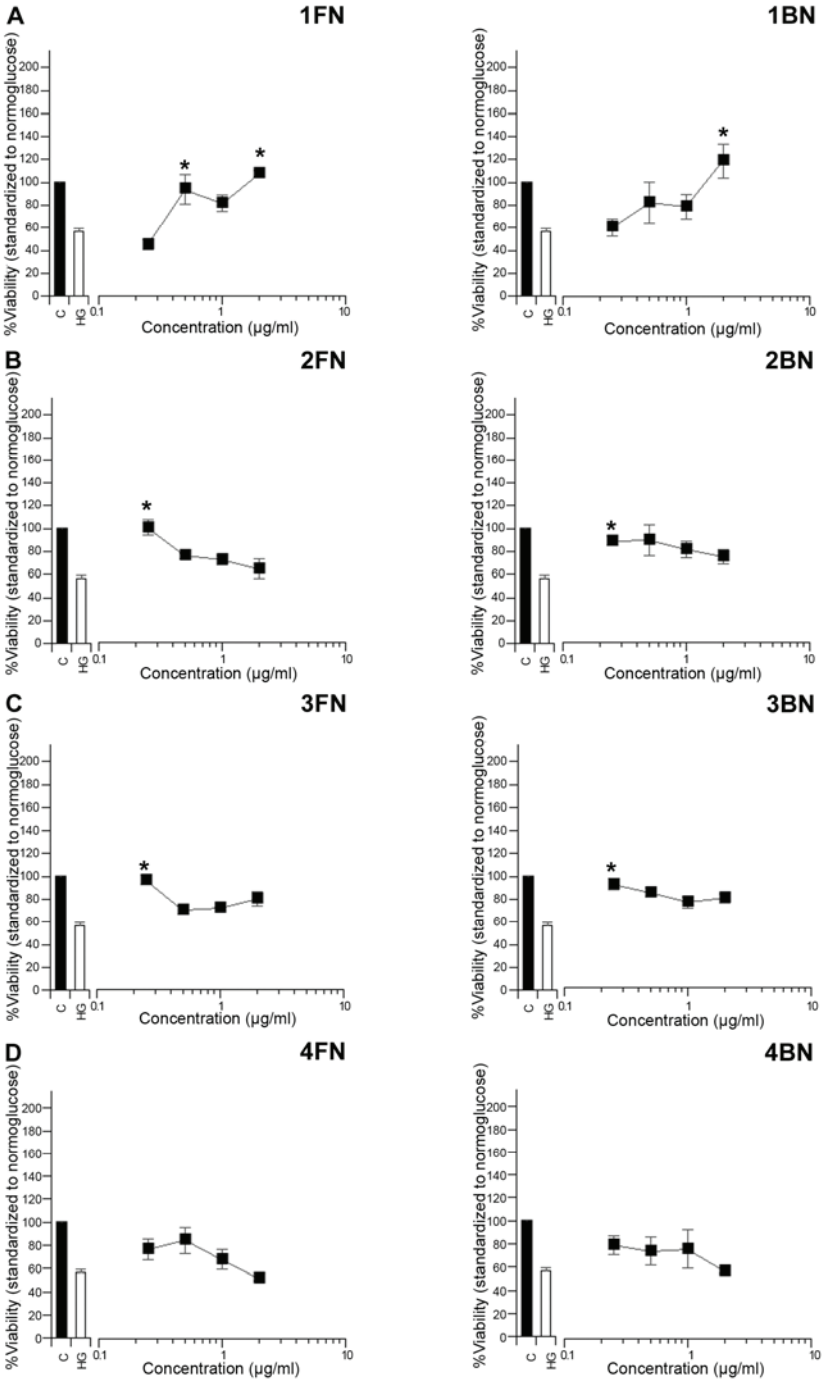

Figure 2 (A-D). Comparison of cytoprotective, mitogenic and cytotoxic activities of *P. mariana* pooled needle extracts from forest and bog populations in a PC12-AC model of high glucose stress. Populations are listed in ascending order from coastal west (**1**) to inland east (**21**) with forest (**F**) and bog (**B**) populations next to one another for comparison. Organ type is specified as the last number in the code; needle (**N**). Therefore each graph has a code identifying population number, habitat type and organ type above it in that order. Bioactivity was assessed using the formazan dye WST which measures mitochondrial dehydrogenase activity. Treatment wells containing extract were standardized to the normoglucose control (C) and compared to this and the high glucose control (HG) for the determination of protective, mitogenic or toxic effects. Student t-test was used in order to determine the significant difference between the normoglucose control (100%) and the high glucose control (56.799%, n=54wells/condition, bar graph, p≤0.05). Anova analysis was employed in order to compare the % viability for each concentration 0.25, 0.50, 1.00 and 2.00ug/ml to the normoglucose and high glucose controls. Differences were deemed significant (*cytoprotection, #mitogenic) when p≤0.05 (n=3 treatment wells/concentration).

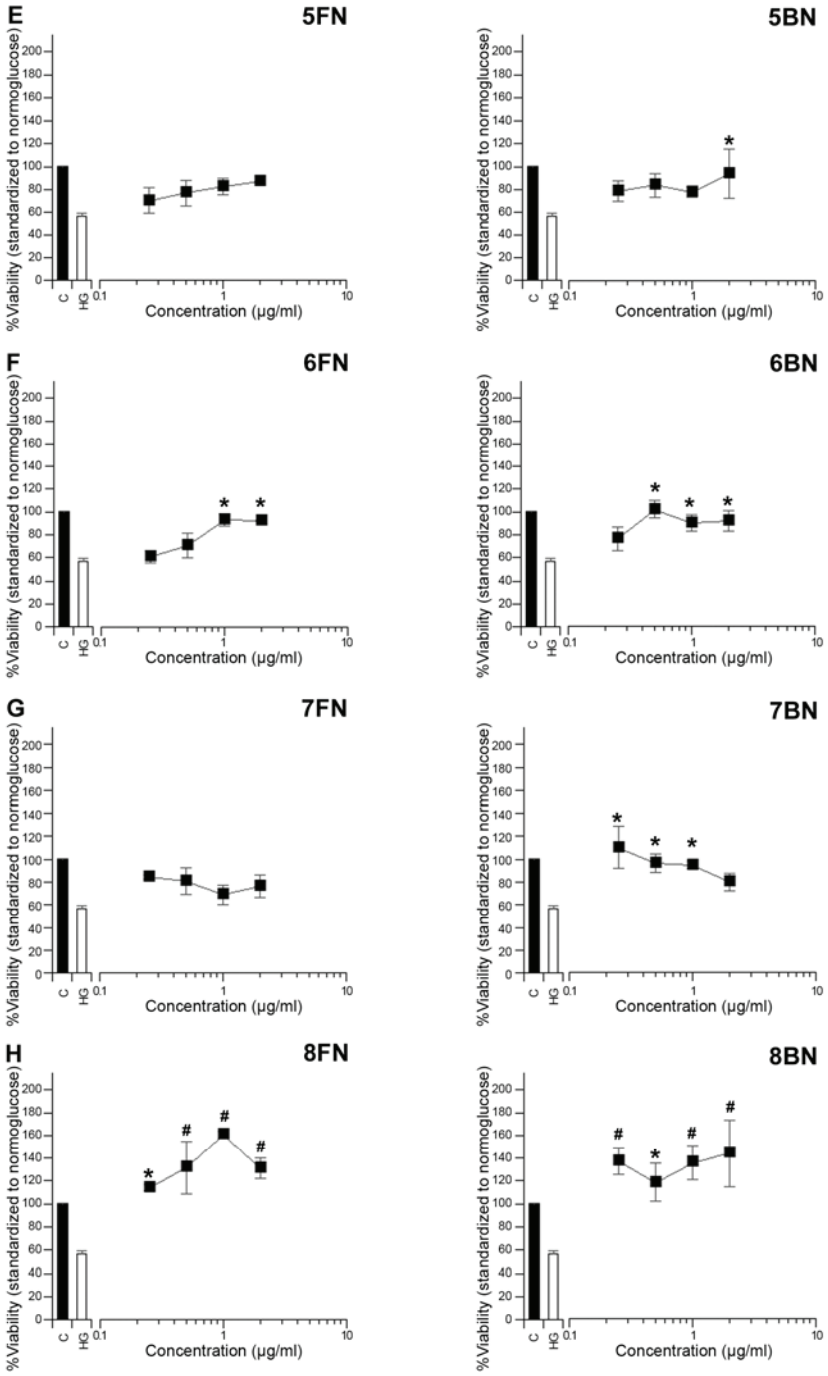

Figure 2 (E-H). Comparison of cytoprotective, mitogenic and cytotoxic activities of *P. mariana* pooled needle extracts from forest and bog populations in a PC12-AC model of high glucose stress. Populations are listed in ascending order from coastal west (**1**) to inland east (**21**) with forest (**F**) and bog (**B**) populations next to one another for comparison. Organ type is specified as the last number in the code; needle (**N**). Therefore each graph has a code identifying population number, habitat type and organ type above it in that order. Bioactivity was assessed using the formazan dye WST which measures mitochondrial dehydrogenase activity. Treatment wells containing extract were standardized to the normoglucose control (C) and compared to this and the high glucose control (HG) for the determination of protective, mitogenic or toxic effects. A students *t*-test was used in order to determine the significant difference between the normoglucose control (100%) and the high glucose control (56.799%, n=54wells/condition, bar graph, $p \leq 0.05$). Anova analysis was employed in order to compare the % viability for each concentration 0.25, 0.50, 1.00 and 2.00ug/ml to the normoglucose and high glucose controls. Differences were deemed significant (*cytoprotection, #mitogenic) when $p \leq 0.05$ (n=3 treatment wells/concentration).

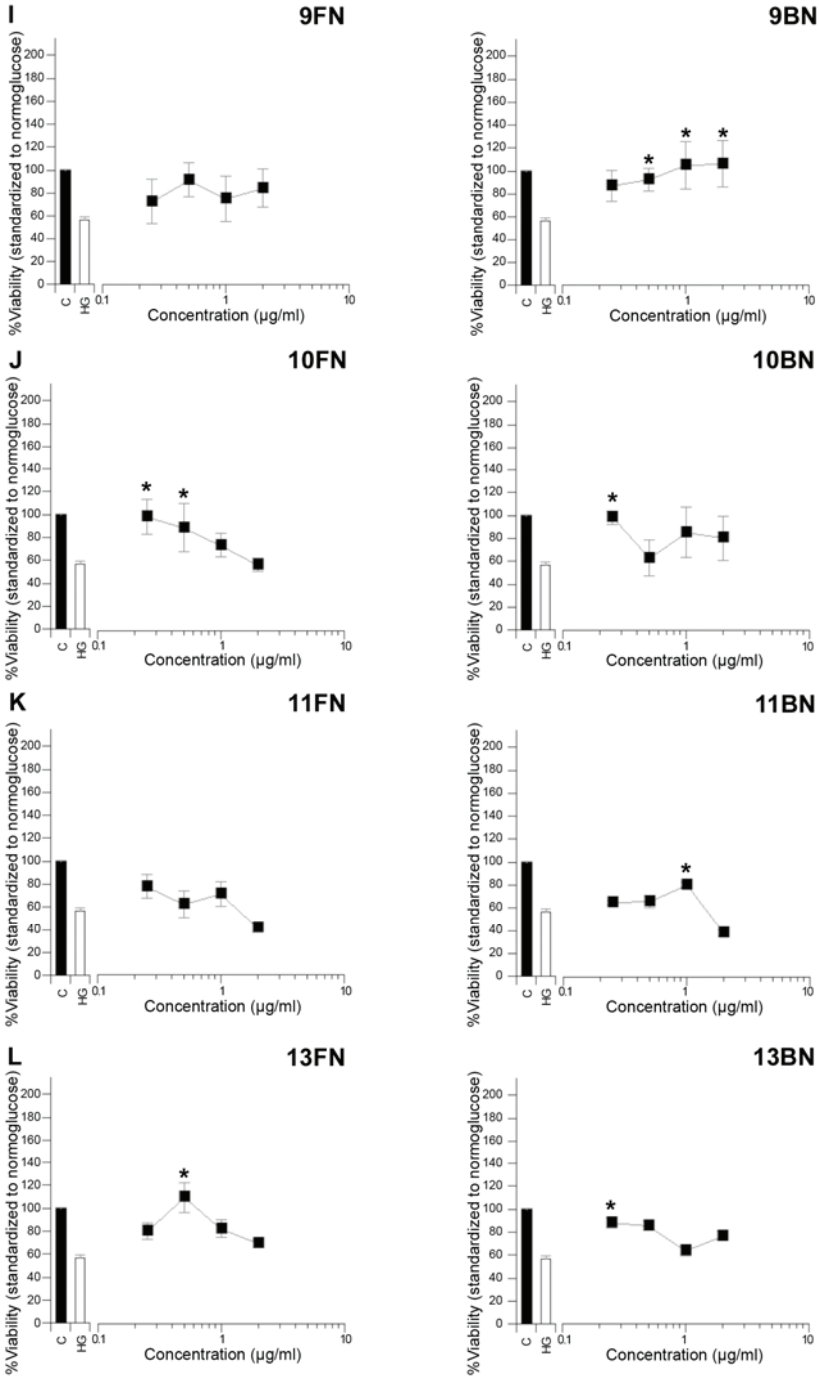

Figure 2 (I-L). Comparison of cytoprotective, mitogenic and cytotoxic activities of *P. mariana* pooled needle extracts from forest and bog populations in a PC12-AC model of high glucose stress. Populations are listed in ascending order from coastal west (**1**) to inland east (**21**) with forest (**F**) and bog (**B**) populations next to one another for comparison. Organ type is specified as the last number in the code; needle (**N**). Therefore each graph has a code identifying population number, habitat type and organ type above it in that order. Bioactivity was assessed using the formazan dye WST which measures mitochondrial dehydrogenase activity. Treatment wells containing extract were standardized to the normoglucose control (C) and compared to this and the high glucose control (HG) for the determination of protective, mitogenic or toxic effects. A students *t*-test was used in order to determine the significant difference between the normoglucose control (100%) and the high glucose control (56.799%, n=54wells/condition, bar graph, p≤0.05). Anova analysis was employed in order to compare the % viability for each concentration 0.25, 0.50, 1.00 and 2.00ug/ml to the normoglucose and high glucose controls. Differences were deemed significant (*cytoprotection, #mitogenic) when p≤0.05 (n=3 treatment wells/concentration).

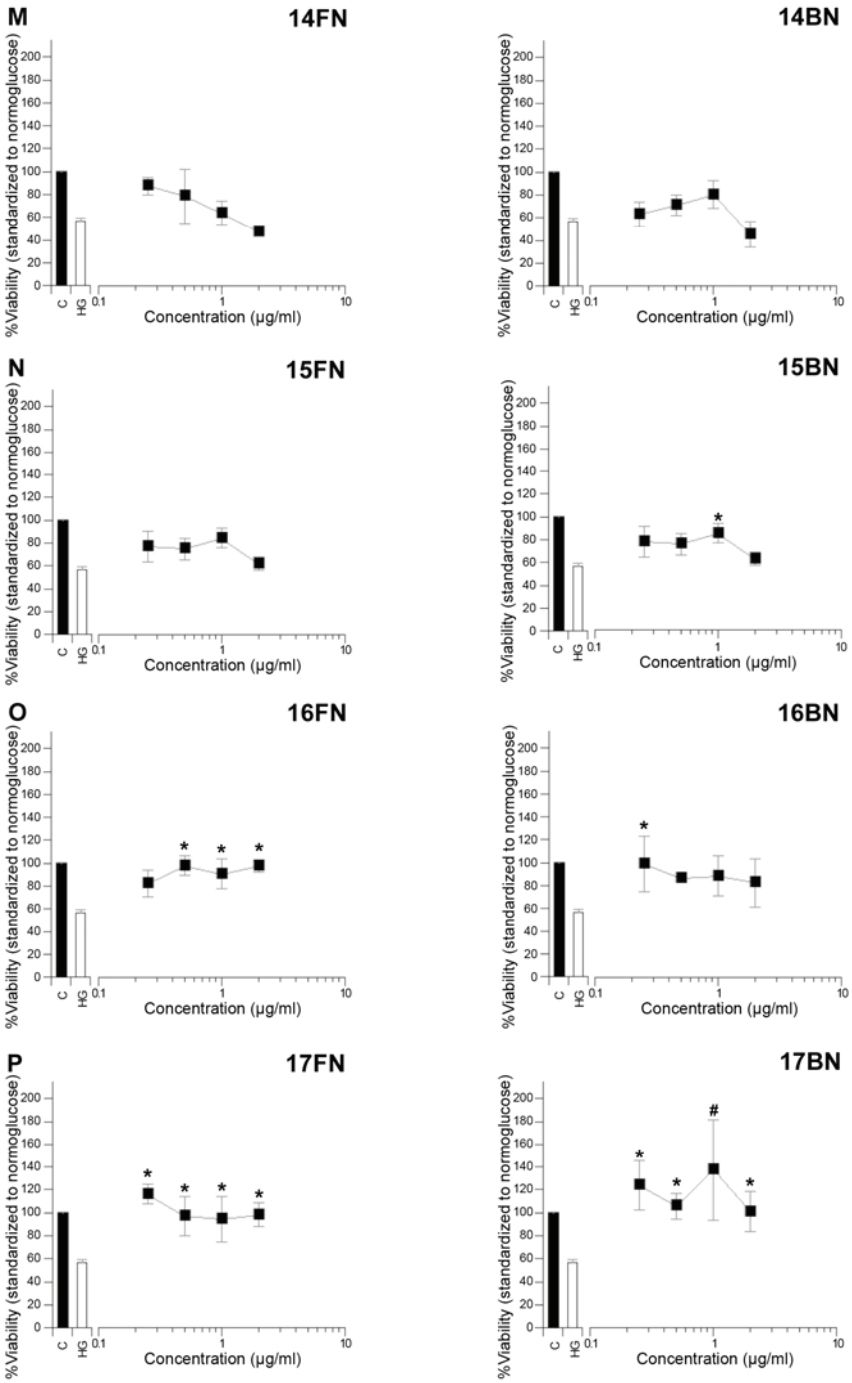

Figure 2 (M-P). Comparison of cytoprotective, mitogenic and cytotoxic activities of *P. mariana* pooled needle extracts from forest and bog populations in a PC12-AC model of high glucose stress. Populations are listed in ascending order from coastal west (**1**) to inland east (**21**) with forest (**F**) and bog (**B**) populations next to one another for comparison. Organ type is specified as the last number in the code; needle (**N**). Therefore each graph has a code identifying population number, habitat type and organ type above it in that order. Bioactivity was assessed using the formazan dye WST which measures mitochondrial dehydrogenase activity. Treatment wells containing extract were standardized to the normoglucose control (C) and compared to this and the high glucose control (HG) for the determination of protective, mitogenic or toxic effects. A students *t*-test was used in order to determine the significant difference between the normoglucose control (100%) and the high glucose control (56.799%, n=54wells/condition, bar graph, p≤0.05). Anova analysis was employed in order to compare the % viability for each concentration 0.25, 0.50, 1.00 and 2.00ug/ml to the normoglucose and high glucose controls. Differences were deemed significant (*cytoprotection, #mitogenic) when p≤0.05 (n=3 treatment wells/concentration).

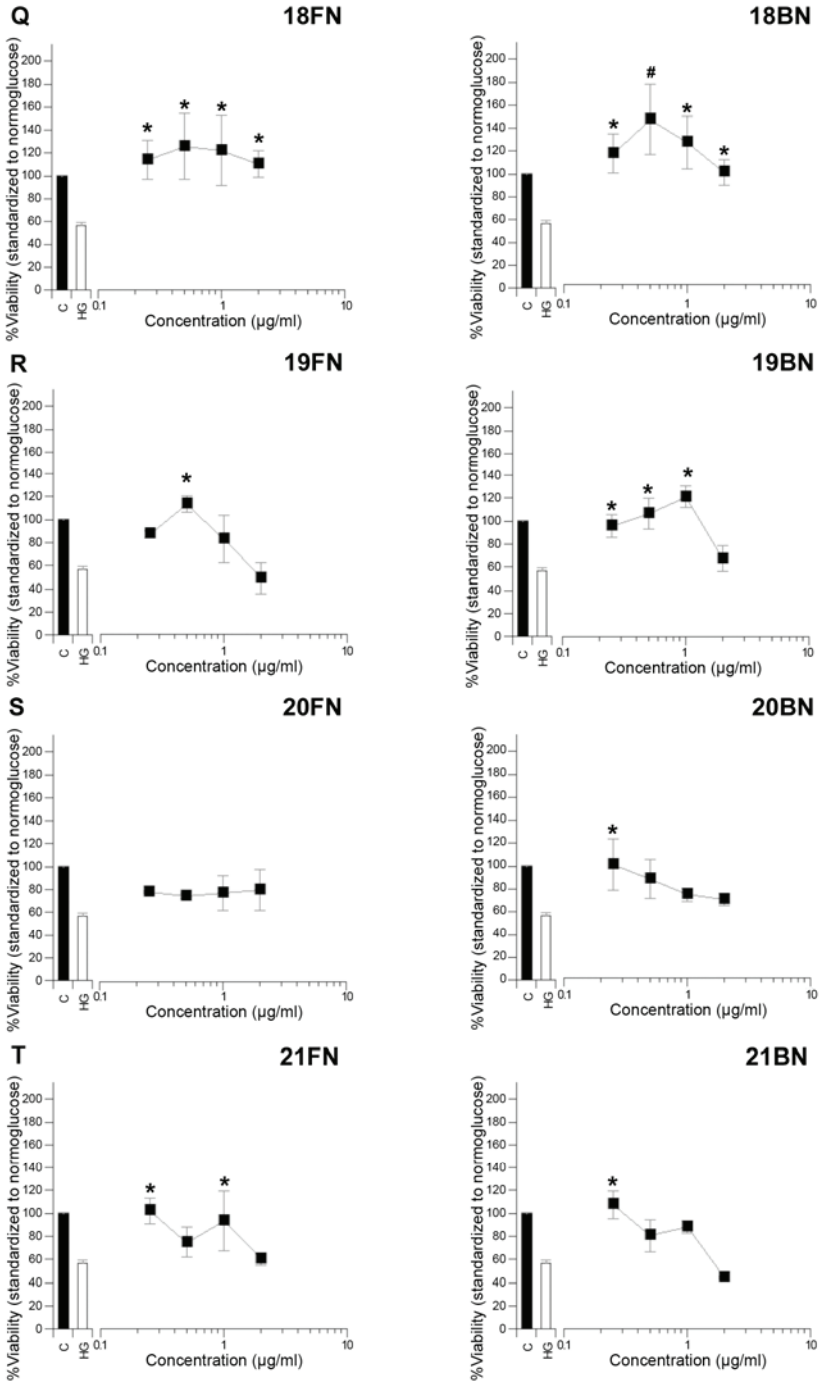

Figure 2 (Q-T). Comparison of cytoprotective, mitogenic and cytotoxic activities of *P. mariana* pooled needle extracts from forest and bog populations in a PC12-AC model of high glucose stress. Populations are listed in ascending order from coastal west (**1**) to inland east (**21**) with forest (**F**) and bog (**B**) populations next to one another for comparison. Organ type is specified as the last number in the code; needle (**N**). Therefore each graph has a code identifying population number, habitat type and organ type above it in that order. Bioactivity was assessed using the formazan dye WST which measures mitochondrial dehydrogenase activity. Treatment wells containing extract were standardized to the normoglucose control (C) and compared to this and the high glucose control (HG) for the determination of protective, mitogenic or toxic effects. A students *t*-test was used in order to determine the significant difference between the normoglucose control (100%) and the high glucose control (56.799%, n=54wells/condition, bar graph, p≤0.05). Anova analysis was employed in order to compare the % viability for each concentration 0.25, 0.50, 1.00 and 2.00ug/ml to the normoglucose and high glucose controls. Differences were deemed significant (*cytoprotection, #mitogenic) when p≤0.05 (n=3 treatment wells/concentration).

Appendix VI

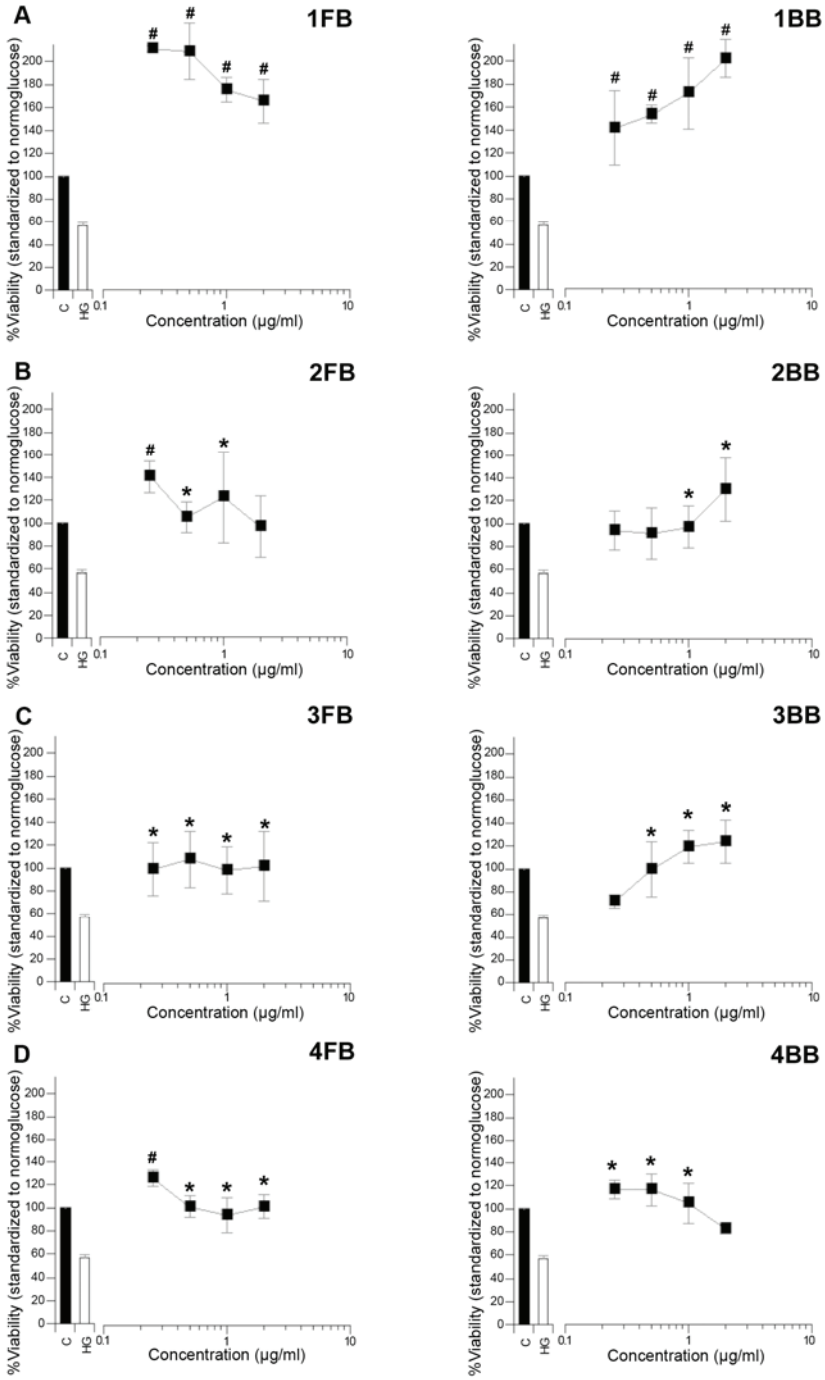

Figure 3 (A-D). Comparison of cytoprotective, mitogenic and cytotoxic activities of *P. mariana* pooled bark extracts from forest and bog populations in a PC12-AC model of high glucose stress. Populations are listed in ascending order from coastal west (**1**) to inland east (**14**) with forest (**F**) and bog (**B**) populations next to one another for comparison. Organ type is specified as the last number in the code; bark (**B**). Therefore each graph has a code identifying population number, habitat type and organ type above it in that order. Bioactivity was assessed using the formazan dye WST which measures mitochondrial dehydrogenase activity. Treatment wells containing extract were standardized to the normoglucose control (C) and compared to this and the high glucose control (HG) for the determination of protective, mitogenic or toxic effects. A students *t*-test was used in order to determine the significant difference between the normoglucose control (100%) and the high glucose control (56.799%, n=54wells/condition, bar graph, $p \leq 0.05$). Anova analysis was employed in order to compare the % viability for each concentration 0.25, 0.50, 1.00 and 2.00ug/ml to the normoglucose and high glucose controls. Differences were deemed significant (*cytoprotection, #mitogenic) when $p \leq 0.05$ (n=3 treatment wells/concentration).

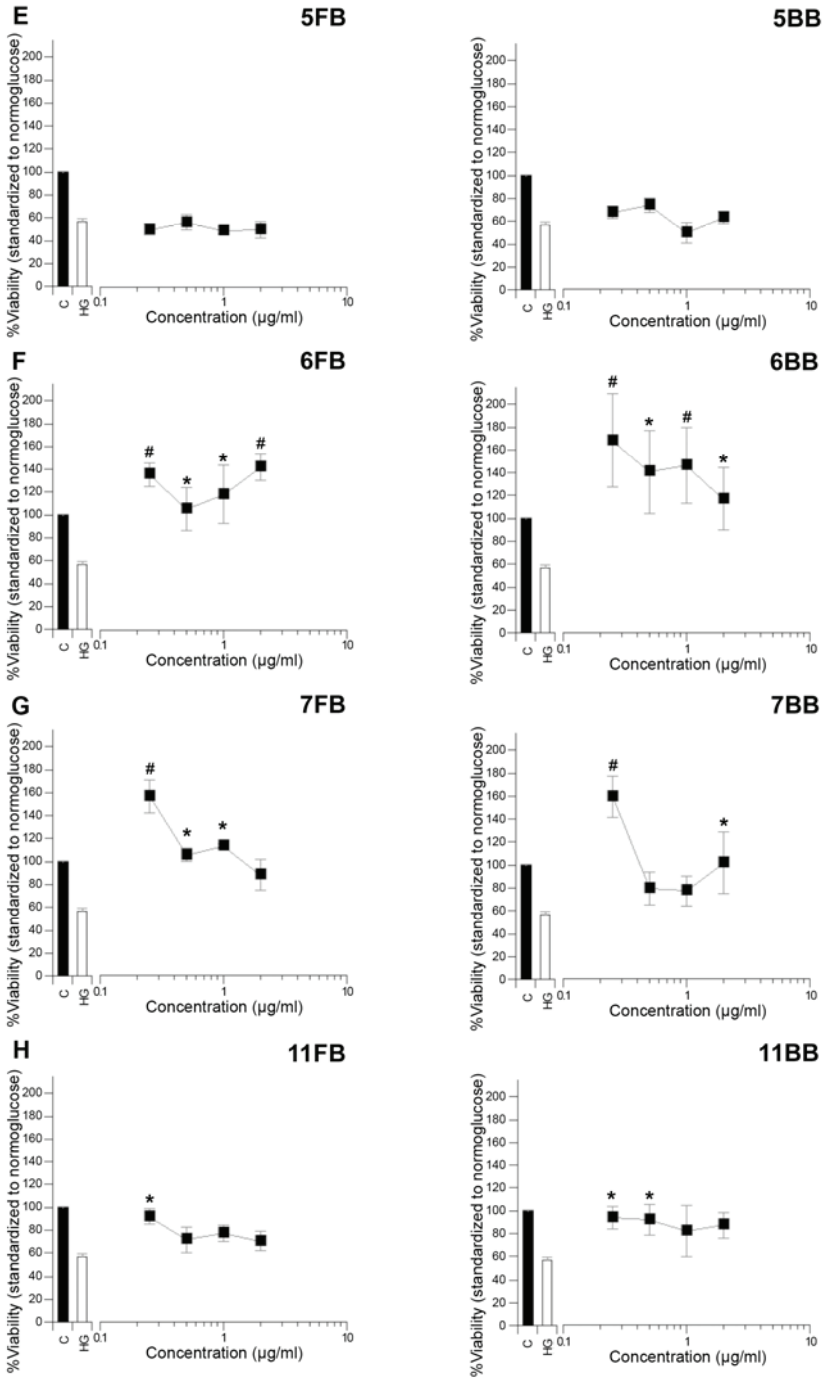

Figure 3 (E-H). Comparison of cytoprotective, mitogenic and cytotoxic activities of *P. mariana* pooled bark extracts from forest and bog populations in a PC12-AC model of high glucose stress. Populations are listed in ascending order from coastal west (**1**) to inland east (**14**) with forest (**F**) and bog (**B**) populations next to one another for comparison. Organ type is specified as the last number in the code; bark (**B**). Therefore each graph has a code identifying population number, habitat type and organ type above it in that order. Bioactivity was assessed using the formazan dye WST which measures mitochondrial dehydrogenase activity. Treatment wells containing extract were standardized to the normoglucose control (C) and compared to this and the high glucose control (HG) for the determination of protective, mitogenic or toxic effects. A students *t*-test was used in order to determine the significant difference between the normoglucose control (100%) and the high glucose control (56.799%, n=54wells/condition, bar graph, $p \leq 0.05$). Anova analysis was employed in order to compare the % viability for each concentration 0.25, 0.50, 1.00 and 2.00ug/ml to the normoglucose and high glucose controls. Differences were deemed significant (*cytoprotection, #mitogenic) when $p \leq 0.05$ (n=3 treatment wells/concentration).

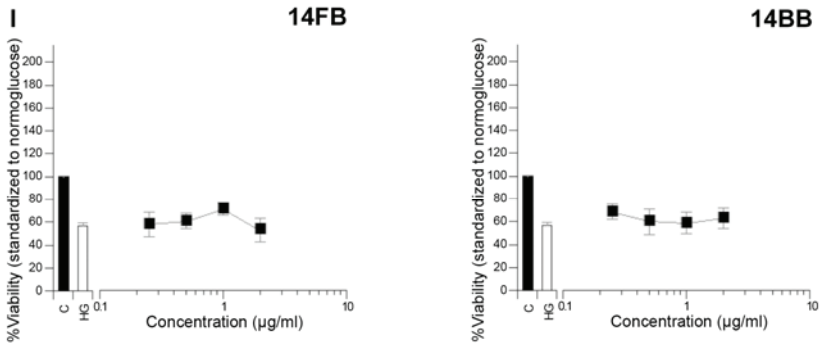

Figure 3 (I). Comparison of cytoprotective, mitogenic and cytotoxic activities of *P. mariana* pooled bark extracts from forest and bog populations in a PC12-AC model of high glucose stress. Populations are listed in ascending order from coastal west (**1**) to inland east (**14**) with forest (**F**) and bog (**B**) populations next to one another for comparison. Organ type is specified as the last number in the code; bark (**B**). Therefore each graph has a code identifying population number, habitat type and organ type above it in that order. Bioactivity was assessed using the formazan dye WST which measures mitochondrial dehydrogenase activity. Treatment wells containing extract were standardized to the normoglucose control (C) and compared to this and the high glucose control (HG) for the determination of protective, mitogenic or toxic effects. A students *t*-test was used in order to determine the significant difference between the normoglucose control (100%) and the high glucose control (56.799%, n=54wells/condition, bar graph, $p \leq 0.05$). Anova analysis was employed in order to compare the % viability for each concentration 0.25, 0.50, 1.00 and 2.00ug/ml to the normoglucose and high glucose controls. Differences were deemed significant (*cytoprotection, #mitogenic) when $p \leq 0.05$ (n=3 treatment wells/concentration).

Appendix VII

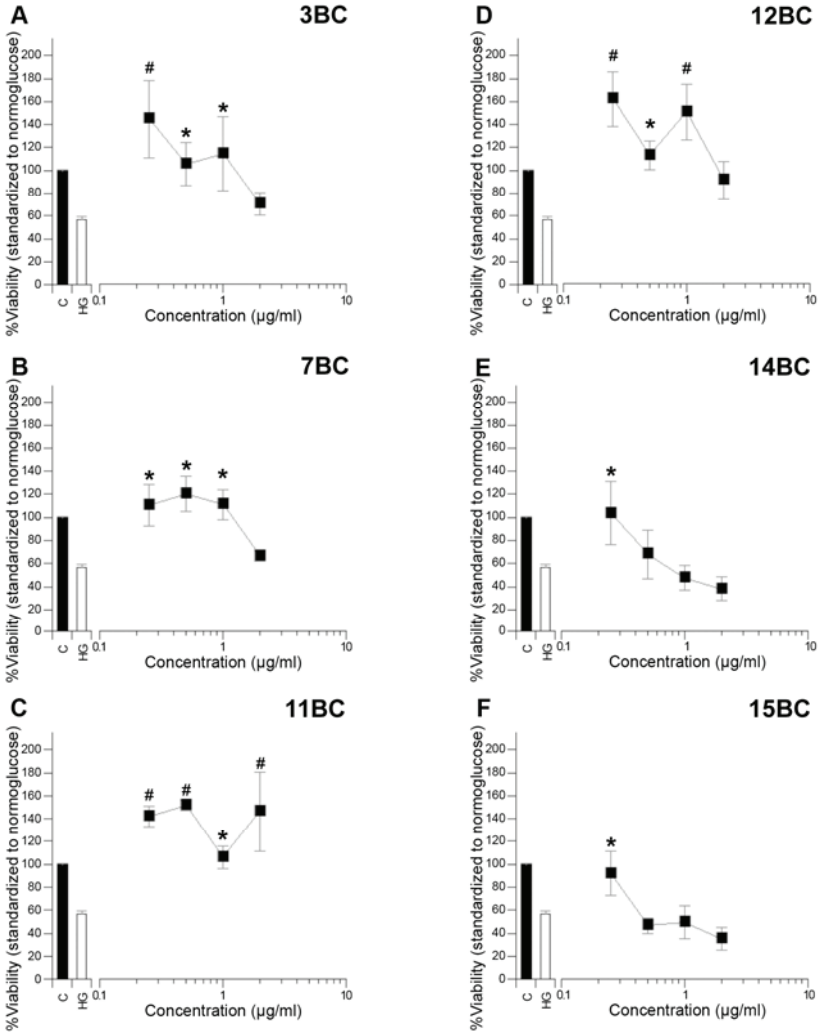

Figure 4 (A-F). Comparison of cytoprotective, mitogenic and cytotoxic activities of *P. mariana* pooled cone extracts from bog populations in a PC12-AC model of high glucose stress. Populations are listed in ascending order from coastal west (**3**) to inland east (**15**) Habitat is the second letter in the code (bog=**B**) while Organ type is specified as the last number in the code (cone=**C**). Therefore each graph has a code identifying population number, habitat type and organ type above it in that order. Bioactivity was assessed using the formazan dye WST which measures mitochondrial dehydrogenase activity. Treatment wells containing extract were standardized to the normoglucose control (C) and compared to this and the high glucose control (HG) for the determination of protective, mitogenic or toxic effects. A students *t*-test was used in order to determine the significant difference between the normoglucose control (100%) and the high glucose control (56.799%, n=54wells/condition, bar graph, $p \leq 0.05$). Anova analysis was employed in order to compare the % viability for each concentration 0.25, 0.50, 1.00 and 2.00ug/ml to the normoglucose and high glucose controls. Differences were deemed significant (*cytoprotection, #mitogenic) when $p \leq 0.05$ (n=3 treatment wells/concentration).

Appendix VIII

ID	Voucher	Date
Waskaganish		
WAS001	*Thuja occidentalis*	July 13 2008
WAS002	*Populus balsamifera*	July 12 2008
see field vouchers	*Picea mariana*	
WAS004	*Alnus incana* spp. *rugosa*	Aug 10 2008
WAS005	*Heracleum maximum*	Aug 6 2008
WAS006	*Sorbus* sp.	Aug 10 2008
WAS007	*Larix laricina*	Aug 10 2008
WAS008	*Rhododendron groenlandicum*	Aug 10 2008
WAS009	*Pinus banksiana*	Aug 11 2008
WAS010	*Picea glauca*	Aug 11 2008
WAS011	*Abies balsamea*	Aug 13 2008
WAS012	*Sarracenia purpurea*	Aug 15 2008
Nemaska		
NEM001	*Rhododendron groenlandicum*	Aug 20 2008
NEM002	*Pinus banksiana*	Aug 20 2008
see field vouchers	*Picea mariana*	
NEM003	*Alnus incana* spp. *rugosa*	Aug 20 2008
see Waskaganish	*Abies balsamea*	
NEM004	*Larix laricina*	Aug 21 2008
not found	*Vaccinium vitis-idaea*	
see Waskaganish	*Populus balsamifera*	Aug 20 2008
NEM006	*Kalmia angustifolia*	Aug 20 2008
NEM007	*Sorbus* sp.	Aug 21 2008
see Waskaganish	*Picea glauca*	

*Herbarium accession numbers and collection dates for voucher specimens from Waskaganish and Nemaska interviews. Three vouchers of each type share the same ID code but are labeled A, B or C (ie NEM001-A). Specimens are kept at the Marie Victorin Herbarium, Université of Montréal (MT)

Appendix VIIII

ID	Voucher	Date
Picea mariana field sites		
PM013	Bog 1	Aug 14 2008
PM014	Forest 1	Aug 14 2008
lost	Bog 2	Aug 10 2008
lost	Forest 2	Aug 10 2008
PM003	Bog 3	Aug 11 2008
PM004	Forest 3	Aug 11 2008
PM009	Bog 4	Aug 13 2008
PM010	Forest 4	Aug 13 2008
PM011	Bog 5	Aug 13 2008
PM012	Forest 5	Aug 13 2008
PM007	Bog 6	Aug 12 2008
PM008	Forest 6	Aug 12 2008
PM005	Bog 7	Aug 12 2008
PM006	Forest 7	Aug 12 2008
PM033	Bog 8	Aug 26 2008
PM034	Forest 8	Aug 26 2008
PM031	Bog 9	Aug 25 2008
PM032	Forest 9	Aug 25 2008
PM019	Bog 10	Aug 21 2008
PM020	Forest 10	Aug 21 2008
PM015	Bog 11	Aug 19 2008
PM016	Forest 11	Aug 19 2008
PM017	Bog 12	Aug 21 2008
PM018	Forest 12	Aug 21 2008
PM025	Bog 13	Aug 23 2008
PM026	Forest 13	Aug 23 2008
PM023	Bog 14	Aug 23 2008
PM024	Forest 14	Aug 23 2008
PM021	Bog 15	Aug 22 2008
PM022	Forest 15	Aug 22 2008
PM027	Bog 16	Aug 24 2008
PM028	Forest 16	Aug 24 2008
PM029	Bog 17	Aug 24 2008
PM030	Forest 17	Aug 24 2008
PM035	Bog 18	Aug 28 2008
PM036	Forest 18	Aug 28 2008
PM037	Bog 19	Aug 29 2008

PM038	Forest 19	Aug 29 2008
PM039	Bog 20	Aug 30 2008
PM040	Forest 20	Aug 30 2008
PM041	Bog 21	Aug 30 2008
PM042	Forest 21	Aug 30 2008

*Herbarium accession numbers and collection dates for voucher specimens from field sites where Picea marians was collected for PC12 bioassays. Specimens are kept at the Marie Victorin Herbarium, Université of Montréal (MT)