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Ethnobotanique de la Nation crie d'Eeyou Istchee et variation géographique des plantes médicinales antidiabétiques

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Ethnobotanique de la Nation crie d'Eeyou Istchee et variation géographique des plantes médicinales antidiabétiques

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Résumé

Le diabète de type 2 affecte en moyenne 29% de la population adulte crie d'Eeyou Istchee (CEI). Afin d'identifier les plantes médicinales possédant un potentiel antidiabétique, des interviews ont été réalisés dans les communautés CEI de Wemindji et Oujé-Bougoumou. Utilisant une approche quantitative, les espèces mentionnées ont été classées et comparées à la pharmacopée des communautés avoisinantes. Seize et 25 plantes ont été mentionné à Wemindji et Oujé-Bougoumou, respectivement. Sept nouvelles espèces de plantes et une de champignon se sont ajoutées à la liste des espèces à potentiel antidiabétique, bien que la plupart de celles mentionnées pendant les interviews soit en communes à la pharmacopée CEI générale, démontrant ainsi leur importance culturelle. Des analyses phytochimiques sur deux de ces espèces, *Rhododendron groenlandicum* et *Sarracenia purpurea*, ont été réalisées à partir d'échantillons récoltés à différents endroits du territoire eeyouch. Bien qu'aucun patron n'ait été détecté dans la variation des composantes biologiquement actives chez *S. purpurea,* les composés phénoliques chez *R. groenlandicum,* particulièrement la (+)-catéchine, l'(-)-epicatéchine et la quercétine-3-galactoside, varient spatialement en fonction de paramètres d'insolation telles la radiation solaire ou la photopériode. Les échantillons de cette dernière espèce, testés *in vitro* dans le bioessai de l'adipogenèse des cellules adipocytes murines 3T3-L1, augmentent l'accumulation intracellulaire des triglycérides, leur conférant ainsi une activité diabétique semblable à la rosiglitazone. Cependant, cette activité était plus faible dans les échantillons à haute teneur en quercétine, cela pouvant ainsi avoir un impact sur la qualité d'un produit de santé naturel fabriqué à partir de cette espèce.

Mots-clés : Diabète de type 2, *Rhododendron groenlandicum, Sarracenia purpurea,* ethnobotanique, Cri d'Eeyou Istchee, Premières Nations, phytochimie, photoinhibition, variations géographiques, adipogenèse, plantes médicinales, produits de santé naturels.

Abstract

Type 2 diabetes has reached epidemic proportions among Canada's aboriginal populations and affects on average 29% of adult Cree of Eeyou Istchee (CEI). In collaboration with the Cree Board of Health and Social Services of James Bay and the CIHR team in Traditional Antidiabetic Aboriginal Medicines, interviews were held in the CEI communities of Wemindji and Oujé-Bougoumou to identify potential antidiabetic plants. Using a quantitative approach, species mentioned were ranked and compared to the pharmacopoeia of other participating communities. Sixteen and 25 plants were mentioned in Wemindji and Oujé-Bougoumou respectively. Seven new plant and one fungal species were added to the list of potential antidiabetic species, although most of those mentioned were common to the general CEI pharmacopoeia, thus supporting the cultural importance that they hold. Phytochemical analyses of two of these species, *Rhododendron groenlandicum* and *Sarracenia purpurea,* were made from accessions harvested throughout Eeyou Istchee. While no pattern was detected in the variation of *S. purpurea'*s biologically active compounds, phenolic compounds from *R. groenlandicum,* specifically (+)-catchin, (-)-epicatechin and quercetin-3-galactoside, varied spatially as a function of insolation parameters such as solar radiation or photoperiod. Samples from the latter, tested *in vitro* in the 3T3-L1 murine adipocytes adipogenesis bioassay, increased the intracellular accumulation of triglycerides, thus conferring it a glitazone-like antidiabetic activity. This activity, however, was weaker in accessions with high quercetin content, which could have an impact on the quality of a natural health product made from this species.

Keywords : Type 2 diabetes, *Rhododendron groenlandicum*, *Sarracenia purpurea*, ethnobotany, Eeyou Istchee Cree, First Nations, phytochemistry, photoinhibition, geographical variations, adipogenesis, medicinal plants, natural health products.

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Liste des abréviations

3T3-L1 : Cellules adipocytaires murines/*Murine adipocyte cells*

ACN : Acétonitrile/*Acetonitrile*

AGE : Produits terminaux de glycation avancée/*Advanced glycation end-products*

AIC : Critère d'information d'Akaike/*Akaike's information criterion*

AMP : Adenosine monophosphate

AMPK : Protéine kinase activée par l'AMP/*AMP-activated protein kinase*

ANOVA : Analyse de variance/*Analysis of variance*

CBHSSJB :Conseil Cri de la santé et des services sociaux de la Baie James/*Cree Board of Health and Social Services of James Bay*

CEI : Cri d'Eyou Istchee/*Cree of Eeyou Istchee*

CIHR-TAAM : Équipe de recherche des IRSC sur les médecines autochtones antidiabétiques/ *CIHR Team in Aboriginal Antidiabetic Medicines*

CTM : Médecine traditionelle crie/*Cree traditional medicine*

DAD : Détecteur à barrette de diode/*Diode array detector*

DMEM : Milieu minimum essentiel de Eagle modifié par Dubelcco/*Dubelcco's modified Eagle's minimal essential medium*

DMSO : Diméthylsulfoxyde/*Dimethyl sulfoxide*

DMX : Dexaméthasone/*Dexamethasone*

DW : Masse sèche/*Dry weight*

ESI : Ionisation par électronébuliseur/*Electrospray ionization*

EtOH : Éthanol/*Ethanol*

FBS : Sérum de veau fœtal/*Fetal bovine serum*

FC : Fréquence de mentions/*Frequency of citation value*

HPLC : Chromatographie en phase liquide à haute performance/*High performance liquid chromatography*

IBMX : 3-isobutyle-1-méthylxanthine/*3-isobutyl-1-methylxanthine*

MANOVA : Analyse de variance multivariée/*Multivariate analysis of variance*

MeOH : Méthanol/*Methanol*

MS : Spectromètrie de masse/*Mass spectrometry*

NCS : Sérum de jeune veau/*Bovine calf serum*

NHP : Produit de santé naturel/*Natural health product*

PBS : Tampon phosphate salin/*Phosphate buffered saline*

PCA : Analyse en composante principale/*Principal component analysis*

PPARγ : Récepteur de type gamma activé par les proliférateurs de peroxysomes/*Peroxisome proliferator-activated receptor gamma*

PTFE : Polytétrafluoroéthylène/*Polytetrafluoroethylene*

RDA : Analyse canonique de redondance/Canonical redundancy analysis

ROS : Espèces réactives d'oxygène/*Reactive oxygen species*

RPM : Rotations par minute/*Rotations per minute*

SIV : Valeur de l'importance syndromique/*Syndromic importance value*

T2D : Diabète de type 2/*Type 2 diabetes*

TG : Triglycérides/*Triglycerides*

UPLC : Chromatographie en phase liquide à ultra-haute performance/*Ultra-high performance liquid chromatography*

UV : Ultraviolet/*Ultraviolet*

VIF : Facteur d'inflation de la variance/*Variance inflation factor*

A mamie de la Martinique et babcia Bożenka
pour leur savoir traditionnel des plantes et des
champignons…
La source de mes inspirations.

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Chapitre 1 - Introduction

En 2003, un projet collaboratif a été mis en place avec des communautés de la Nation crie d'Eeyou Istchee (CEI), le Conseil Cri de la Santé et des Services Sociaux de la Baie James et ses professionnels de la santé (*Cree Board of Health and Social Services of James Bay*, CBHSSJB) ainsi que des chercheurs des Universités McGill, d'Ottawa et de Montréal. L'équipe de recherche des IRSC sur les médecines autochtones antidiabétiques (*CIHR Team in Aboriginal Antidiabetic Medicines,* CIHR-TAAM) a pour but la recherche des plantes médicinales ayant un potentiel antidiabétique. Abordant la problématique du diabète chez les Cris en respectant leur culture et leur territoire, le CIHR-TAAM travaille de façon étroite et éthique avec les membres des communautés CEI afin de trouver des solutions adaptées à leur culture.

L'évaluation scientifique des plantes médicinales de la pharmacopée CEI comprend, entre autres, des analyses phytochimiques, des essais toxicologiques et des expériences pharmacologiques *in vitro* et *in vivo*. Plusieurs de ces plantes ont montré un potentiel antidiabétique et ces résultats ont suscité un intérêt soutenu de la part des Cris dans tout le territoire Eeyouch. Quatre des neuf communautés CEI ont déjà fait part de leur savoir traditionnel depuis la création du CIHR-TAAM. Mais durant l'été 2010, j'ai été invité à discuter avec les aînés et les Conseils de bande de Wemindji et d'Oujé-Bougoumou de leur participation à ce projet. Puis, j'ai été appelé à compléter les études ethnobotaniques au sein de ces deux communautés et à examiner davantage la phytochimie et la pharmacologie de certaines espèces clés.

1.1 Introduction au diabète

Le diabète est une affection chronique caractérisée par l'hyperglycémie, soit une concentration trop élevée du glucose dans le sang. Cela est causé par une faible sécrétion d'insuline généralement reliée à des problèmes chez les cellules β des tissus pancréatiques (Prentki and Nolan, 2006; Agence de la santé publique du Canada, 2011).

La maladie a atteint des dimensions pandémiques. En 2011, le nombre total de personnes atteintes était estimé à 366 millions, un nombre représentant 8.3 % de la population

globale. Cette incidence risque d'augmenter jusqu'à 552 millions de personnes en 2030 (Whiting et al., 2011). Au Canada, c'est un problème en croissance : 2,4 millions, soit 6,8 % de la population canadienne, vivaient avec le diabète en 2008/9, représentant ainsi une augmentation de 70% depuis 1998/9 (Agence de la santé publique du Canada, 2011). Les visites fréquentes aux cliniques et hôpitaux, l'hospitalisation liée aux complications, la pharmacothérapie, l'invalidité à court et à long termes, ainsi que la perte de la productivité constituent un fardeau économique important. En 2000, les coûts totaux associés au diabète étaient estimés à 2,5 milliards de dollars (Agence de la santé publique du Canada, 2011).

Il existe trois principales formes du diabète, soit 1) le diabète gestationnel se manifestant durant la grossesse, 2) le diabète de type 1, d'origine génétique, qui résulte de l'endommagement irréversible des cellules β par une réaction auto-immunitaire, et 3) le diabète de type 2 (Agence de la santé publique du Canada, 2011).

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1.2 Diabète de type 2

Représentant plus de 90 % des cas, le diabète de type 2 (T2D) est la forme la plus courante de diabète (Tiwari and Rao, 2002; Agence de la santé publique du Canada, 2011). Aussi appelé diabète sucré non-insulinodépendant (DSNID), il se manifeste généralement suite à l'insulinorésistance des tissus cibles (Bennett et al., 1992; Agence de la santé publique du Canada, 2011). Dans les stades primaires de l'affection, les cellules β du pancréas sont portées à produire de grandes quantités d'insuline pour compenser une sensibilité réduite et maintenir la normoglycémie (Prentki and Nolan, 2006). Cette persistance mène éventuellement à la défaillance des cellules β, impliquant la diminution, voir l'interruption de la production d'insuline (Prentki and Nolan, 2006).

La pathologie du T2D est bien connue. L'insulinorésistance, l'hyperinsulinémie, la sécrétion altérée de l'insuline ainsi que l'absorption et le transport réduits du glucose à travers l'action de l'insuline sont tous caractéristiques de la maladie (Tiwari and Rao, 2002). La fatigue et faiblesse, la soif et miction excessive, la perte de poids inexpliquée, les maladies parodontales, la néphropathie, la rétinopathie, l'amputation des membres, la cardiopathie

ischémique, les maladies cardio- et cérébro-vasculaires, les complications neuropathiques menant à la perte de sensation, la dysfonction sexuelle ainsi que l'augmentation des infections et ulcérations des pieds sont tous des symptômes et complications du T2D découlant du métabolisme altéré du glucose (Giugliano et al., 1996; Tiwari and Rao, 2002; Johansen et al., 2005; Rahimi et al., 2005; Agence de la santé publique du Canada, 2011). Malgré cela, l'étiologie derrière plusieurs de ces problèmes confrontent toujours les chercheurs, médecins et patients (Tiwari and Rao, 2002; Prentki and Nolan, 2006).

Pour ces raisons, le traitement du T2D est compliqué (Tiwari and Rao, 2002). Le contrôle et le maintien des mécanismes d'homéostasie dans les niveaux du glucose sanguin impliquent parfois des injections d'insuline, mais plus couramment l'utilisation de médicaments oraux tels la metformine, les composés sulfonylurés, les thiazolidinediones, les méglitinides et les inhibiteurs d'α-glucosidases (Agence de la santé publique du Canada, 2011).

1.3 Diabète chez les autochtones

Le T2D était autrefois rare chez les Premières Nations du Canada (Thouez et al., 1990; Agence de la santé publique du Canada, 2011), mais depuis plus d'un demi-siècle, le nombre de cas a considérablement augmenté (Brassard and Robinson, 1995; Young et al., 2000). Le diabète atteint maintenant des proportions épidémiques dans certaines communautés autochtones (Agence de la santé publique du Canada, 2011) et ces dernières sont considérées parmi les groupes à haut risque mondialement (WHO and IDF, 2004; Yu and Zinman, 2007). Aussi, l'incidence du diabète est trois à cinq fois plus élevée que la moyenne nationale (Young et al., 2000; Agence de la santé publique du Canada, 2011).

Les Cris d'Eeyou Istchee de la région de la Baie James du nord du Québec sont particulièrement touchés par le T2D (Robinson, 1988; Brassard et al., 1993a; Brassard et al., 1993b; Brassard and Robinson, 1995; Dannenbaum et al., 1999). Les premiers cas rapportés en 1983 n'affectaient que 2,9% des CEI (Thouez et al., 1990), mais la maladie est maintenant

un problème de santé majeur. En 2009, l'incidence du T2D, ajusté pour l'âge, chez les CEI adultes était de 29 % (Kuzmina et al., 2010).

L'inaccessibilité de certains services de santé n'aide pas à containdre ces taux élevés de complications chez les communautés autochtones. L'apparition est souvent précoce et la détection tardive. On attribut cependant *i)* aux changements rapides du mode de vie et de l'alimentation*, ii)* aux prédispositions génétiques et *iii*) à la faible adhérence aux traitements actuels comme étant les raisons principales expliquant cette incidence élevée.

En premier lieu, le mode de vie traditionnel nomade n'incluait pas un historique de maladie chronique**.** Il se caractérisait par une activité physique et une alimentation saine, incluant poissons et plantes (Berkes and Farkas, 1978; Robinson, 1988). La transition de l'arc à flèche aux couteaux et fusils, ainsi que la pression commerciale associée à la traite de la fourrure (Robinson, 1988), aboutit à la surexploitation de la faune et facilite l'introduction de la diète Européenne (Berkes and Farkas, 1978). Des carences nutritives ont été associées à ces changements dans l'alimentation (Berkes and Farkas, 1978; Hoffer et al., 1981) allant jusqu'à d'importants cas de malnutrition au début du 20e siècle (Moore et al., 1946; Vivian et al., 1948). Amplifiés par la sédentarisation (Boston et al., 1997), ces changements conduisent à l'obésité et celle-ci devient alors un déterminant important du T2D dans les communautés de la Baie James (Brassard and Robinson, 1995), comme ailleur dans le monde (Yu and Zinman, 2007).

Il est entendu que l'alimentation traditionnelle ne contenait pas les hydrates de carbone raffinés que l'on retrouve maintenant (Gittelsohn et al., 1996) dans leur diète. Les problèmes encourus par cette nouvelle stratégie alimentaire sembleraient être exacerbés par des prédispositions génétiques (Ritenbaugh and Goodby, 1989; Neel, 1999; Hegele et al., 2003; Chakravarthy and Booth, 2004; Yu and Zinman, 2007). L'hypothèse du génotype vigoureux, soit le *thrifty genotype* proposé par Neel (1999), stipule que les gènes associés ont été sélectionnés dans les sociétés chasseurs-cueilleurs afin de maintenir plus efficacement les réserves durant les cycles de famines et d'abondances alimentaires. Paradoxalement, la nourriture est de nos jours aisément accessible dans les communautés CEI et ne nécessite qu'un petit voyage au magasin. Associée aux changements rapides dans le mode de vie des populations autochtones, cette adaptation évolutive nuirait plus qu'elle ne ferait du bien en

menant aux désordres métaboliques tels l'obésité et le T2D (Ritenbaugh and Goodby, 1989; Boston et al., 1997; Chakravarthy and Booth, 2004).

Sans s'attarder sur les détails de la théorie du génotype vigoureux et du débat sous-jacent, Hegele et al. (2003) mettent en évidence le lien génétique au T2D dans les populations Oji-Cree de l'Ontario et du Manitoba. Un polymorphisme spécifique du gène *HNF1A* a été décrit dans cette population, d'où la mutation G319S encodée dans le gène. Cette mutation serait associée à une augmentation substantielle des risques du T2D (Hegele et al., 2003).

Finalement, une étude parmi cette population indique qu'il existe une dichotomie entre le monde autochtone et non-autochtone par rapport à l'alimentation, les maladies et la médecine (Gittelsohn et al., 1996). Le T2D est souvent perçu comme une « maladie de l'homme blanc » introduite tout comme la tuberculose et la variole dans le passé (Boston et al., 1997; Young et al., 2000). Ainsi, 90 % des autochtones diabétiques ont consulté un professionnel de santé, selon une enquête auprès des peuples autochtones (EAPA) en 1991; cependant, une enquête de la santé régionale auprès des Premières Nations et Inuits montre que 80 % de ceux-ci croyaient que le système de santé a besoin d'être amélioré (Young et al., 2000). Une autre étude, portant sur l'intervention alimentaire et l'activité physique dans les communautés CEI, a montré le peu d'impact de telles interventions, ce qui remet en question les approches jusqu'alors utilisées pour interpeler le problème du T2D (Gray-Donald et al., 2000). Même s'ils indiquent qu'ils vont suivre les conseils des professionnels de la santé, les membres de la communauté crie conceptualisent toujours le traitement relié au diabète dans une perspective crie (Boston et al., 1997).

C'est donc avec un succès limité que ces approches vouées à des stratégies alimentaires ont été mises en place parmi les populations autochtones (Boston et al., 1997; Gray-Donald et al., 2000). Il s'ensuit que le défi du T2D devra être résolu avec une compréhension approfondie de la perception autochtone vis-à-vis de la médecine contemporaine (Gray-Donald et al., 2000).

1.4 Approches alternatives

La prise en compte de la culture et du langage sont des recommandations qui doivent être adoptées au sein des cliniques visant à combattre la maladie parmi les peuples autochtones (Meltzer et al., 1998). Cela aura pour effet d'augmenter la participation des membres des communautés autochtones quant à la médecine allochtone (Robinson, 1988; Boston et al., 1997; Meltzer et al., 1998; Bisset et al., 2004; Yu and Zinman, 2007).

Une étude menée dans un centre de santé autochtone urbain de Milwaukee, Wisconsin, montre qu'une grande portion (38 %) des patients fréquentait un guérisseur (Marbella et al., 1998). Bien qu'un tiers de ces patients recevait cependant des conseils différents de la part des médcins et des guérisseurs, 61 % avaient une meilleure opinion des conseils de ces derniers et 15 % en informaient le médecin de ces visites (Marbella et al., 1998). Pareillement, la perspective CEI considère la médecine occidentale séparée des concepts et des approches cries sur la santé et ne rapporte pas régulièrement ces pratiques alternatives (Boston et al., 1997).

Les chercheurs donnent donc raison à l'intégration des médecines traditionnelles aux approches modernes courantes (Niezen, 1997; Young et al., 2000). Bien que l'utilisation de la médecine traditionnelle et conventionnelle en tandem soit fréquemment employée dans les pays en développement (Bodeker, 2001), au Canada, c'est une approche originale qui doit débuter par l'intermédiaire de la recherche en ethnobotanique (Young et al., 2000).

1.5 Ethnobotanique

L'origine végétale de la galégine, précurseur de l'une des drogues antidiabétiques les plus prescrites mondialement, la metformine, met en lumière l'importance d'une approche ethnobotanique dans le domaine de la santé. En effet, plus de 42 millions de prescriptions ont été faites en 2009 aux États-Unis, le classant au 10e rang des drogues les plus distribuées du pays (SDI/Verispan and VONA, 2010). Cette drogue hypoglycémiante (Marles and Farnsworth, 1995) est dérivée de la guanidine isolée du *Galega officinalis* L. (Fabaceae) à la fin du 19e siècle. Les parties aériennes de cette plante étaient utilisées médicalement en

Europe médiévale pour traiter une panoplie de symptômes et malaises, dont certains sont liés au T2D (Bailey and Day, 2004).

Il va de soi que la médecine traditionnelle CEI contient un savoir approfondi des plantes médicinales (Marshall et al., 2003; Fraser, 2006; Leduc et al., 2006; Marshall, 2006; Cosset and Mansion, 2009; Downing, 2010). Ces espèces boréales sont utilisées communément par les diverses Nations cries à travers la forêt boréale canadienne (Holmes, 1884; Strath, 1903; Beardsley, 1941; Arnason et al., 1981; Zieba, 1990; Siegfried, 1994; Marles et al., 2008; Moerman, 2009; Uprety et al., 2012). Plusieurs de ces espèces sont utilisées pour traiter des complications et des symptômes pouvant être associés au T2D. Il existe désormais une compréhension limitée, notamment chez les aînés, par rapport à l'association entre ces problèmes et le T2D (Boston et al., 1997). Afin de prioriser l'évaluation des plantes à potentiel antidiabétique, Leduc et al. (2006) ont développé un indice permettant de mesurer l'importance d'une plante par rapport à une maladie. La valeur de l'importance syndromique (*syndromic importance value,* SIV), tenant compte *i)* du consensus, *ii)* du nombre de symptômes pour lesquels une plante est citée et *iii)* de l'importance de ces symptômes pour le diabète, a été utilisée au sein du CIHR-TAAM dans les communautés CEI de Mistissini (Leduc et al., 2006), Whapmagoostui (Fraser, 2006; Fraser et al., 2007), Nemaska et Waskaganish (Downing, 2010).

Grâce à cette approche ethnobotanique quantitative, une multitude de plantes ont été identifiées pour des évaluations antidiabétiques poussées. Une grande portion de ces espèces proviennent de la famille des Ericaceae et des Pinaceae, mais aussi des Cupressaceae, Betulaceae, Lycopodiaceae, Salicaceae, Sarraceniaceae, Rosaceae, Typhaceae, Poaceae, Empetraceae et Apiaceae (Fraser, 2006; Leduc et al., 2006; Downing, 2010). Quoique la phytochimie et la pharmacologie de la majorité de ces espèces aient été peu étudiées, certains représentants de ces familles, tels les *Vaccinium* spp. des Ericaceae et les *Salix* spp. des Salicaceae, sont tout de même bien connus pour leurs bienfaits sur la santé.

1.6 Pharmacologie

Les plantes médicinales CEI, résultant de ces études ethnobotaniques, ont fait l'objet de plusieurs bioessais pour évaluer leur potentiel antidiabétique (Spoor et al., 2006; Fraser et al., 2007; Harbilas et al., 2009; Harris et al., 2011). Ainsi, le thé du Labrador, *Rhododendron groenlandicum* (Oeder) Kron & Judd (**fig 1.1a**) stimule l'adipogenèse des cellules adipocytaires murines 3T3-L1 possiblement par l'activation du récepteur de type gamma activé par les proliférateurs de peroxysomes (*peroxisome proliferator-activated receptor gamma*, PPARγ) (Spoor et al., 2006; Ouchfoun, 2011). La sarracénie pourpre, *Sarracenia purpurea* L. (**fig 1.1b**), quant à elle, stimule l'absorption du glucose par les cellules musculaires C2C12 (Spoor et al., 2006; Muhammad et al., 2012) et protège les pré-neurones sympathiques PC12-AC de la toxicité glycémique (Spoor et al., 2006; Harris et al., 2012).
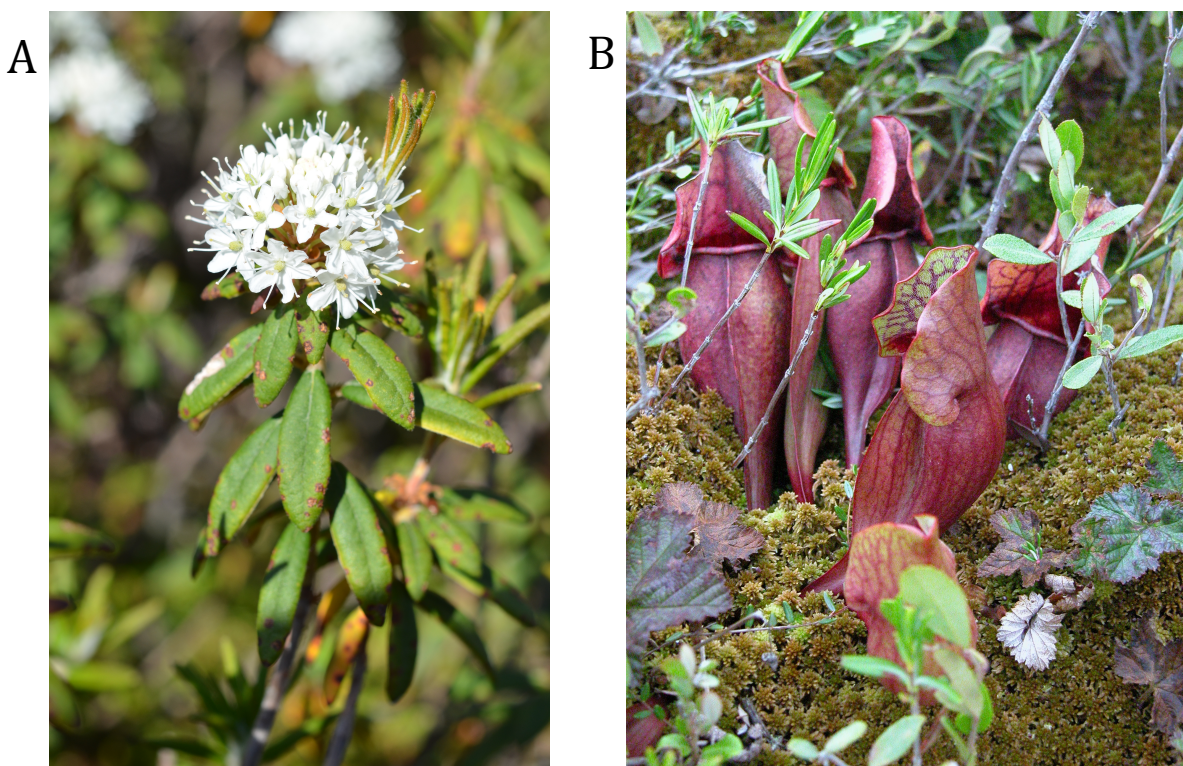
Figure 1.1 Image de *Rhododendron groenlandicum*, thé du Labrador, en fleurs (**A**) et *Sarracenia purpurea*, sarracénie pourpre (**B**).

Dans le premier cas, le mécanisme d'action améliore l'insulinorésistance conférant au *R. groenlandicum* l'activité antidiabétique imputée aux thiazolidinediones comme la rosiglitazone (Grimaldi, 2001; MacDougald and Mandrup, 2002; Hansen and Kristiansen,

2006; Rosen and MacDougald, 2006; Ouchfoun, 2011). Dans le deuxième, le mécanisme d'action, analogue à la metformine, passerait par la protéine kinase activée par l'AMP (*AMP-activated protein kinase,* AMPK) en réponse aux désordres mitochondriaux (Martineau, Adeyiwola-Spoor, et al., 2010).

Le fractionnement guidé par bioessais des extraits bruts de ces plantes dévoile que les composés phénoliques sont en partie responsables de leur activité antidiabétique. La (+)-catéchine et l'(-)-épicatéchine, stimulant l'adipogenèse, a été isolée du *R. groenlandicum* (Ouchfoun, 2011), alors que la quercétine-3-*O*-galactoside, stimulant l'absorption du glucose, a été isolée chez *S. purpurea* (Muhammad et al., 2012). Le fractionnement des extraits bruts de cette plante par le test de toxicité glycémique a également démontré l'activité cytoprotectrice de la quercétine-3-*O*-galactoside sur les cellules PC12-AC (Harris et al., 2012). Il semblerait que ces espèces posséderaient une activité antidiabétique primaire, soit une activité hypoglycémiante, et secondaire, soit la protection contre les complications indirectes.

1.7 Phytochimie

Il devient de plus en plus évident que plusieurs des symptômes et des complications associés au T2D sont causés par les stress oxydatifs liés à l'hyperglycémie sur les systèmes micro- et macro-vasculaires (Giugliano et al., 1996; Tiwari and Rao, 2002; Johansen et al., 2005; Rahimi et al., 2005). L'auto-oxydation du glucose, entre autres, mènerait à la production de radicaux libres et le déséquilibre ainsi créé dans les réactions d'oxydo-réductions cellulaire contribuerait à augmenter la production d'espèces réactives d'oxygène (*reactive oxygen species,* ROS) (Tiwari and Rao, 2002; Johansen et al., 2005; Rahimi et al., 2005).

Bien que l'activité antidiabétique soit connue pour plusieurs composés phénoliques (Marles and Farnsworth, 1995), leurs propriétés antioxydantes le sont davantage, tout particulièrement chez les flavones, flavonoles et flavonoïdes (Harborne et al., 1999). Les recherches scientifiques montrent de plus en plus le bienfait des antioxydants dans la gestion des complications associées au diabète (Marles and Farnsworth, 1995; Rahimi et al., 2005; Harris et al., 2011). La production élevée de produits terminaux de glycation avancée

(*advanced glycation end-products,* AGE) chez les diabétiques, par exemple, contribue au développement des complications micro- et macro-vasculaires (Hegab et al., 2012; Yamagishi et al., 2012). Harris et al. (2011) montrent cependant une corrélation entre le contenu phénolique d'une plante et sa capacité à réduire la production d'AGE. Ils montrent de plus que ce dernier est corrélé à l'activité antioxydante de la plante.

Les composés phénoliques possèdent des structures chimiques qui leur permettent d'inactiver les ROS et d'agir comme antioxydant (Haslam, 1996; Habtemariam, 1997; Rice-Evans et al., 1997; Takahama and Oniki, 1997; Pietta et al., 1998; Plumb et al., 1998; Kähkönen et al., 1999). Cette activité pharmacologique tient de leur fonction physiologique chez la plante qui est elle-même susceptible au dommage oxydatif. Malgré la nécessité de recevoir de l'énergie lumineuse, les plantes peuvent souffrir de la photoinhibition lorsque la densité du flux de photons photosynthétiquement actifs surpasse ce qui est nécessaire à l'assimilation du CO_2 (Carvalho and Amâncio, 2002). L'augmentation des ROS, produits par cet excès d'énergie, est de plus susceptible à d'autres facteurs environnementaux, telles les températures extrêmes, qui réduisent la capacité photosynthétique des photosystèmes (Dixon and Paiva, 1995; Carvalho and Amâncio, 2002; Close et al., 2003; Carvalho et al., 2006; Solovchenko and Merzlyak, 2008; Bhattacharjee, 2011). Récemment, maintes études ont élucidé les fonctions antioxydantes des composés phénoliques dans la protection contre les stress oxydatifs induits par ces facteurs environnementaux (Dixon and Paiva, 1995; Takahama and Oniki, 1997; Pietta et al., 1998; Close et al., 2003; Solovchenko and Merzlyak, 2008; Faisal and Anis, 2009; Bhattacharjee, 2011). Il a été également montré que la lumière induit l'expression des gènes responsables aux voies biosynthétiques des flavonoïdes (Hartmann et al., 2005; Matus et al., 2009) et que cette expression est modulée, d'autre part, par la température et l'azote (Lillo et al., 2008; Usadel et al., 2008).

Plusieurs des plantes médicinales cries, tels *R. groenlandicum* et *S. purpurea*, sont abondantes et largement distribuées sur le territoire Eeyouch et la zone boréale du Canada. Le vaste territoire dans lequel les CEI puisent leurs ressources médicinales, s'étend du 49e au 55e parallèle nord. Ainsi, des différences marquées dans la durée du jour (**fig. 1.2**) ainsi que dans les conditions climatiques peuvent être observées entre ces extrémités nord-sud. Étant donné les relations étroites entre le rôle physiologique des composés phénoliques dans la plante et les

conditions climatiques, nous nous attendons à ce que cela joue sur la normalisation et le contrôle de qualité des produits de santé naturels provenant de ce territoire et *a fortiori* de la forêt boréale. Jusqu'à présent, il existe peu d'études concernant la variation phytochimique, particulièrement dans la composition de composantes pharmacologiquement actives, sur de grands gradients géographiques. Des corrélations positives entre la concentration phénolique et la latitude ont été montrées chez les Cupressaceae, les Ericaceae et les Betulaceae, soit *Juniperus communis* (Martz et al., 2009a), *Vaccinium myrtillus* (Martz et al., 2010) et *Betula pubescens* (Stark et al., 2008), respectivement. Bien que le rôle des facteurs environnementaux, tels la luminosité, la température et la qualité du sol, ait été mentionné (Stark et al., 2008; Martz et al., 2010), aucune étude à notre connaissance ne s'attarde sur le rôle et l'impact de ces facteurs, autant abiotiques que biotiques.

1.8 Objectifs du mémoire

Les neuf communautés CEI sont dispersées sur un vaste territoire dans le nord du Québec entre les 49e et 55e parallèles nord. Caractéristique de la forêt boréale, la diversité végétale varie néanmoins sur ce vaste territoire qui chevauche la toundra au nord et la forêt mixte au sud (Farrar, 2005). Cependant, les études ethnobotaniques réalisées au sein du CIHR-TAAM représentent le savoir traditionnel de moins de la moitié des communautés, ce qui ne peut pas être interprété comme une représentation complète du savoir CEI. De plus, à l'instar du savoir qui change selon différents environnements, l'arsenal de défense des plantes est également apte à répondre aux mêmes changements. Les aînés rapportent que le potentiel médicinal des plantes n'est pas identique sur la totalité du territoire. Cependant, de telles variations dans le profil phytochimique et leur activité biologique peuvent avoir un impact sur la normalisation et le contrôle de qualité des produits de santé naturels.

Afin de fournir de meilleures recommandations pour le développement de traitements antidiabétiques culturellement adaptés aux CEI, trois objectifs principaux divisés en quatre articles sont présentés dans ce mémoire.

Objectif 1 : Identifier les plantes à potentiels antidiabétiques provenant de la pharmacopée traditionnelle de Wemindji et d'Oujé-Bougoumou et les comparer à la pharmacopée des autres communautés CEI.

Objectif 2 : Déterminer si des variations spatiales (latitudinales) peuvent être observées parmi les composantes biologiquement actives de deux plantes médicinales CEI:

    i)   le thé du Labrador, *Rhododendron groenlandicum;*

    ii)   la sarracénie pourpre, *Sarracenia purpurea*.

Objectif 3 : Déterminer si ces variations phytochimiques sont pharmacologiquement significatives.

Considérant les différences dans la durée du jour sur le territoire Eeyouch (**fig 1.2**) et l'intensification des conditions climatiques extrêmes dans le nord, l'hypothèse suivante est postulée :

Hypothèse : Le contenu phénolique de ces deux plantes sera plus élevé dans les populations exposées à de plus hauts niveaux de stress associés à l'ensoleillement, comme la radiation solaire ou la photopériode.
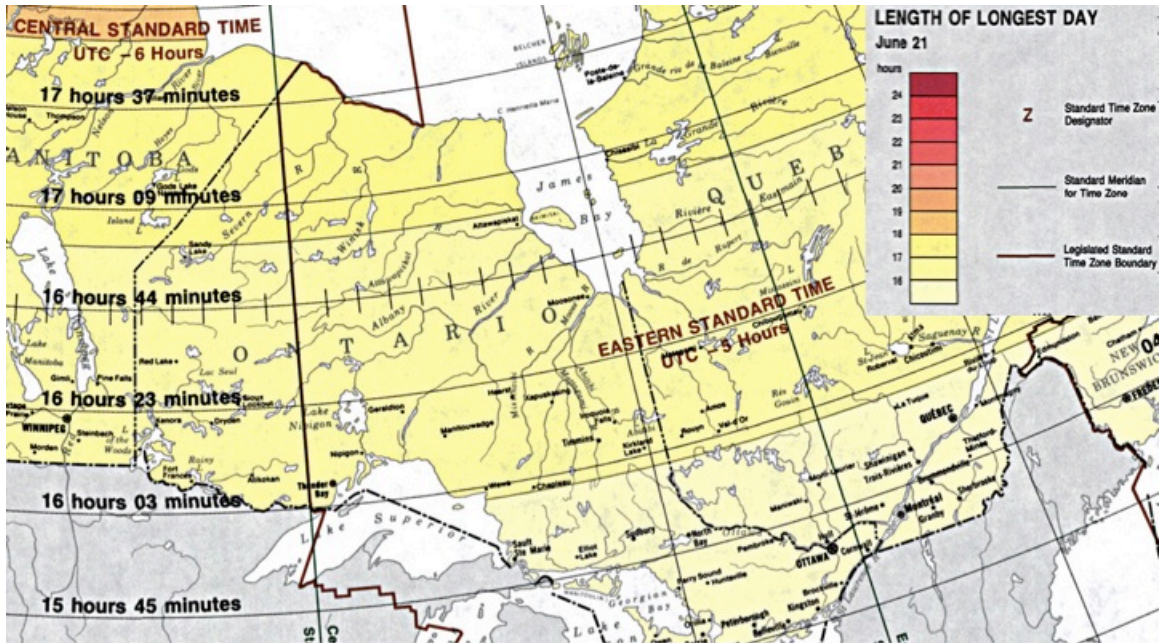
Figure 1.2. Variation spatiale de la durée du jour, mesurée lors du solstice d'été, dans la région de la Baie James et dans le sud du Canada. Figure adaptée de l'Atlas National du Canada, 5e édition (National Research Council, 1995).

Chapitre 2

Potential antidiabetic plants from the Eeyou Istchee Cree pharmacopoeia of Wemindji and Oujé-Bougoumou, Northern Quebec

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2.1 Abstract

Type 2 diabetes (T2D) has reached epidemic proportions in aboriginal communities across Canada with an average age-adjusted incidence of 29% of the population in some nations. In collaboration with the Cree Board of Health and Social Services of James Bay (CBHSSJB) and the CIHR Team in Traditional Antidiabetic Aboriginal Medicines (CIHR-TAAM), surveys were conducted in two Eeyou Istchee Cree communities to identify potential antidiabetic plants used within the traditional Cree pharmacopoeia. 52 Elders were interviewed using a list of 15 symptoms in association with T2D. Altogether, one fungal species and 28 plant species were identified by community members. Species were ranked based on their Syndromic Importance Value calculated using the frequency of citation by informants, the number of symptoms they were mentioned for and the importance of that symptom to T2D. Results concur with those obtained from past ethnobotanical studies conducted in other communities of the Eeyou nation. Although many of the same species were cited in these studies and have since been tested and shown to possess antidiabetic potential, 7 new species were identified. This study not only contributes to the edification of the general pharmacopoeia of the Eeyouch, but also demonstrates once again the importance of valuable quantitative tools in ethnobotany for bridging traditional and modern medicinal knowledge in the preliminary development of culturally sound therapeutic approaches.

Le diabète de type 2 (T2D) a atteint un niveau épidémique chez les communautés autochtones à travers le Canada et il affecte en moyenne 29% de la population dans certaines régions. En collaboration avec le Conseil Cri de la santé et des services sociaux de la Baie James et l'Équipe de recherche sur les médecines autochtones antidiabétiques des IRSC, des interviews ont été réalisés chez deux communautés cries de la Nation d'Eeyou Istchee. De ces deux communautés, 52 ainés ont été interviewés au sujet de l'usage traditionnel des plantes médicinales en utilisant une liste de 15 symptômes associés au T2D. Un champignon et 28 espèces végétales ont été identifiés par les membres de la communauté. Les espèces ont été classées selon la Valeur de leur Importance Syndromique calculée selon la fréquence des mentions, le nombre de symptômes pour lesquels les espèces ont été nommées ainsi que l'importance des symptômes au T2D. Les résultats concordent avec ceux d'études antérieures menées dans les autres communautés d'Eeyou Istchee. Plusieurs des espèces citées ont depuis été testées et montrent un potentiel antidiabétique. Nous avons tout de même identifié sept nouvelles espèces provenant de la pharmacopée crie. Cette étude souligne la nécessité d'utiliser des outils quantitatifs en ethnobotanique pour faire le pont entre les connaissances médicinales traditionnelles et modernes pour le développement préliminaire d'approches thérapeutiques culturellement adaptées.

2.2 Introduction

Diabetes is a chronic disease that has reached pandemic proportions. In 2011, the number of people affected by diabetes was estimated at 366 million and this number is expected to rise to 522 million people in 2030 (Whiting et al., 2011). Many ethnic groups are at risk, especially the indigenous peoples of the Americas (WHO and IDF, 2004), where transition from a traditional diet and lifestyle to a modern high glycemic diet and sedentary lifestyle are contributing risk factors to diabetes.

In Canada, diabetes is a growing concern with health care cost projected at $8.14 billions by 2016 (Ohinmaa et al., 2004). While it affected 6.8% of the total Canadian population in 2008/09, the incidence among First-Nations' population on reserves, is three times higher than the national average, and is considered by Health Canada to have reached epidemic proportions (Public Health Agency of Canada, 2011). With an age-adjusted prevalence of 29% (Kuzmina et al., 2010) the Cree of Eeyou Istchee (CEI) of Northern Québec are severely affected. Moreover, in northern regions of the country, where communities are often isolated, access to health care services and medical resources are often limited.

Because of long standing traditions in local medicinal practices and overall traditional knowledge, it has been suggested that traditional medicines, such as the Cree traditional medicine (CTM), may be used in the prevention and complementary treatment of diabetes (Marles and Farnsworth, 1995; Ryan et al., 2000; Young et al., 2000). This is particularly relevant since studies have revealed that current approaches to treating diabetes are culturally inappropriate in Cree communities (Boston et al., 1997; Johnston, 2002). Low compliance has led to higher levels of type 2 diabetes (T2D) complications and a resulting diminution in the quality of life. Since 2003, the CIHR Team in Aboriginal Antidiabetic Medicine (CIHR-TAAM) has been working in a multidisciplinary fashion with researchers, health care professionals, the Cree Board of Health and Social Services of James Bay (CBHSSJB) and CEI community members with the goal of identifying traditional medicinal plants and assessing their safety and mode of action.

An ethnobotanical approach to developing culturally appropriate therapies is thus warranted. Knowledgeable Healers from the communities of Mistissini (Leduc et al., 2006), Whapmagoostui (Fraser, 2006; Fraser et al., 2007), Nemaska and Waskaganish (Downing, 2010) participated in studies identifying traditional medicinal plants for T2D. The CIHR-TAAM has attempted to translate traditional knowledge into scientific knowledge through continuous discourse and cultural dialogue (Cuerrier et al., 2012). This collaboration has allowed the ethnobotanical prioritization and ranking of species for the pharmacological screening of their antidiabetic potential (Haddad et al., 2012). However, these studies represent less than half the CEI communities and cannot be considered a complete

representation of the CEI pharmacopoeia. The collaboration of all or most communities is thus essential in obtaining a better understanding of the CEI pharmacopoeia not only in providing appropriate recommendations for the research in antidiabetic plants but also ensuring that these are culturally relevant to all the CEI.

Prior to 1940, diabetes was rare amongst the Aboriginal population in North America (Thouez et al., 1990; Public Health Agency of Canada, 2011). Because the high occurrence of diabetes in CEI communities is relatively recent (first cases reported in 1983 with a 2.87 % prevalence (Thouez et al., 1990)), in-depth knowledge and understanding of T2D is poor. A novel approach, developed by Leduc et al. (2006), consisted of breaking down the disease into easily discernible and significant symptoms is employed in our study to a) rank traditional medicinal plants for research, b) identify plant species with strong associations to specific symptoms, and c) quantitatively compare communities. CEI resides primarily in the boreal forest region and we therefore expected major medicinal plants commonly used amongst Cree communities to be cultural keystone species, abundant and widely distributed within that region. However, the territory is vast, extending from the 49th to the 55th parallel, thus witnessing the overlap in niches of a variety of northern and southern plant species, as well as neighbouring cultural influences at the edge of the Cree territory. Hence, we reasonably expect specific uses and ranking to vary amongst communities.

Therefore the objectives of this study were to identify traditional medicinal plants from the CEI communities of Wemindji and Oujé-Bougoumou that have potential antidiabetic properties. In continuing the CIHR-TAAM collaborative research in antidiabetic plants, we present not only the first ethnobotanical study of Oujé-Bougoumou, but also, the first ethnobotanical reports of the latest communities to have joined the team. We compared our results to those from four other communities and report important medicinal plants commonly used by the CEI. In doing so, we report new boreal plant species that are added to the general CEI pharmacopoeia.

2.3 Materials and Methods

2.3.1 Study Site

The Eeyou Istchee is a large region in northern Québec also known as the James Bay region. It borders Nunavik in the north at the 55th parallel, and extends south to Abitibi-Témiscamingue and la Mauricie at the 49th parallel. From the James Bay coast in the west, it ranges to its eastern border, the Saguenay-Lac-Saint-Jean region. In the nine Eeyou Istchee communities, two dialects are spoken. These can be roughly categorized as the northern (or coastal) and the southern (or inland) dialects. Wemindji, at the 53rd parallel, is a coastal community speaking the northern dialect. Situated at the mouth of the Maquatua river where it flows into James Bay, it is a small community of 1,267 inhabitants relocated in the late 50's from Old Factory, a small island 30km to the south (Scott et al., 2009). Inland, Oujé-Bougoumou sits on the shores of Lake Opemisca, not too far from Chibougamau and Chapais at the 50th parallel. Designed by the Métis architect Douglas Cardinal and built in 1992, it is one of the youngest and smallest communities, with a population of 725 people (Statistique Canada, 2012). It is also one of the most progressive, gaining international recognition by winning a UNESCO award for its construction based on criterion of sustainable development.

2.3.2 Interviews

Participants were identified by community members as possessing a great deal of knowledge in traditional herbal medicine. Interviews took place in each community for a period of one month. They began in June 2011, starting with Wemindji, and July for Oujé-Bougoumou. The average age of participants was 72 (n=27) years in Wemindji and 66 (n=22) in Oujé-Bougoumou. While the average age in Oujé-Bougoumou might be slightly lowered by two participants in their mid to late 40's, this, nonetheless, reflects the relatively young age of the community's members. Couples were interviewed in pair and in one case, two siblings preferred to be interviewed together. These were consequently counted as n=1. One Métis and one Inuit, who now live in Wemindji, were also known for their knowledge in herbal remedies. While these two were interviewed, they were not included in the calculation of SIVs

in order to keep strictly to the Cree pharmacopoeia and, consequently, the information they shared will simply be mentioned in the discussion.

The survey used the same semi-structured questionnaire previously used by Leduc et al. (2006), Fraser et al. (2007) and Downing (2010) in the aforementioned communities. The survey is structured around 15 symptoms and complications in association with T2D (**table 2.1**). Survey and methods were approved by the Université de Montréal ethics committee, « Commité d'éthique de la recherche de la faculté des arts et de sciences (CÉRFAS) » (**appendix I**). Informed consent was obtained prior to the interview and participants were informed that they were not obligated to answer all questions and that they could stop the interview at any time. Four voucher specimens were collected for each plant cited and used to confirm their identity. Specimens will be donated to the Cree Cultural Institute and the local school for educational purposes, while the others will remain in the Marie-Victorin (MT) and University of Ottawa (OTT) herbaria (**appendix II**).

2.3.3 Ranking

Each plant was ranked based on their frequency of citation (FC; Ladio and Lozada, 2004) which reveals their relative importance and their syndromic importance value (SIV; Leduc et al., 2006). Due to the inclusion of personalized disease symptoms into the equation, the SIV ranking enables the comparison of the plants' potential importance to the treatment of a specific illness, such as T2D in this case. It is this measure of importance that is used to compare pharmacopoeias amongst communities and rank all these as one overall population. The SIV is calculated using the following equation,

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

where "F" is the total number of interviews, "*f*" is the frequency of citations and "S" is the number of symptoms in the survey (*i.e.* 15) for which each contributes a value *"s"* of 0 or 1 and has a specific degree of association to T2D, "*w*" ((0 < *w* < 1, $\sum w = 1$), table 2.1). Thus "$\sum ws$" is the sum of all weighted symptoms for which the plant was mentioned, whereas $\sum ws$

= 1 if a plant was mentioned for all 15 symptoms, and "∑*wf*" is the sum of the symptom weight multiplied by the amount of times it was mentioned for that use (Leduc et al., 2006).

2.3.4 Data Analysis

Results were analyzed using both univariate and multivariate statistical analysis. Spearman rank correlations and matrix comparisons such as Mantel tests and data level comparison tests as per Leduc et al. (2006) and Downing (2010) to compare ethnobotanical results from one community to the next. Wemindji and Oujé-Bougoumou are thus compared against one another as well as against Whapmagoostui, Waskaganish, Nemaska and Mistissini. In order to determine the degree of association between plant species and specific symptoms within the survey list, correspondence analysis was performed (Leduc et al., 2006).

Non-parametric Spearman rank correlations were used to evaluate the similarity amongst species ranking (i.e., importance) in each community. These analyses were performed on SIV results in order to keep with the methodology first employed by Leduc et al. (2006). This measure of importance also proved more informative than FC rankings as the latter ranked many plant species equally (see also the comparison of different methods in Araújo et al., 2008). Only species in common to both communities being compared were included in the analysis so that they would be ranked 1 to *n*, whereas *n* = the number of species in common.

Matrices were produced with symptoms as objects and species as descriptors for each community and then compared using multivariate statistical tests. Mantel tests enabled us to compare distance matrices from two communities based on the null hypotheses that these were no more similar that two randomly generated matrices. For these, raw data were transformed into presence/absence of species mentions (1 or 0). Euclidean distances were calculated amongst symptoms prior to obtaining the Mantel statistic and significance was obtained by permutation tests (100,000 permutations). For data level comparison tests, raw data, corresponding to the number of mentions for each plant species at any given symptom, were employed. These tests use Manhattan differences via 10,000 permutations of rows, columns or both (developed by Podani, see Leduc et al., 2006). This enabled us to further explore how communities resembled one another without relying on prior modification of the data into

distance or similarity matrices. Matrices were constructed by including solely the plant species which appeared in both communities being compared.

Correspondence analyses were performed on two-dimensional contingency tables constructed similarly to those employed in data level comparison tests. All species mentioned more than once in the survey were included and the data in each cell correspond to the number of mentions. Those which appeared once were considered outliers and removed from the table. Results were visualized by three-dimensional scatter diagrams representing both species and symptoms. Species are said to be closely associated to a symptom if both points lie in proximity of one another. This holds true if a) the species had the highest frequency of citation for the symptom and b) the symptom had the highest frequency of citation for the particular species (Leduc et al., 2006).

In order to compare plant uses of the CEI with neighbouring First Nations from the same linguistic family, all the species compiled since the beginning of these surveys were traced back into the scientific literature (Arnason et al., 1981; Marles et al., 2008; Moerman, 2009; Uprety et al., 2012). Plant uses from Wemindji, Oujé-Bougoumou and the compiled results of all 6 communities were compared against uses by the Algonquin, Atikamekw, Innu and Ojibway. A generalized matrix for the Algonquian linguistic family was also constructed by summing up the uses from all those First Nations. CEI plant uses were also compared to those of the Nunavik Inuit based on the literature (Cuerrier and Elders of Kangiqsualujjuaq, 2011; Cuerrier and Elders of Kangiqsujuaq, 2011; Cuerrier et al., 2011). Because a reliable measure of consensus and frequency based on the literature could not be made, data were summarized as presence/absence of species mention and analyzed using Mantel tests.

Spearman rank correlations, Mantel tests and correspondence analysis were all performed using R (R Development Core Team, 2012). Data level comparison tests were performed using the algorithm programmed by Podani (see Leduc et al., 2006).

2.4 Results

2.4.1 Ranking

In Wemindji, 22 interviews were completed with 28 Elders. Because two of the informants were not Cree, they where excluded from the ranking calculations thus providing *n* = 20 interviews. Sixteen plants were listed, one of which, *Populus tremuloides* (**table 2.2** and **2.3**), is new to the project (species names and family are presented in **appendix IV**). While most of the plants cited by both non-Cree participants belong to the Wemindji Cree pharmacopoeia, *Empetrum nigrum, Rhododendron tomentosum* Harmaja*, Chamerion angustifolium* subsp. *angustifolium,* and *Salix* spp. were unique to them. Furthermore, *Sorbus decora* was cited by the Métis Elder, while *Andromeda polifolia, Honckenia peploides* and *Fucus* spp. were cited by the Inuit Elder. *Kalmia angustifolia, Abies balsamea, Rhododendron groenlandicum, Picea mariana* and *Sphagnum fuscum* were the top 5 species according to SIV ranking (**Table 2.2**). With the exception of *K. angustifolia,* which is replaced by *Larix laricina,* these species also hold the top 5 spots with the FC ranking (**table 2.3, figure 2.1a**).

As for Oujé-Bougoumou, 18 interviews were completed with 24 Elders mentioning 25 plants (**table 2.4** and **2.5**). *Prunus pensylvanica, Cornus stolonifera, Rubus pubescens, Ribes lacustre, Salix humilis* var. *humilis* and an unknown plant by the Cree name achikaashwaashkw are new to the project. Interestingly, their rankings are low. A fungus which could not be identified to species level, *Boletus* spp., was also new to the project and is the only mention of a fungal species in all 6 communities. The fruits of *R. pubescens* and *R. lacustre* were given by one of the youngest participants in response to the symptom of general weakness, whereas *C. stolonifera* was given by the second youngest participant who learned the practice from his mother, an Elder of the nearby community of Waswanipi. For both *Boletus* spp. and *C. stolonifera,* traditional Cree names could not be given. *Populus tremuloides* was also cited and bears the same Cree name as *Populus balsamifera*, as is the case in Wemindji. *Plantago major* was also cited by a few participants and is new to the project. Because it is an introduced plant in the region, it was omitted from the analysis. *Larix laricina, R. groenlandicum, Sorbus* spp., *Alnus incana* subsp. *rugosa* and *A. balsamea* were the top 5 species according to SIV ranking (**table 2.4**). With the exception of *Sorbus* spp.,

which is replaced by *Sarracenia purpurea*, these species also hold the top 5 spots with the FC ranking (**table 2.5, figure 2.1b**).

In both communities, informants claimed certain species could be used specifically for general diabetes. In Wemindji, these were *R. groenlandicum, Vaccinium vitis-idaea, Vaccinium angustifolium* and *Gaultheria hispidula. Rhododendron groenlandicum* was mentioned in Oujé-bougoumou as well as *R. tomentosum*. Elders from that community also stressed the general health benefits associated with the consumption *V. angustifolium* and *G. hispidula* berries.

Other plant-based medicinal preparations made from store bought foods and materials were frequently mentioned in both communities. These involve various preparations made from flour, rice, oats, tea, bread, peanut butter or even pipe tobacco. Although they were frequently cited and are worth mentioning as part of the Cree pharmacopoeia, these were not included in our analyses as they are not native, nor do they grow readily in the boreal forest region.

Species of the animal kingdom are also an important faction of the Cree pharmacopoeia. Preparations made with the grease of beaver, *Castor canadensis,* black bear, *Ursus americanus,* Canada goose, *Branta canadensis,* either alone or as adjuvants to plant medicinal preparations, were also mentioned. Beaver castoreum, ***wishna***, regarded as a particularly strong medicine, is frequently employed and mentioned. Other animal species appearing in the surveys are mink, seal, otter, mice and fish such as walleye, pike and whitefish.

When the data from all communities are combined together, there are a total of 32 plant species, excluding mosses, cited for the 15 diabetes associated symptoms. *Rhododendron groenlandicum, L. laricina, R. tomentosum, P. mariana* and *Picea glauca* remained among the top 5 plants (**table 2.6**), as reported in Downing (2010), and differed from that study only in *P. glauca* and *P. mariana* switching ranks.

2.4.2 Community Comparison

Results of Spearman rank correlations, Mantel tests and data level comparison tests are presented in **table 2.7**. Oujé-Bougoumou possessed significant similarities in plant SIV ranking to Whapmagoostui ($r = 0.78$, $p = 0.017$) and Mistissini ($r = 0.60$, $p = 0.02$). Although Wemindji and Oujé-Bougoumou possessed a relatively high Spearman's r, it was not statistically significant ($r = 0.53$, $p = 0.079$).

Mantel tests using presence-absence matrices of plant uses enable us to compare how similar they are between communities. Contrary to results from the Spearman's rank correlation, Wemindji and Oujé-Bougoumou were significantly similar regardless of a small correlation factor ($r = 0.21$, $p = 0.036$), as were Wemindji and Nemaska ($r = 0.19$, $p = 0.041$). As with the Spearman's rank correlation, the similarity between Oujé-Bougoumou and Mistissini was stronger, as well as highly significant ($r = 0.43$, $p = 0.0019$).

Results from data level comparison tests were considerably more liberal, with Wemindji and Nemaska being the only pair of communities not showing significant similarities based upon low Manhattan differences. These results conflict with those from the Mantel test between Wemindji and Nemaska. Although the Mantel test suggests that plant uses are similar between these two communities, raw counts are used in the data level comparison test. It may be that where uses are similar in the former test, the consensus factor, represented by high counts of specific species to specific symptoms, may not be strong enough to warrant similarities in the latter. The opposite appears to apply as well. Whereas general plant uses between two communities may differ based on Mantel results, the consensus factor, where similarities exist for specific uses, may be high enough to appear significant in data level comparison tests.

With regards to all three tests, only Oujé-Bougoumou and Mistissini seemed to be truly similar to one another.

2.4.3 Symptom-Species Associations

Symptom-species associations for each community are summarized in **table 2.8**. In Wemindji (**figure 2.2a**) four plant species were strongly associated with symptoms in three

distinct groupings. Both poplar species, *P. balsamifera* and *P. tremuloides*, were strongly associated with the treatment of abscesses and boils, the latter being mentioned more frequently than the former. *Juniperus communis* was strongly associated to the symptom of frequent urination and *V. vitis-idaea* was the only species that could be associated with two symptoms: blurry vision and increased thirst.

In Oujé-Bougoumou, species-symptom associations did not appear as evident as those in Wemindji. Nonetheless, seven species closely linked to symptoms formed three distinct groupings (**figure 2.2b**). *Vaccinium angustifolium* was associated to general weakness whereas *S. purpurea, L. laricina* and *A. balsamea* were associated with infections. For back and kidney pains, three plants came out, namely, *K. angustifolia, R. groenlandicum* and *P. pensylvanica*, the latter being mentioned only once for that symptom. Although this grouping is visually discernible, the species that can be associated with back and kidney pains had higher frequency of mentions for other symptoms that were popularly treated by a variety of plant species as well. These were not highlighted by the correspondence analysis as both species and symptoms play a role in the algorithm. The association of a frequently cited plant to a symptom will inherently be weighted by other species which are just as frequently cited.

When all six communities are analyzed together, *J. communis, V. vitis-idaea, P. balsamifera* and *P. tremuloides* remained strongly associated with frequent urination, blurry vision and abscesses and boils, just as they were when analyzing the community of Wemindji separately. *Sarracenia purpurea* was associated with symptoms of infections as in Oujé-Bougoumou and *A. incana* subsp. *rugosa* was the only species that could be associated with two symptoms intricately related: abscesses, boils and infections (**table 2.9, figure 2.3**).

2.5 Discussion

During the interview process, Cree Elders often referred to morphologically similar plants interchangeably by giving them the same name and usage. Taxa in which this has been noted include *Sorbus* spp. (*S. americana* and *S. decora*), blueberries, herein referred to as *V. angustifolium,* but encompassing also *V. myrtilloides* and *V. boreale,* and clubmosses, herein

referred to as *Lycopodium clavatum,* but also encompassing *L. annotinum* Leduc et al. (2006) reported the same for *Alnus* spp. (*A. incana* subsp. *rugosa* and *A. viridis* subsp. *crispa*) among the Mistissini Cree, although it has since been rectified to include only *A. incana* subsp. *rugosa* (Cuerrier, *verbatim).* Indeed, Elders from Wemindji and Oujé-Bougoumou clearly specified as **utuuspii** the species whose branches and inner bark turns red upon bruising and cutting, which is consistent with *A. incana* subsp. *rugosa.* Although no distinction was made in the identity and uses of the aforementioned species, *Populus tremuloides* and *P. balsamifera* were distinguished as separate plants even though they have identical names (**mitus**) and similar uses (**table 2.2** and **2.4**). In fact, a few Elders from Mistissini distinguish *P. balsamifera* by the name **mash-mitus** as opposed to **mitus** (Cuerrier, *verbatim*). Surprisingly, this name did not receive any mention in Oujé-Bougoumou, a neighbouring community sharing the same dialect. Given their lack of popularity within the community's pharmacopoeia, it may be that the name has simply not been retained in the community or that it is simply vernacularly distinct to Mistissini.

The frequency of citation, as a basic measure of informant consensus, reveals the relative importance of species (Ladio and Lozada, 2004). High FC values reflect top SIV ranking order between species and these consensuses support the idea that these species are culturally important to the CEI. However, similar ranking orders between these two methods (**figure 2.1**) is expected as consensus is also implied in the SIV calculation. Yet differences in the order of certain species point to differences that exist between the FC and SIV calculations. First, it assumes, like the SIV method, that the more biologically effective a plant is, the more agreement there will be concerning its use, but it also assumes that specialized knowledge is not present (Araújo et al., 2008). In spite of a relatively low FC ranking in Wemindji, *K. angustifolia* was the top ranking species when using the SIV method. It is used in the community much like *R. groenlandicum* but is believed to be stronger. Nonetheless, the fact that the plant is toxic (Hill, 1986) is known to the community members who constantly advise that its use be undertaken with great caution. Risk versus benefit assessment may lead to informants preferring the use of a safer plant and perhaps being more selective in sharing this knowledge. Furthermore, the FC method is less informative than the SIV and does not distinguish as many ranks amongst species. When comparing different quantitative methods of

ranking medicinal plants, it was shown that the SIV approach allowed the identification of plant species with high levels of biologically active compounds (Araújo et al., 2008). Combining symptoms, and their relevance, to the consensus factor not only narrows the search through specialized knowledge, but provides a seemingly more informative index to identifying biologically active plants and compounds.

Although these are the first mentions of *P. tremuloides, Betula papyrifera, R. lacustre, P. pensylvanica, Typha latifolia* and *Boletus* spp. in interviews conducted within the scope of the CIHR-TAAM project, *B. papyrifera* and *T. latifolia* had been mentioned in Mistissini at a meeting held during the cultural gathering (2008) and at another meeting in Chibougamau in 2009. Furthermore, *B. papyrifera* has already been reported in the CEI pharmacopoeia for treating symptoms employed in this study. It is reported in both the communities of Chisasibi (Marshall, 2006) and Waskaganish (Marshall et al., 2003) for treating abscesses, boils, wounds and skin infections such as impetigo and scabies. This highlights the unique contributions in knowledge that each community can bring as well as the importance of including as many communities as possible in completing the Cree pharmacopoeia. Interestingly, *B. papyrifera* did not arise in Downing's (2010) ethnobotany of Waskaganish, nor did cattails (*T. latifolia*), which are also reported in Waskaganish for skin infections (Marshall et al., 2003). Moreover, Jacques Rousseau noted the use of *P. pensylvanica* and *P. tremuloides* for medicinal purposes by the Mistissini Cree (Cosset and Mansion, 2009). Although these are not being specified for uses related to T2D, it does provide evidence nonetheless of their presence in the CEI pharmacopoeia.

Differences in the usage of plant species are the result of many factors. The distribution of plant species, for one, explains why some species are employed in certain communities, and not in others. *Thuja occidentalis* reaches its northern limits around Waskaganish and Oujé-Bougoumou (Chambers, 1993), while *S. purpurea* is absent from the area of Whapmagoostui (Forest and Legault, 1977). Where distribution is not a factor, unique local histories and inherent subcultures may play an important role. It may also simply be that plant species are not used in the same way (Johns et al., 1990) by different groups. Nonetheless, these differences do not undermine the cultural relevance and value of these plants towards the CEI as it is evidenced by intercommunity comparisons.

When compiling the data from all the communities, the highest ranked species are commonly used in most communities and often ranking high in each of them. Among the top 10, only two species were absent from more than two communities. *Rhododendron tomentosum*, a close relative of the popular *R. groenlandicum*, was mentioned solely in Whapmagoostui, yet ranked 3rd overall and *J. communis* ranked 10th (**table 2.6**). Even if mentioned for many symptoms, some of these highly ranked species seemed strongly associated to specific ones (**table 2.8** and **2.9**). *Alnus incana* subsp. *rugosa* is acknowledged by at least half the participating communities to be effective against skin infections, abscesses and boils, while *Juniperus communis* was strongly associated with the symptom of frequent urination in the community of Wemindji. Similarities in uses between this community and Whapmagoostui means that this remained true when combining the knowledge from all participating communities. Although contrary to the idea that Juniper cones act as a diuretic (Stanić et al., 1998; DiPasquale, 2008), CEI traditional uses suggest a general application of this species to ailments associated with voiding of urine.

Alternatively, species with low SIV values need not be regarded as being less important, particularly if they are strongly associated with certain symptoms. Such is the case of *V. vitis-idaea* for blurry vision. This symptom was generally associated with the culturally relevant problem of snow blindness or photokeratitis, overexposure by UV irradiation to the cornea and conjunctiva (Cullen, 2002) causing damage, even destruction, of epithelial cells and significant stromal swelling (Bergmanson, 1990; Cullen, 2002). *Vaccinium vitis-idaea*, however, is known to possess anti-inflammatory activity (Tunón et al., 1995; Markov et al., 2011), which may play a role in attenuating the damaging effect of extensive ultra-violet exposure to the eyes. Also, blurry vision, like general weakness, may also be caused by the hypoglycemic state found in diabetics, as a side effect to therapy, such as insulin or sulphonylurea, seeking to restablish normoglycemia (Hepburn et al., 1993; Henderson et al., 2003). Consequently, spikes in blood sugar, caused by the absorption of those naturally found in the fruit, might give temporary relief of this symptom. In brief, even if certain species may eventually show low antidiabetic potential, strong bioactivity to certain symptoms and complications can nonetheless increase the quality of life of diabetics if taken appropriately.

Similarly, species mentioned in selected communities should not be regarded as less important. The absence of *R. tomentosum* from other communities is due to its natural distribution, but knowledge of its medicinal uses exists outside of Whapmagoostui. One community member from Oujé-Bougoumou kept in the freezer a container filled with the aerial parts of *R. tomentosum* which had been obtained from Whapmagoostui and used it for medicinal purposes. On the other hand, community members from Wemindji had a name for the species with claims that it grew on the islands within James Bay. Indeed, a study of the flora of Wemindji and its surroundings (Blondeau, 2009) does not report *R. tomentosum*. Nor does it report *K. angustifolia,* which is commonly used in Wemindji (**table 2.2** and **2.3**). Although we could not confirm this species in the immediate surroundings of the community, we managed to find it in the greater region (MTR-2011-086; MT and OTT herbarium), an area which was not included in Blondeau's (2009) study. Naturally, community members may travel long distances to have access to hunting territories not only inland, but also into James Bay. Many of the islands are characterised by the similar arctic and boreal ecological elements (Bussières et al., 2008). The Manitounuk Islands, in Hudson Bay, is where *R. tomentosum* is found (Deshaye and Cayouete, 1988). In venturing out on islands further off the coast, it may in fact be possible to find populations of this species in the Wemindji region. If that is the case, evaluating these populations' susceptibility to potential harvesting, as it has been done for *R. groenlandicum* (Tendland et al., 2012), will be indispensable.

The above situation provides evidence of the transfer of knowledge that exists within the CEI nation. But not only are knowledgeable Elders from certain communities known by others, there is also a certain openness to knowledge coming from outside. In Wemindji, where an Inuit and Métis Elder became fixtures, they were known for their mastery of medicinal plants and consulted by Cree community members. Although many of the plants cited by these two are common to the CEI pharmacopoeia, many others such as *C. angustifolium* and *Honckenya peploides* seem to reflect knowledge unique to these Elders' cultural background (Cuerrier and Hermanutz, 2012).

In Oujé-Bougoumou, there were frequent mentions of the current cultural influences from the Ojibway as well as from the neighbouring Innu of Mashteuiatsh, consequently highlighting the differences in the naming and usage of plants. Interactions between the Innu

from Lac Saint-Jean and the Oujé-Bougoumou Crees are well documented. Whereas many families have hunting territories in proximity to rivers leading into the lake, many had historically adopted the habit of reprovisioning at the Mashteuiatsh trading post instead of Mistissini, thus creating close relationships between certain families (Frenette, 1985). This interaction with members of neighbouring First Nations may explain the presence of a considerable number of outliers: species with infrequent mentions, low SIV values and/or specific to certain communities. This is particularly supported by strong similarities, from Mantel test results, in plant uses between Oujé-Bougoumou and the Innu-Naskapi ($r = 0.42$, $p = 0.013$) and the Ojibway ($r = 0.49$, $p = 0.0067$, **table 2.10**).

Medicinal plant uses by the community of Wemindji were also statistically similar to uses by the Innu-Naskapi ($r = 0.36$, $p = 0.0048$). Based upon the literature, of the 33 plant species mentioned in the Cree pharmacopoeia, all but *A. polifolia, E. nigrum, Leymus mollis, R. pubescens* and *Vaccinium uliginosum* possessed medicinal uses by First Nations of the Algonquian linguistic family for key T2D associated symptoms identified in this study. As an Algonquian nation, it is not surprising that CEI uses were statistically similar to those of culturally and linguistically related nations (**table 2.10**).

Cultural influence is a basic assumption, which can certainly be made about certain plant uses in Whapmagoostui, the northernmost CEI community, which is often mistaken for Kuujjuarapik, the adjacent Inuit community. Although no significant similarity was statistically detected between Cree and Inuit uses (**table 2.10),** *P. mariana, P. glauca, L. laricina, J. communis, E. nigrum, V. uliginosum, V. vitis-idaea, Salix planifolia, R. tomentosum* and *R. groenlandicum* were common to both pharmacopoeia. Furthermore, plant species unique to Whapmagoostui and mentioned by Wemindji's Inuit Elder are all present in the ethnobotany of Kuujjuarapik and other Inuit communities (Cuerrier and Elders of Kangiqsualujjuaq, 2011; Cuerrier and Elders of Kangiqsujuaq, 2011; Cuerrier et al., 2011).

While some knowledge is specific to certain families and contribute to local specificities in plant uses, the loss of knowledge resulting from traumatic experiences felt by some communities may also be a contributing factor to these differences. These include residential schools, relocations and other collateral implications from the major hydro projects of the 1970's (Berkes, 1981; Kirmayer et al., 2000). Whereas uses and traditional knowledge

of certain plant species may have been borrowed, some communities may also act as a refuge for knowledge from a once larger and extentsive CEI pharmacopoeia.

Plant uses by the aboriginal people of Canada's boreal forest for diabetes are not very common. Aside from a few documented cases, this appears to be a reflection of the modern day rise in T2D affecting the CEI and other aboriginal people. Of the species appearing in our study, *P. balsamifera, P. tremuloides* and *Ribes americanum*, a close relative of *R. lacustre* cited here, are mentioned specifically for diabetes (Marles et al., 2008; Uprety et al., 2012). Using quantitative methods and breaking down an illness into basic symptoms is an effective approach in bridging the gap between traditional knowledge and modern ailments, as our study has shown. However, it cannot be used solely by itself. Even in plants where SIV values are low, it is argued that the lack of direct information does not imply biological inactivity (Moerman, 2007). Although this measure is appropriate for assessing the cultural relevance of a medicinal plant, one must also consider the complex nature of relationships existing with plant species. Berries, such as those of *V. angustifolium, V. vitis-idaea, V. uliginosum* and *E. nigrum,* are primarily considered as food by the CEI and are consequently ranked poorly. Yet the scientific literature abounds with studies supporting the health benefits and antidiabetic potential of many of these berries (**table 2.11**) due in part to an abundance of phenolic compounds (Martineau et al., 2006; McIntyre et al., 2008; Grace et al., 2009; Graf et al., 2010; Hohtola, 2010; Sancho and Pastore, 2012).

Many of these berries are abundant and were once an important part of the Cree diet (Anonymous, 1971; Berkes and Farkas, 1978). Blueberries are still culturally important and celebrated in certain communities, such as Oujé-Bougoumou, with yearly festivals. Nonetheless, the CEI would greatly benefit from a significant reintroduction of these berries into their diet. Just as the Cree Trapper's Association manages programs established to support the Cree Hunters in their activities, similar programs should be created for the gathering of wild berries, which, in the case of abundant species, could potentially open the door to an economically viable commerce (Määttä-Riihinen et al., 2005). One needs only consider the case of *V. vitis-idaea,* strongly associated with blurry vision (**table 2.8** and **2.9**), which is harvested commercially in Scandinavian countries (Ihalainen et al., 2003), as well as in

Newfoundland and Labrador, and can be harvested from unmanaged natural stands (Penney et al., 1997).

While studies show there is a consumer interest in boreal forest products of aboriginal origin (Boxall et al., 2003; Murray et al., 2005), there already exists a line of successful products marketed by aboriginal and non-aboriginal groups alike (BMC - Boreal Medicinal Canada, 2011; Avataq Cultural Institute, 2012; Lise Watier Cosmétiques, 2012; Produits es Bois Inc., 2012). This raises questions about the concept of commercialization of non-timber forest products, which is not always well accepted amongst aboriginal people. Without exception, CEI Elders consider themselves custodians of the land where knowledge provided by the creator is to be used for human health and wellbeing (Cuerrier et al., 2012), hence, a responsibility of stewardship (Sioui, 2012). However, this may locally render accessible medicinal products to those whose lifestyle or physical and health limitations may not allow the personal collection of these materials and in periods and times when they cannot be collected. Elders should thus be concerned with and consulted on potential strategies towards making CEI medicine more accessible.

Many of the plants mentioned in the ethnobotany of Wemindji and Oujé-Bougoumou, particularly amongst the high ranking ones, have undergone more in depth studies since the creation of the project in 2003 (Haddad et al., 2012). The screening process has revealed that many of these plants are active *in vitro* in various antidiabetic bioassays (Spoor et al., 2006; Fraser et al., 2007; Harbilas et al., 2009). The highest ranking plant of the CEI, *R. groenlandicum*, has been shown in multiple *in vitro* assays to possess antioxidant activity (Dufour et al., 2007; Fraser et al., 2007) a strong adipogenic activity and slight activity in glucose uptake (Spoor et al., 2006), all of which play an important role in the pathology of T2D. In a diet-induced obese mouse model, it has also been shown to possess hypoglycemic and anti-obesity properties *in vivo* (Ouchfoun, 2011). In the same model, *L. laricina* effectively decrease glycemia levels, improve insulin resistance, and decrease abdominal fat pad and body weight (Harbilas et al., 2012a), while *P. balsamifera* was also shown to reduce glycemia, weight gain, retroperitoneal fat pad and liver as well as improve insulin sensitivity (Harbilas et al., 2012b).

In keeping with providing recommendations for the application of these traditional medicines for the treatment of T2D, multiple studies have been realized on the impact of harvest as well as the seasonal and geographical variations in biologically active compounds. It was shown that harvesting of *R. groenlandicum* was sustainable if leaves from the previous growing season were collected (Tendland et al., 2012) and that concentrations in active compounds varied geographically in response to environmental variables (Rapinski et al., in preparation(a)). Concentrations in the active compounds of *R. tomentosum* and *V. angustifolium* leaves and stem varied throughout the growing season to reach optimum concentration at the end of summer or beginning of fall, in turn affecting these plants' bioactivity (McIntyre et al., 2008; Black et al., 2011). These studies show the need for knowledge of harvesting time and place in the production of reliable standardized and sustainable products. Surely, strong consideration should be taken of the impact of harvesting these plants, especially when heavy impact harvesting methods, such as removing the bark, are used.

Evidently, treatment of T2D with drugs does not cure the disease, but does indeed increase the quality of life of those affected. That is true even among a demographic group which is compliant and is used to the current health care approach. Thus, careful attention to the diet of diabetics is necessary and in agreement with the opinions of Elders and other community members. While this study focuses on exploring medicinal plants and their antidiabetic potential, Elders are quick to stress that food and medicine go hand in hand, and when one talks about food, one talks about plants and animals alike. During the interview process, animals were often cited for the treatment of many symptoms (data not shown). More importantly, however, is the report by community members affected by T2D, and disciplined in checking their blood glucose levels, that extended periods of feeding oneself with traditional meats effectively has a hypoglycemic effect. While this important observation has yet to be verified in a controlled study, they certainly cannot be ignored.

2.6 Conclusion

We clearly show in this study that many of the plants in the CEI pharmacopoeia are generally and culturally important to Wemindji, Oujé-Bougoumou and its neighboring communities. Many of these top ranking species identified within the CTM have shown pharmacological activity and potential benefit to treat TD2. While our results demonstrate the potential benefit of using multidisciplinary approaches in medicinal plant research, they also support the ongoing research on these plants, especially pertaining to high ranking species. Furthermore, special attention should be given to *Vaccinium* spp., which, although scoring low in the SIV ranks, are readily available in the CEI territory and are known for their health benefits. Continuing work on selected species identified here may help provide better recommendations for the development of sustainable and alternative therapies, but much thought and discussion must be devoted to ensuring that these are culturally and ethically appropriate.

2.7 Acknowledgment

2.8 Tables and Results

Table 2.1 List of 15 symptoms and complications used in semi-structured questionnaires. The weight (*w*) represents their degree of association to type 2 diabetes and is used in the SIV calculation.

Symptom	*w*
1. Abcesses/boils	0.0838
2. Increased appetite	0.0681
3. Back/kidney pain	0.0419
4. Diarrhea	0.0419
5. Foot numbness/sores	0.0942
6. Headache	0.0366
7. Heart/chest pain	0.0733
8. Skin infections; slow healing	0.0942
9. Inflammation/swelling	0.0471
10. Rheumatism/arthritis	0.0366
11. Sore/swollen limbs	0.0681
12. Increased thirst	0.0785
13. Increased urination	0.0890
14. Blurred vision	0.0838
15. General weakness	0.0628
$\sum w$	1

Table 2.2 Species mentioned by informants in Wemindji in decreasing order of SIV ranking. 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.

SIV	Rank	Latin	Cri	S	I	O
0,0265	1	*Kalmia angustifolia*	ᐅᔅᒋᔅᒋᐱᐧᒀ uschischipikw-h	11	9	L
0,0232	2	*Abies balsamea.*	ᐃᔮᔒᐦᑦ iyaashiht	8	20[1]	Ca, L, S
0,0212	3	*Rhododendron groenlandicum*	ᑳᒋᒑᐱᒄ kaachichaapikw	12[1,1,2]	13[1,2]	L
0,0169	4	*Picea mariana*	ᐃᔮᐦᑎᒄ iiyaahtikw	7[1]	16[1]	Ca, W, S, L, C
0,0168	5	*Sphagnum fuscum*	ᐊᐧᐋᔒᔥᒌᔥ awaashishchiish	7	15	A
0,0157	6	*Larix laricina*	ᐙᒋᓈᑭᓐ waachinaakin	9[1,1,2]	19[1,2]	Ca, S, B, L, Br
0,0128	7	*Betula papyrifera*	ᐧᐃᔥᑯᐃ wishkui	6	1	S
0,0120	8	*Picea glauca*	ᒥᓂᐦᐄᒄ minihiikw	6[1]	8[1]	S, L, Ca, C
0,0114	9	*Populus tremuloides*	ᒦᑐᔅ miitus	6[1,1]	11[1]	Ca, B, CaA, L
0,0099	10	*Vaccinium vitis-idaea*	ᐧᐄᓯᒋᒥᐤ wiisichimin	4[2]	11[1,2]	F
0,0065	11	*Populus balsamifera*	ᒦᑐᔅ miitus	2	4	Ca, CaA
0,0055	12	*Juniperus communis.*	ᑳᐦᑳᒋᐄᒥᓈᐦᑎᒄ kaahkaachiiminaahtikw	3[1]	8[1]	C
0,0044	13	*Alnus incana* subsp. *rugosa*	ᐅᑐᔅᐲ utuspii	1	8	B, Ca, Br
0,0029	14	*Typha latifolia*	ᐅᒋᔥᒁᔪᐦᒡᑐᔫᐦ uchishkwaayuushkushiuh	1	1	R
0,0027	15	*Vaccinium angustifolium*	ᐄᔨᒥᐤ iiyimin	2[2]	2[2]	F
0,0027	15	*Vaccinium uliginosum*	ᓂᒋᑯᒥᐤ nichikumin	2[2]	2[2]	F

L = leaf/needles, Br = branch, S = sap/gum, W = wood, B = bark, C = cone, Ca = cambium, A = aerial part, CaA= cambium ash, F = fruit, R = root

1 = (S) Symptom mentioned by the Métis informant and not the Cree; (I) Métis informant included

2 = (S) Symptom mentioned by the Inuit informant and not the Cree; (I) Inuit informant included

Table 2.3 Species mentioned by informants in Wemindji in decreasing order of rank based on the FC method. Consensus is represented by the frequency of citations calculated over the total number of interviews.

Species	% Frequency	Rank
Abies balsamea	95	1
Larix laricina	85	2
Picea mariana	75	3
Sphagnum fuscum	75	3
Rhododendron groenlandicum	55	4
Populus tremuloides	50	5
Vaccinium vitis-idaea	45	6
Kalmia angustifolia	45	6
Alnus incana subsp. *rugosa*	40	7
Juniperus communis	35	8
Picea glauca	35	8
Populus balsamifera	20	9
Vaccinium angustifolium	5	10
Vaccinium uliginosum	5	10
Betula papyrifera	5	10
Typha latifolia	5	10

Table 2.4 Species mentioned by informants in Ouje-Bougoumou 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.

SIV	Rank	Latin	Cree	S	I	O
0.0214	1	*Larix laricina*	·ᐊᒋᓈᐲᑲᓐ waachinaakan	8	13	Ca, Br, C, L
0.0210	2	*Rhododendron groenlandicum*	ᑲᒋᒉᐸᒄ kaachichepakw	9	11	L, Br
0.0187	3	*Sorbus* spp.	ᒪᔥᑯᒥᓈᓈᐦᑎᒄ maskuminaanaahtikw	9	5	B, Ca, F, Br
0.0171	4	*Alnus incana* subsp. *rugosa*	ᐅᑑᔥᐲ utuuspii	7	7	B, Ca, L
0.0144	5	*Abies balsamea*	ᐄᓈᔥᑦ iinaasht	5	14	Ca, S, C, L
0.0131	6	*Kalmia angustifolia*	ᐅᔥᒋᔥᒋᐸᒄ uschischipakw	6	3	L
0.0103	7	*Prunus pensylvanica*	ᑰᐦᐄᒥᓈᓐ kuuhiiminaanh	4	2	Ca, B
0.0098	8	*Betula papyrifera*	ᐅᔥᑯᐃ ushkui	4	3	Ca, S
0.0095	9	*Sarracenia purpurea*	ᐊᔨᑲᑖᔥ ayikataash	3	11	L, R
0.0079	10	*Gaultheria hispidula*	ᐱᔦᐅᒥᓈᓐ piyeuminaanh	3	2	F, L
0.0079	11	*Picea glauca*	ᒥᓈᐦᐄᒄ minahiikw	3	5	L, S, C, R
0.0078	12	*Picea mariana*	ᐄᓈᐦᑎᒄ iinaahtikw	3	5	W, C
0.0060	13	*Pinus banksiana*	ᐅᔥᒋᔥᒄ uschisk	2	3	C, L
0.0049	14	*Thuja occidentalis*	ᒫᔥᒋᔥᒄ maaschiisk	2	2	C
0.0048	15	*Salix humilis* var. *humilis*	ᓃᐲᔒᐊᐦᑎᒃ niipiishiaahtik	1	1	B
0.0048	16	*Vaccinium vitis-idaea*	·ᐄᔥᒋᒥᓈᓐ wiisachiminaanh	2	2	F, L
0.0048	16	?	ᐊᒋᑲᔥᐗᔥᒄ achikaashwaashkw	2	2	L
0.0041	16	*Cornus stolonifera*	?	2	1	Ca
0.0036	17	*Vaccinium angustifolium*	ᐄᓂᒥᓈᓐ iiniminaanh	2	3	F, L
0.0033	18	*Lycopodium clavatum*	ᐹᔥᒋᓈᐦᒀᓐ paaschinaahkwaan	1	1	L
0.0033	18	*Boletus* spp.	?	1	1	A
0.0030	19	*Populus tremuloides*	ᒦᑐᔥ miitus	1	1	Ca
0.0026	20	*Populus balsamifera*	ᒦᑐᔥ miitus	1	1	L
0.0022	21	*Rubus pubescens*	ᐊᔫᔥᑲᓐ ayuuskan	1	1	F
0.0022	21	*Ribes lacustre*	ᔖᐳᒥᓇᒡ shaapuminach	1	1	F
0.0013	22	*Andromeda polifolia*	·ᐄᔥᒋᐸᒄ wiisachipakw	1	1	L

L = leaf/needle, Br = branch, S = sap/gum, W = wood, B = bark, C = cone, Ca = cambium, A = aerial part, F = fruit, R = root

Table 2.5 Species mentioned by informants in Oujé-Bougoumou in decreasing order of rank based on the FC method. Consensus is represented by the frequency of citations calculated over the total number of interviews.

Species	% Frequency	Rank
Abies balsamea	77.78	1
Larix laricina	72.22	2
Rhododendron groenlandicum	61.11	3
Sarracenia purpurea	61.11	3
Alnus incana subsp. *rugosa*	38.89	4
Picea glauca	27.78	5
Picea mariana	27.78	5
Sorbus spp.	27.78	5
Betula papyrifera	16.67	6
Kalmia angustifolia	16.67	6
Pinus banksiana	16.67	6
Vaccinium angustifolium	16.67	6
Gaultheria hispidula	11.11	7
Prunus pensylvanica	11.11	7
Thuja occidentalis	11.11	7
Vaccinium vitis-idaea	11.11	7
Achikaashwaashkw	11.11	7
Andromeda polifolia	5.56	8
Cornus stolonifera	5.56	8
Lycopodium clavatum	5.56	8
Populus tremuloides	5.56	8
Populus balsamifera	5.56	8
Salix humilis var. *humilis*	5.56	8
Rubus pubescens	5.56	8
Ribes lacustre	5.56	8
Boletus spp.	5.56	8

Table 2.6 List of 32 plant species and one fungal species mentioned by informants from six Eeyou Istchee Cree communities in decreasing order of their global SIV ranks. The ranking of species in each community is compared. Zero indicates that a species was not mentioned in that community. Communities are numbered (1) Whapmagoostu, (2) Waskaganish, (3) Mistissini, (4) Nemaska, (5) Wemidnji and (6) Oujé-Bougoumou.

Rank	SIV	Species	Community rank					
			1	2	3	4	5	6
1	0.0377	*Rhododendron groenlandicum*	1	5	1	6	3	2
2	0.0370	*Larix laricina*	2	3	2	3	6	1
3	0.0359	*Rhododendron tomentosum*	3	0	0	0	0	0
4	0.0352	*Picea mariana*	5	4	4	1	4	12
5	0.0347	*Picea glauca*	4	1	14	10	8	11
6	0.0321	*Kalmia angustifolia*	6	0	12	9	1	6
7	0.0312	*Sorbus* spp.	8	12	5	8	0	3
8	0.0245	*Abies balsamea*	0	2	3	4	2	5
9	0.0240	*Alnus incana* subsp. *rugosa*	0	9	6	2	13	4
10	0.0203	*Juniperus communis*	7	0	0	0	12	0
11	0.0180	*Pinus banksiana*	13	6	9	7	0	13
12	0.0178	*Salix* spp.	10	0	7	0	0	15
13	0.0167	*Betula papyrifera*	0	0	0	0	7	8
14	0.0165	*Vaccinium vitis - idaea*	11	0	13	0	10	16
15	0.0132	*Sarracenia purpurea*	0	11	8	0	0	9
16	0.0117	*Populus balsamifera*	0	10	0	5	11	20
17	0.0100	*Gaultheria hispidula*	0	0	15	0	0	10
18	0.0099	*Populus tremuloides*	0	0	0	0	9	19
19	0.0099	*Prunus pensylvanica*	0	0	0	0	0	7
20	0.0087	*Lycopodium clavatum*	0	0	10	0	0	18
21	0.0083	*Vaccinium angustifolium*	0	0	11	0	15	17
22	0.0073	*Thuja occidentalis*	0	7	0	0	0	14
23	0.0072	*Leymus mollis*	12	0	0	0	0	0
24	0.0046	*Heracleum maximum*	0	8	0	0	0	0
25	0.0046	Achikaashwaashkw	0	0	0	0	0	16
26	0.0039	*Cornus stolonifera*	0	0	0	0	0	16
27	0.0032	*Boletus* spp.	0	0	0	0	0	18
28	0.0030	*Empetrum nigrum*	14	0	0	0	0	0
29	0.0028	*Typha latifolia*	0	0	0	0	14	0
30	0.0026	*Vaccinium uliginosum*	0	0	0	0	15	0
31	0.0021	*Rubus pubescens*	0	0	0	0	0	21
31	0.0021	*Ribes lacustre*	0	0	0	0	0	21
32	0.0012	*Andromeda polifolia*	0	0	0	0	0	22

Table 2.7 Community pharmacopoeia comparisons as evaluated by three statistical methods, Spearman rank correlation test, Mantel analysis and data level comparisons. Spearman rank correlation test was a measurement of how similarly plants were ranked between communities ($p < 0.5$ considered similar). The Mantel analysis evaluated whether the same plants were used for the same symptoms ($p < 0.05$ were considered similar) while data level comparison tests compared the number of times each plants was mentioned ($p < 0.05$, were considered similar). Significant results are shown shaded.

Comparison	Spearman		Mantel		Data level comparisons		
	r	*p*	*r*	*p*	**Row**	**Column**	**Both**
Wemindji/Whapmagoostui	0.5000	0.2667	0.1068	0.1252	0.0001	0.0051	0.0000
Wemindji/Waskaganish	0.4643	0.3024	0.0455	0.2956	0.0439	0.0413	0.0092
Wemindji/Nemaska	-0.2619	0.5364	0.1857	0.0408	0.1057	0.3520	0.1288
Wemindji/Mistissini	0.3500	0.3586	0.1694	0.0547	0.0254	0.0098	0.0013
Wemindji/Oujé-Bougoumou	0.5315	0.0793	0.2061	0.0357	0.0000	0.0000	0.0000
Oujé-Bougoumou/Whapmagoostui	0.7833	0.0172	-0.0402	0.5831	0.0260	0.0122	0.0002
Oujé-Bougoumou/Waskaganish	0.1273	0.7138	0.1091	0.2318	0.0042	0.0512	0.0026
Oujé-Bougoumou/Nemaska	0.1152	0.7588	0.1377	0.1800	0.0309	0.0874	0.0263
Oujé-Bougoumou/Mistissini	0.6000	0.0204	0.4342	0.0019	0.0001	0.0000	0.0000

Table 2.8 Species-symptom associations obtained by comparing three-dimensional perceptual maps resulting from correspondence analysis of the species-use contingency table for Wemindji (fig 2.2a) and Oujé-Bougoumou (fig 2.2b).

Group	Symptom	Species	# mentions
1	Abscesses/Boils	*P. balsamifera*	3
		P. tremuloides	9
2	Frequent urination	*J. communis*	7
3	Blurry vision	*V. vitis-idaea*	8
4	Increased thirst	*V. vitis-idaea*	1

Oujé-Bougoumou

Group	Symptom	Species	# mentions
1	General weakness	*V. angustifolium*	2
		R. groenlandicum	4
2	Back/kidney pains	*P. pensylvanica*	1
		K. angustifolia	3
		S. purpurea	8
3	Infections	*L. laricina*	10
		A. balsamea	14

Table 2.9 Species-symptom associations obtained by comparing three-dimensional perceptual maps **(fig 2.3)** resulting from correspondence analysis of the species-use contingency table for six Eeyou Istchee Cree communities.

Group	Symptom	Species	Communities (# mentions)
1	Increased urination	*J. communis*	Whap(34), Wem(7)
2	Blurred vision	*V. vitis-idaea*	Whap(15), Wem(8)
3	Infections	*S. purpurea*	Mist(6), OJ(8)
		A. incana subsp. *rugosa*	Mist(5), Nem(1), Wem(8), OJ (6)
		A. incana subsp. *rugosa*	Wask(13), Nem(13), OJ(1)
4	Abscesses/boils	*P. balsamifera*	Wask(7), Nem(3), Wem(3)
		P. tremuloides	Wem(9), OJ(1)

Table 2.10 Community pharmacopoeia comparisons against litterature based surveys for surrounding First Nations of the Algonquian linguistic familly and Inuit. Results from Mantel analysis evaluated whether the same plants were used for the same symptoms ($p < 0.05$ were considered similar and are shaded).

Comparison	*r*	*p*
CEI/Algonquin	0.185	0.148
CEI/Attikkamek	0.006	0.461
CEI/Innu	0.193	0.098
CEI/Ojibway	0.224	0.082
CEI/Algonquian	0.299	0.002
CEI/Inuit (Nunavik)	-0.132	0.899
Oujé-Bougoumou/Algonquin	0.268	0.097
Oujé-Bougoumou/Attikkamek	-0.008	0.483
Oujé-Bougoumou/Innu	0.420	0.013
Oujé-Bougoumou/Ojibway	0.439	0.007
Oujé-Bougoumou/Algonquian	0.214	0.032
Oujé-Bougoumou/Inuit (Nunavik)	0.195	0.086
Wemindji/Algonquin	0.150	0.107
Wemindji/Attikkamek	0.173	0.108
Wemindji/Innu	0.358	0.005
Wemindji/Ojibway	0.064	0.267
Wemindji/Algonquian	0.329	0.006
Wemindji/Inuit (Nunavik)	0.059	0.272

Table 2.11 Literature review of the known pharmacological activity of berries used by the Cree of Eeyou Istchee pertaining to the pathology of type 2 diabetes

Species	Pharmacological activity	Reference
Empetrum nigrum	Antioxidant activity	Fraser et al., 2007; Ogawa et al., 2008
Gaultheria hispidula	Antioxidant activity	Fraser et al., 2007; Harbilas et al., 2009
	Hypoglycemic activity	Harbilas et al., 2009; Nistor Baldea et al., 2010
	Anti-glycation activity	Harris et al., 2011
	Cytoprotection against glucose toxicity and deprivation	Harbilas et al., 2009
Vaccinium angustifolium	Antioxidant activity	Boivin et al., 2007; Mizuno and Rimando, 2009; Papandreou et al., 2009; Del Bo' et al., 2010; Joseph et al., 2010
	Anti-glycation activity	McIntyre et al., 2008
	Hypoglycemic activity	Martineau et al., 2006; Grace et al., 2009; Graf et al., 2010; Hohtola, 2010
	Adipogenic, insulinotropic and proliferative (pancreatic β cells) activity	Martineau et al., 2006
	Cytoprotection against glucose toxicity	Martineau et al., 2006; Hohtola, 2010
	Insulinosensitizing and anti-obesogenic activity	Hohtola, 2010
Vaccinium vitis-idaea	Antioxidant activity	Kahkonen et al., 2001; Korotkova et al., 2003; Määttä-Riihinen et al., 2005; Wang et al., 2005; Fraser et al., 2007; Yang et al., 2011
	Anti-glycation activity	Beaulieu et al., 2010; Harris et al., 2011
	Hypoglycemic activity	Harbilas et al., 2009; Eid et al., 2010; Nistor Baldea et al., 2010
	Adipogenic activity	Harbilas et al., 2009
	Anti-inflammatory activity	Markov et al., 2011
Vaccinium uliginosum	Antioxidant activity	Fraser et al., 2007
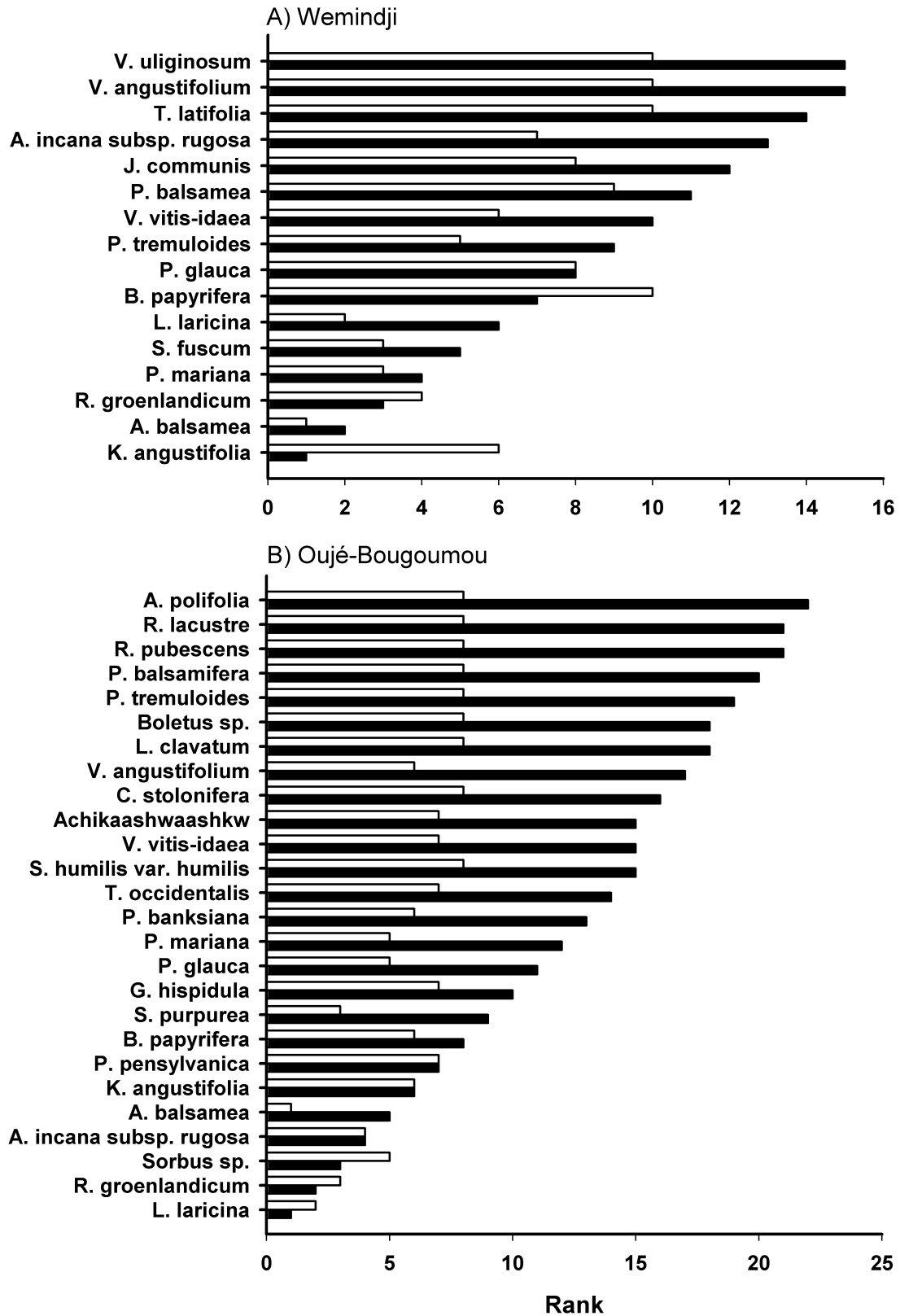
Figure 2.1 Ranking comparison between SIV (black) and FC (white) for the 16 plants mentioned in Wemindji **(A)** and the 26 species mentioned in Oujé-Bougoumou **(B).**

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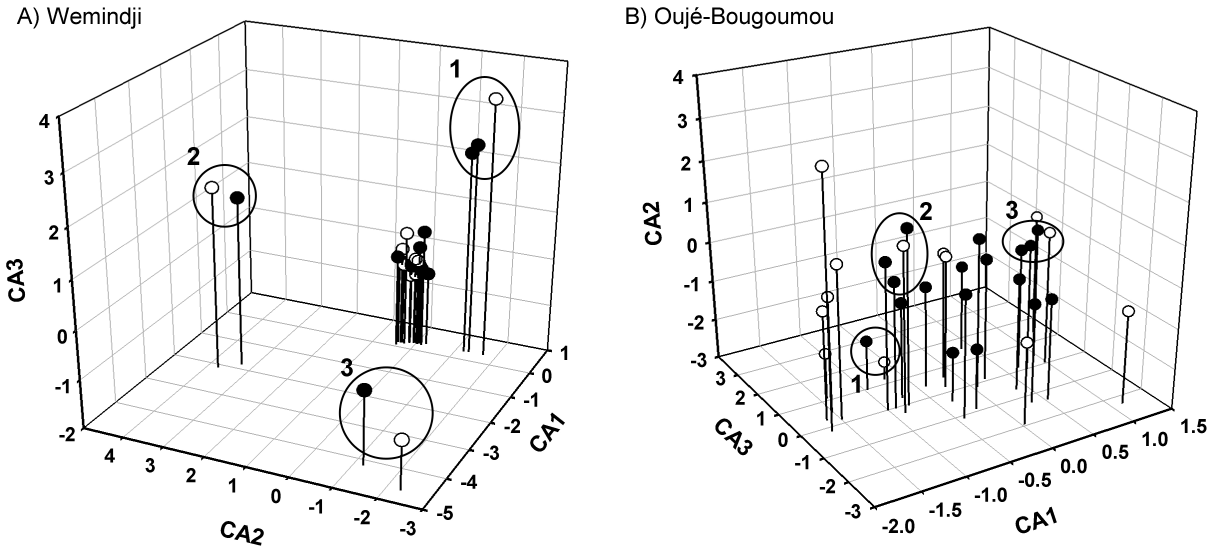
Figure 2.2 Three-dimensional scatterplots from correspondence analysis of plant species (●) and symptoms (**O**) for Wemindji (A) and Oujé-Bougoumou (B). Symptoms and species located in proximity to one another are deemed to be highly associated. Interpretations of scatterplots are summarized in **table 2.8**.
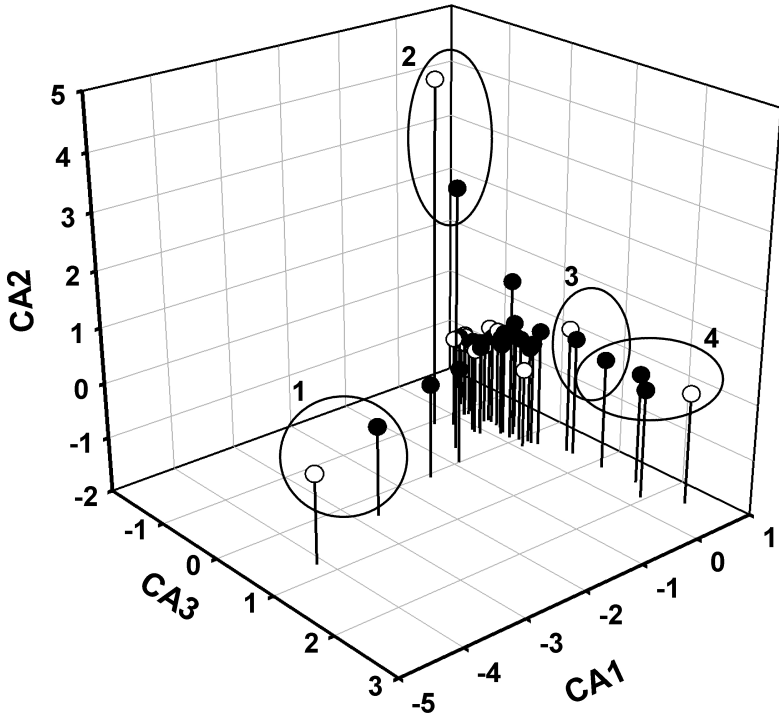
Figure 2.3 Three-dimensional scatterplots from correspondence analysis of plant species (●) and symptoms (**O**) for all six communities combined. Symptoms and species located in proximity to one another are deemed to be highly associated. Interpretations of scatterplots are summarized in **table 2.9**.

Chapitre 3

Environmental trends in the variation of biologically active phenolic compounds in Labrador Tea, *Rhododendron groenlandicum,* from Northern Quebec, Canada.

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3.1 Abstract

The impact of several environmental factors was studied on the production of phenolic compounds in a North American medicinal plant, Labrador tea (*Rhododendron groenlandicum*). Leaves were harvested in 2006 and 2010 over a latitudinal gradient in Northern Quebec, and known phenolic markers were quantified by HPLC-DAD. The concentration of selected compounds expressed a quadratic relationship with latitude where they were higher between the 51-53rd parallel of Northern Quebec and lower in the peripheral northern and southern. Major variations were observed in the following marker compounds: (+)-catechin, (-)-epicatechin, quercetin-3-galactoside and an undetermined quercetin-glycoside. The variation in phenolic compounds was best explained by insolation parameters such as solar radiation and photoperiod in the month of June.

Key words: Phenolic compounds, traditional medicine, insolation, photoinhibition, Cree Nation of Eeyou Istchee.

3.2 Introduction

Rhododendron groenlandicum (Oeder) Kron & Judd*,* Labrador tea, from the Ericaceae, is a common plant growing in Canada's boreal forests with a long history of traditional uses

among indigenous populations. Its recorded and/or current uses are reported for the Inuit (Cuerrier and Hermanutz, 2012) and First Nations of the Algonquian, Salish, Waskashane and Tsimshianic linguistic families (Arnason et al., 1981; Zieba, 1990; Siegfried, 1994; Marles et al., 2008; Moerman, 2009; Uprety et al., 2012). Its widespread use is perhaps due to its abundance, wide distribution, medicinal properties and aromatic flavour.

A high incidence of type 2 diabetes (T2D), reaching an average of 29%, has been reported in the Cree Nation of Eeyou Istchee (CEI) communities (Kuzmina et al., 2010), and the CIHR Team on antidiabetic aboriginal medicines (TAAM) was formed to undertake collaborative research with Cree Healers to study traditional medicinal plants for their antidiabetic potential. Within the pharmacopoeia of the CEI, Labrador tea was mentioned for the treatment of a wide range of symptoms related to diabetes (Marshall et al., 2003; Fraser, 2006; Leduc et al., 2006; Marshall, 2006; Downing, 2010). In pharmacological studies, *R. groenlandicum* was shown also to posses *in vitro* glitazone-like activity comparable to rosiglitazone in the lipid accumulation of the 3T3-L1 preadipocytes (Spoor et al., 2006).

Flavonoids, catechins and triterpenes are biologically active components that play a key role in the protection of plants from the oxidative stress induced by environmental factors (Dixon and Paiva, 1995; Close et al., 2003; Solovchenko and Merzlyak, 2008). Stressors such as solar irradiation, cold temperatures and low soil nutrients vary considerably over the large CEI territory, which extends midway through Quebec from the James Bay coast, and these have been shown to induce the expression of flavonoid pathway genes (Hartmann et al., 2005; Lillo et al., 2008; Usadel et al., 2008; Matus et al., 2009). Variation in medicinal efficacy has been observed by CEI Elders who report northern populations to be "stronger medicine" (Cuerrier, *verbatim*).

Indeed, studies have shown the important role that phenolic compounds play in reducing solar-induced damage to plants. High quantities of UV-absorbing polyphenols in epidermal and hypodermal layers of leaves and branches (Li et al., 1993; Dixon and Paiva, 1995) screen the sun's damaging rays before they reach the photosystems. Li et al. (1993) were the first to demonstrate a direct link between flavonoids and UV protection in *Arabidopsis* mutants. Although solar radiation is essential for photosynthesis, plants may suffer from photoinhibition when photosynthetically active photon flux density is in excess of what is

necessary for CO_2 assimilation (Carvalho and Amâncio, 2002). Such excess in energy due to high light can generate an increase in reactive oxygen species (ROS) such as H_2O_2 as shown in various acclimatization studies (Carvalho and Amâncio, 2002; Carvalho et al., 2006). UV radiation has also been shown to increase ROS production (Bhattacharjee, 2011). Oxidative damage due to ROS and photodamage due to high levels of UV-A and UV-B radiation include dimerization of lipids, enzymes, proteins, pigments, nucleic acids, DNA and RNA (Dixon and Paiva, 1995; Pietta et al., 1998; Solovchenko and Merzlyak, 2008; Faisal and Anis, 2009; Bhattacharjee, 2011). Plants are thus equipped with antioxidant systems, scavenging ROS through enzymatic and non-enzymatic mechanisms whose activity has been shown to increase with insolation parameters (Carvalho and Amâncio, 2002; Carvalho et al., 2006; Faisal and Anis, 2009). Of the non-enzymatic mechanisms, phenolic compounds are well-known antioxidants repeatedly demonstrated to play a key protective role against ROS.

Phenolic compounds are free or cell wall-bound compounds (Nara et al., 2006) whose chemical structure allows them to efficiently inactivate ROS (Takahama and Oniki, 1997) and act as antioxidants (Haslam, 1996; Habtemariam, 1997; Rice-Evans et al., 1997; Pietta et al., 1998; Plumb et al., 1998; Kähkönen et al., 1999). Due to absorption in the ultraviolet range of the electromagnetic spectrum, some compounds, such as kaempferol glycosides, may act as UV filters, while others are radical scavengers or chelators of metals that prevent the Fenton reaction (Takahama and Oniki, 1997).

The antioxidant capacity of phenolic compounds to UV-induced ROS in plant tissues has proven to be transferable to animal tissues as well. Compounds isolated from *Dalbergia odorifera* T. Chen (Fabaceae) showed antioxidant activities and helped mediate oxidative damage in rat lenses exposed to UV irradiation (Yu et al., 2007). Such antioxidant activities from exogenous plant-based compounds in humans have a favourable impact on chronic diseases such as T2D (McCune and Johns, 2007; Sancho and Pastore, 2012) and total phenolics generally tend to correlate positively with antioxidant activity in *in vitro* studies (Pietta et al., 1998; Fraser et al., 2007; Harris et al., 2011).

The biological activity of many plants, including Labrador tea, identified in the CEI pharmacopoeia for the treatment of symptoms related to T2D (Spoor et al., 2006; Harbilas et al., 2009; Saleem et al., 2010), has been attributed to phenolic compounds (Eid et al., 2010;

Guerrero-Analco et al., 2010; Harris et al., 2012; Muhammad et al., 2012; Shang et al., 2012). Yet the abundance and wide distribution of these plants across Eeyou Istchee and the boreal zone of Canada pose potential problems for quality control both for current traditional use and in the event that they may be transformed into natural health products (NHPs) for future use in communities. The territory occupied by the CEI lies between the 50th and 55th parallel North, and a marked difference can be observed in the increase of day length between the extremities of the territory (National Research Council, 1995). Cree Elders and Healers have noted variations in the medicinal potential of plants within this territory. We hypothesize that such observations in *R. groenlandicum* reflect changes in its phenolic content, which increases as a function of latitude, and that these are positively related to insolation parameters such as solar radiation and photoperiod.

To date, there have been few studies addressing the question of geographical variations in the phytochemistry of plants, especially biologically active compounds. Positive correlations between phenolic concentration and latitude have been shown in the Cupressaceae, Ericaceae and Betulaceae, or *Juniperus communis* (Martz et al., 2009a), *Vaccinium myrtillus* (Martz et al., 2010) and *Betula pubescens* (Stark et al., 2008), respectively. But fewer studies address the question of the environmental factors responsible for these changes. In the present study, we report variations in the phenolic content of compounds quantified from *R. groenlandicum*, as well as a link between environmental factors and phytochemical concentrations over a large latitudinal gradient.

3.3 Materials and Methods

3.3.1 Sampling

Mature leaves of *R. groenlandicum* were sampled during the summers of 2006 and 2010 around the communities of Mistissini, Nemaska, Waskaganish, Eastmain, Wemindji and Whapmagoostui, thus covering the entire north-south gradient in Eeyou Istchee. Waskaganish was the only CEI community that was not sampled in 2006. Samples from the Ottawa region, south of the CEI territory, were also collected in 2010. This effectively doubles the latitudinal

gradient, extending it into the Great Lakes St-Lawrence forest region characterized by a greater diversity of deciduous hardwood trees (Farrar, 2005). Ten accessions, each containing leaves from multiple (~ 5) individual plants, were collected within a 50 km radius around each community. In 2010, additional samples, collected along the James Bay Highway and in the Ottawa region, were included in the statistical analyses involving geographic coordinates. Samples were air dried and preserved in paper bags at room temperature. Representative voucher specimens from each community are deposited at the Marie-Victorin Herbarium of the University of Montréal (MT) and the University of Ottawa Herbarium (OTT) (**Appendix III**).

3.3.2 Extraction

Plant material was thoroughly dried at 35°C over night in a commercial food dehydrator (Nesco® Professional Food and Jerky Dehydrator). Samples were milled through a Wiley Mill with at 40 mesh and extracted overnight in at 25 mL/g of 80 % EtOH by orbital shaking at room temperature at 250 RPM. The pellet was extracted overnight in 15 mL 80 % EtOH. The pooled supernatants (adjusted to 50.0 mL in a volumetric flask) were centrifuged for 15 min at 1828 x *g* at room temperature. An aliquot (1 mL) of the centrifuged extract was prepared for High Performance Liquid Chromatography hyphenated with Diode Array Detector (HPLC-DAD) by filtering through a 20 μm PTFE filter. Extracts were kept at -20°C and sonicated before analysis. Unfiltered crude extracts were dried using a speedVac. Trace water was removed by lyophilization using a SuperModuylo freeze dryer and stored at -80°C for future use.

3.3.3 Chemicals and standards

(+)-Catechin **(1)**, chlorogenic acid **(2)**, (-)-epicatechin **(3)**, *p*-coumaric acid **(4)**, rutin **(5)**, quercetin-3-galactoside **(6)**, quercetin-3-glucoside **(7)**, quercetin-3-rhamnoside **(12)**, myricetin **(13)** and quercetin **(14)** were purchased from Sigma-Aldrich (Oakville, Ontario, Canada) and Extrasynthese (Genay, France). HPLC grade water, acetonitrile, and formic acid (99% purity) were purchased from Sigma-Aldrich.

3.3.4 Identification and Quantification

The identification and quantification of phenolic compounds listed in **table 3.1** in the crude extracts were based on a validated method (Saleem et al., 2010). Briefly, a 10 µl of each extract was injected through an autosampler and detected by DAD at 290 nm, band width 4, reference off. The separations were performed on a Luna C18 column (250 x 4.6 mm, 5 µM particle size). Peak identification was undertaken by co-chromatographic comparison of the spectral data adopted in our in-house metabolomics spectral library (Saleem et al., 2010). A standard curve was constructed by injection of serially diluted marker compounds in methanol. The quantification was based on peak height and area. The quantitation of putatively identified quercetin-glycosides was done based on the calibration curve of quercetin-galactoside. Each sample was analyzed in triplicate and averaged to account for instrumental variation.

3.3.5 Environmental data

Estimates for over 190 environmental and climatic variables were provided by the Canadian Forest Services of Natural Resources Canada. Values were generated by spatially continuous climatic models adjusted by ANUSPLIN, a non-parametric multivariate technique for the noise-reduction of multiple variable data. Interpolation of surfaces is calculated by smoothing algorithms and taking into consideration the effect of altitude. For the most part, these surfaces are generated from a 30 years data span collected by meteorological stations across the country. Using each sample's geographical coordinates at a resolution of 4 decimal-degrees, environmental and climatic estimates were obtained for each collection site. Monthly estimates for solar radiation (Mjoules) and photoperiod (h) were obtained from data of the 1961-1990 period. Other variables including evapotranspiration and climatic moisture index (CMI) were available for the 1971-2000 period while monthly and annual estimates for such variables as temperature and precipitation were also available for the first collection year of 2006. Details of the protocols generating these estimates are described by McKenney et al. (2007a,b).

3.3.6 Statistical analysis

Univariate and multivariate statistical analyses were performed using R statistical language (R Development Core Team, 2012). Because sampling sites are concentrated around specific communities, these were considered as a grouping factor in analysing how phenolic content varied from region to region. Specific compounds were analyzed separately by parametric analysis of variance (ANOVA) and post-hoc Tukey multiple comparisons to elucidate how each varied. To elucidate potential chemotypes and important markers, phenolic profiles of *R. groenlandicum* were analyzed by performing a multivariate analysis of variance (MANOVA) using a partial-RDA approach (Anderson and Legendre, 1999). Following the significant results in the interaction between communities and harvest years in a 2-way factorial analysis, 1-way analyses were performed for each year thus including supplementary samples for the analysis of 2010. Post-hoc pairwise comparisons were realized by performing an RDA for each pair of community and adjusting the α value using Holm's correction. In order to facilitate the visualization of relationships among communities, we opted for dendrograms using complete linkage analysis to ensure that the fusion of each community is based on their most distant pairs.

The spatial pattern in the variation of phenolic compounds was further examined by using the geographic coordinates as a continuous variable. Polynomial regressions were used to analyze the spatial variation in the sum of quantified phenolic markers in order to assess the general trend of phenolic compounds. Determination of polynomial equations for fitted values were realized based on Legendre and Legendre (1998). Regression models for both years were subsequently compared by analysis of covariance (ANCOVA), specifying latitude and year as a continuous and grouping variable respectively.

Furthermore, the phytochemical profile of *R. groenlandicum* was spatially analyzed by canonical redundancy analysis (RDA) in order to identify the important varying markers. Spatial representations of the multivariate data were obtained by interpolating the first canonical axis over the constricting geographical area of the sampling sites. Interpolation was performed by ordinary kriging using *v.krige.py,* a command from the GRASS environment (GRASS Development Team, 2012), using R software functions in the background.

Intermediate values were estimated by adjusting the best-fit theoretical model, automatically detected in the command script, to empirical variograms generated for each spatial analysis.

Simple and multiple linear regressions were used to determine which climatic and environmental variables best explained the spatial variation observed in the sum of quantified phenolic markers. In a similar fashion, the phytochemical profile of *R. groenlandicum* was analyzed by RDA with these variables to determine which of these best explained the variations of important phenolic markers. Because of the large quantity of variables available, they were selected for a most parsimonious model explaining the maximum variation in the phenolic profile. Selection was performed using both information-theoretics and hypothesis-testing approaches. Automated routines for forward, backward and stepwise selection were realized using Akaike's information criterion (AIC) (Akaike, 1973) as the selection criteria. Forward selection was also performed using adjusted R^2 and α level as selection criteria in a hypothesis-testing approach (Blanchet et al., 2008). Results from all four methods were taken into consideration in the selection of final variables to be modelled and the variance inflation factor (VIF) calculated for each of these to evaluate the severity of multicolinearity.

Statistical significance of multivariate analyses was determined by non-parametric tests using 100,000 permutations and $\alpha = 0.05$, while the statistical significance of univariate analyses was determined using the normal distribution after verifying that the data met the required assumptions.

3.4 Results and discussion

3.4.1 Phenolic content and profile

Representative HPLC-DAD chromatographs of *R. groenlandicum* leaf extracts (**fig. 3.1)** show that of 10 known and identified phenolic compounds, all but *p*-coumaric acid, rutin and myricetin, which were detected in trace amounts, were quantified and subsequently analyzed. Three of these compounds, (+)-catechin, (-)-epicatechin and quercetin, have been found responsible for the adipogenic activity of *R. groenlandicum* in bioassay-guided fractionation (Ouchfoun, 2011). Four unknown, yet significantly present, quercetin-glycosides

were detected through chromatographs (**fig. 3.1)** and also quantified. These compounds, including quercetin-glycosides, have been found in a multitude of boreal plants, most notably members of the Ericaceae such as *Gaultheria hispidula, Kalmia angustifolia, Rhododendron tomentosum, Vaccinium vitis-idaea* and *V. angustifolium* (Harris et al., 2007; McIntyre et al., 2008; Saleem et al., 2010; Black et al., 2011; Ferrier et al., 2012). These species also appear in CEI pharmacopoeia and, like *R. groenlandicum,* have been screened by the CIHR-TAAM and shown to possess antioxidant activity and antidiabetic potential (Spoor et al., 2006; McIntyre et al., 2008; Harbilas et al., 2009; Black et al., 2011; Ferrier et al., 2012).

The sum of quantified compounds varied between 21.3 – 33.9 mg/g DW in 2006 (**fig. 3.2a**), averaging 28.8 mg/g DW. In 2010, concentrations were lower, averaging 21.1 mg/g DW and varying between 12.6 – 29.2 mg/g DW (**fig. 3.2b**). **Figure 3.2** clearly shows that roughly two thirds of accessions were within the mid-range: 64 % and 71.4 % in 2006 and 2010, respectively, and a lower number of accessions was observed at the high and low concentration ranges. This is useful for considering the plant as an NHP, since the pharmacological activity of these compounds are well documented and are known to possess antidiabetic, antioxidant, anti-inflammatory and even antibiotic properties (Gray and Flatt, 1997; Harborne et al., 1999).

The most abundant compound was quercetin-3-galactoside, followed by quercetin-glycoside 4, (+)-catechin, chlorogenic acid and (-)-epicatechin (**fig. 3.3**). Although means differed between sampling years, the ranking of concentrations were relatively similar. In 2006, all but quercetin-3-galactoside varied geographically between communities while in contrast, only (+)-catechin, (-)-epicatechin, quercetin, quercetin-3-galactoside and quercetin-glycoside 1 varied significantly in 2010 (results are presented in **table 3.2**). Of the most abundant compounds, only (+)-catechin and (-)-epicatechin remained significantly variable during both harvest years (**fig. 3.4)**. Although not as clear in 2010, variations in metabolite concentrations, in both cases, follow a bell-shaped pattern where concentration means are higher towards the centre of the sampling gradient.

The relationship between the phytochemical profiles of plants collected in the different communities are further elucidated in **figure 3.5c** and **3.6c**. How these differed from one community to the others were not identical between harvest years ($p=5.1 \times 10^{-4}$). Nonetheless,

canonical relationships were significant in 2006 ($p = 1 \times 10^{-5}$, $R^2_{adj} = 0.32$, **fig. 3.5a** and **b**) and 2010 ($p = 1 \times 10^{-5}$, $R^2_{adj} = 0.17$, **fig. 3.6a** and **b**), and show in both cases that the phenolic profiles of *R. groenlandicum* from Eastmain, Wemindji and Nemaska were similar. Regardless of statistical differences, the clustering of samples resulting from canonical analyses (**fig. 3.5a** and **3.6a**) do not appear distinct enough to propose various chemotypes, but rather a differentiation towards chemoraces. This is particularly true for the northern plant accessions of Whapmagoostui, which lies in the transitional taïga forest region. Located between two distinct ecosystems, the boreal forest and the tundra, changes in environment, climate and species composition may play an important role in driving this differentiation. The same can be said about *R. groenlandicum* populations in Ottawa as they grow in isolated remnants of a once more extensive boreal forest interspersed within the mixed Laurentian forest region. However, a supplementary harvest for that area is necessary to verify this assertion.

When considering the theory that the impact of abiotic parameters, leading to the ecological distinctiveness of these forest regions, acts in the differentiation of chemotypes, this may explain why accessions from the boreal forest were generally not statistically different as their environment might be too similar. Even so, Mistissini's relationship to other communities differs between harvest years (**fig. 3.5c** and **3.6c**). This may simply be caused by yearly fluctuations of local climatic and environmental variables, for which the effect is suppressed in the calculations of estimates. Regardless, important markers explaining the spatial variations in the phenolic profile of *R. groenlandicum* were (+)-catechin, (-)-epicatechin, quercetin-3-galactoside and quercetin-glycoside 4 (**fig. 3.5b** and **3.6b)**, all of which rank amongst the most abundant compounds (**fig. 3.3**). Quercetin-3-rhamnoside was ranked as a less-important marker, yet its relationship to geographical variations was inversely proportional to the general trend (**fig. 3.4**) and only chlorogenic acid showed some discrepancy by acting as a major compound in 2006. Of these compounds, (+)-catechin and (-)-epicatechin seem like the most important in supporting the claim of a chemotype differentiation in the Whapmagoostui populations where concentrations are lower. This may have important implications in the antidiabetic potential of *R. groenlandicum* as these two compounds have been shown *in vitro* to promote adipogenesis (Ouchfoun, 2011). From an NHP perspective, this suggests that harvesting should take place within the boreal forest.

3.4.2 Spatial patterns and variation

A geographic variation was observed for the sum of quantified phenolics that, although not varying linearly in 2006 (p = 0.802) or 2010 (p = 0.101), showed a significant quadratic relationship with latitude. Concentrations peaked at midlatitudes but were lower at highest and lowest latitudes (**fig. 3.7**). The regression equations of fitted values for 2006 and 2010 were

$$\hat{y} = -2253.86 + 86.30x - 0.81x^2 \; (p = 1.48 \times 10^{-6}, R^2_{adj} = 0.425)$$

and

$$\hat{y} = -353.18 + 14.78x - 0.15x^2 \; (p = 0.000715, R^2_{adj} = 0.158)$$

respectively. The quantitative scales in concentrations were statistically different ($p < 2.2 \times 10^{-16}$) as observed in **figure 3.2**, but for both years, the sum of quantified phenolics reached a maximum concentration at mid range of the sampling area.

When the RDA results of all phenolic compounds were interpolated (f**ig. 3.8**), the spatial variation in the phytochemical profile of *R. groenlandicum* continued to reflect quadratic relationships with latitude. These canonical relationships were globally very highly significant in 2006 (p = 1 x 10^{-5}, R^2_{adj} = 0.33) and 2010 (p = 3 x 10^{-5}, R^2_{adj} = 0.13). Redundancy analysis produced two canonical axes in 2006 (λ_1 = 0.952, λ_2 = 0.640) of which the first was very highly significant (p = 1 x 10^{-5}) and explained 21.7% of the variance in the phytochemical profile. As for 2010, two canonical axes were also produced (λ_1 = 0.641, λ_2 = 0.088) of which the first was again very highly significant (p = 1 x 10^{-5}) and explained 13.4 % of the variance in the phytochemical profile. Interpolation of the first canonical axis (**fig. 3.8**) illustrates the general variation of phenolic compounds across the sampling gradient, whereas concentrations are highest between the 51st and 53rd parallel. This corresponds to the region where lie the communities of Wemindji, Nemaska and Waskaganish as discussed in the previous section.

Our results differ from those obtained by Martz (2009a; 2010) and Stark et al. (2008). Although, positive relationships between phenolic concentrations and latitude were observed in *V. myrtillus* (Martz et al., 2010)*,* this is the only other report of this kind for a species of Ericaceae. Other studies have shown various geographical trends in the variations of phenolic compounds from other species. Both positive and negative correlations with latitude were observed for specifc phenols in *B. pubescens* (Stark et al., 2008). Positive relationships with altitude were observed in species of Dennstaedtiaceae, *Pteridium caudatum* and *Pteridium arachnoideum* (Alonso-Amelot et al., 2004, 2007), as well as a species of Jungermanniaceae, *Jungermannia exsertifolia* subsp. *cordifolia* (Arróniz-Crespo et al., 2006), while a negative one was observed in the Asteraceae *Scorzoneroides helvetica* (Zidorn, 2009). Spatial correlations of different natures have also been observed in alkane, alkaloid and terpene concentrations from various other species (Camp, 1949; Lincoln and Langenheim, 1976, 1979, 1981; Nicholls and Bohm, 1982, 1983; Dodd and Poveda, 2003).

The discovery of quinine and related alkaloids in the bark of *Cinchona* spp. (Rubiaceae) is one of the major ethnobotanical contributions to modern medicine in the treatment of malaria. The quest for high-yielding trees has led to Camp's (1949) study in the latitudinal and altitudinal variations of *Cinchona* alkaloid concentrations. His study showed geographic variations whereas concentrations related positively with altitude rather than latitude, an effect attributed to cooler temperatures in the high mountains of the Andes. Alternatively, a negative relationship was observed between the flavone, orientin and latitude in *Lupinus sericeus* (Fabaceae) collected along a 1500 km north-south transect (Nicholls and Bohm, 1982, 1983). Studies for both plants suffered, however, from taxonomic difficulties in species identification. This is illustrated by the number of debated taxa amongst the *L. sericeus* complex at the time of the study (Fleak, 1971), by speciation into subspecies (Nicholls and Bohm, 1982, 1983) and by hybridization among *Cinchona* spp. (Camp, 1949).

One of the better documented studies in the geographical variations of secondary metabolites in a given species is that of *Satureja douglasii* (Lincoln and Langenheim, 1976, 1979, 1981) of the Lamiaceae. Sampled between latitudes 34ºN and 50ºN in western North-America, the pattern of monoterpene variations in mature leaves supports geographically distinct chemotypes in *S. douglasii* (Lincoln and Langenheim, 1976). Discontinuities in the

linear gradients with regards to latitude suggest the presence of significant differences, as we observed in our own results. Although diverse, spatial patterns can clearly be distinguished in the variation of plant phytochemicals. This diversity may be attributed to the fact that latitude acts as a marker for underlying abiotic factors, as Camp's (1949) study and our results further suggest.

3.4.3 Environmental and climatic relationships

Of over 190 climatic and environmental variables, selection of the most parsimonious models explaining variations in the phenolic profile of *R. groenlandicum* included factors related to insolation as most important and significant (**fig. 3.9**). For 2006, the best-fit model included solar radiation for the months of March and June ($p = 1 \times 10^{-5}$, $R^2_{adj} = 0.33$, **fig. 3.9a**) for which partial p values were all statistically significant ($p < 0.05$). Redundancy analysis produced two canonical axes explaining cumulatively 36.1% of the variation of the phytochemical profile. The canonical axes were statistically significant ($\lambda_1 = 1.034$, $p_1 = 1 \times 10^{-5}$ and $\lambda_2 = 0.552$, $p_2 = 8 \times 10^{-5}$) and individually explained 23.5% and 12.6% of the variation respectively. Important markers correlated well with climatic variables. For instance, along the main canonical axis, (+)-catechin, (-)-epicatechin and chlorogenic acid were best associated to March solar radiation. Along the secondary canonical axis, quercetin-3-galactoside and quercetin-glycoside 4 related better to June solar radiation.

For 2010, the best-fit model included monthly photoperiod averages for the months of June and September ($p = 0.001$, $R^2_{adj} = 0.09$, **fig. 3.9b**) for which partial p values were all statistically significant ($p < 0.05$). Redundancy analysis produced two canonical axes ($\lambda_1 = 0.316$, $\lambda_2 = 0.167$), explaining cumulatively 12.3% of the variation in the phytochemical profile, of which the first was significant ($p = 0.00265$) in explaining 8.4% of the variance. Important markers, such as (+)-catechin and (-)-epicatechin, correlated well with climatic variables, notably associating with the June photoperiod, while quercetin-3-galactoside related best to the September photoperiod. Quercetin-3-rhamnoside, for its part, related negatively to June photoperiod. Unlike the results for 2006, the unidentified quercetin-glycoside 4 and chlorogenic acid did not present themselves as important markers.

In a similar fashion to the RDA results, the sum of quantified compounds in 2006 (**fig. 3.10a**) is related to the solar radiation in June ($p = 0.00388$, $R^2_{adj} = 0.149$). When removing compounds which did not vary significantly with environmental variables, the sum of quantified compounds in 2010 (**fig. 3.10b**) was related to the monthly photoperiod averages for the month of June ($p = 0.00467$, $R^2_{adj} = 0.11$), thus corroborating multivariate results shown in **figure 3.9**. Similarly, the variation could be better explained in both cases when the same explanatory variables ($R^2_{adj} = 0.414$; see **fig. 3.9**) are analysed with March solar radiation in 2006 ($p = 2.25 \times 10^{-6}$) and September monthly photoperiod in 2010 ($R^2_{adj} = 0.146$; $p = 0.00324$).

Solar radiation and photoperiod during the month of June were the most significant and important environmental variables explaining the variation in the phenolic profile of *R. groenlandicum* for 2006 and 2010 samples, respectively. Coinciding with the summer solstice, June photoperiod is at its longest, giving higher levels of solar radiation than any other month of the year. Although preliminary, March solar radiation and September photoperiod results suggest, however, that the concentration of certain phenolic compounds reflect stresses during other periods of the year. This may represent a long-term adaptation to predictable and cyclical events such as the melting of snow and ice in late winter and at the beginning of spring, as it is suggested by the analysis of the 2006 accessions, or the sudden drops in temperature at the beginning of fall, as suggested by the 2010 analysis. In both cases, these represent periods where photosystem activity is either inhibited or reduced by factors such as temperature, all while still being exposed to potentially harming levels of solar energy.

In *S. douglasii*, based upon environmental factors such as insolation and herbivory, high-yielding chemotypes tended to occur under low light-high herbivore pressure, while low-yielding chemotypes tended to occur under high light-low herbivore pressure (Lincoln and Langenheim, 1979). While this relationship with light intensity contradicts the results obtained in our study, this may be attributed to the quantification of a different phytochemical class, since terpenes are known to act as a deterrent to herbivory (Harborne, 2001), thus, also highlighting the role biotic factors may also play.

Furthermore, Nicholls and Bohm (1983) have shown that variations in flavonoid concentrations in *L. sericeus* disappeared when plants from geographically distinct

populations were grown under controlled and uniform conditions of day length and temperature. The Lincoln and Langenheim's (1979, 1981) studies suggest otherwise however, as they argue that constant exposure to certain ecological variables specific to the habitat may lead to the genetic selection of monoterpenoid chemotypes; this may not be the case amongst phenolic compounds as evidenced by Nicholls and Bohm (1983). *Rhododendron groenlandicum* can be found growing indiscriminately in open bogs and in the forest understory. Delayed production and accumulation of phenolics have been shown in *V. myrtillus* growing in forest compared to high light sites (Martz et al., 2010). Although habitat/abiotic parameters were not taken into account in this study, such consideration may eliminate some of the statistical variation obtained and does not rule out possible genetic adaptations towards phenolic concentrations and insolation.

Indeed, a high variance in the data is expected as biological mechanisms are naturally affected by complex interactions between multiple biotic and abiotic factors. Seasonal variation in phenolic concentrations has been detected in other members of the Ericaceae such as *Vaccinium angustifolium* (McIntyre et al., 2008) and *Rhododendron tomentosum* (Black et al., 2011). Circunmcribing the sampling time to a larger window of operation, as was the case for the 2010 harvest, may result in a larger variance thus explaining smaller R^2 values. Another possible bias lays in the accuracy of the environmental data and the means by which they were obtained (McKenney et al., 2007a,b), as these factors can change considerably from year to year. Utilizing estimates obtained from observations over long periods of time is useful in pulling out underlying trends, but does not explain yearly variation properly. Regardless of these sources of variation, observed trends were supported by two years of harvest suggesting a cyclical and long-term adaptation by plants to conditions of insolation.

The role of measuring instruments must also be considered in interpreting these results. Photoperiod was measured using a Campbell-Stokes heliograph, a ground recording instrument which does not measure visible sunshine, but bright sunshine as determined by the intensity of focused sun rays reaching the instrument (Environment Canada, 2011). Consequently, bright sunshine, and inherently, solar radiation, is reduced by diffusion through atmospheric turbidity and cloud factors. It has thus been suggested that enhanced evapotranspiration rates over large bodies of water may result in bright sunshine decline in

coastal regions as they increase cloud formation (Pallé and Butler, 2001). This may explain why Whapmagoostui, which lies on the banks of Hudson Bay and experiences frequent fogs, possesses the lowest bright sunshine period while it should be highest. This supports the quadratic relationship observed between phenolic concentrations and latitude because bright sunshine does vary with latitude as visible sunshine does.

Similar trends in the variation of phenolic profile and the lack of distinct chemotypes from one year to the next in boreal regions seem to downplay the genotypic involvement in predetermining the phenolic profile of *R. groenlandicum*. It seems more probable that these variations depend simply on annual changes in environmental factors. Low phenolic concentrations in Whapmagoostui accessions may alternatively be constrained by increasingly stressful conditions. Since the species distribution reaches into its northern limits (Hébert and Thiffault, 2011), it may be investing more into the production of primary metabolites rather than secondary metabolites to ensure basic survival. Furthermore, plants collected around the Ottawa region were ecological outliers; the surrounding natural habitat in the Ottawa region is characterized by the Great Lakes St-Lawrence forest region, whereas *R. groenlandicum* grows in isolated vestiges of a formely more extensive boreal forest, such as the Mer Bleu and Alfred bogs. Phytochemically, phenolic concentrations were low yet solar radiation during the summer months was considerably higher than those in Eeyou Istchee. Similarly, temperature was also higher, a factor that is known to increase photosynthetic capacity and productivity as long as it does not converge to the extreme (Chabot and Chabot, 1977; Pastenes and Horton, 1996; Solovchenko and Merzlyak, 2008). Due to unique climatic conditions, their cumulative stress may not be important enough in enhancing photoinhibition of photosystems, thus suppressing the need to produce greater quantities of phenolic compounds for the purpose of photoprotection. Unfortunately, the lack of samples filling the gap between Ottawa and the southernmost boreal communities does not allow us to adequately assess this hypothesis and draw firm conclusions on this observed phenomenon. It is also possible that isolation in environmentally distinct regions may also have contributed to distinct genotypes. Needless to say, genetic or common garden studies on all plant populations may further elucidate the mechanisms behind the phytochemical variations of *R. groenlandicum*.

Although specific climatic variables explaining the variation in the phytochemical profile of *R. groenlandicum* differ between harvest years, variation partitioning of these best-fit models with spatial variables, *i.e.* the quadratic model, produced similar results (**fig. 3.11**). For both years, the unique contribution of each group of variables (fraction [a+b] and [b+c]) to variation in the phytochemical profiles is statistically significant, yet, when the effect of one over the other is taken into consideration, they no longer are ([a] and [c]). Because most of the variation is explained by spatial and climatic variables together ([b]), this confirms that the variation explained by the latitudinal relationship is the same as that which is explained by climatic variables, notably, factors of insolation.

Because the phenolic compounds quantified here were identified by bioassay-guided isolation, variations in their concentration should have an impact on the pharmacological activity of *R. groenlandicum*. Ferrier et al. (2012) showed relationships between total phenolics and anti-glycation end products inhibition within the *Vaccinium* genus, which varied with latitude. As well, McIntyre et al. (2008) and Black et al. (2011) reported variations in pharmacological activities associated with seasonal variations in the phenolic concentrations of plants from the Ericaceae, *V. angustifolium* and *R. tomentosum,* respectively. Although biologically active constituents in *R. groenlandicum* were found to vary geographically, pharmacological bioassays need to be performed in order to properly assess the pharmacological significance of these observations.

3.5 Conclusion

Our study demonstrates a relationship between phenolic compound quantities and latitude, which is an indirect marker for environmental parameters that directly affect plant physiology. The quantitative variations in the biologically active phytochemicals of *R. groenlandicum* relate best to insolation parameters such as solar radiation and photoperiod, notably during the month of June. The reason for such observations may be explained by the role that phenolic compounds play as protective agents against photoinhibition and photodamage. In short, latitude acts as a geographical marker translating the effect of environmental variables affecting *R. groenlandicum'*s photosynthesis needs. Major markers to

which these trends may be attributed are (+)-catechin, (-)-epicatechin, quercetin-3-galactoside and quercetin-glycoside 4, whereas concentrations were highest between the 51-53rd parallel and lowest in the peripheral distribution area of this species. In providing appropriate recommendations for the harvest of *R. groenlandicum* in quality controlled NHPs, our results show that medicinal quality is similar over most of the Cree communities except the most northerly area Whapmagoostui where a differentiation to a distinct chemorace seems to occurs.

3.6 Acknowledgements

3.7 Tables and Figures

Table 3.1 List of compounds identified and quantified from the leaves of *Rhododendron groenlandicum.* Concentration means ± SD for all samples collected in 2006 and 2010 and references for studies corroborating its presence in *R. groenlandicum*

Peak #	Compound	Concentration (mg/g DW)		Reference
		2006	2010	
1	(+)-Catechin	4.98 (1.04)	2.16 (0.64)	Ouchfoun, 2011; Saleem et al., 2010
2	Chlorogenic acid	3.17 (0.77)	1.53 (0.55)	Ouchfoun, 2011; Saleem et al., 2010; Spoor et al., 2006
3	(-)-Epicatechin	2.73 (0.78)	1.78 (0.68)	Ouchfoun, 2011; Saleem et al., 2010
4	*p*-Coumaric acid	T	T	
5	Rutin	T	T	
6	Quercetin-3-galactoside	6.49 (0.97)	5.50 (1.26)	Ouchfoun, 2011; Saleem et al., 2010
7	Quercetin-3-glucoside	1.81 (0.27)	1.42 (0.36)	Ouchfoun, 2011; Saleem et al., 2010
8	Quercetin-glycoside 1	0.68 (0.19)	0.51 (0.22)	
9	Quercetin-glycoside 2	1.52 (0.25)	0.89 (0.30)	
10	Quercetin-glycoside 3	1.36 (0.20)	1.02 (0.24)	
11	Quercetin-glycoside 4	5.53 (0.88)	4.67 (1.02)	
12	Quercetin-3-rhamnoside	0.38 (0.36)	1.41 (0.82)	Saleem et al., 2010; Scott et al., 2006
13	Myricetin	T	T	Saleem et al., 2010
14	Quercetin	0.12 (0.03)	0.09 (0.05)	Chartier, Staub, & Goetz, 2005; Ouchfoun, 2011
	Total	28.76 (3.09)	21.08 (3.77)	

Table 3.2 ANOVA results analyzing concentration variations of phenolic compounds in *Rhododendron groenlandicum* collected around various communities in 2006 and 2010. Significant results are determined at $\alpha = 0.05$ and are shaded.

Compound	2006		2010	
	p-value	R^2_{adj}	*p-value*	R^2_{adj}
(+)-Catechin	2.17×10^{-06}	0.454	2.72×10^{-11}	0.562
Chlorogenic acid	0.00761	0.195	0.131	0.0572
(-)-Epicatechin	1.94×10^{-06}	0.457	7.2×10^{-12}	0.580
Quercetin-3-galactoside	0.147	0.0607	0.0406	0.102
Quercetin-3-glucoside	0.0151	0.167	0.0933	0.0710
Quercetin-glycoside 1	0.0147	0.168	0.0367	0.106
Quercetin-glycoside 2	0.0361	0.129	0.113	0.0632
Quercetin-glycoside 3	0.000114	0.343	0.575	-0.0173
Quercetin-glycoside 4	1.63×10^{-06}	0.461	0.1228	0.0599
Quercetin-3-rhamnoside	0.00939	0.274	0.217	0.0350
Quercetin	0.00865	0.190	3.25×10^{-10}	0.526
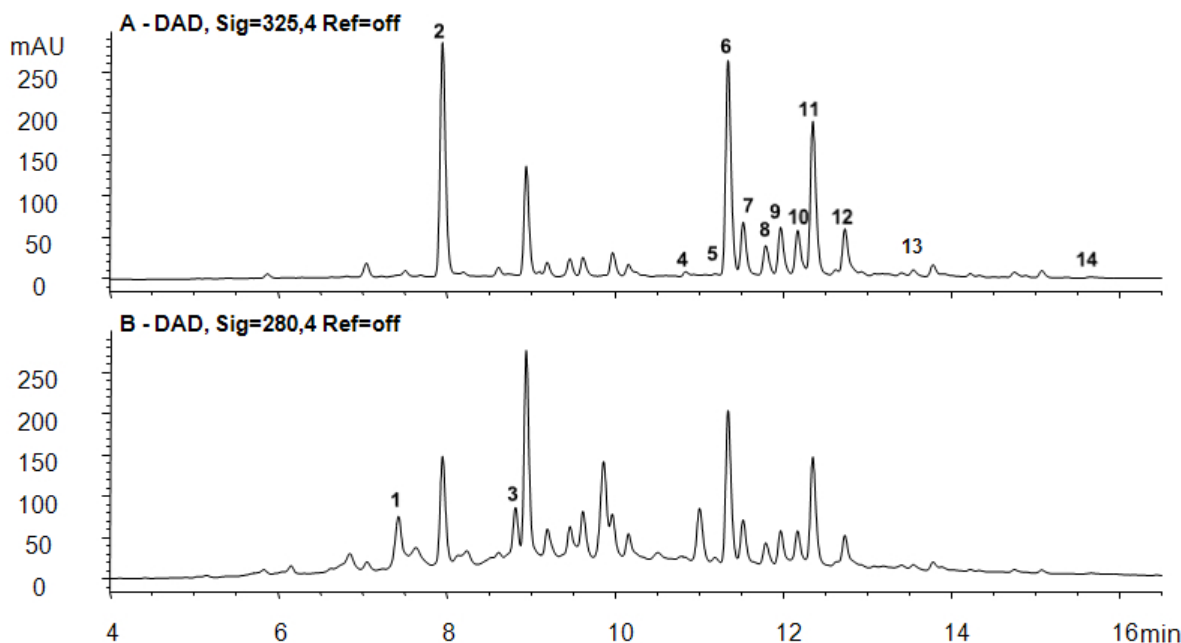
Figure 3.1 Sample HPLC chromatogram with DAD at 325 and 280 nm of *R. groenlandicum* leaves. Numbers represent compounds as follows : (+)-catechin **(1)**, chlorogenic acid **(2)**, (-)-epicatechin **(3)**, *p*-coumaric acid **(4)**, rutin **(5)**, quercetin-3-galactoside **(6)**, quercetin-3-glucoside **(7)**, quercetin-glycoside 1 **(8)**, quercetin-glycoside 2 **(9),** quercetin-glycoside 3 **(10)**, quercetin-glycoside 4 **(11),** quercetin-3-rhamnoside **(12)**, myricetin **(13)** and quercetin **(14)**.
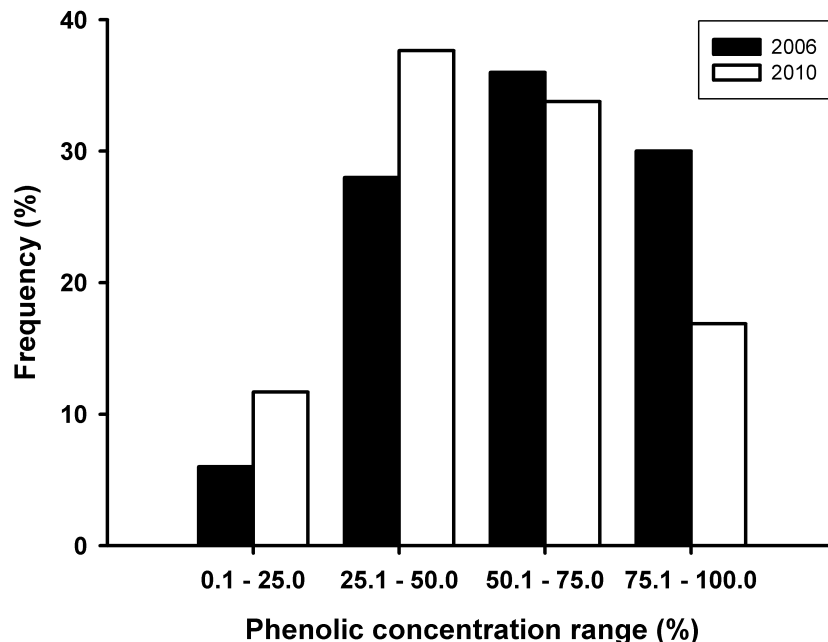
Figure 3.2 Frequency (%) of accessions found within various ranges of phenolics concentrations (% calculated from the lowest to highest concentration) expressed as the sum of all quantified compounds for **A)** 2006 (*n* = 50) and **B)** 2010 (*n* = 77).
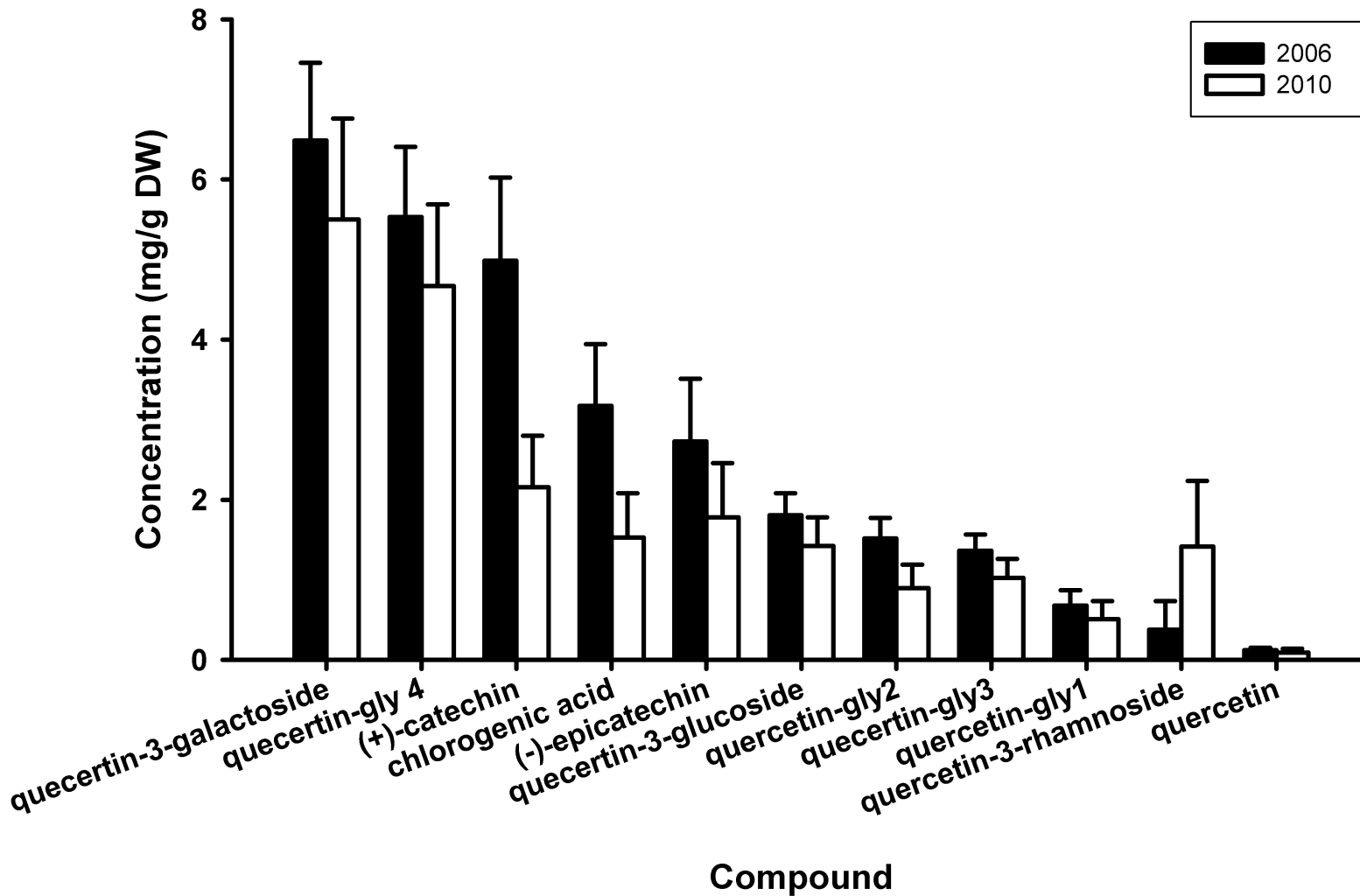
Figure 3.3 Concentration means ± SD (mg/g DW) for each quantified marker from 2006 (*n* = 50) and 2010 (*n* = 77) harvests.

Figure 3.4 Phytochemical variation of two of the most abundant compounds ((+)-catechin and (-)-epicatechin). Mean concentration ± SD (*n* = 10) in *R. groenlandicum* leaf extracts collected from Northern Quebec and Eastern Ontario is shown. **A)** 2006 (5 locations) and **B)** 2010 (7 locations). Different letters indicate significant differences amongst communities for each compound at α = 0.05.

Figure 3.5 RDA ordination biplots, in type 2 scaling, of concentration variations in the phenolic profile of *R. groenlandicum* leaves as a function of community of origin for the 2006 harvest. **A)** Symbols represent sampling sites from the communities of Mistissini (▲), Nemaska (●), Eastmain (■), Wemindji (◆) and Whapmagoostui (▼). Centroids for these sites are drawn out by ellipses. **B)** Phenolic markers are represented by arrows. **C)** Similarities between community based on phenolic profile is elucidated by complete linkage cluster analysis. Communities which do not share a common letter represent significant differences in profile concentrations as determined by post-hoc pairwise comparisons with Holm's correction. Numbers represent compounds as follows : (+)-catechin **(1)**, chlorogenic acid **(2)**, (-)-epicatechin **(3)**, quercetin-3-galactoside **(6)**, quercetin-3-glucoside **(7)**, quercetin-glycoside 1 **(8)**, quercetin-glycoside 2 **(9),** quercetin-glycoside 3 **(10)**, quercetin-glycoside 4 **(11),** quercetin-3-rhamnoside **(12)** and quercetin **(14)**.
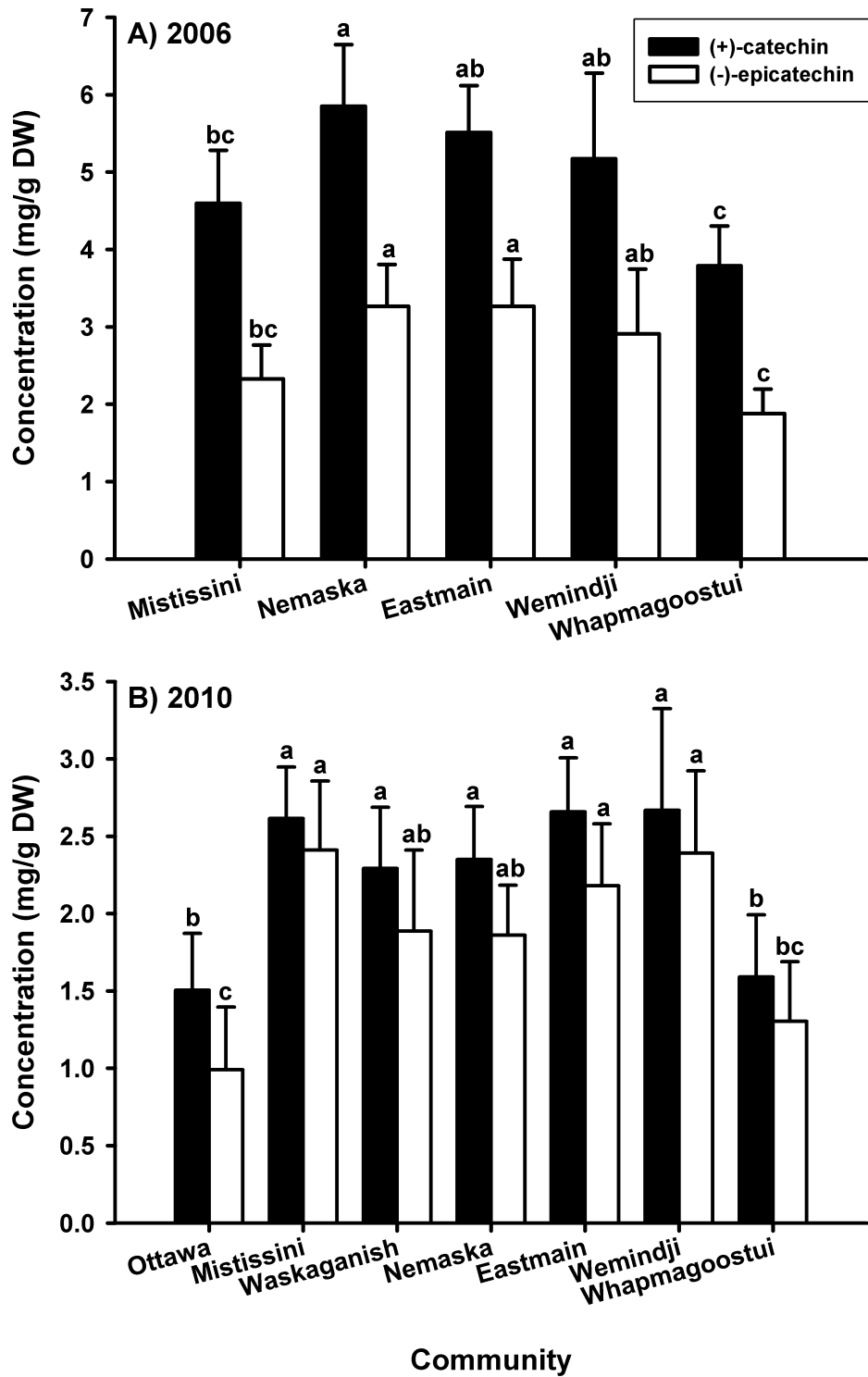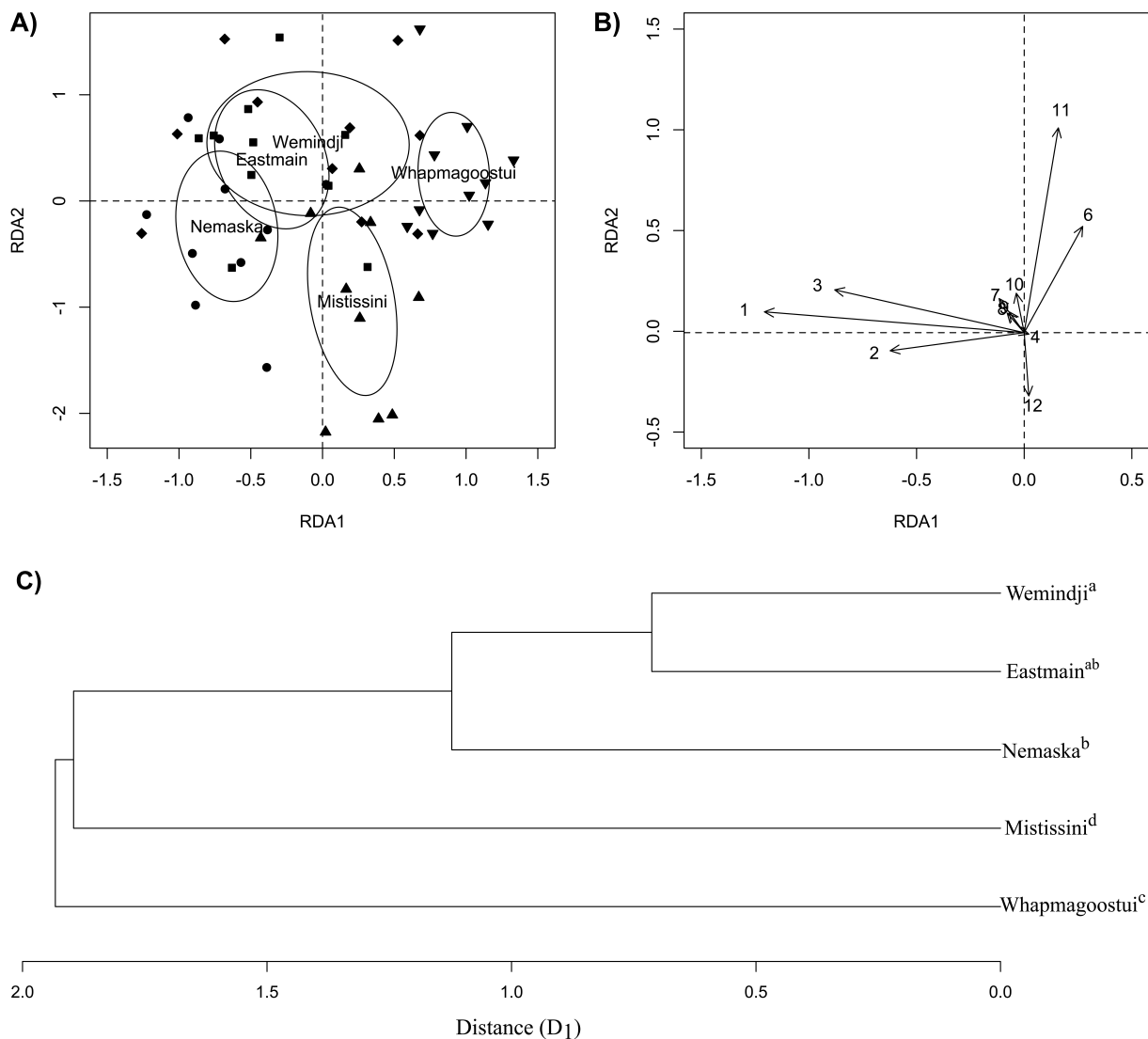
Figure 3.6 RDA ordination biplots, in type 2 scaling, of concentration variations in the phenolic profile of *R. groenlandicum* leaves as a function of community of origin for the 2010 harvest. **A)** Symbols represent sampling sites from the communities of Ottawa (○), Mistissini (▲), Nemaska (●), Waskaganish (□), Eastmain (■), Wemindji (◆) and Whapmagoostui (▼). Centroids for these sites are drawn out by ellipses. **B)** Phenolic markers are represented by arrows. **C)** Similarities between community based on phenolic profile is elucidated by complete linkage cluster analysis. Communities which do not share a common letter represent significant differences in profile concentrations as determined by post-hoc pairwise comparisons with Holm's correction. Numbers represent compounds as follows : (+)-catechin **(1)**, chlorogenic acid **(2)**, (-)-epicatechin **(3)**, quercetin-3-galactoside **(6)**, quercetin-3-glucoside **(7)**, quercetin-glycoside 1 **(8)**, quercetin-glycoside 2 **(9),** quercetin-glycoside 3 **(10)**, quercetin-glycoside 4 **(11),** quercetin-3-rhamnoside **(12)** and quercetin **(14)**.
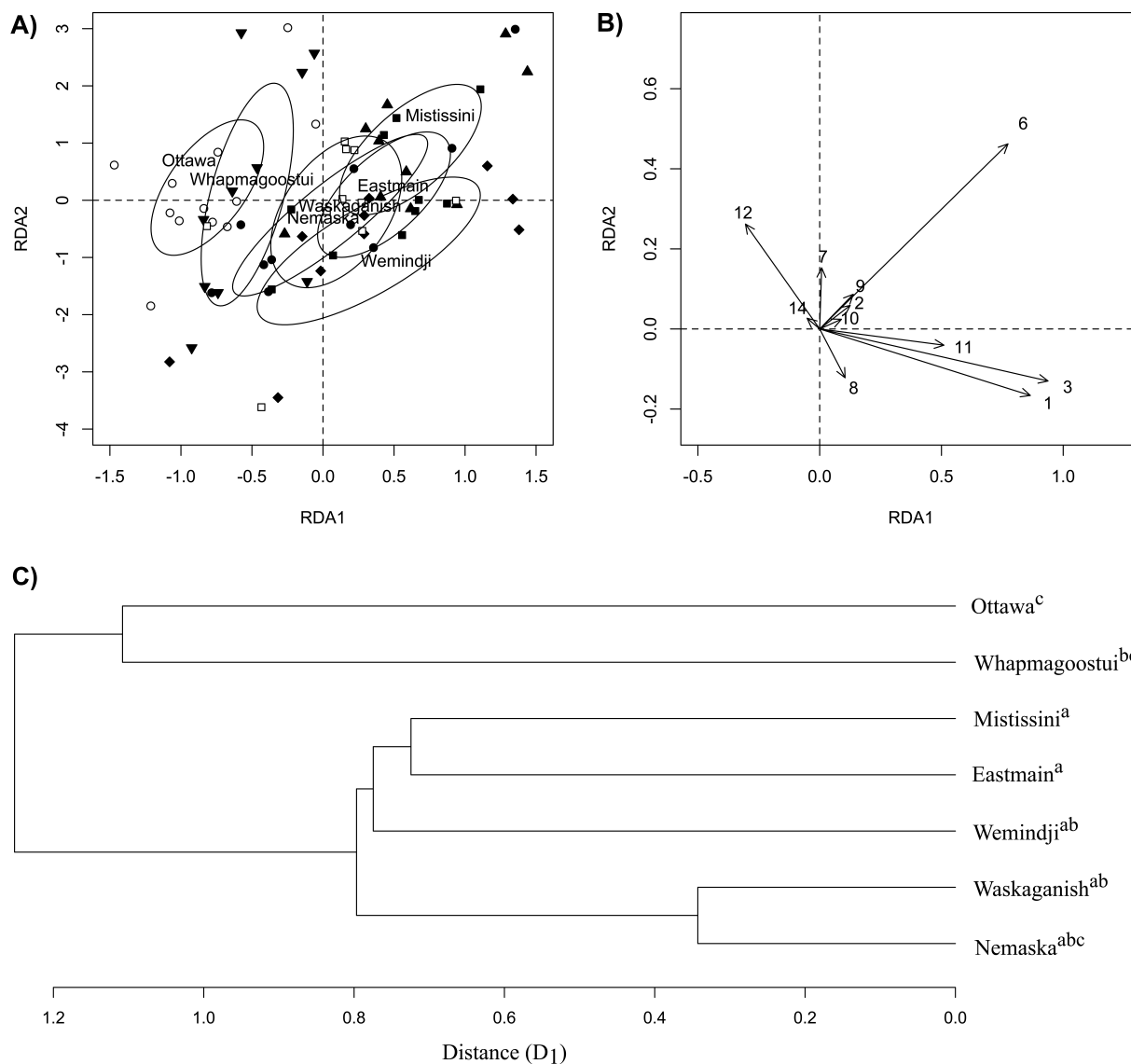
Figure 3.7 Concentration of the sum of quantified phenolics in *R. groenlandicum* leaves as a function of latitude for **A)** 2006 (*n* = 48) and **B)** 2010 *(n* = 77) samples. Total phenolics were determined as the sum of all quantified phenolic compounds. Curves reprensent the significant quadratic relationships in the spatial distribution of total phenolic concentrations determined by parametric polynomial regressions ($\alpha = 0.05$).
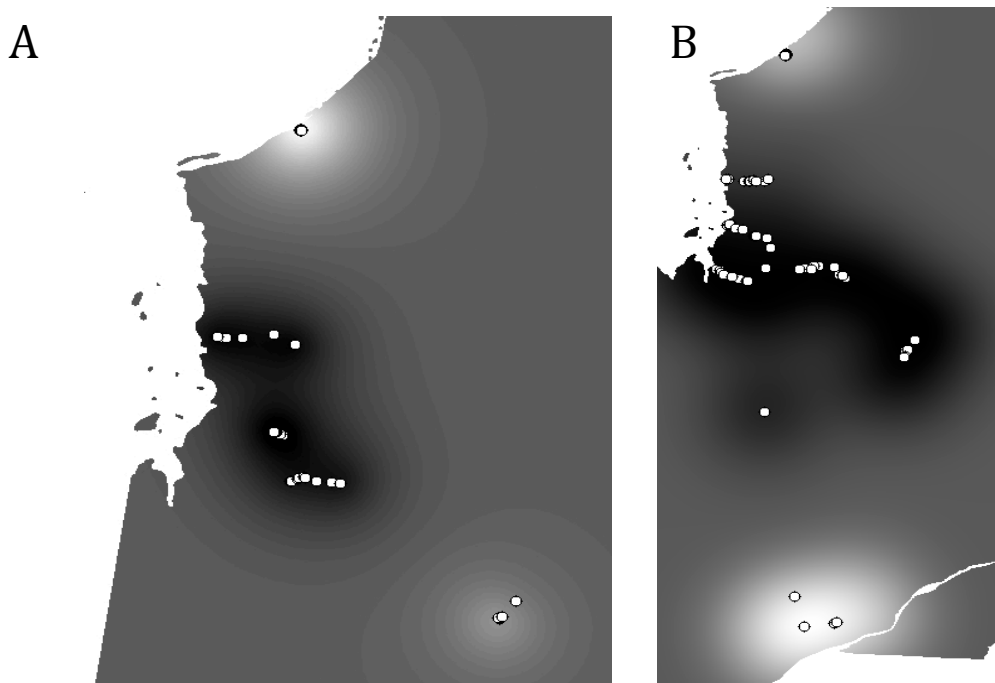
Figure 3.8 Maps showing high (black background) and low (white background) concentrations of phenolic compounds for the two harvest years **A)** 2006 and **B)** 2010. Shading data are based on the assessment of the first canonical axis (RDA1) resulting from the RDA of the phytochemical profile in *R. groenlandicum* constrained by the spatial polynomial (*i.e.* the quadratic relationship with latitude). Interpolation by kriging was performed with fitted site scores and circles represent sampling sites. The north-south direction is parallel to the vertical axis of the maps and covers a larger area in **(B)**.
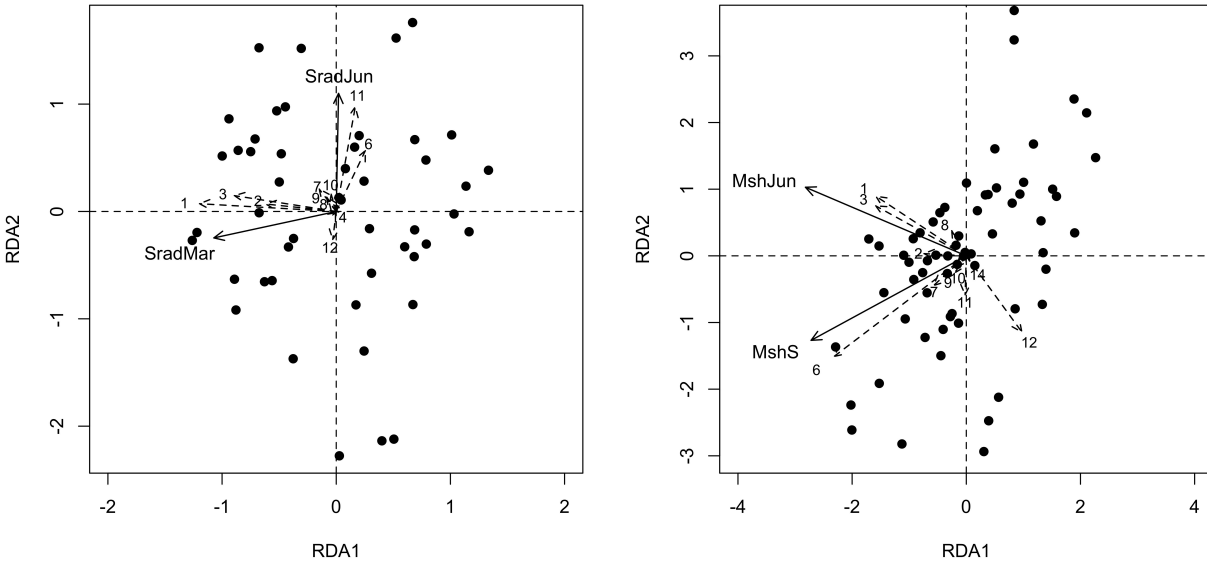
Figure 3.9 RDA ordination biplots in type 2 scaling representing the variation in the phytochemical profile of *R. groenlandicum* leaves as a function of solar radiation (Mjoules) for the 2006 harvest (a) and monthly bright sunshine photoperiod averages (h) for the 2010 harvest (b). Samples from Ottawa were not included in the analysis for 2010. Full lines represent explanatory variables and dashed lines represent quantified phenolic compounds.
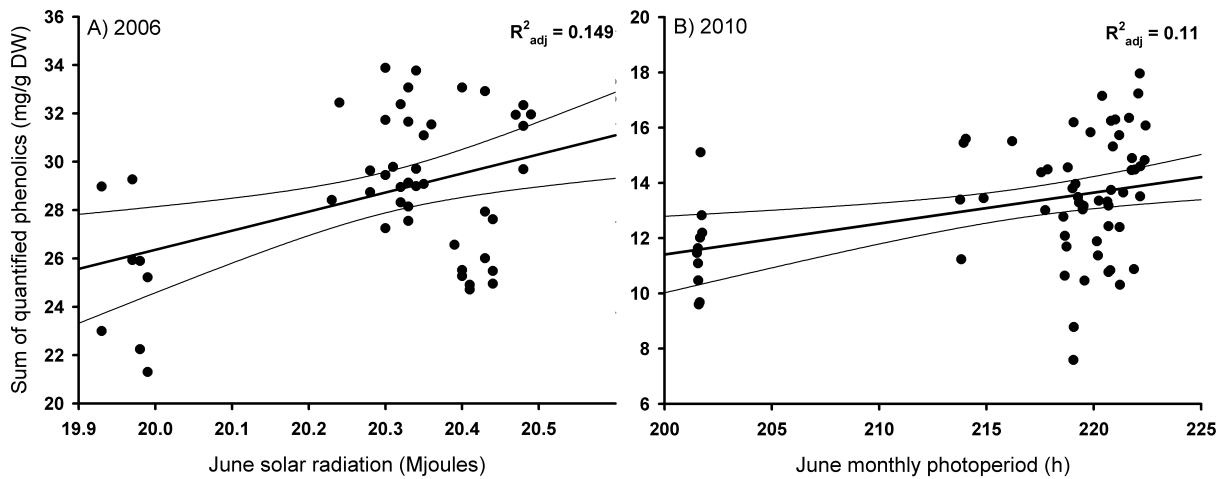
Figure 3.10 Variation of the sum of quantified compounds in relation to averages of **A)** June solar radiation in 2006 and **B)** June monthly bright sunshine photoperiod in 2010. Samples from Ottawa were excluded from the analysis for 2010 and only significantly varying compounds were including in calculating the sum of phenolics.

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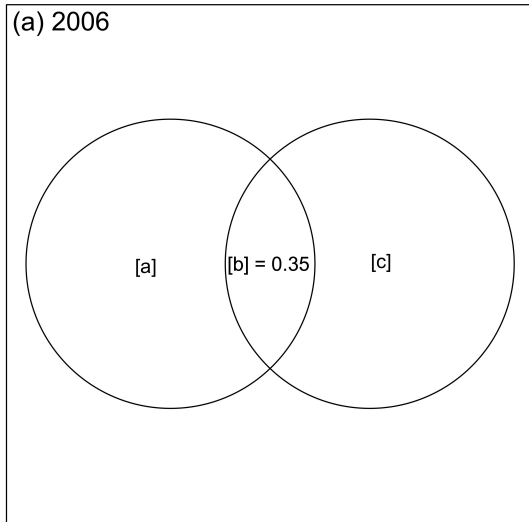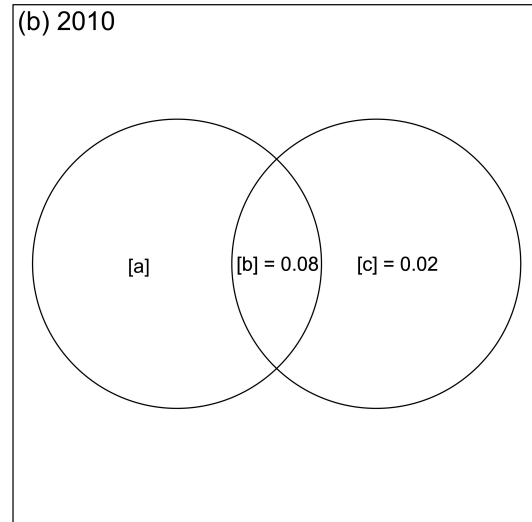
Figure 3.11 Variation partitionning between spatial and environmental variable groups for 2006 (a) and 2010 (b) harvests. Fraction a = spatial variables (latitude), b = spatial variables + environmental variables, c = environmental variables (insolation).

Chapitre 4

Variations in the phytochemistry of biologically active compounds in pitcher plant, *Sarracenia purpurea,* from Northern Quebec, Canada.

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4.1 Abstract

A survey of the phytochemistry of biologically active compounds in a North American medicinal plant, *Sarracenia purpurea* L., was conducted for two harvest years. Leaves were harvested from wild populations in 2006 and 2010 over a latitudinal gradient in Northern Québec, and known biologically active compounds were quantified by UPLC-MS. Major quantified compounds were the terpenes, morronoside, ursolic acid and betulinic acid, as well as the phenolics quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, taxifolin-3-*O*-glucoside and (-)-epicatechin. Morronoside was the most abundant marker and the only one to vary geographically during both harvest years. Variations in the content of these compounds did not reflect a distinct spatial pattern nor could the underlying effect of environmental and climatic factors be discerned. Despite the patchy nature of the distribution of populations, no chemotypes could be distinguished. Baseline data on the content level of pharmacologically active compounds in *S. purpurea* reported here provide reference values for possible quality assurance programs in the implementation of locally and culturally appropriate approaches to treating type 2 diabetes.

Key words: Sarracenia purpurea, phenolics, terpenes, phytochemical variations, traditional medicine, type 2 diabetes, Cree Nation of Eeyou Istchee.

4.2 Introduction

Sarracenia purpurea L. (Sarraceniaceae), pitcher plant or ***Aygadash*** in Cree, is a common plant growing in the sphagnum bogs and peaty barrens of Canada's boreal forests (Scoggan, 1978). It has a long history of traditional uses among indigenous populations. Its recorded and/or current uses are primarily reported for First Nations groups of the Algonquian linguistic family and the Iroquois (Arnason et al., 1981; Zieba, 1990; Siegfried, 1994; Marles et al., 2008; Moerman, 2009).

A high incidence (29%) of type 2 diabetes (T2D) has been reported in communities of the Cree Nations of Eeyou Istchee (CEI) (Kuzmina et al., 2010), and the CIHR Team on Antidiabetic Aboriginal Medicines (CIHR-TAAM) was formed to undertake collaborative research with Cree Healers to study traditional medicinal plants for their anti-diabetic potential. Within the pharmacopoeia of the CEI, *S. purpurea* is used to treat a range of symptoms related to diabetes (Marshall et al., 2003; Leduc et al., 2006; Marshall, 2006; Downing, 2010). In laboratory studies, it was shown to possess *in vitro* pharmacological activity comparable to metformin in the glucose uptake of C2C12 myotubes and significantly protected against glucose toxicity and deprivation of PC12-AC neurones (Spoor et al., 2006; Harris et al., 2012; Muhammad et al., 2012).

The CEI, however, occupy a very large territory lying between the 50th and 55th parallel. Although sphagnum bogs and peaty barrens are widely distributed within the region, they are highly fragmented with a patchy distribution (Schwaegerle and Schaal, 1979). This creates challenges to gene flow that may lead to substantial levels of genetic differentiation between populations (Schwaegerle and Schaal, 1979) and inbreeding depression due to self-pollination (Ne'eman et al., 2006). In keeping with CIHR-TAAM goals of developing culturally adapted therapies for the treatment of T2D in the communities, this situation is not without problems in ensuring the quality control of natural health products (NHPs).

Little is known about the phytochemical composition of *S. purpurea,* particularly concerning its biologically active constituents. Yet its antidiabetic potential promises possible alternative approaches to treating diabetes in the CEI. Establishing the nature of

phytochemical variation in the plant is of interest in assessing the practicability of employing this resource in a controlled and standardized manner.

The objective of this study was to determine the phytochemical variation in *S. purpurea* populations. Because of the wide territory within which pitcher plant occurs, we hypothesized that such variation might be attributed to environmental variables and may be reflected in distinct chemotypes. We report spatial and temporal variations of important pharmacologically active markers.

4.3 Materials and Methods

4.3.1 Sampling

Mature leaves of *S. purpurea* were sampled during the summers of 2006 and 2010 around the communities of Mistissini, Nemaska, Waskaganish, Eastmain and Wemindji, thus covering most of the entire north-south gradient in Eeyou Istchee. Waskaganish was the only CEI community that was not sampled in 2006. Samples from the Ottawa region, south of the CEI territory, were also collected in 2010. This collection effectively doubles the latitudinal gradient, extending it into the Great Lakes St-Lawrence forest region characterized by a greater diversity of deciduous hard wood trees (Farrar, 2005). Ten accessions, each containing leaves from multiple (~5) individual plants, were collected within a 50 km radius around each community. Representative voucher specimens from each community are deposited at the Marie-Victorin Herbarium of the Université de Montréal (MT) and the University of Ottawa Herbarium (OTT) (**Appendix III**).

4.3.2 Extraction

Plant material was thoroughly dried at 35°C overnight in a commercial food dehydrator (Nesco® Professional Food and Jerky Dehydrator). Samples were milled through a Wiley Mill with at 40 mesh and extracted overnight in 25 mL/g of 80 % EtOH by orbital shaking at room temperature at 250 RPM. The pellet was extracted overnight in 15 mL of 80 % EtOH. The pooled supernatants (adjusted to 50 mL in a volumetric flask) were centrifuged

for 15 min at 1828 x *g* at room temperature. An aliquot (1 mL) of the centrifuged extract was prepared for chromatographic analysis by filtering through a 20 μm PTFE filter. Extracts were kept at -20°C and sonicated before analysis. Unfiltered crude extracts were dried using a speedVac. Trace water was removed by lyophilization using a SuperModulo freeze dryer and stored at -80°C for future use.

4.3.3 Chemicals and standards

(+)-Catechin **(1)**, morronoside **(2)**, (-)-epicatechin **(3)**, taxifolin-3-glucoside **(4)**, quercetin-3-galactoside **(5)**, quercetin-3-glucoside **(6)**, kaempferol-3-rutinoside **(7)**, betulinic acid **(8)**, ursolic acid **(9)** and rutin (**10**) were purchased from Sigma-Aldrich (Oakville, Ontario, Canada) and Extrasynthese (Genay, France). LC-MS grade water, acetonitrile, and formic acid (99.9% purity) were purchased from Fisher (Fisher Scientific, Ottawa, Ontario).

4.3.4 Identification and Quantification

The identification of compounds listed in **table 4.1** was carried out on a Shimadzu Ultra-High Performance Liquid Chromatography hyphenated with Mass Spectrometer (UPLC-MS) system (Mandel scientific company Inc, Guelph, Ontario), which consisted of LC30AD pumps, a CTO20A column oven, a SIL-30AC autosampler and a LCMS-2020 mass spectrometer. Briefly, the separation of the phenolic compounds was carried out on a phenomenex Kinetex™ C18 column (100 x 2.1mm, 2.6μm particle size, phenomenex, Mississauga, Ontario) with a gradient elution method (Method A). The initial solvent was 86% solvent A (0.1% formic acid) and 14% solvent B (70%ACN+ 30%MeOH), the ratio remained at 86:14 for 5.5 min and then changed to 65:35 in 2 min. The column was then washed with 100% B for 2 min and change back to the initial condition. The flow was set at 0.4 ml/min with a column temperature at 55 °C. The separation of terpenes was carried out on the same column with an isocratic elution method (Method B). Marker compounds were eluted with a 0.4 ml/min flow of 62.5% ACN for 5 min. The column temperature was set at 55 °C.

Peak identifications were undertaken by co-chromatographic comparison of the retention time and mass data with commercially available purified compounds. Appropriate and corresponding standard curves were built by injecting dilutions of standard compounds

stock solutions. These curves were then used to quantify the amount of each marker compound. Each sample was analyzed in triplicate and averaged to account for instrumental variation.

4.3.5 Environmental data

Estimates for over 190 environmental and climatic variables were provided by the Canadian Forest Services of Natural Resources Canada. Values were generated by spatially continuous climatic models adjusted by ANUSPLIN, a non-parametric multivariate technique for the noise-reduction of multiple variable data. Interpolation of surfaces is calculated by smoothing algorithms and taking into consideration the effect of altitude. For the most part, these surfaces are generated from a 30 years data span collected by meteorological stations across the country. Using each sample's geographical coordinates at a resolution of 4 decimal-degrees, environmental and climatic estimates were obtained for each collection site. Monthly estimates for solar radiation (Mjoules) and photoperiod (h) were obtained from data of the 1961-1990 period. Other variables including evapotranspiration and climatic moisture index (CMI) were available for the 1971-2000 period while monthly and annual estimates for such variables as temperature and precipitation were also available for the first collection year of 2006. Details of the protocols generating these estimates are described by McKenney *et al.* (2007a,b).

4.3.6 Statistical analysis

The statistical analyses performed on the obtained data were based on the procedure described in Rapinski et al. (in preparation(a)).

One-way or two-way analysis of variance (ANOVA) were carried out to determine differences in the concentrations of different compounds and how each of those differed from one community to the next. Tukey's multiple-comparison tests were performed *post-hoc* when initial ANOVA's were statistically significant. All results were expressed as mean ± SD and considered to be statistically significant using α = 0.05.

To elucidate potential chemotypes and important markers, the phytochemical profiles of *S. purpurea* were analyzed by performing a multivariate analysis of variance (MANOVA)

using a partial-RDA approach (Anderson and Legendre, 1999) if more than one compound varied significantly among communities. Ellipses around the centroid for each community facilitate the visualization of relationships amongst these. These relationships were determined by post-hoc pairwise comparisons done by performing a canonical redundancy analysis (RDA) for each pair of community and adjusting the α value using Holm's correction.

Simple and multiple linear regressions were used to determine which climatic and environmental variable best explained the variation observed in quantified compounds from *S. purpurea*. Because of the large quantity of variables available, they were selected for a most parsimonious model explaining the maximum variation in the phenolic profile. Selection was performed using both an information-theoretics and hypothesis-testing approach. Automated routines for forward, backward and stepwise selection were generated using Akaike's information criterion (AIC) (Akaike, 1973) as the selection criteria. Forward selection was also performed using adjusted R^2 and α level as selection criterion in a hypothesis-testing approach (Blanchet et al., 2008). Results from all four methods were taken into consideration in the selection of final variables to be modelled and the variance inflation factor (VIF) calculated for each of these to evaluate the severity of multicolinearity.

Statistical significance of multivariate analyses was determined by non-parametric tests using 100,000 permutations and α = 0.05 while statistical significance of univariate analyses was determined using the normal distribution after verifying that the data met, or was transformed to meet, the assumptions required. Univariate and multivariate statistical analyses were performed using R statistical language (R Development Core Team, 2012).

4.4 Results and Discussion

Representative UPLC-MS-ESI chromatographs of *S. purpurea* leaf extracts are shown in **figure 4.1**. Of all 10 compounds, only the terpenes morronoside, ursolic acid and betulinic acid, as well as the phenolic compounds (-)-epicatechin, quercetin-3-*O*-galactoside, taxifolin-3-*O*-glucoside and quercetin-3-*O*-glucoside were found in sufficient quantifiable quantities for both sampling years (**table 4.2**). Rutin was not detected in the 2006 harvest but was found in

trace amounts in 2010. Kaempferol-3-*O*-rutinoside was found in trace amounts both years, while (+)-catechin could only be quantified in 2010. The remainder of compounds were either found in low concentrations one year, or in trace amount the other year.

Taxifolin-3-*O*-glucoside, rutin, kaempferol-3-*O*-rutinoside, morronoside and quercetin-3-*O*-galactoside were active compounds isolated from *S. purpurea* by bioassay-guided fractionation on glucose uptake in C2C12 mouse muscle cells (Muhammad et al., 2012). All of these, with the exception of taxifolin-3-*O*-glucoside, significantly potentiated glucose uptake whereas quercetin-3-*O*-galactoside exhibited the highest maximal efficacy (Muhammad et al., 2012). Morronoside and quercetin-3-*O*-galactoside were also the active compounds in protecting against glucose toxicity (Harris et al., 2012). Phenolic compounds are some of the most common bioactive compounds detected in the boreal forest plant species of the CEI pharmacopoeia (Spoor et al., 2006; Saleem et al., 2010), particularly in genera, such as *Vaccinium* (McIntyre et al., 2008; Beaulieu et al., 2010; Ferrier et al., 2012) and *Rhododendron* (Rapinski et al., in preparation(a); Black et al., 2011), of the Ericaceae. The terpene betulinic acid is one of the major bioactive compounds reported in *Betula papyrifera* (Pisha et al., 1995; Takada and Aggarwal, 2003; Tan et al., 2003; Cichewicz and Kouzi, 2004; Ehrhardt et al., 2004; Jeremias et al., 2004; Krasutsky, 2006; Lavoie and Stevanovic, 2007; Thibeault et al., 2007; Gauthier et al., 2010) and has been extensively studied for anti-inflammatory, antiviral, antibiotic and anticancer properties (see Krasutky (2006) for an extensive review). Ursolic acid is known for similar properties (Harborne et al., 1999) and has been identified in *Rhododendron tomentosum* (Wollenweber and Kohorst, 1984)*, R. groenlandicum* (Hooper and Chandler, 1984; Wollenweber and Kohorst, 1984; Dufour et al., 2007; Ikeda et al., 2008; Jangra et al., 2010) and *V. vitis-idaea* (Fokina et al., 1988; Kondo et al., 2011).

The most abundant compound was morronoside, followed closely by quercetin-3-*O*-galactoside, then betulinic acid, taxifolin-3-*O*-glucoside, ursolic acid, quercetin-3-*O*-glucoside and (-)-epicatechin (**fig. 4.2**), where concentrations were considerably higher for the first two. With the exception of quercetin-3-*O*-glucoside, mean concentration for each compound was higher in 2010 than in 2006 and maintained a similar ranking order. Furthermore, the sum of quantified compounds, calculated from those which were common to both sampling years

(**table 4.2**), be it phenolics, terpenes or both, ranged over greater concentrations in 2010 than in 2006 (**fig. 4.3**). Nonetheless, **figure 4.3** clearly shows that the content of bioactive compounds are normally distributed with most accessions containing average quantities. These distributions were similar for both sampling years and also show that fewer accessions possessed a phenolic content in the upper range than in the lower.

An ANOVA of the concentration of compounds by locality is presented in **table 3** with each year analysed separately. Morronoside was the only compound to vary among localities for both sampling years (**fig. 4.4**), but relationships between communities were completely different each year. Whereas Mistissini was the region with the lowest concentrations in 2006, it was amongst the highest in 2010 and vice-versa for Nemaska and Eastmain. Waskaganish had the lowest concentrations of all the regions sampled. In 2010, (+)-catechin, (-)-epicatechin, quercetin-3-O-galactoside and ursolic acid also varied among localities (**fig. 4.5**). How concentrations of (-)-epicatechin varied from one community to the next reflect the relationships observed in morronoside. On the other hand, those observed for the other two compounds were similar to morronoside's pattern in 2006 with a distinct spike in concentrations in Eastmain.

The contribution of large concentrations of morronoside appears to weigh importantly in determining how terpene content, and total compound content, vary from one community to the next as patterns mirror one another in both years (**fig. 4.4** and **4.6**). This is evident in the 2006 collection as it was the only compound varying significantly. An RDA ($p = 1x10^{-5}$, R^2_{adj} = 0.462) of quantified compounds from 2010 samples support this idea further (**fig. 4.7**). Morronoside appeared in the analysis as the most important marker, strongly associated with the first canonical axis ($\lambda = 81.861$) which was highly significant ($p = 1x10^{-5}$) and explained 45.36 % of the variance in the phytochemical profile. Quercetin-3-O-galactoside was the only other important marker that could explain some of the variance in the phytochemical profile but associations to any of the canonical axes were weak. Consequently, the geographical trends shown for quercetin-3-O-galactoside did not appear to be great enough to affect the general quantified phenolic content as they remained statistically similar in each community (**fig. 4.6**).

Our results do not show temporally distinct geographical pattern in the phytochemistry of *S. purpurea*. Coupled with the inconsistent nature of certain compounds varying one year but not the other, there is no support for the presence of distinguished chemotypes. Although this is the first study of this type for this species, a study on the phytochemistry of isolated wild populations of North American ginseng, *Panax quinquefolius* (Araliaceae), also failed to show distinguishing chemotypes in its northern range (Assinewe et al., 2003), though there is evidence of a chemotype in the far south of its range. Bees and flies are known floral visitors and pollinators to *S. purpurea* (Ne'eman et al., 2006) and their contribution to gene flow may be enough in attenuating the distinction towards chemoraces. The reduction of migrating genotypes on a glacial front and time since colonization of the region by the species may as well play a role (Soltis et al., 1997). Alternatively, variations in the phytochemical profile of *S. purpurea* may be primarily dependant on other factors than genetics.

Although we found some relationships between bioactive compounds with environmental and climatic factors, no distinct pattern was conspicuous. For example, step-wise regression showed that morronoside, the most abundant and important marker in explaining the phytochemical variation in *S. purpurea*, correlated best with minimum December temperature in 2006 ($p = 0.001$, $R^2_{adj} = 0.273$), in contrast to mean diurnal ranges and April mean monthly sunshine hours together in 2010 ($p = 0.001$, $R^2_{adj} = 0.6387$). The lack of consistent trends with climatic factors does not enable us to make significant biological interpretations of content based on these factors. Furthermore, the considerable change in the geographical pattern of morronoside from one year to the other suggests that its concentration is simply not dependent on long-term climatic patterns. These patterns are in contrast to latitudinal and climatic parameters that correlate with phenolic content in *Rhododendron groenlandicum* (Ericaceae) as found in a separate chapter in this thesis. In comparing the preferred environment of the two species, the effect of the pitcher plant's aquatic habitat may affect its response to climatic influences as opposed to the terrestrially inclined *R. groenlandicum*.

Although the role of morronoside in the plant is not well studied, it is known that iridoid glycosides are important in defense reactions against insects and mammals (Martz et al., 2009b) but have also been shown to vary in response to abiotic factors. Concentrations

have been shown to increase in the flower buds of *Lonicera japonica,* of the Caprifoliaceae (Ning et al., 2012), and decrease in *Menyanthes trifoliate,* of the Menyanthaceae (Martz et al., 2009b), in relation to UV radiation, a relationship which our results have failed to suggest in one form or another. Wang et al. (2010) showed, on the other hand, that drought stress increased iridoid glycoside content in the root of *Scrophularia ningpoensis* (Scrophulariaceae). It was observed during ongoing fieldwork that a considerable number of *S. purpurea* plants from study sites around Mistissini had died. Drought may explain the considerable increase in morronoside contents in the Mistissini samples; however, 2010 environmental and climatic data are not yet available to confirm this suspicion.

Alternatively, the emulsion (or acid) hydrolysis of morronoside is also known to derive sarracenin (Miles et al., 1976; Baldwin and Crimmins, 1982), a key compound of *S. purpurea* (Newman et al., 2000). The role of the volatile monoterpene sarracenin was initially thought as an intermediate in the biosynthesis of certain monoterpenes and indole alkaloids (Miles et al., 1976), though more recently, it has been suggested to play a role in carnivorous plants in attracting insect prey (Jaffé et al., 1995). As a product of sarracenin's biosynthetic pathway, morronoside may in fact be largely dependent on biotic factors for which underlying climatic trends do not act as proper markers of this variation.

In a similar study of the phytochemistry of bioactive compounds in *R. groenlandicum,* we found quercetin-3-*O*-galactoside, the second most abundant compounds in *S. purpurea,* to be a major marker in explaining geographical trends in the species phenolic profile (Rapinski et al., in preparation(a)). Latitudinal trends were found to act as markers for translating the effect of environmental variables, notably, insolation parameters. However, the absence of such trends in the phenolic compounds from *S. purpurea* quantified here fail to support such a conclusion. This may be attributed to the presence of anthocyanins responsible for the red pigmentation of *S. purpurea* (Sheridan and Mills, 1998a; b; Sheridan and Griesbach, 2001). It is presumed that the presence of these compounds in carnivorous plants play a role in increasing insect capture rates, yet studies both support (Schaefer and Ruxton, 2008) and refute (Rodenas, 2012) this hypothesis. Needless to say, ample studies show that anthocyanins play an important protective role in reducing photoinhibition and photobleaching of chlorophyll under light-induced stress conditions (see Steyn et al. (2002), for a comprehensive

review of the subject). They were shown to increase in the carnivorous plant *Pinguicula vulgaris* (Lentibulariaceae) under high UV-B radiation, which was subsequently found to be less susceptible to photoinhibition (Mendez et al., 1999). This suggests that *S. purpurea* may not need to produce the phenolic compounds, quantified here, in response to light-induced stress, as it was shown in *R. groenlandicum* (Rapinski et al. in preparation(a)), since there already is an elevated presence of anthocyanins.

Nonetheless, anthocyanin deficient individuals exist (Sheridan and Mills, 1998a) and a range in the intensity of leaf pigmentation can be clearly observed. Individuals with little or no pigmentation may be more reliant on the production of phenolics compounds, such as those identified here, to fill the photoprotective role that anthocyanins would normally play in *Sarracenia purpurea*. Although this has not been taken into account in our study, it may explain why concentrations in the species' phenolic compounds do not follow a consistent pattern from one sampling year to the other. For the purpose of producing standardized NHPs from wildcrafted *S. pupurea* plants, the role and influence which anthocyanins play, if any, in the production of targeted phenolic compounds should be evaluated.

Our study shows morronoside to be a major compound in the phytochemical profile of biologically active constituents in *S. pupurea*. It is a promising pharmacologically active compound, not only for its antidiabetic activity, but also for its anti-osteoporosis activity (Dinda et al., 2011). However, the content variability observed in wild populations may represent challenges for ensuring standardized NHPs. Nonetheless, concentrations reported here (**table 4.2**) are higher than those reported in other pharmacologically active plants, such as the antioxidant Cornelian cherry fruits, *Cornus officinalis* (Cornaceae), reported to contain between 9.94 – 17.07 mg/g DW of morronoside (West et al., 2012).

4.5 Conclusion

Our study underlines morronoside as a major component in the phytochemistry of bioactive compounds in *S. purpurea*. Although other major compounds were found to vary within samples of the second harvest year, morronoside remains the most important marker in

explaining geographical variations. No distinct chemotypes were apparent despite the patchy nature of *S. purpurea*'s distribution and the content of pharmacologically active compounds in random collections appear to have a normal distribution around the median of their expected range. Nonetheless, the lack of predictive patterns, geographical, environmental or climatic, in the content of these compounds has important implications in ensuring a standard use for the treatment of T2D in Cree communities, particularly because wildcrafting is, so far, the only harvest method used by CEI Elders and Healers. Cultivation of *S. pupurea* under controlled growing conditions might produce more constant levels of bioactive compounds.

4.6 Acknowledgements

4.7 Tables and Figures

Table 4.1 Identification of marker compounds from the leaves of *Sarracenia purpurea* by UPLC-MS.

Peak #	Compound	Separation Method	Retention time (min)	Detected Mass	Ionization Method
1	(+)-Catechin	A	1.61	291 ($M+H^+$)	ESI-(+)
2	Morroniside	A	1.77	429 ($M+Na^+$)	ESI-(+)
3	(+)-Epicatechin	A	2.10	291 ($M+H^+$)	ESI-(+)
4	Taxifolin-3-glucoside	A	2.47	465 ($M-H^+$)	ESI-(-)
5	Quercetin 3-galactoside	A	4.81	487 ($M+H^+$)	ESI-(+)
6	Quercetin 3 -glucoside	A	5.30	487 ($M+H^+$)	ESI-(+)
7	Kaempferol-3-rutinoside	A	7.28	593 ($M-H^+$)	ESI-(-)
8	Betulinic acid	B	3.24	455 ($M-H^+$)	ESI-(-)
9	Ursolic acid	B	3.48	455 ($M-H^+$)	ESI-(-)
10	Rutin	A	5.09	609 ($M-H^+$)	ESI-(-)

Table 4.2 List of compounds identified and quantified from the leaves of *Sarracenia purpurea,* geographical concentration means ± SEM and references for studies corroborating its presence in *S. purpurea.*

Peak #	Compound	Concentration (mg/g DW)		Reference
		2006	**2010**	
1	(+)-Catechin	T	0.11 (0.08)	
2	Morronoside	21.22 (8.98)	22.83 (11.38)	Jensen et al., 1975; Harris et al., 2012; Muhammad et al., 2012
3	(-)-Epicatechin	0.54 (0.13)	0.94 (0.40)	Harris et al., 2012
4	Taxifolin-3-O-glucoside	3.20 (1.75)	5.80 (3.23)	Muhammad et al., 2012
5	Quercetin-3-O-galactoside	11.87 (3.40)	21.92 (6.15)	Romeo et al., 1977; Harris et al., 2012; Muhammad et al., 2012
6	Quercetin-3-O-glucoside	0.72 (0.25)	0.42 (0.24)	
7	Kaempferol-3-O-rutinoside	0.13 (0.04)	T	Romeo et al., 1977
8	Betulinic acid	5.18 (1.19)	6.47 (1.35)	
9	Ursolic acid	2.53 (0.51)	4.13 (0.73)	
10	Rutin	ND	T	Muhammad et al., 2012
	Total	28.76 (3.09)	21.08 (3.77)	

Table 4.3 ANOVA of concentration of phenolic compounds in *Sarracenia purpurea* by collection site near 5 Eeyou Istchee communities in 2006 and 2010. Significant results are determined at α = 0.05 and are shaded.

Compound	2006		2010	
	p-value	R^2_{adj}	*p-value*	R^2_{adj}
(+)-Catechin	NA	NA	0.0033[†]	0.207[†]
Morronoside	0.00314	0.259	2.86×10^{-10}	0.583
(-)-Epicatechin	0.744	-0.0472	7.30×10^{-6}	0.382
Taxifolin-3-O-glucoside	0.913	-0.0678	0.523	-0.0134
Quercetin-3-O-galactoside	0.950	-0.0729	0.00288	0.211
Quercetin-3-O-glucoside	0.856	-0.0607	0.8915	-0.0600
Kaempferol-3-O-rutinoside	NA	NA	NA	NA
Betulinic acid	0.738	-0.0465	0.638	-0.0275
Ursolic acid	0.101	0.0866	0.0438	0.111
Total quantified phenolics	0.994	-0.0811	0.148	0.0568
Total quantified terpenes	0.0100	0.206	0.001	0.587
Total quantified compounds	0.0244	0.163	0.001	0.384

[†]Log transformation
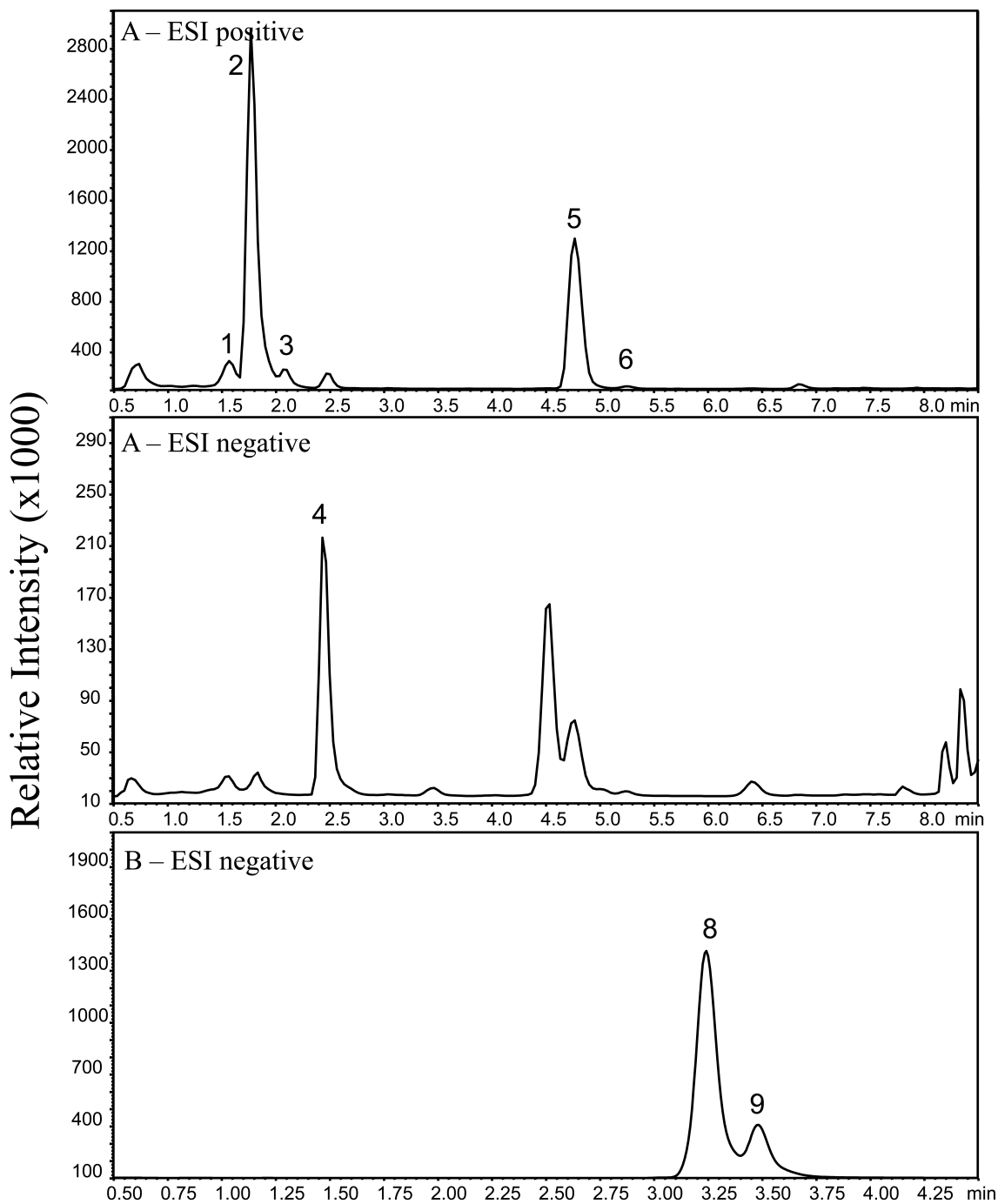
Figure 4.1 Sample UPLC chromatogram separaration methods **A** and **B** using positive and negative ionization methods **(table 1)** of *S. pupurea* leave extracts. Numbers represent compounds as follow: (+)-catechin **(1)**, morronoside **(2)**, (-)-epicatechin **(3)** taxifolin-3-glucoside **(4)**, quercetin-3-galactoside **(5)**, quercetin-3-glucoside **(6)**, betulinic acid **(8)** and ursolic acid **(9).**
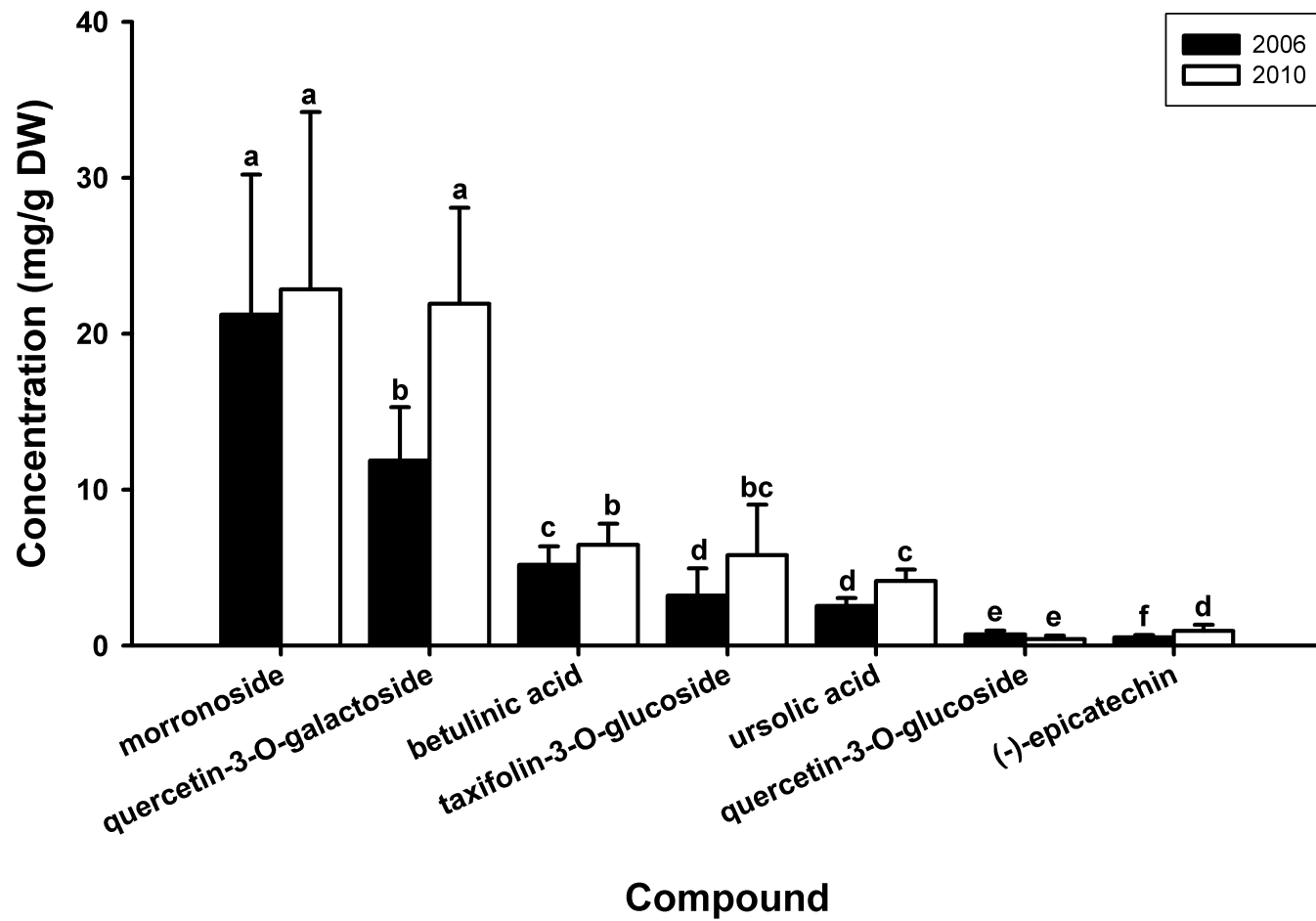
Figure 4.2 Concentration means ± SD (mg/g DW) for each quantified marker in *S. purpurea* leaves from 2006 (*n* = 40) and 2010 (*n* = 60) harvests. Mean contents, within each year, accompanied by the same letter (a-f) are not significantly different in Tukey's multiple-comparison tests.
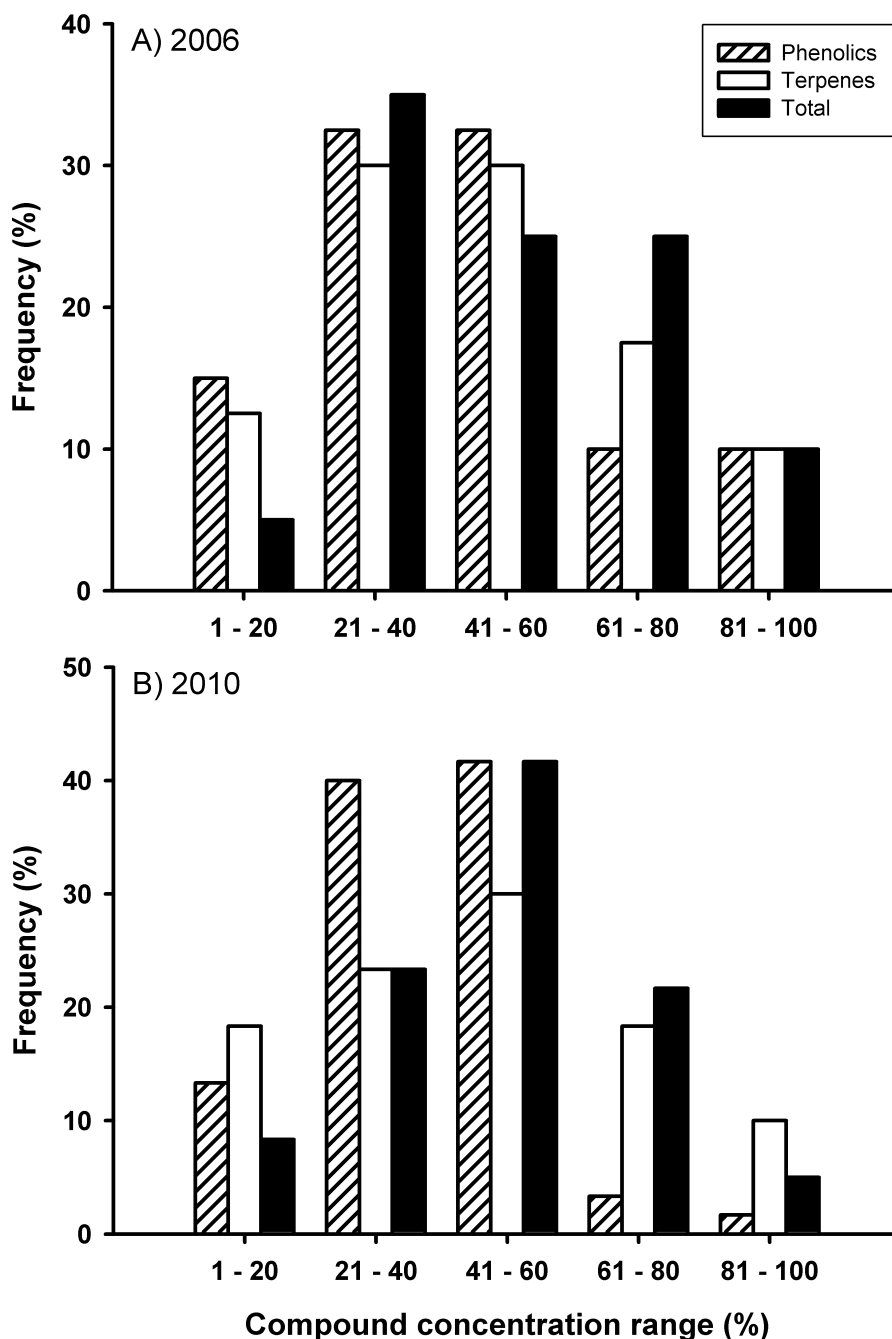
Figure 4.3 Frequency (%) of accessions found within various ranges of phenolics concentrations (% calculated from the lowest to highest concentration) expressed as the sum of all quantified compounds, phenolics and terpenes for 2006 ($n = 40$) and **B)** 2010 ($n = 60$). In 2006, phenolic content ranged from 7.05 – 28.14 mg/g DW (mean = 16.33 mg/g DW), terpene content from 12.25 – 48.01 mg/g DW (mean = 28.94 mg/g DW) and the total from 22.77 – 67.67 mg/g DW (mean = 45.26 mg/g DW). In 2010, phenolic content ranged from 9.64 – 61.02 mg/g DW (mean = 29.08 mg/g DW), terpene content from 11.16 – 59.98 mg/g DW (mean = 33.43 mg/g DW) and the total from 30.21 – 98.18 mg/g DW (mean = 62.51 mg/g DW).
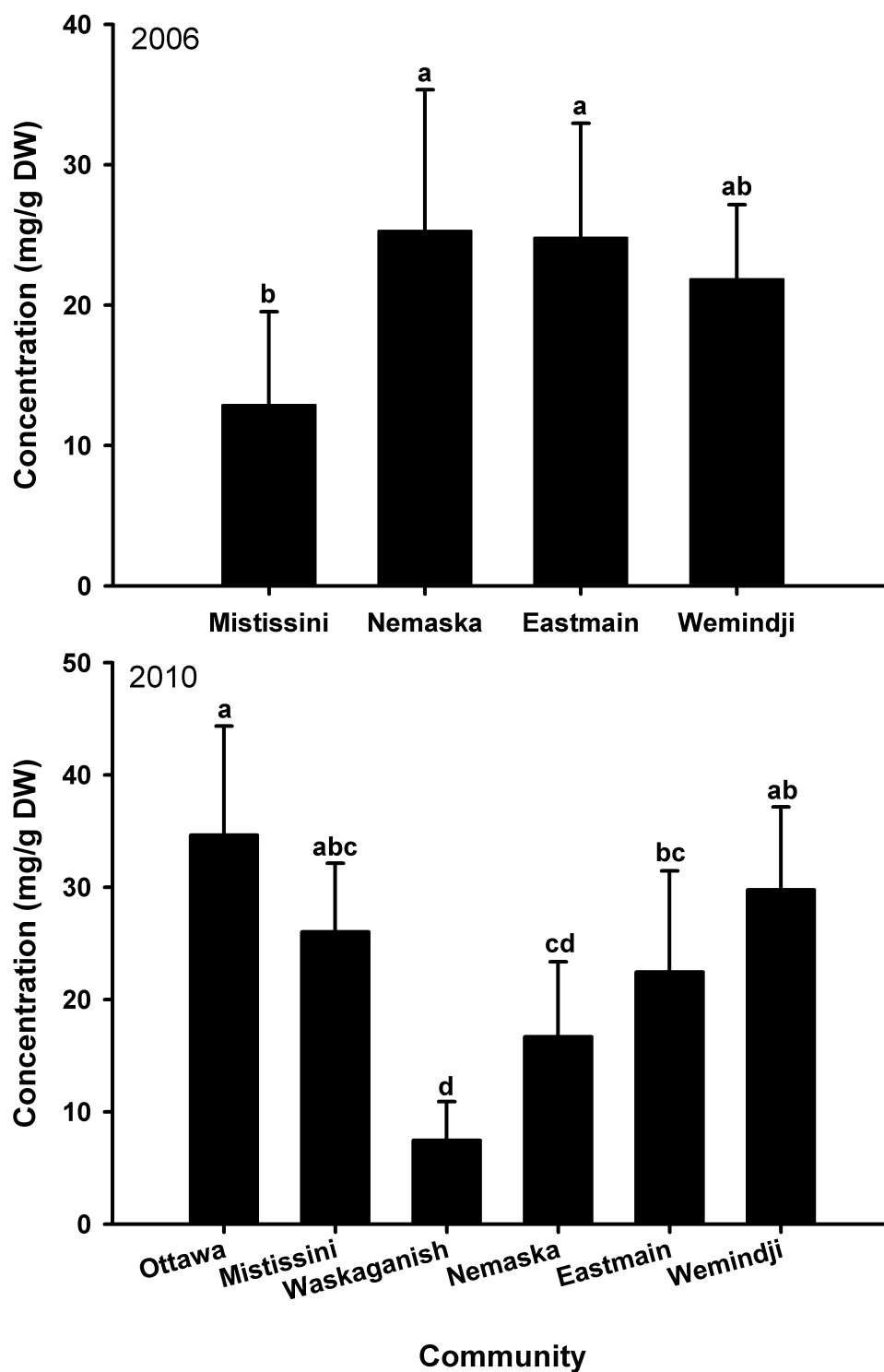
Figure 4.4 Phytochemical variation of morronoside, the most abundant compound, in **(A)** 2006 (4 locations) and **(B)** 2010 (6 locations). Mean concentration ± SD (n = 10) in *S. purpurea* leaf extracts collected from Northern Quebec and Eastern Ontario is shown. Different letters (a-d) indicate significant differences between communities in Tukey's multiple-comparison tests.
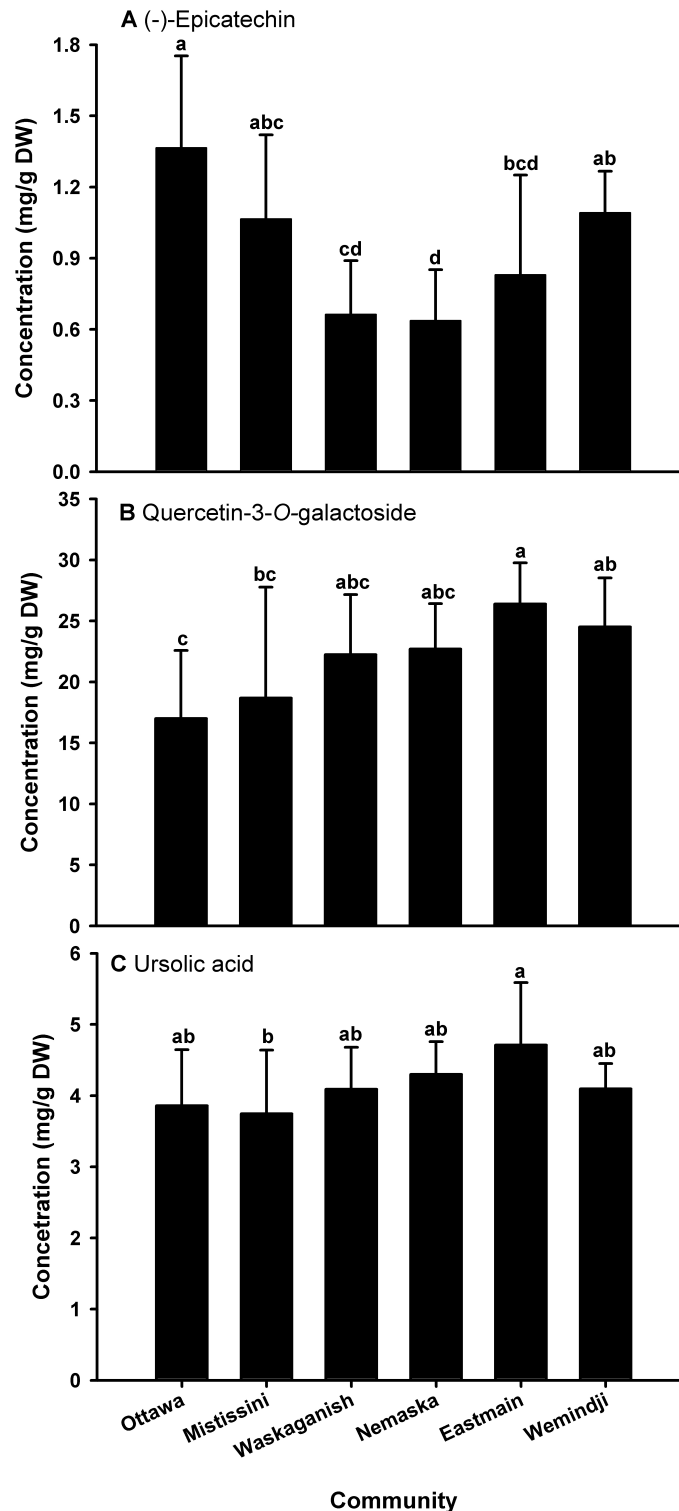
Figure 4.5 Phytochemical variation of **(A)** (-)-epicatechin, **(B)** quercetin-3-*O*- galactoside and **(C)** ursolic acid for the 2010 sampling year. Mean concentration ± SD ($n = 10$) in *S. purpurea* leaf extracts collected from Northern Quebec and Eastern Ontario is shown. Different letters (a-d) indicate significant differences between communities in Tukey's multiple-comparison tests.
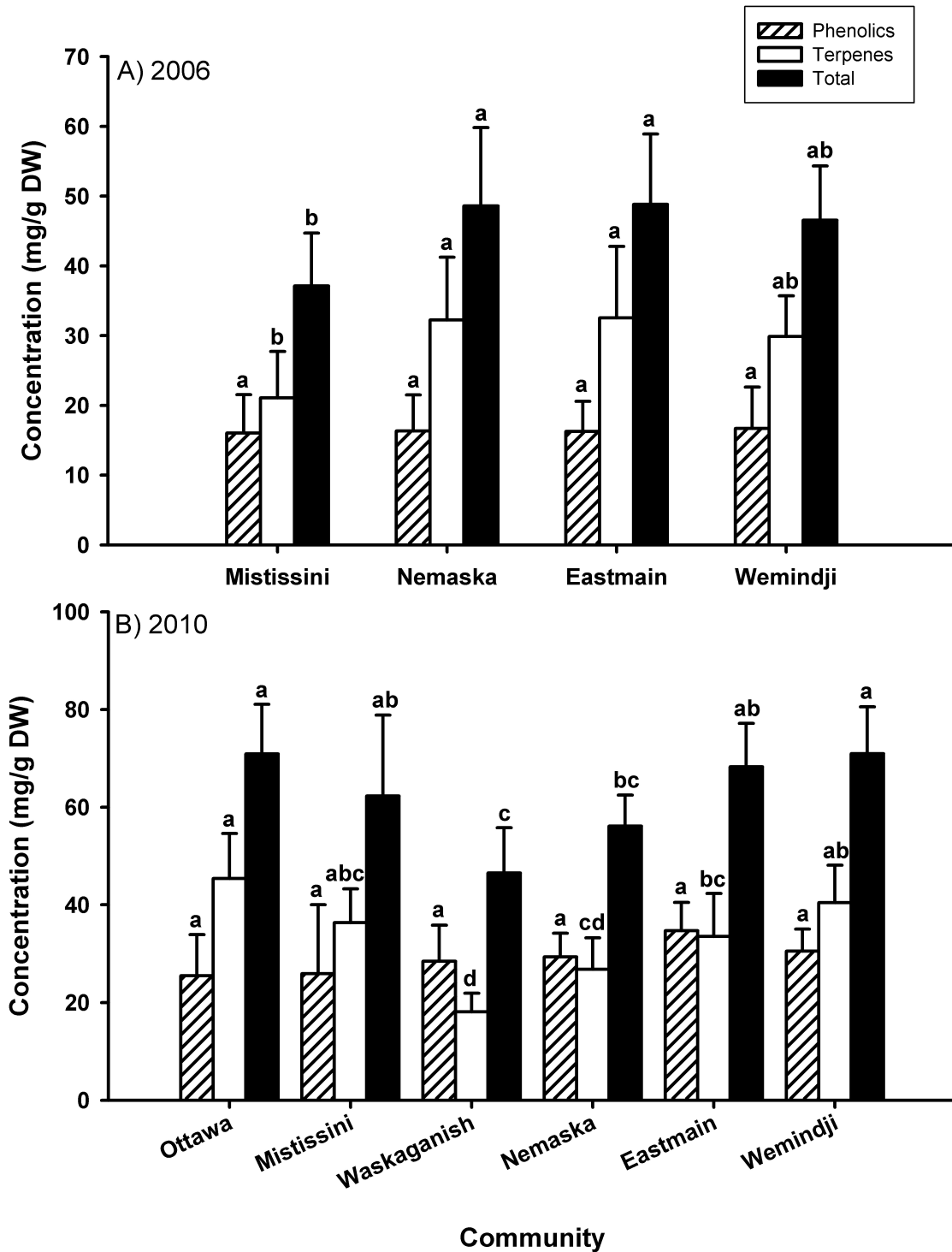
Figure 4.6 Phytochemical variation of the sum of all quantified phenolics ((-)-epicatechin, taxifolin-3-*O*-glucoside, quercetin-3-*O*-galactoside and quercetin-3-*O*-glucoside), terpenes (morronoside, betulinic acid and ursolic acid) and total compounds (phenolics and terpenes) in **(A)** 2006 and **(B)** 2010. Mean concentration ± SD (*n* = 10) in *S. purpurea* leaf extracts collected from Northern Quebec and Eastern Ontario is shown. Different letters (a-d) indicate significant differences between communities in Tukey's multiple-comparison tests.
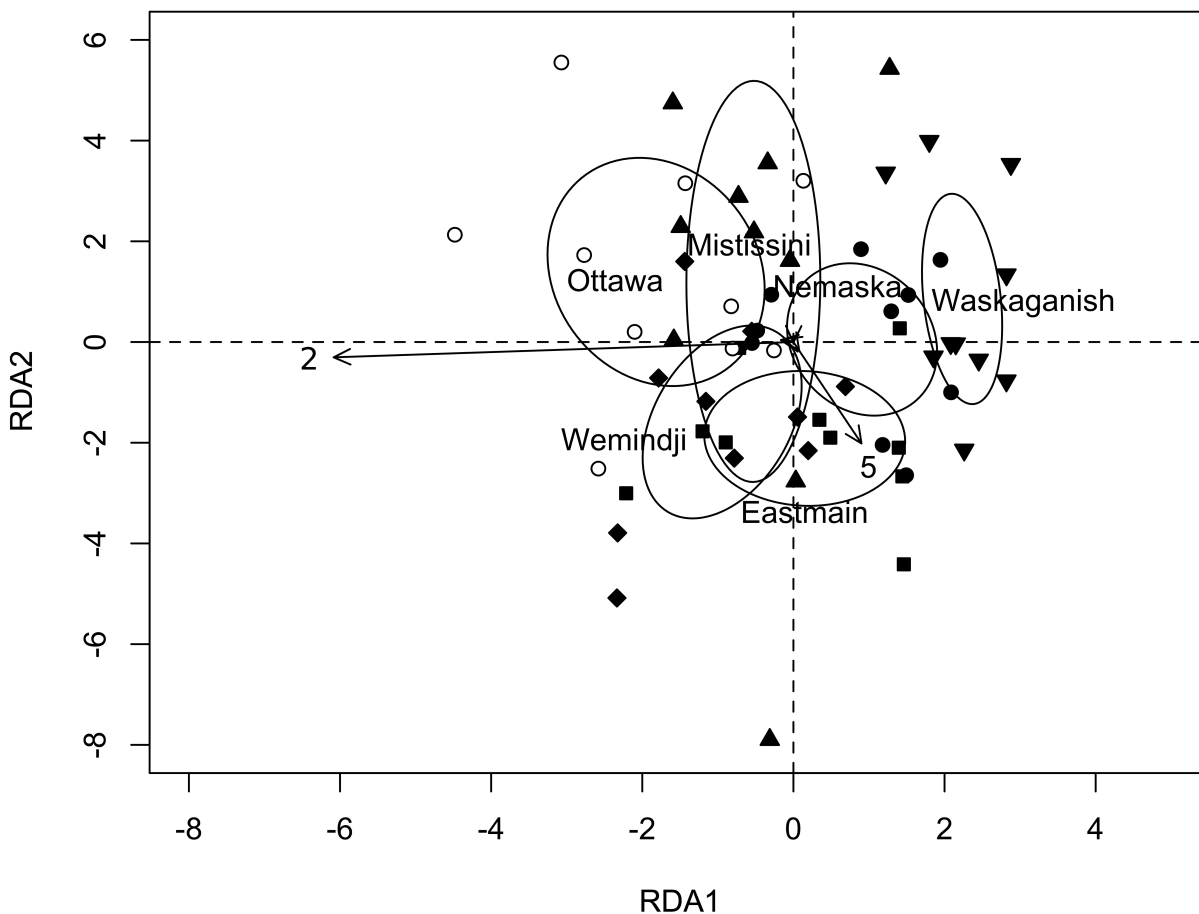
Figure 4.7 RDA ordination biplots, in type 2 scaling, of concentration variations in the profile of bioactive constituents in *S. purpurea* leaves as a function of community of origin for the 2010 harvest. Results from the 2006 harvest are not presented here because morronoside was the only varying compound. Symbols represent sampling sites from the communities of Ottawa (○), Mistissini (▲), Nemaska (●), Waskaganish (▼), Eastmain (■) and Wemindji (♦). Centroids for these sites are drawn out by ellipses. Markers included in the analysis were those which could be quantified for both sampling years and are represented by the following numbers: morronoside **(2)**, (-)-epicatechin **(3)** taxifolin-3-glucoside **(4)**, quercetin-3-galactoside **(5)**, quercetin-3-glucoside **(6)**, betulinic acid **(8)** and ursolic acid **(9).** Arrows for all but morronoside (**2**) and quercetin-3-galactoside (**5**) appear absent as scores remained close to the origin.

Chapitre 5

Adipogenic activity of wild populations of *Rhododendron groenlandicum*, a medicinal shrub of the James Bay Cree traditional pharmacopeia.

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5.1 Abstract

The traditional medicinal plant, Labrador tea (*Rhododendron groenlandicum*; Ericaceae)*,* present in the pharmacopoeia of the Cree of Eeyou Istchee, has shown glitazone-like activity in the 3T3-L1 adipogenesis bioassay. This activity has been attributed to phenolic compounds, which have been shown to vary in this plant as a function of insolation parameters. The goal of this study was to determine if these changes in phenolic content were pharmacologically significant. Leaves were harvested in 2006 throughout the James Bay region of Northern Quebec and ethanol extracts were tested *in vitro* using the 3T3-L1 murine cell line adipogenesis bioassay. This traditional medicinal plant was found active in the assay. However, there was no detectable spatial pattern in the accumulation of intracellular triglycerides, suggesting that such patterns previously observed in the phenolic profile of Labrador tea were not pharmacologically significant. Nonetheless, a reduction in the adipogenic activity was observed and associated with higher concentrations of quercetin for which selected environmental variables did not appropriately explain its variation.

Key words: Phenolic compounds, traditional medicine, type 2 diabetes, adipogenesis, Cree Nation of Eeyou Istchee.

5.2 Introduction

In a previous study on the phytochemistry of the North American medicinal plant, *Rhododendron groenlandicum* (Oeder) Kron & Judd (Ericaceae), Labrador tea, we found the concentration of biologically active compounds to vary on a latitudinal scale in Northern Quebec's Hudson and James Bay region (Rapinski et al., in preparation(a)). Labrador tea is a common species in Canada's boreal forest. More importantly, it is a popular medicinal plant found in the traditional pharmacopoeia of indigenous populations from the Algonquian, Salish, Waskashane, Tsimshianic and Eskimo-Aleut linguistic families (Arnason et al., 1981; Zieba, 1990; Siegfried, 1994; Marles et al., 2008; Cuerrier and Elders of Kangiqsualujjuaq, 2011; Cuerrier and Elders of Kangiqsujuaq, 2011; Cuerrier et al., 2011; Cuerrier and Hermanutz, 2012).

In ethnobotanical studies conducted in six communities of the Cree Nation of Eeyou Istchee (CEI), we found *R. groenlandicum* to be the top-ranked plant species used for the treatment of symptoms associated with type 2 diabetes (T2D) (Fraser, 2006; Leduc et al., 2006; Downing, 2010; Rapinski et al., in preparation(b)). The inherent cultural relevance of this species to CEI traditional medicine (CTM) warrants further investigation into its antidiabetic potential.

The CIHR Team on Antidiabetic Aboriginal Medicines (CIHR-TAAM), formed through collaborative work between CEI communities, the Cree Board of Health and Social Services of James Bay (CBHSSJB) and Canadian academic researchers, has screened many of the multiple plants present in the CEI pharmacopoeia (Spoor et al., 2006; Fraser et al., 2007; Harbilas et al., 2009; Harris et al., 2011). Of these, *R. groenlandicum* was shown to possess *in vitro* glitazone-like activity comparable to rosiglitazone in an adipogenic assay measuring the lipid accumulation of differentiating 3T3-L1 preadipocytes (Spoor et al., 2006).

The antidiabetic drug rosiglitazone induces an increase in the sensitivity to insulin, acting as a PPARγ receptor agonist (Fang et al., 2008); the expression of this transcription factor being particularly implicated in the differentiation of adipocytes (Grimaldi, 2001;

MacDougald and Mandrup, 2002; Hansen and Kristiansen, 2006; Rosen and MacDougald, 2006) and in insulin sensitivity (Fang et al., 2008). Hence, it plays a critical role in the pathogenesis of T2D. The action of PPARγ is an improvement in the absorption of fatty acids in differentiated adipocytes which store them as triglycerides (TG) (Fang et al., 2008). Hence, adipocytes provide storage for fatty substances that would otherwise accumulate in tissues such as skeletal muscle and liver, thereby contributing to metabolic disorders such as insulin resistance (Rosen and MacDougald, 2006).

The pharmacological activity of *R. groenlandicum* has been attributed to phenolic compounds (Spoor et al., 2006; Harbilas et al., 2009; Saleem et al., 2010). Bioassay-guided fractionation using adipogenesis of 3T3-L1 murine cells confirmed that specific phenolics are the most active compounds (Ouchfoun, 2011). In developing culturally appropriate approaches to treating T2D in the CEI communities, the variation of these compounds in *R. groenlandicum* has important implications in ensuring the quality control of traditional medicinal plants or to develop standardized natural health products (NHPs).

In this study, we assessed potential variations in the antidiabetic potential of *R. groenlandicum*. Our objective was to determine if the phytochemical variations observed in the species' phenolic profile (Rapinski et al., in preparation(a)) are biologically significant. We evaluated the *in vitro* pharmacological activity of crude extracts from various localities using the adipogenesis bioassay and hypothesized that high concentrations of phenolic compounds would result in a stronger adipogenic activity.

5.3 Materials and Methods

5.3.1 Sampling

Mature leaves of *R. groenlandicum* were sampled during the summer of 2006 around the communities of Mistissini, Nemaska, Eastmain, Wemindji and Whapmagoostui, thus covering much of the north-south gradient in Eeyou Istchee. Five accessions, each containing leaves from multiple individual plants, were collected within a 50 km radius around each community. Samples were air dried and preserved in paper bags at room temperature.

5.3.2 Extraction

The extraction method was previously described in Rapinski et al. (in preparation(a)). Plant material was thoroughly dried at 35°C overnight in a commercial food dehydrator (Nesco® Professional Food and Jerky Dehydrator). Samples were milled through a Wiley Mill with at 40 mesh and extracted overnight in at 25 mL/g of 80 % EtOH by orbital shaking at room temperature at 250 RPM. The pellet was extracted overnight in 15 mL 80 % EtOH. The pooled supernatants (adjusted to 50.0 mL in a volumetric flask) were centrifuged for 15 min at 1828 x g at room temperature. An aliquot (1 mL) of the centrifuged extract was prepared for High Performance Liquid Chromatography coupled with Diode Array Detector (HPLC-DAD) by filtering through a 20 μm PTFE filter. Extracts were kept at -20°C and sonicated before analysis. Unfiltered crude extracts were dried using a speedVac. Trace water was removed by lyophilization using SuperModulo freeze dryer and stored at -80°C.

5.3.3 Cell lines, chemicals, biochemicals and standards

For the identification and quantification of phenolic markers, (+)-Catechin **(1)**, chlorogenic acid **(2)**, (-)-epicatechin **(3)**, *p*-coumaric acid **(4)**, rutin **(5)**, quercetin-3-galactoside **(6)**, quercetin-3-glucoside **(7)**, quercetin-3-rhamnoside **(12)**, myricetin **(13)** and quercetin **(14)** were purchased from Sigma-Aldrich (Oakville, Ontario, Canada) and Extrasynthese (Genay, France). HPLC grade water, acetonitrile, and formic acid (99% purity) were purchased from Sigma-Aldrich.

For cell culture and adipogenesis, pre-adipocyte 3T3-L1 cell line was purchased from the American Type Culture Collection (ATCC; Manassas, VA). Dexamethasone (DMX), bovine pancreatic insulin, 3-isobutyl-1-methylxanthine (IBMX), Dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (Oakville, ON). Rosiglitazone from was obtained Alexis Biochemicals (Hornby, ON).

Dubelcco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS) and bovine calf serum (NCS) were from Wisent Inc (St-Bruno, QC). AdipoRed reagent was purchased from Combrex Bio Science Walkersville Inc (Walkersville, MD).

5.3.4 Identification and Quantification

The method for identifying and quantifying the phenolic compounds in the samples presented here have been previously described in Rapinski et al. (in preparation(a)). Briefly, a 10 µL of each extract was injected through an autosampler and detected by DAD at 290 nm, band width 4, reference off. The separations were performed on a Luna C18 column (250 x 4.6 mm, 5 µM particle size). Peak identification was undertaken by co-chromatographic comparison of the spectral data adopted in our in-house metabolomics spectral library (Saleem et al., 2010). A standard curve was constructed by injection of serially diluted marker compounds in methanol. The quantification was based on peak height and area. The quantitation of putatively identified quercetin-glycosides was achieved based on calibration curve of quercetin-3-galactoside. Each sample was analyzed in triplicate and averaged to account for instrumental variation.

5.3.5 Cell culture

3T3-L1 murine pre-adipocyte cells were grown to confluence in 24-well plates in DMEM proliferation medium containing 10% FBS. Media was changed every 2 days. At 24 h post-confluence (day 0), cells were induced to differentiate with a short-term differentiation medium of DMEM supplemented with 10 % FBS, 1 µM DMX, 250 µM IBMX and 500 nM insulin. After 48 h, the media was replaced with DMEM containing 10 % FBS and 500 nM insulin for long-term differentiation. Cells were differentiated for a total of 5 days with media change every 2 days. *Rhododendron groenlandicum* extracts (75 µg/mL) and rosiglitazone (10 µM; positive control) were dissolved in DMSO and added to the cells as of day 0 of differentiation. The final concentration of DMSO was kept at 0.1 % throughout the differentiation period.

5.3.6 Adipogenesis

We measured intracellular TG content at day 5 of differentiation using the AdipoRed reagent according to the manufacturer's instructions. Methods have been previously described in Spoor et al. (2006) and Harbilas et al. (2009). In short, wells were washed twice with phosphate-buffered saline (PBS) before 1 mL of PBS containing 30 µl of AdipoRed reagent

was added to each well and incubated for 15 minutes at room temperature. AdipoRed becomes fluorescent when partitioned in a hydrophobic compartment. The fluorescence of each well was measured with a Wallac Victor2 fluorimeter (Perkin-Elmer, St-Laurent, QC) at an excitation wavelength of 485 nm and an emission wavelength of 572 nm. The results were reported as percentage of the vehicle control, 0.1 %DMSO.

5.3.7 Environmental data

Estimates for insolation variables were provided by the Canadian Forest Services of Natural Resources Canada. Values were generated by spatially continuous climatic models adjusted by ANUSPLIN, a non-parametric multivariate technique for the noise-reduction of multiple variable data. Interpolation of surfaces is calculated by smoothing algorithms and taking into consideration the effect of altitude. These surfaces are generated from a 30 years data span collected by meteorological stations across the country. Using each sample's geographical coordinates at a resolution of 4 decimal-degrees, estimates were obtained for each collection site. Monthly estimates for solar radiation (Mjoules) were obtained from data of the 1961-1990 period. Details of the protocols generating these estimates are described by McKenney et al. (2007a,b).

5.3.8 Statistical analysis

To reduce interassay variation, TG content was normalized relative to each assay's vehicle control, 0.1% DMSO, set at 100%. *Rhododendron groenlandicum* and rosiglitazone always induced significant increases in activity as verified by the fact that the 95% confidence interval of the mean activity (quadruplicate determinations) did not include the 100% adipogenic activity reference ($p<0.05$). Differences between communities were analyzed by one-way analysis of variance. The relationships between TG content and compounds were analyzed by multiple and simple linear regressions. To represent the adipogenic activity of *R. groenlandicum* and the quantified compounds, principal components analysis (PCA) was performed on the matrix of these compounds using the correlation matrix. Individual samples were scored onto the PCA axes and represented with the vectors for each compounds. TG content was subsequently projected onto the principal components in order to interpret the

dimensions of variability. Finally, we partitioned the variation in the adipogenic activity of *R. groenlandicum* between the two sets of variables: compounds and insolation factors. This was done using a partial-redundancy analysis (partial-RDA) approach (Borcard and Legendre, 1992; Meot et al., 1998). All analyses were performed using R statistical language (R Development Core Team, 2012). Results are reported as means ± SD and statistical significance set at $\alpha = 0.05$.

5.4 Results and Discussion

The phytochemistry of the same *R. groenlandicum* accessions has already been described and discussed in greater length in Rapinski et al. (in preparation(a)). Here, we present the results of a sub-sample of 2006 accessions in the adipogenesis bioassay.

The glitazone-like activity of *R. groenlandicum* to increase the accumulation of intracellular TG in 3T3-L1 adipocytes was measured at day 5 of differentiation. Extracts increased adipogenesis, with an average content of TG of 159.0 % that of DMSO (**fig. 5.1**) with a 95% confidence interval of 138.8 – 179.1% of DMSO. The adipogenic activity of *R. groenlandicum* was half of the positive control, rosiglitazone. This is lower than what has previously been reported for this species. Spoor et al. (2006) reported the stimulation of adipogenesis to be comparable to rosiglitazone, while later determinations measured an activity representing two-thirds that of the antidiabetic drug (Ouchfoun, 2011). With the exception of a few samples, which inhibited adipogenesis (**fig. 5.2**), our results nonetheless confirm the antidiabetic potential of this species. It is important to consider the fact that previous determinations of activity were carried out using extracts prepared from large quantities of source material (large number of individual plants) collected a few years prior to the material used in the present studies. Hence, inter-individual variations were absent and different climatic conditions may have prevailed. This can explain, at least in part, the differences in adipogenic potential observed between the studies.

There were no statistically distinct spatial patterns detected in the pharmacological activity of *R. groenlandicum*. None of the communities sampled possessed accessions which

significantly increased intracellular TG more than the others (p = 0.348, **fig. 5.3**). We have previously found that biologically active phenolics were greater in collections made around the communities of Nemaska, Eastmain and Wemindji and that this variation followed a quadratic latitudinal trend (Rapinski et al., in preparation(a)). The adipogenic activity of *R. groenlandicum* followed a similar trend, as can be observed in **figure 5.3**, albeit statistical significance of a polynomial relationship was not achieved (p = 0.170), possibly due to high variability. This suggests that variations in the phytochemical profiles, observed in Rapinski et al. (in preparation(a)), may be pharmacologically relevant, but further studies will be necessary to confirm this point.

Indeed, we found that quantified compounds explained considerable variability obtained in this species' pharmacological activity (p = 0.0279, R^2_{adj} = 0.491). The phytochemical profile and distribution of *R. groenlandicum* could be reconstructed into three principal components explaining 69.92% of the variation, whereby some major markers were shown to be highly correlated (**fig. 5.4**). When projecting intracellular TG onto this plot, it did not appear well correlated with the bulk of these markers. The only marker for which a significant relationship with TG appears to exist is quercetin (**fig. 5.4b** and **5.4c**). **Figure 5.5** further illustrates the linear correlation (p = 0.0458, R^2 = 0.162) whereby the adipogenic activity of *R. groenlandicum* decreases as the concentration of quercetin in the sample increases. This is consistent with observations from our own group (Ouchfoun, 2011) where pure quercetin was found to inhibit adipogenesis in a dose-dependent manner. The activity of quercetin is well studied and has also been consistently shown by others to be a potent inhibitor of adipocyte differentiation and adipogenesis (Iwashita et al., 2001; Hsu and Yen, 2007; Fang et al., 2008; Yang et al., 2008).

Our results suggest that while the geographical location does not appear to have a statistically significant impact on the adipogenic activity of crude extracts of localized *R. groenlandicum* samples*,* variations in active compounds do explain a significant proportion of variability in pharmacological activity. We have shown that insolation parameters, such as solar radiation, could significantly explain some of the variation in the species' phenolic compounds (Rapinski et al., in preparation(a)). When the variation in TG content was partitioned, a significant proportion of the variation was explained by these environmental

variables and this converged with the variation explained by the phenolic compounds (**table 5.1, fig 5.6**). Indeed, while the relationship with compounds was statistically significant, the unique contribution of phenolics were no longer evident when the effect of environmental variables were taken into account.

This confirms the caveat that environmental variables play an underlying role in affecting the content of biologically active compounds. Conversely, quercetin, the only significant compound related to changes in the adipogenic activity of *R. groenlandicum,* was not found to be strongly associated with environmental variables (Rapinski et al., in preparation(a)). This may have contributed to reduce the relationship of variability between adipogenic activity and phenolic content when environmental factors were taken into account.

On the other hand, our results provide support for the hypothesis that synergistic interactions may occur between compounds. For instance, the inhibitory action of *Hibiscus sabdariffa* (Malvaceae) was greater than the sum of its parts when polyphenols had been fractionated, isolated and tested individually (Herranz-López et al., 2012). More importantly, in bioassay-guided fractionation experiments, the activity of crude Labrador tea extracts was higher than that of each active compound tested individually (Ouchfoun, 2011). Finally, quercetin and resveratrol, together, decreased lipid accumulation considerably more than each of these used separately at the same dose (Yang et al., 2008).

Many of the compounds quantified in this paper have shown adipogenic activity in some form or another. Quercetin-3-glucoside has been found toxic to adipocytes at relatively low concentration (50 μM) but was not found to affect adipogenic activity (Hsu and Yen, 2007). Quercetin-3-rhamnoside has been found inactive at low concentrations, but was found to inhibit at high concentrations and chlorogenic acid has been found to inhibit intracellular triglycerides accumulation (Hsu and Yen, 2007). Content variations of some of these, particularly (+)-catechin and (-)-epicatechin, have been explained by environmental variables (Rapinski et al., in preparation(a)). Although the individual effect of these compounds were not detected in our study, it does not undermine the role they may play when found in a cocktail of substances.

5.5 Conclusion

We have previously shown that latitude acted as a marker for the impact of environmental variables on phytochemical concentrations (Rapinski et al., in preparation(a)). Therefore, a trend could possibly exist between abiotic factors and concentration of targeted secondary metabolites, but a larger sample size might be needed to detect it. There may also be other environmental, climatic and even biotic factors that were not taken into account, which explain the changes in quercetin content. These may better explain the ecophysiological processes affecting the antidiabetic potential of *R. groenlandicum.* Increase in the adipogenic potential of this traditional medicine was associated primarily with lower concentrations of quercetin, but the cause for its variation will require further investigation. Nonetheless, our results do not provide enough evidence to justify the idea that specific accessions of Labrador tea may have reproducibly better adipogenic potential than others along a latitudinal gradient. Conversely, our study implies that random harvesting of *R. groenlandicum* in the Eeyouch territory of Northern Quebec should not have a major impact on the quality of traditional preparations or NHPs made from this plant.

5.6 Acknowledgements

recognition to Jonathan Ferrier who also provided comments, ideas and support. Finally, thank you to S. Daigle and P. Legendre for statistical advice.

5.7 Tables and Figures

Table 5.1 Variation partitioning of the adipogenic activity of *R. groenlandicum* leaf extracts explained by the content in biologicaly active compounds and the effect of insolation parameters. The variation of each fractions are represented in **figure 5.6**. Asterix (*) indicate significant fractions at $\alpha = 0.05$.

Fractions	R^2_{adj}	*p*
[a+b] = Compounds	0.491	0.0272 *
[b+c] = Insolation	0.145	0.0700
[a+b+c] = Compounds + Insolation	0.476	0.0555
[a] = Compounds|Insolation	0.331	0.0948
[b]	0.160	Not testable
[c] = Insolation|Compounds	-0.0156	0.473
[d] = Unexplained	0.524	Not testable
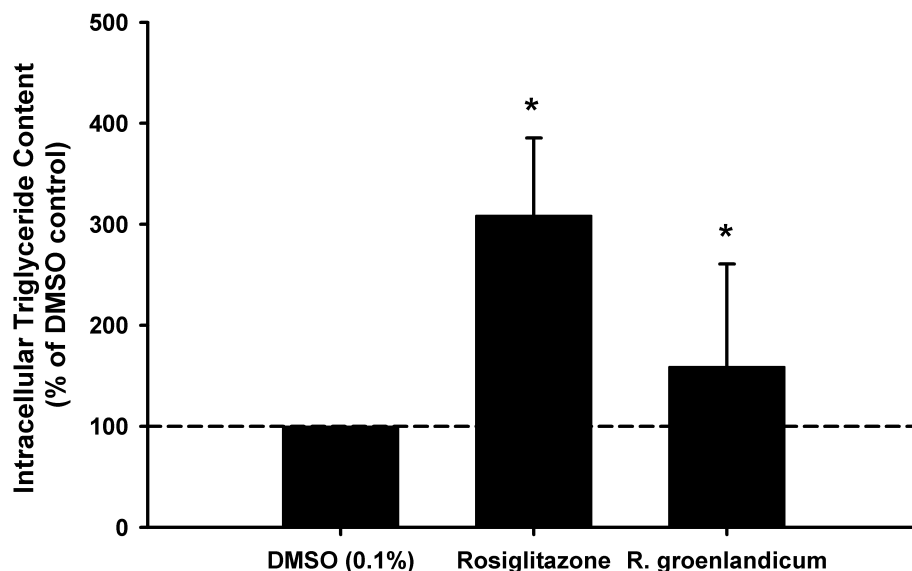
Figure 5.1 Effect of *R. groenlandicum* crude leaf extracts from Northern Quebec on lipid accumulation. Intracellular triglyceride content was measured by AdipoRed fluorescence, in live 3T3-L1 murine adipocytes incubated with plant extracts for 5 days post-differentiation. Means ± SD (n = 4 for rosiglitazone, n=100 for *R. groenlandicum*) are normalized to the vehicle control (0.1% DMSO). Asterix (*) indicate significant differences with respect to the DMSO control at α = 0.05.
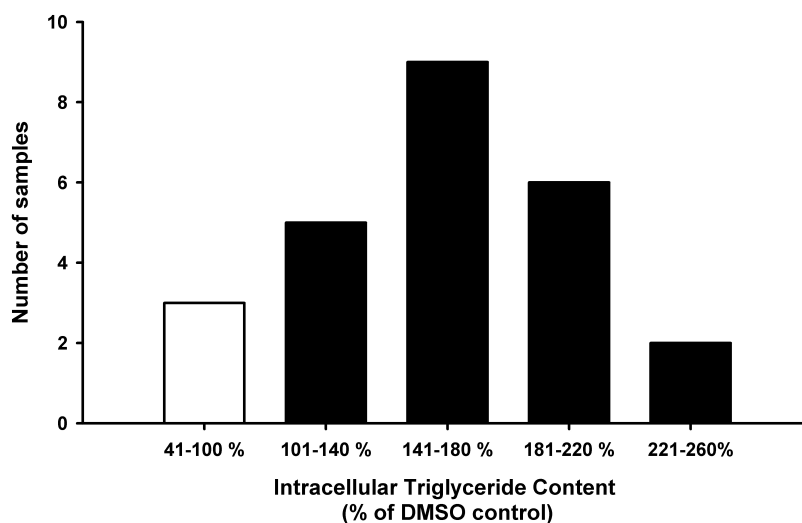
Figure 5.2 Frequency distribution of the adipogenic activity from 25 samples of *R. groenlandicum* leaves collected throughout Northern Quebec. Intracellular triglyceride content was measured by AdipoRed fluorescence, in live 3T3-L1 murine adipocytes incubated with plant extracts for 5 days post-differentiation. Triglyceride content was normalized to the vehicle control (0.1% DMSO). Samples with content levels below 100% (in white) were considered inhibitory and decreased lipid accumulation.

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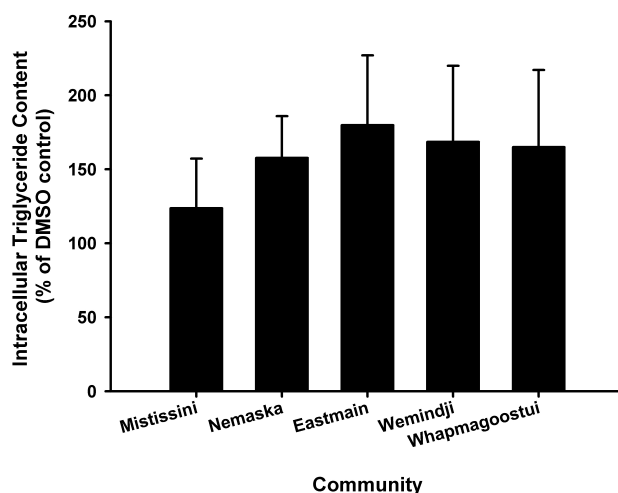
Figure 5.3 Effect of *R. groenlandicum* crude leaf extracts prepared from accessions collected around five communities in Northern Quebec on lipid accumulation. Intracellular triglyceride content was measured by AdipoRed fluorescence, in live 3T3-L1 murine adipocytes incubated with plant extracts for 5 days post-differentiation. Means ± SD (n = 5) are normalized to the vehicle control (0.1% DMSO). There were no significant differences between communities (*p* = 0.348).
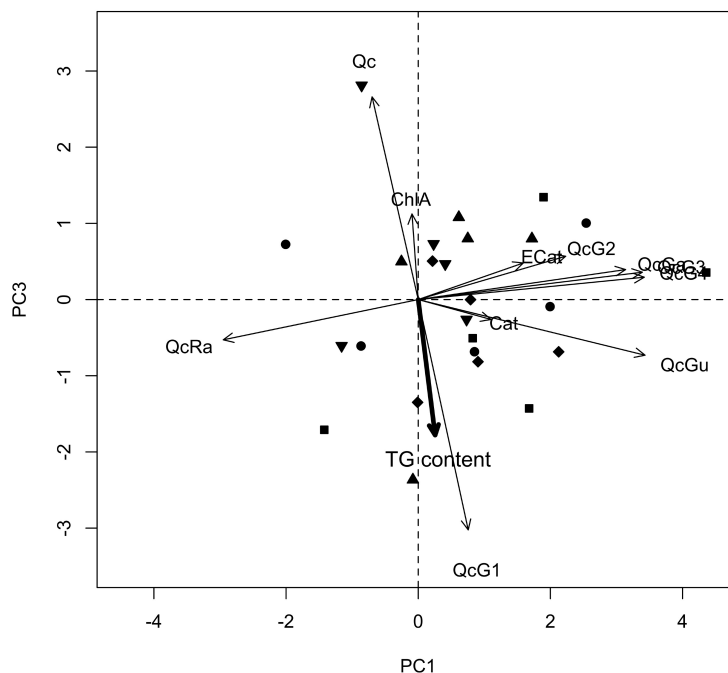
Figure 5.4 Principal component analysis biplot of 11 phenolic compounds in *R. groenlandicum* leaves. Solid lines represent relative loadings of these variables on axes 1, 2 and 3. TG content (bold arrow) was selected as a supplementary variable and plotted onto principal components generated from the phytochemical markers. Scores for individual samples are represented by symbols for the communities of Mistissini (▲), Nemaska (●), Eastmain (■), Wemindji (◆) and Whapmagoostui (▼).

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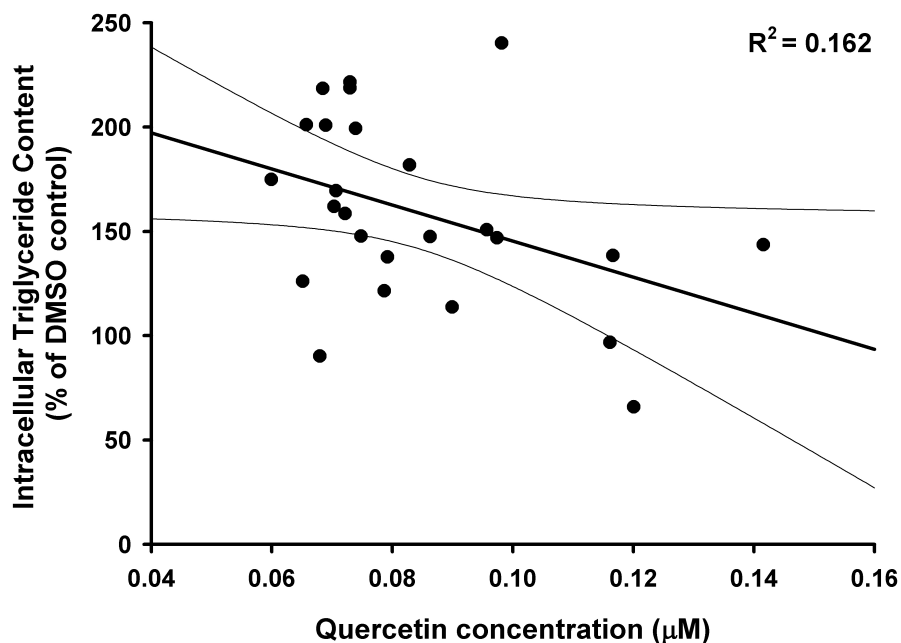
Figure 5.5 Intracellular triglycerides content of 3T3-L1 murine adipocytes exposed to 75 μg/ml of *R. groenlandicum* leaf extracts collected from various locations. Quercetin concentrations in crude extract were significantly and negatively associated with the species' adipogenic activity (*p* = 0.0458). Triglyceride contents are normalized to the vehicle control (0.1% DMSO).
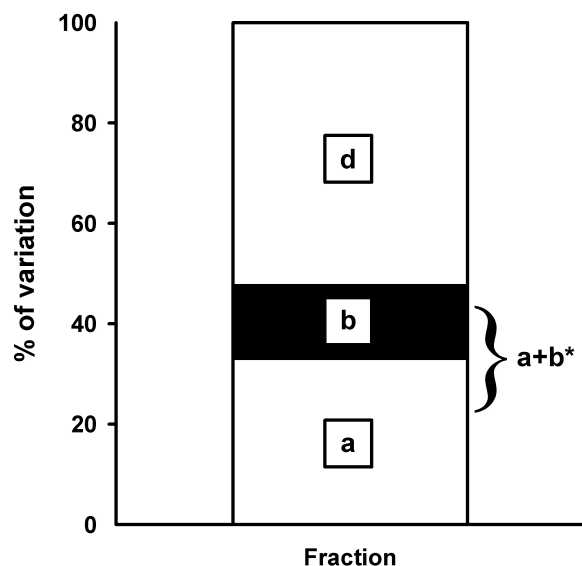
Figure 5.6 Percentages of variation in the adipogenic activity of *R. groenlandicum* leaf extracts explained by the content in biologicaly active compounds and the effect of insolation parameters. a = compounds, b = compounds + insolation, d = undetermined. Fraction c = insolation is not shown as the percentage of variation explained was not significant (see **table 5.1**). Asterix (*) indicate significant fractions at α = 0.05.

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Chapitre 6 - Conclusion

De façon générale, nous pouvons dire que nous avons rencontré les objectifs que nous nous étions fixés, soit 1) identifier les plantes à potentiel antidiabétique provenant de la pharmacopée traditionnelle de Wemindji et d'Oujé-Bougoumou et de les comparer à la pharmacopée des autres communautés CEI, 2) déterminer si des variations spatiales (latitudinales) peuvent être observées parmi les composantes biologiquement actives de deux plantes médicinales CEI, et 3) déterminer si ces variations phytochimiques sont pharmacologiquement significatives.

L'intégration des communautés de Wemindji et Oujé-Bougoumou à l'équipe CIHR-TAAM a été réalisée avec succès suite aux interviews menés auprès de leurs Aînés et Guérisseurs. À l'aide d'entretiens semi-dirigés basés sur les symptômes et complications associés au T2D, 16 et 25 plantes ont été respectivement mentionnées dans les communautés de Wemindji et d'Oujé-Bougoumou. Ces études, conformt à celles menées dans les communautés de Mistissini, Nemaska, Waskaganish et Whapmagoostui dans le cadre du projet de l'équipe CIHR-TAAM, sont les premières investigations de ce genre pour ces communautés. En plus d'ajouter sept nouvelles espèces et un champignon à la liste des espèces à potentiel antidiabétique, plusieurs des plantes médicinales traditionnelles importantes à Wemindji et Oujé-Bougoumou sont couramment utilisées à travers le territoire Eeyouch. Les espèces qui ressortent selon leurs valeurs de SIV globales représentent des plantes médicinales culturellement importantes aux CEI. Cela en fait des plantes candidates appropriées pour le développement d'approches thérapeutiques culturellement adaptées. Le thé du Labrador, *Rhododendron groenlandicum,* se classe en première position suite à la classification par SIV pour le traitement du T2D. Cela témoigne de son importance culturelle et vient justifier la nécessité d'évaluer sa diversité phytochimique aux fins éventuelles de production de produits de santé naturels normalisés et de contrôle de qualité des préparations traditionnelles.

Effectivement, la teneur en composantes biologiquement actives trouvée dans les feuilles du *R. groenlandicum* varie spatialement en fonction de la latitude. Étant donné la nature antioxydante des composés phénoliques ainsi identifiés, l'hypothèse que ces derniers varient positivement en fonction de paramètres d'insolation, telles la radiation solaire ou la photopériode a été vérifiée en utilisant des techniques analytiques et statistiques avancées et

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innovatrices. Ces résultats, reproduits sur deux années de collecte et montrés pour la première fois sur un tel gradient, sont significatifs en ce qu'ils viennent appuyer davantage le rôle physiologique que jouent les composés phénoliques dans la protection contre la photoinhibition et le photodommage chez les plantes.

Les études sur *R. groenlandicum* font aussi le lien entre les facteurs environnementaux, qui influencent la production de composantes biologiquement actives dans les plantes, et leur effet pharmacologique. Toutefois, l'analyse phytochimique de la sarracénie pourpre, *Sarracenia purpurea*, une autre plante importante de la pharmacopée CEI, montre que ses composantes ne sont pas affectées par les mêmes facteurs. Cela témoigne des relations complexes qui existent entre les espèces végétales et leur environnement. Quoique des études approfondies nous permettent de faire des prédictions touchant le développement de produits de santé naturels, elles ne peuvent être généralisées à tous les cas.

Afin de voir si la variation phytochimique du *R. groenlandicum* est biologiquement significative, les échantillons du *R. groenlandicum* ont été évalués par bioessai *in vitro*; en l'occurrence, l'adipogenèse. Quoique le thé du Labrador stimule généralement l'entreposage intracellulaire des triglycérides, son profil d'activité adipogénique ne reflète pas en général les tendances observées dans les variations de son profil phénolique. Cependant, des échantillons en haute teneur de quercétine ont malgré tout une activité biologique réduite suggérant que des facteurs, abiotiques ou biotiques, menant à ces variations peuvent tout de même avoir un impact significatif sur l'action pharmacologique de la plante. Cela n'appuie pas l'hypothèse voulant que l'activité pharmacologique de *R. groenlandicum* soit plus élevée suite à un contenu plus élevé en composés phénoliques. De toute évidence, l'on doit aussi considérer le rôle complexe des relations synergistiques et antagonistiques qui peuvent exister entre les diverses composantes d'un extrait brut. Il faudra attendre des études plus fines visant des molécules précises afin de mieux cerner le rôle de certains phénols en lien avec l'environnement tant abiotique que biotique. Ces études devront être couplées à des analyses pharmacologiques.

Les études présentées dans ce mémoire représentent les premières investigations approfondies et si étendues sur la phytochimie et la pharmacologie de plantes médicinales canadiennes. À l'exception des populations de *R. groenlandicum* provenant de

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Whapmagoostui, où il semble y avoir une différentiation du profil phytochimique, l'absence de chémotypes indique que la phytochimie du *R. groenlandicum* et *S. purpurea* est assez constante. En conséquence, ces espèces peuvent être utilisées comme produit de santé naturel sans crainte sur tout le territoire Eeyouch. Malgré tout, les variations observées peuvent justifier le recours éventuel à la normalisation, surtout si l'usage thérapeutique de la plante chez les diabétiques cris prend beaucoup d'ampleur.

Les résultats de l'analyse phytochimique du *R. groenlandicum* appuient l'hypothèse que son contenu phénolique sera plus élevé dans les populations exposées à de plus hauts niveaux de stress associés à l'ensoleillement, comme la radiation solaire ou la photopériode. Les résultats du *S. purpurea,* quant à eux, ne viennent pas la supporter. Cela pourrait s'expliquer par les effets de l'environnement aquatique de cette plante. Par ailleurs, la production de ses composantes pourrait être induite par des facteurs qui n'ont pas été mesurés dans cette étude. Il semble, par exemple, que le niveau d'eau dans les tourbières soit crucial pour cette espèce (Tendland, 2011).

Table 6.1 Description des modèles animaux *in vivo* et des bioessais *in vitro* utilisés pour évaluer le potentiel antidiabétique des plantes médicinales de la pharmacopée CEI. L'ordre de présentation des modèles réflète le plan de priorisation des espèces en fonction de leur activité antidiabétique selon Haddad et al. (2012).

ID	Modèle	Description
1	*in vivo*	Souris DIO C57BL/6 (activité hypoglycémiante et anti-obésité)
2	*in vivo*	Rats STZ (activité hypoglycémiante; diabète type 1)
3	*in vivo*	Souris KK-A^y (activité hypoglycémiante; diabète type 2)
4	*in vivo*	Rats pré-diabétiques résistants à l'insuline (sensibilisation à l'insuline)
5	*in vivo*	Rats normaux
6	*in vitro*	Sécrétion d'insuline dans les cellules β pancréatiques β TC-tet
7	*in vitro*	Prolifération des cellules β pancréatiques β TC-tet
8	*in vitro*	Absorption du glucose par les myotubes C2C12
9	*in vitro*	Absorption du glucose par les adipocytes 3T3-L1
10	*in vitro*	Production du glucose dans les cellules hépatiques H4IIE
11	*in vitro*	Adipogenèse des adipocytes 3T3-L1
12	*in vitro*	Absorption intestinale du glucose dans les cellules Caco-2
13	*in vitro*	Produits terminaux de glycation avancée
14	*in vitro*	Toxicité glycémique des pré-neurones sympathiques PC12-AC
15	*in vitro*	Carence glycémique des pré-neurones sympathiques PC12-AC

Table 6.2 Activité antidiabétique des plantes médicinales de la pharmacopée CEI évaluée par expériences *in vitro* et *in vivo* décrites dans le **tableau 6.1**. Voir les références pour plus de détails sur la méthodologie et les résultats.

Rang	Espèce	Activité	ID	Références
1	*R. groenlandicum*	Hypoglycémiante	1, 8, 9	Ouchfoun, 2011; Spoor et al., 2006
		Adipogénique	11	Ouchfoun, 2011; Spoor et al., 2006
		Antiglycation	13	Harris et al., 2011
		Anti-obésité	1	Ouchfoun, 2011
2	*L. laricina*	Hypoglycémiante	1, 8	Harbilas, et al., 2012a; Spoor et 2al., 2006
		Insulino-sensibilisatrice	1	Harbilas et al., 2012a
		Anti-obésité	1	Harbilas et al., 2012a
		Adipogénique	11	Shang et al., 2012; Spoor et al., 2006
3	*R. tomentosum*	Hypoglycémiante	5, 9, 12	Harbilas et al., 2009; Nistor Baldea et al., 2010
		Adipogénique	11	Harbilas et al., 2009
		Cytoprotective	14, 15	Harbilas et al., 2009
		Antiglycation	13	Harris et al., 2011
4	*P. mariana*	Hypoglycémiante	5, 9, 12	Nistor Baldea et al., 2010; Spoor et al., 2006
		Adipogénique	11	Spoor et al., 2006
		Cytoprotective	14	Downing, 2010; Spoor et al., 2006
		Antiglycation	13	Harris et al., 2011
5	*P. glauca*	Hypoglycémiante	12	Nistor Baldea et al., 2010
		Cytoprotective	14, 15	Harbilas et al., 2009; Harris et al., 2008
		Antiglycation	13	Harris et al., 2011
6	*K. angustifolia*	Adipogénique	11	Harbilas et al., 2009
		Antiglycation	13	Harris et al., 2011

7	*S. decora*	Hypoglycémiante	2, 3, 4, 8, 12	Guerrero-Analco et al., 2010; Nistor Baldea et al., 2010; Spoor et al., 2006; Vianna et al., 2011
		Insulino-sensibilisatrice	4	Vianna et al., 2011
		Cytoprotective	14	Spoor et al., 2006
8	*A. balsamea*	Hypoglycémiante	8, 9	Spoor et al., 2006
		Anti-glycation	13	Harris et al., 2011
9	*A. incana* subsp. *rugosa*	Anti-obésité	11	Martineau, Hervé, et al., 2010; Martineau, Muhammad, et al., 2010
		Hypoglycémiante	8, 9, 12	Nistor Baldea et al., 2010; Spoor et al., 2006
		Cytoprotective	15	Spoor et al., 2006
10	*J. communis*	Hypoglycémiante	12	Nistor Baldea et al., 2010
		Anti-glycation	13	Harris et al., 2011
11	*P. banksiana*	Hypoglycémiante	9, 12	Nistor Baldea et al., 2010; Spoor et al., 2006
		Adipogénique	11	Spoor et al., 2006
		Cytoprotective	15	Spoor et al., 2006
		Anti-glycation	13	Harris et al., 2011
12	*S. planifolia*	Hypoglycémiante	9, 12	Harbilas et al., 2009; Nistor Baldea et al., 2010
		Cytoprotective	15	Harbilas et al., 2009
		Anti-glycation	13	Harris et al., 2011
14	*V. vitis-idaea*	Hypoglycémiante	8, 9, 12	Eid et al., 2010; Harbilas et al., 2009; Nistor Baldea et al., 2010
		Adipogénique	11	Harbilas et al., 2009
		Anti-glycation	13	Beaulieu et al., 2010; Harris et al., 2011
15	*S. purpurea*	Cytoprotective	14, 15	Harris et al., 2012; Spoor et al., 2006
		Hypoglycémiante	8, 9, 10,	Muhammad et al., 2012; Nistor Baldea et al., 2010;

			12	Spoor et al., 2006
		Anti-glycation	13	Harris et al., 2011
16	*P. balsamifera*	Anti-obésité	1, 11	Harbilas et al., 2012b; Martineau, Hervé, et al., 2010; Martineau, Muhammad, et al., 2010
		Hypoglycémiante	1, 12	Harbilas et al., 2012b; Nistor Baldea et al., 2010
		Insulino-sensibilisatrice	1	Harbilas et al., 2012b
		Cytoprotective	14	Harbilas et al., 2009
		Anti-glycation	13	Harris et al., 2011
17	*G. hispidula*	Hypoglycémiante	9, 12	Harbilas et al., 2009; Nistor Baldea et al., 2010
		Cytoprotective	14, 15	Harbilas et al., 2009
		Anti-glycation	13	Harris et al., 2011
20	*L. clavatum*	Hypoglycémiante	12	Nistor Baldea et al., 2010
		Adipogénique	11	Harbilas et al., 2009
		Cytoprotective	14	Harbilas et al., 2009
21	*V. angustifolium*	Hypoglycémiante	8, 9	Martineau et al., 2006
		Adipogénique	11	Martineau et al., 2006
		Cytoprotective	14	Martineau et al., 2006
		Insulinotrope	6	Martineau et al., 2006
		Proliférative	7	Martineau et al., 2006
		Anti-glycation	13	McIntyre et al., 2008

Outre leur activité antioxydante évaluée dans maints modèles expérimentaux (Spoor et al., 2006; Fraser et al., 2007; Harbilas et al., 2009; Harris et al., 2011), plusieurs des plantes médicinales citées dans les communautés de Wemindji et Oujé-Bougoumou ont déjà été testées à l'aide d'une plateforme étendue de bioessais *in vitro* et de modèles animaux *in vivo* (**tableau 6.1**). Le résultat de ces expériences témoigne du potentiel antidiabétique que possèdent les plantes de la médecine traditionnelle crie (**tableau 6.2**) et, conséquemment, de la possibilité de les intégrer dans des approches culturellement adaptées pour aider à gérer le DT2.

L'usage sécuritaire et efficace de ces plantes dans des régimes de médecine traditionnelle dépend toutefois de plusieurs facteurs. Il faut, d'une part, s'assurer d'une mise en place qui tienne compte des différents intervenants du milieu de la santé cri (projet en cours) et, d'autre part, du développement durable de cette ressource. Ainsi, Tendland et al. (2012) décrivent expérimentalement les seuils de surexploitation du *R. groenlandicum* et modélisent ainsi le temps de réhabilitation chez les populations sauvages afin d'assurer le renouvèlement de la ressource. Les études sur la variabilité phytochimique, quant à elles, permettent de développer des produits de santé naturels de qualité, efficaces et normalisés.

La variabilité géographique des composantes phytochimiques est bien documentée, tel qu'est le cas chez *Abies balsamea* (Zavarin and Snajberk, 1972; Lester, 1974). Il existe également quelques études cherchant à élucider les tendances et les gradients qui les soutendent (**tableau 6.3**). Cependant, les gradients géographiques sont souvent variés et contradictoires, comme on le remarque dans ce mémoire en comparant les résultats pour *R. groenlandicum* et *S. purpurea*. De plus, les liens de causalité avec les facteurs biotiques et abiotiques soupçonnés d'être impliqués sont rarement mesurés ni testés efficacement et relèvent généralement de la spéculation.

Deux hypothèses s'opposent pour expliquer de manière générale les variations géographiques qui existent chez les métabolites secondaires (Zidorn, 2009). Premièrement, plus le climat est extrême, moins la pression sélective par l'herbivorie est prononcée. Ainsi, la quantité et diversité de composantes bioactives devraient diminuer de l'équateur vers les pôles. Deuxièmement, les extrêmes climatiques par rapport à la radiation solaire et à la

température, par exemple, sont des facteurs de stress sur les plantes. On observerait donc l'effet opposé; les plantes ayant recours aux composantes bioactives pour se protéger.

Ces hypothèses ne peuvent cependant pas être généralisées à toutes les composantes, car elles ne possèdent pas toutes les mêmes fonctions physiologiques. Cela étant dit, les structures spatiales sont plutôt des marqueurs expliquant des relations biotiques ou abiotiques. Les études qui s'y rattachent doivent donc être réalisées et interprétées cas par cas en prenant en considération les groupes de composantes ciblés, l'impact de la variabilité génétique, les interactions avec les autres métabolites présents ainsi que leur rôle dans les voies biosynthétiques.

Finalement, un suivi des résultats obtenus de l'analyse phytochimique du *S. purpurea* est tout indiqué pour mieux les comprendre dans un contexte de pharmacologie. Comme il a été fait pour *R. groenlandicum*, l'analyse pharmacologique par bioessai *in vitro*, soit une étude tenant compte de l'absorption du glucose en présence de la sarracénie (Spoor et al., 2006; Muhammad et al., 2012), éluciderait le rôle pharmacologique des variations phytochimiques observées. Quant au *R. groenlandicum,* des études contrôlées en serre sont nécessaires afin d'élucider davantage le rôle de la radiation solaire et de la photopériode sur la production de composantes biologiquement actives.

De plus, la compilation des données ethnobotaniques révèle des espèces végétales possédant une importance culturelle élevée par rapport à la médecine traditionnelle des CEI. Plusieurs de ces plantes ont démontré un potentiel antidiabétique (**tableau 6.2**) et bénéficieront d'études plus poussées comme les études cliniques. Malgré leur faible classement, une attention particulière devrait tout de même être portée aux fruits sauvages, tels *Vaccinium uliginosum, Empetrum nigrum* et *Prunus pensylvanica,* pour lesquels les bienfaits ont déjà été explorés scientifiquement. Puisque ces fruits sont principalement perçus et appréciés comme aliments, ils peuvent être utilisés pour remplacer d'autres sources alimentaires de glucides raffinés, tels les sucreries et les boissons gazeuses. Leur intégration dans des approches culturellement adaptées pour traiter le DT2 pourrait donc être facilitée. Des études ethnobotaniques ayant pour objectif d'identifier les fruits sauvages couramment consommés et recherchés permettraient de mieux cerner cette approche.

Table 6.3 Liste des études portant sur les gradients géographiques et la phytochimie de diverses espèces végétales. Les variables spatiales associées à ces tendances sont indiquées suivi des facteurs abiotiques ou biotiques déduits ou testés pour expliquer ces corrélations.

Espèce	Famille	Métabolite	Corrélation[1]	Facteur abiotique/biotique	Référence
Cinchona spp.	Rubiaceae	Alcaloïdes	Altitude (+)	Température	Camp, 1949
Lupinus sericeus	Fabaceae	Flavonoles	Latitude (-)		Nicholls and Bohm, 1982, 1983
Satureja douglasii	Lamiaceae	Monoterpènes	Latitude	Luminosité et herbivorie	Lincoln and Langenheim, 1976, 1979
Sanguinaria canadensis	Papaveraceae	Alcaloïdes	Altitude (-)	Herbivorie et pathogénie	Salmore and Hunter, 2001
Pteridium caudatum	Dennstaedtiaceae	Polyphénoles	Altitude (+)	Radiation solaire	Alonso-Amelot et al., 2004
Pteridium arachnoideum	Dennstaedtiaceae	Polyphénoles	Altitude (+)	Radiation solaire	Alonso-Amelot et al., 2004, 2007
		Alkanes	Altitude (x^2)	Température et aridité	Dodd and Poveda, 2003
Juniperus communis	Cupressaceae	Polyphénoles	Latitude (+)		Martz et al., 2009
		Terpènes	Altitude (+)		Martz et al., 2009
			Latitude (+)		
Vaccinium myrtillus	Ericaceae	Polyphénoles	Altitude (+)	Luminosité, température et qualité du sol	Martz et al., 2010
Jungermannia exsertifolia subsp. *cordifolia*	Jungermanniaceae	Polyphénoles	Altitude (+)	Radiation solaire	Arróniz-Crespo et al., 2006
Betula pubescens	Betulaceae	Flavonoïdes	Latitude (+/-)[2]	Température et [P]	Stark et al., 2008
Scorzoneroides helvetica	Asteraceae	Flavonoles	Altitude (-)		Zidorn, 2009

[1](+) = relation positive, (-) = relation négative, (x^2) = relation quadratique
[2]Gradients de quercétine et ses dérivées sont opposés à ceux d'apigénine et de naringénine

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Annexe I – Certificat d'éthique

University **ṁ**
de Montréal

Faculté des arts et des sciences
Vice-décanat à la recherche

No de certificat : CÉRFAS-2010-11-120-A

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 et en vertu des documents qui lui ont été fournis, a examiné le projet de recherche suivant et conclu qu'il respecte les règles d'éthique énoncées dans la *Politique sur la recherche avec des êtres humains* de l'Université de Montréal :

Titre : ***Ethnobotanique des Cris de la Nation d'Eeyou Istchee ainsi que la variation phytochimique des plantes médicinales***

Requérant : ***RAPINSKI, Michel (code permanent RAPM17108502), étudiant à la maîtrise, Département de sciences biologiques***
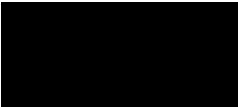
sous la direction de :

CUERRIER, Alain, professeur associé, Département de sciences biologiques

Tout changement anticipé au protocole de recherche devra être communiqué au CÉRFAS qui en évaluera l'impact au chapitre de l'éthique.

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

Selon les exigences éthiques en vigueur, **un suivi annuel est minimalement exigé afin de maintenir la validité de ce certificat**, et ce, jusqu'à la fin du projet. Le questionnaire de suivi peut être consulté sur la page Web du CÉRFAS.

Jean Leclair, président
Comité d'évaluation accélérée

Deirdre Meintel, présidente
CÉRFAS

Date de délivrance : 2010 / 08 / 16
AAAA / MM / JJ

Date d'échéance* : 2012 / 01 / 01
AAAA / MM / JJ
*correspond à la date prévue de fin du projet

Certificat prolongé jusqu'au : 2013 / 08 / 16

Katia Maliantovitch, secrétaire, CERFAS

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Annexe II – Spécimens d'herbier : ethnobotanique de Wemindji et Oujé-Bougoumou

Spécimen	# de récolte	Herbier*
Wemindji		
Abies balsamea	MTR-2011-080	MT; OTT; a; b
Alnus incana subsp. *rugosa*	MTR-2011-079	MT; OTT; a; b
Betula papyrifera	MTR-2011-087	MT; OTT; a; b
Gaultheria hispidula	MTR-2011-077	MT; OTT; a; b
Juniperus communis	MTR-2011-031	MT; b
Juniperus communis	MTR-2011-030	MT; OTT; a
Kalmia angustifolia	MTR-2011-086	MT; OTT; a; b
Larix laricina	MTR-2011-071	MT; OTT; a; b
Picea glauca	MTR-2011-033	MT; OTT; a; b
Picea mariana	MTR-2011-074	MT; b
Picea mariana	MTR-2011-055	MT; OTT, a
Populus balsamifera	MTR-2011-062	MT; OTT; a; b
Populus tremuloides	MTR-2011-061	MT; OTT; a; b
Rhododendron groenlandicum	MTR-2011-054	MT; OTT; a; b
Sphagnum fuscum	MTR-2011-035-D	MT
Typha latifolia	MTR-2011-085	MT; OTT; a; b
Vaccinium angustifolium	MTR-2011-056	MT; OTT; a; b
Vaccinium uliginosum	MTR-2011-042	MT; OTT; a; b
Vaccinium vitis-idaea	MTR-2011-068	MT; OTT; a; b
Espèces mentionées par le participant inuit ou métis:		
Andromeda polifolia	MTR-2011-036	MT; OTT; a
Chamerion angustifolium	MTR-2011-073	MT; OTT; a; b
Honckenya peploides	MTR-2011-046	MT; OTT
Empetrum nigrum	MTR-2011-059	MT; OTT; a; b
Rhododendron tomentosum	Non trouvé	
Salix arctophila	MTR-2011-043	MT; OTT; a
Sorbus decora	MTR-2011-065	MT; OTT; a; b
Ouje-Bougoumou		
Abies balsamea	MTR-2011-128	MT; OTT; a; c
Alnus incana subsp. *rugosa*	MTR-2011-107	MT; OTT; a; c
Andromeda polifolia[1]	MTR-2011-036	MT; OTT; a; c
Betula papyrifera	MTR-2011-104	MT; OTT; a; c

Cornus stolonifera	MTR-2011-143	MT; OTT; a; c
Gaultheria hispidula	MTR-2011-109	MT; a
Gaultheria hispidula	MTR-2011-110	MT; OTT; a; c
Kalmia angustifolia	MTR-2011-126	MT; OTT; a; c
Larix laricina	MTR-2011-089	MT; OTT; a; c
Lycopodium annotinum	MTR-2011-146	MT; OTT; a; c
Lycopodium clavatum	Non trouvé	
Picea glauca	MTR-2011-129	MT; OTT; a; c
Picea mariana	MTR-2011-088	MT; OTT; a; c
Pinus banksiana	MTR-2011-127	MT; OTT; a; c
Plantago major	MTR-2011-114	MT; OTT; a; c
Populus balsamifera	MTR-2011-139	MT; OTT; a; c
Populus tremuloides	MTR-2011-120	MT; OTT; a; c
Prunus pensylvanica	MTR-2011-144	MT; OTT; a; c
Rhododendron groenlandicum	MTR-2011-103	MT; OTT; a; c
Ribes lacustre	MTR-2011-145	MT; OTT; a; c
Rubus pubescens	MTR-2011-111	MT; OTT; a; c
Salix humilis var. *humilis*	MTR-2011-132	MT; OTT; a; c
Sarracenia purpurea	MTR-2011-100	MT; OTT; a; c
Sorbus americana	MTR-2011-142	MT; OTT; a; c
Sorbus decora[1]	MTR-2011-065	MT; OTT; a; b
Thuja occidentalis	MTR-2011-121	MT; OTT; a; c
Vaccinium angustifolium	MTR-2011-141	MT; OTT; a; c
Vaccinium myrtilloides	MTR-2011-140	MT; OTT; a; c
Vaccinium vitis-idaea	Non trouvé	

* Les spécimens d'herbier sont déponsés dans l'herbier Marie-Victorin du Jardin Botanique de Montréal (MT), l'herbier de l'Université d'Ottawa (OTT), le Centre de Culture Crie *Aanischaaukamikw* (a), l'école Maquatua de Wemindji (b) et Waapihtiiwewan de Oujé-Bougoumou (c).
[1]Spécimen récolté à Wemindji

Annexe III – Spécimens d'herbier : phytochimie de *Rhododendron groenlandicum* et *Sarracenia purpurea*

# de récolte	Lieux	Herbier
Rhododendron groenlandicum		
MTR-2010-001	Ottawa	OTT
MTR-2010-025	Mistissini	MT; OTT
MTR-2010-003	Nemaska	MT; OTT
MTR-2010-017	Waskaganish	MT; OTT
MTR-2010-018	Eastmain	MT; OTT
MTR-2010-021	Wemindji	MT; OTT
MTR-2010-008	Whapmagoostui	MT; OTT
Sarracenia purpurea		
MTR-2010-002	Ottawa	OTT
MTR-2010-023	Mistissini	MT; OTT
MTR-2010-006	Waskaganish	MT; OTT
MTR-2010-022	Wemindji	MT; OTT
MTR-2010-020	Eastmain	MT; OTT
MTR-2010-004	Nemaska	MT; OTT

* Les spécimens d'herbier sont déponsés dans l'herbier Marie-Victorin du Jardin Botanique de Montréal (MT) et l'herbier de l'Université d'Ottawa (OTT).

Annexe IV – Liste des espèces avec autorité et famille

Espèce	Famille
Plantae	
Abies balsamea (L.) Mill.	Pinaceae
Alnus incana subsp. *rugosa* (Du Roi) R. T. Clausen	Betulaceae
Alnus viridis subsp. *crispa* (Ait.) Turrill	Salicaceae
Andromeda polifolia L.	Ericaceae
Betula papyrifera Marsh.	Betulaceae
Chamerion angustifolium (L.) Holub	Onagraceae
Cornus stolonifera Michx.	Cornaceae
Empetrum nigrum L.	Ericaceae
Gaultheria hispidula (L.) Muhl.	Ericaceae
Heracleum maximum W. Bartram	Apiaceae
Honckenya peploides (L.) Ehrh.	Caryophyllaceae
Juniperus communis L.	Cupressaceae
Kalmia angustifolia L.	Ericaceae
Larix laricina (Du Roi) K. Koch	Pinaceae
Leymus mollis (Trin.) Pilg.	Poaceae
Lycopodium annotinum L.	Lycopodiaceae
Lycopodium clavatum L.	Lycopodiaceae
Picea glauca (Moench) Voss	Pinaceae
Picea mariana (Mill.) BSP	Pinaceae
Pinus banksiana Lamb.	Pinaceae
Plantago major L.	Plantaginaceae
Populus balsamifera L.	Salicaceae
Populus tremuloides Michx.	Salicaceae
Prunus pensylvanica L. f.	Rosaceae
Rhododendron groenlandicum (Oeder) Kron & Judd	Ericaceae
Rhododendron tomentosum Harmaja	Ericaceae
Ribes lacustre (Pers.) Poir.	Grossulariaceae
Rubus pubescens Raf.	Rosaceae
Salix humilis Marsh. var. *humilis*	Salicaceae
Sarracenia purpurea L.	Sarraceniaceae
Sorbus americana Marsh.	Rosaceae
Sorbus decora C. K. Schneid.	Rosaceae
Sphagnum fuscum (Schimp.) Klinggr	Sphagnaceae
Thuja occidentalis L.	Cupressaceae
Typha latifolia L.	Typhaceae
Vaccinium angustifolium Ait.	Ericaceae

Vaccinium boreale I.V. Hall & Aalders	Ericaceae
Vaccinium myrtilloides Michx.	Ericaceae
Vaccinium uliginosum L.	Ericaceae
Vaccinium vitis – idaea L.	Ericaceae