Evaluation of the antidiabetic potential of selected medicinal plant extracts from the Canadian boreal forest used to treat symptoms of diabetes: part II

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Abstract: Among the Cree of northern Quebec, the disproportionately high rate of diabetic complications is largely due to the cultural inadequacy of modern therapies for type 2 diabetes. To establish culturally adapted antidiabetic treatments, our team identified several candidate plant species used by the Cree to treat symptoms of diabetes. An initial study focused on 8 species and revealed that most possess significant in vitro antidiabetic activity. The purpose of the present study was to assess a further 9 species identified through the ethnobotanical survey. Crude plant extracts were screened for (*i*) potentiation of basal and insulin-stimulated glucose uptake by skeletal muscle cells (C2C12) and adipocytes (3T3-L1); (*ii*) potentiation of glucose-stimulated insulin secretion by pancreatic β cells (βTC); (*iii*) potentiation of adipogenesis in 3T3-L1 cells; (*iv*) protection against glucose toxicity and glucose deprivation in PC12-AC neuronal precursor cells; and (*v*) diphenylpicrylhydrazyl (DPPH) oxygen free radical scavenging. Four species potentiated basal glucose uptake in muscle cells or adipocytes, one species being as potent as metformin. Adipogenesis was accelerated by 4 species with a potency roughly half that of rosiglitazone. Five species protected PC12-AC cells against glucose toxicity and 4 protected against glucose deprivation. Five species exhibited antioxidant activity comparable to ascorbic acid. However, no species increased insulin secretion. The present study revealed that *Gaultheria hispidula*, *Rhododendron tomentosum*, and *Vaccinium vitis-idaea* exhibit a promising profile of antidiabetic potential and are good candidates for more in-depth evaluation.

Key words: diabetes mellitus, natural products, traditional medicine, glucose uptake, cytoprotection, antioxidant activity, polyphenolics.

Résumé : Chez les Cris du Québec, un taux disproportionnellement élevé de complications de diabète dû à une résistance culturelle aux produits pharmaceutiques amplifie ce problème. Afin de développer des traitements antidiabétiques culturellement adaptés à cette population, notre équipe a identifié plusieurs espèces de plantes utilisées par les Cris pour traiter les symptômes de diabète. Une première étude de criblage sur 8 espèces a révélé que plusieurs d'entre elles possèdent un potentiel antidiabétique significatif. L'objectif de la présente étude est d'évaluer 9 autres espèces également identifiées par notre équipe. Nous avons testé leur potentiel dans différents tests dont (*i*) la stimulation de la prise de glucose par des cellules musculaires squelettiques (C2C12) et par les adipocytes (3T3-L1); (*ii*) l'amplification de la sécrétion d'insuline stimulée par le glucose dans des cellules β-pancréatiques (βTC); (*iii*) la stimulation de l'accumulation de lipides dans des cellules 3T3-L1 en différentiation; (*iv*) la protection de cellules pré-neuronales PC12-AC contre la toxicité du glucose ou

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la privation du glucose; (*v*) l'effet piégeur de radicaux libres du DPPH. Nous avons démontré que 4 espèces ont augmenté le transport de glucose basal, dont l'un ayant un effet similaire à la metformin, dans les C2C12 ou dans les 3T3-L1. Quatre espèces ont accéléré la différentiation des 3T3-L1 avec une puissance environ de moitié celle de rosiglitazone. Cinq ont protégé les cellules PC12-AC contre la toxicité au glucose et 4 contre la privation du glucose. Cinq ont démontré une activité antioxydante comparable à celle de l'acide ascorbique. Aucune a augmenté la sécrétion d'insuline. En conclusion la majorité des espèces de plantes étudiées possèdent une forme d'activité antidiabétique in vitro, qui peut être utilisée pour faire un choix rational de plantes, telles que *Gaultheria hispidula*, *Rhododendron tomentosum*, *Vaccinium vitis-idaea*, pouvant faire l'objet d'études plus poussées.

Mots-clés : diabète sucré, produits naturels, médecine traditionnelle, capture de glucose, protection cellulaire, activité antioxydante, polyphénols.

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Introduction

Type 2 diabetes is a global health problem. In Canada alone, the prevalence of diabetes has increased by 65% between 1995 and 2005 (Lipscombe and Hux 2007) to approximately 2 million cases, and it is projected that by 2030 this number will rise to over 3 million (WHO 2007). As is the case for aboriginal populations throughout the world, the prevalence of type 2 diabetes has reached even more alarming levels amongst Canadian First Nations, such as the Cree of Eeyou Istchee (CEI) of northern Quebec. The latter have an incidence rate that is at least twice as high as the rest of the Canadian population (Brassard et al. 1993; Kuzmina and Dannenbaum 2004; Légaré 2004). This is most likely caused by such factors as recent adoption of a sedentary lifestyle and nontraditional diet (Berkes and Farkas 1978; Hegele 2001), as well as a genetic predisposition towards obesity (Neel 1999; Skyler 2004). More importantly, the rate of diabetic complications within this population, including nephropathy, peripheral neuropathy, and microvascular disorders, is disproportional to the rate of diabetes. This is likely due in no small part to the lack of consideration of cultural awareness and accepted practice that is implicit in drug-based therapies, with a resulting poor management of hyperglycemia (Boston et al. 1997). Clearly, new strategies are needed to address this specialized issue and provide culturally acceptable alternatives through collaboration between basic scientists, health care professionals, and CEI elders and healers (Brassard et al. 1993).

One solution may be found within the rich pharmacopoeia of the Canadian First Nations. Although diabetes is a new disease for this population, it is nevertheless likely that products from their own traditional medicine possess antidiabetic activity. Indeed, antihyperglycemic effects and other antidiabetic activities have been identified in thousands of plant species worldwide (Marles and Farnsworth 1995). Our team therefore used a collaborative ethnobotanical approach to identify plant species that are used by the Cree to treat symptoms related to diabetes (Leduc et al. 2006). A first screening study was performed on 8 relevant medicinal plant species identified in a survey of the Cree Nation of Mistissini (Spoor et al. 2006). This study revealed that, in vitro, most selected species exhibited some form of antidiabetic activity mediated through a variety of physiological mechanisms including increased glucose uptake, accelerated adipocyte differentiation, and cytoprotection against glucose toxicity (Spoor et al. 2006).

A second ethnobotanical survey has been conducted in the nation of Whapmagoostui of northern Quebec. The survey identified many of the same species employed in Mistissini, as well as new species. The ethnobotanical data was combined to produce a list of 17 relevant species for screening, 8 of which were the subject of our first screening study. The present study assesses the antidiabetic potential of the remaining 9 medicinal plant species used in Mistissini and (or) Whapmagoostui. This represents the first testing of their antidiabetic activity using integrative cell-based assays.

Materials and methods

Plant materials

The 17 Cree medicinal plant species identified as being relevant to the treatment of symptoms of diabetes are listed in Table 1. Their predicted importance, based on frequency of citation during interviews and number and importance of symptoms for which they were cited, is listed as a syndromic importance value (SIV) as previously reported (Leduc et al. 2006). The species *Abies balsamea*, *Alnus incana*, *Larix laricina*, *Picea mariana*, *Pinus banksiana*, *Rhododendron groenlandicum*, *Sarracenia purpurea*, and *Sorbus decora* have been the subject of a first screening study (Spoor et al. 2006) for plants identified through an ethnobotanical study in the Cree Nation of Mistissini. As a result of the second ethnobotanical study in the Whapmagoostui First Nation, additional plant species were integrated and the resulting ranking modified. Table 1 presents the combined ranking, based on the new SIVs. The species *Gaultheria hispidula*, *Juniperus communis*, *Kalmia angustifolia*, *Lycopodium clavatum*, *Picea glauca*, *Populus balsamifera*, *Rhododendron tomentosum*, *Salix planifolia*, and *Vaccinium vitis-idaea* are the subject of the present study. Table 1 also lists the specific organ part tested, in accordance with the ethnobotanical data. At least 5 samples of each specimen were collected in a culturally respectful manner in the regions of Mistissini and Whapmagoostui in northern Quebec, Canada. Dr. Alain Cuerrier, taxonomist at the Montreal Botanical Garden, ascertained the botanical identity of the plant species, and voucher specimens were deposited in the Marie-Victorin herbarium of the Montreal Botanical Garden in Montréal, Quebec, Canada. The collected plant samples were air dried and sent to the University of Ottawa, where they were cleaned and separated into plant organ parts. Plant

Table 1. List of CEI medicinal plant species used in the treatment of symptoms of diabetes.

Species	Abbreviation	Common name	Cree name	Family	Plant part	Ethnobotanical Source	Ethnobotanical Ranking	SIV
Abies balsamea (L.) Mill.	*A. balsamea*	Balsam fir	Inaast	Pinaceae	Inner bark	M	8	0.254
Alnus incana subsp. *rugosa* (Du Roi) R.T. Clausen	*A. incana*	Speckled alder	Atuuspiih	Betulaceae	Inner bark	M	11	0.163
***Gaultheria hispidula* (L.) Muhl.**	***G. hispidula***	**Creeping snowberry**	**Piyeumanaan**	**Ericaceae**	**Leaves[a]**	**M**	**17**	**0.040**
***Juniperus communis* L.**	***J. communis***	**Ground Juniper**	**Kaakaachuminatuk**	**Cupressaceae**	**Berries**	**W**	**4**	**0.353**
***Kalmia angustifolia* L.**	***K. angustifolia***	**Sheep laurel**	**Uischichipukw**	**Ericaceae**	**Leaves**	**M, W**	**9**	**0.247**
Larix laricina Du Roi (K. Koch)	*L. laricina*	Tamarack	Waatinaakan	Pinaceae	Inner bark	M, W	3	0.409
***Lycopodium clavatum* L.**	***L. clavatum***	**Common clubmoss**	**Pastinaakwaakin**	**Lycopodiaceae**	**Whole plant**	**M**	**15**	**0.088**
***Picea glauca* (Moench) Voss**	***P. glauca***	**White spruce**	**Minhiikw**	**Pinaceae**	**Needles**	**M, W**	**6**	**0.275**
Picea mariana (P. Mill.) BSP	*P. mariana*	Black spruce	Iinaatikw	Pinaceae	Cones	M, W	5	0.347
Pinus banksiana Lamb.	*P. banksiana*	Jack pine	Uschisk	Pinaceae	Cones	M, W	14	0.088
***Populus balsamifera* L.**	***P. balsamifera***	**Balsam poplar**	**Mash-mitush**	**Salicaceae**	**Inner bark**	**M**	**16**	**0.049**
Rhododendron groenlandicum (Oeder) Kron and Judd	*R. groenlandicum*	Labrador tea	Kaachepukw	Ericaceae	Leaves	M, W	2	0.507
***Rhododendron tomentosum* (Stokes) Harmaja subsp. *subarcticum* (Harmaja) G. Wallace**	***R. tomentosum***	**Northern Labrador tea**	**Wiisichipukw**	**Ericaceae**	**Leaves**	**W**	**1**	**0.656**
***Salix planifolia* Pursh**	***S. planifolia***	**Tealeaf willow**	**Piyeuwaatikw**	**Salicaceae**	**Inner Bark**	**M, W**	**10**	**0.188**
Sarracenia purpurea L.	*S. purpurea*	Pitcher plant	Ayikataas	Sarraceniaceae	Whole plant	M	13	0.092
Sorbus decora (Sarg.) C.K. Schneid.	*S. decora*	Showy mountain ash	Maskumanaatikw	Rosaceae	Inner bark	M, W	7	0.262
***Vaccinium vitis-idaea* L.**	***V. vitis-idaea***	**Mountain cranberry**	**Wiishichimanaanh**	**Ericaceae**	**Berries**	**M, W**	**12**	**0.112**

Note: SIV, syndromic importance value; M, Mistissini; W, Whapmagoostui. The 9 species in bold are the subject of the present study. Combined ethnobotanical ranking of the 17 species is based on the SIVs, calculated according to Leduc et al. (2006).

[a]Berries not available.

material was ground using a Wiley Mill (Arthur H. Thomas, Swedesboro, USA) with a 2-millimetre filter, and extracted in 80% ethanol (10 mL/g dry material) twice for 24 h on a mechanical shaker and then filtered with Whatman paper. Both extracts were combined, and were then dried using a rotary evaporator followed by lyophilization. *Trigonella foenum-graecum* L. (fenugreek), used as a positive control for glucose uptake studies in 3T3-L1 adipocytes, was purchased from Lone Wolf Herb (Phippen, Canada) and was extracted using the same method. *Lactuca sativa* (iceberg lettuce), used as a negative control in the free radical scavenging assay, was purchased in a local market and was also extracted similarly. All lyophilized extracts were conserved at 4 °C in a desiccator and protected from light.

Total phenolics

Total phenolics were measured by the Folin-Ciocalteau method (Singleton and Rossi 1965), modified to reduce volumes as previously described (Farsi et al. 2008; Spoor et al. 2006). Briefly, extract was added to freshly diluted Folin-Ciocalteau reagent (BDH, Toronto, Canada), and the reaction was allowed to proceed to equilibrium for 5 min before adding a solution of 7.5% anhydrous $NaHCO_3$. After a 2 h incubation at room temperature, the absorbance of the mixture was measured at 725 nm. All the tests were conducted in triplicate. Quercetin (Sigma-Aldrich, St. Louis, USA) was used as a standard, and 80% ethanol was used as a blank. The results are expressed as micrograms of quercetin equivalents per milligram of extract (μg/mg).

Phytochemical characterization of extracts

Basic characterization of extracts consisting of identification and quantitation of a small number of phytochemical markers was performed by high-performance liquid chromatography (HPLC) separation and comparison of UV absorbance spectra to a custom metabolomic library of 131 purified or commercially prepared reference compounds, as described elsewhere (Harris et al. 2007*a*; Martineau et al. 2006; Spoor et al. 2006). Briefly, HPLC was performed by using an Agilent 1100 HPLC system (Palo Alto, USA) with photodiode array detector and an atmospheric pressure chemical ionization (APCI) quadrupole mass-selective detector (MSD) mass spectrometer with a range of 50–1500 atomic mass units. All extracts were analyzed using a high carbon load ODS-AM packing column (YMC, Kyoto, Japan) (100 mm × 2 mm ID, 3 μm particle size). Column temperature was maintained at 50 °C and flow rate was 0.3 mL/min. Optimized elution conditions included a mobile phase system of methanol and aqueous trifluoroacetic acid (0.05%; pH 3.4). For the analysis of *P. balsamifera* crude extract, additional resolution was obtained by using a Gemini column (150 mm × 3 mm ID, 3.5 μm particle size) (Phenomenex, USA) at a flow rate of 0.5 mL/min with acetonitrile and water as solvents. MS detection was performed in both positive and negative ionization modes. Preliminary identification of compounds was then performed by matching UV spectra to that of metabolomic library entries. A positive identification was defined by similarity greater than 95% between the absorbance spectrum of an unknown peak and the library entry, as determined by the Agilent Chemstation software (B.01.03). Identifications were further corroborated by comparing fragmentation patterns with reference compounds and by performing co-chromatography.

Cell culture

The C2C12 murine skeletal myoblasts and the 3T3-L1 murine preadipocyte cell lines were acquired from the American Type Culture Collection (ATCC), Manassas, USA. Murine pancreatic β-cell (βTC) lines were provided by Dr. Shimon Efrat, Tel Aviv University, Tel Aviv, Israel. PC12-AC cells, a clonal derivative of the PC12 pheochromocytoma murine cell line that can be differentiated into a sympathetic peripheral neuronal phenotype, were obtained from the ATCC with clonal lines developed in the Bennett laboratory. Cell culture media were purchased from Invitrogen Life Technologies (Burlington, Canada) and Wisent (St. Bruno, Canada). Other reagents were purchased from Sigma-Aldrich (Oakville, Canada), unless otherwise specified below. All cell lines were cultured in a humidified incubator in a 5% CO_2 : 95% air atmosphere at 37 °C. The C2C12 myoblasts were proliferated in high-glucose Dulbecco's modified Eagle medium (HG-DMEM) supplemented with 10% fetal bovine serum (FBS), 10% horse serum (HS), and penicillin–streptomycin antibiotics. The C2C12 myoblasts were proliferated until 80% confluence, and were then passaged or differentiated for a period of 7 days into multinucleated myotubes in HG-DMEM containing 2% HS and antibiotics. The 3T3-L1 preadipocytes were proliferated until 80% confluent in HG-DMEM, 10% FBS, and antibiotics. Cells were then passaged or differentiated into adipocytes for 2 days in proliferation medium supplemented with 250 μmol/L 3-isobutylmethylxanthine (IBMX), 1 μmol/L dexamethasone (DMX), and 500 nmol/L insulin and an additional 7 to 10 days in proliferation medium supplemented with 500 nmol/L insulin. At the end of the differentiation period, more than 90% of the cells contained lipid droplets that could be viewed under low power magnification. This protocol was slightly altered for the adipogenesis experiment, whereby differentiation was initiated 1 day post-confluence and total differentiation time was 5 to 6 days. The βTC cell line was grown in HG-DMEM supplemented with 15% FBS, 2.5% HS, and antibiotics. Assays were performed when cells reached approximately 80% confluence. The PC12-AC cell line was cultured in RPMI 1640 medium containing 11 mmol/L glucose, 10% HS, 5% FBS, and antibiotics.

Determination of maximum nontoxic extract concentrations

We selected maximum nontoxic concentrations for each plant species in each bioassay to maximize the potential responses while minimizing potential toxic effects. The maximum nontoxic concentration of extracts was determined by treating differentiated C2C12 cells with extract concentrations ranging from 5 to 200 μg/mL for 24 h and assessing toxicity visually as the presence of any morphological changes or the incorporation of the trypan blue vital dye. Although toxicity studies were based on a 24-hour incubation period with each extract, morphology was assessed during the longer incubation periods with the extracts in adipocytes to ensure that no signs of toxicity were missed. The highest concentration that did not induce toxicity was

then tested on 3T3-L1 and βTC cells and adjusted as necessary. Concentrations selected are listed in Table 2, and these concentrations were used in all the bioassays involving these cell lines. Glucose toxicity and deprivation assays, as well as the diphenylpicrylhydrazyl (DPPH) assay, were performed on a range of concentrations, as described below. Extracts were solubilized in dimethyl sulfoxide (DMSO), filter sterilized, aliquoted, and stored at –20 °C. Aliquots were thawed freshly and dissolved in experimental medium at 1:1000, for a final DMSO concentration of 0.1%.

Table 2. Concentration of plant extracts used in glucose uptake, adipogenesis, and insulin secretion assays in the C2C12, 3T3-L1, and βTC cell lines.

Extract	C2C12, μg/mL	3T3-L1, μg/mL	βTC, μg/mL
G. hispidula	25	25	25
J. communis	5	5	5
K. angustifolia	50	50	50
L. clavatum	100	100	100
P. glauca	125	125	125
P. balsamifera	100	100	25
R. tomentosum	50	50	50
S. planifolia	25	25	25
V. vitis-idaea	200	200	200

Glucose uptake bioassay

To determine whether plant extracts had insulin-like or insulin-sensitizing effects on glucose uptake, C2C12 myotubes and 3T3-L1 adipocytes were treated with the maximum nontoxic concentration of plant extracts and rate of glucose uptake was determined by measuring the incorporation over time of ^{3}H-labelled deoxyglucose, a nonmetabolizable analogue of glucose. Both the C2C12 and 3T3-L1 cell lines express GLUT-1 and GLUT-4 glucose transporters when differentiated and exhibit insulin-regulated glucose uptake (Berti and Gammeltoft 1999; Calderhead et al. 1990; Klip and Paquet 1990). Cells grown in 12-well plates to 80% confluence and then differentiated as described above were treated with either vehicle (0.1% DMSO) alone, or with extracts solubilized in vehicle, or with a positive control solubilized in vehicle, for 1 h or 18 h. Metformin at 400 μmol/L (Kumar and Dey 2002) was used as the positive control for the C2C12 cell line, and fenugreek seed ethanolic extract at 75 μg/mL (Vats et al. 2002) was used for the 3T3-L1 cell line. Two hours before the 1 h treatment, differentiation medium was replaced with serum-free medium. The 1 h treatment was also performed in serum-free medium. The first 15 h of the 18-hour treatment were performed in complete differentiation medium. The medium was then replaced with serum-free medium containing fresh treatment for the remaining 3 h of the treatment. After the 18-hour treatment, cells were rinsed twice with warm KPB (Krebs phosphate buffer, in mmol/L: 20 Hepes, 4.05 Na_2HPO_4, 0.95 NaH_2PO_4, pH 7.4, 136 NaCl, 5 glucose, 4.7 KCl, 1 $CaCl_2$, and 1 $MgSO_4$) and were treated in the absence of insulin or the presence of 1 nmol/L (physiological range) or 100 nmol/L (supraphysiological range) insulin in KPB for 30 min in the presence or absence of extract or controls at 37 °C. Cells were then rinsed twice in warm glucose-free KPB and incubated in glucose-free KPB containing 0.5 μCi/mL 2-deoxy-D-[1-^{3}H]-glucose (TRK-383, Amersham Biosciences, Baie d'Urfé, Canada) for exactly 10 min at 37 °C. Cells were then placed on ice and rapidly rinsed 3 times in ice-cold glucose-free KPB. Cells were lysed in 500 μL of 0.1 mol/L NaOH for 30 min and scraped. The lysates, along with 1 mL of water, were each added to 4 mL of Ready-Gel 586601 liquid scintillation cocktail (Beckman Coulter, Fullerton, USA), and incorporated radioactivity was measured in a scintillation counter.

Insulin-secretion assay

The potential of the extracts to modulate glucose-stimulated insulin secretion (GSIS), and glucose-independent secretion was determined in βTC cells. These cells release insulin in response to physiological concentrations of glucose in a dose-dependent manner. Cells were seeded in 12-well plates, grown until 80% confluence, and treated with extracts or vehicle for 18 h at 37 °C, conditions in which other antidiabetic plant species (Benhaddou-Andaloussi et al. 2008), including some from the boreal forest ecosystem (Martineau et al. 2006), have demonstrated measurable effects on insulin secretion and βTC cell proliferation. The cells were then rinsed twice and preincubated with KRB (Krebs–Ringer buffer, in mmol/L: 10 Hepes, 25 $NaHCO_3$, 2 NaH_2PO_4, 1 KH_2PO_4, pH 7.4, 118 NaCl, 5 KCl, 2.5 $CaCl_2$, 1 $MgSO_4$, and 0.1% fatty acid-free BSA) for 1 h at 37 °C in the presence or absence of vehicle or extract (Benhaddou-Andaloussi et al. 2008; Fleischer et al. 1998; Hohmeier et al. 2000; Martineau et al. 2006; Spoor et al. 2006). The medium was then replaced with KRB containing 0.5 mmol/L IBMX plus extract or vehicle, and either 2, 6, 10, or 16 mmol/L glucose (GSIS), or 2 mmol/L glucose plus 45 mmol/L potassium, a non-fuel secretagogue (buffer adjusted to 45 mmol/L KCl and 79 mmol/L NaCl). After 1 h at 37 °C, media were collected and analyzed for insulin content as measured by radioimmunoassay (RIA) (Linco Research, St. Charles, USA) according to manufacturer's instructions (described below). Three replicates were performed for each experimental condition. The cells treated with extracts or vehicle and 2 mmol/L glucose were lysed with 1.25% HCl in 75% ethanol overnight at 4 °C, to extract intracellular insulin. The cells were then scraped and the lysates centrifuged at 12 000 rpm (13 400*g*) 5 min at 4 °C. Finally the supernatant was collected and stored at –80 °C until assayed for insulin by RIA. RIA was performed by using a Linco rat insulin assay kit, according to the manufacturer's instructions. Briefly, the samples were thawed and then diluted into phosphate assay buffer (50 mmol/L Na_2HPO_4, pH 7.5, 25 mmol/L EDTA, 1% BSA RIA-grade, and 0.01% thimerosal) to which radioactive ^{125}iodide and primary antibody against rat insulin (No. 1013) were added (all Linco Research). The reaction took place in 12 mm × 75 mm polypropylene RIA tubes for 24 h at 4 °C, after which precipitating reagent (Linco, No. 2020) was added for 20 min at 4 °C, and then the mixture was centrifuged at 5350*g* for 20 min at 4 °C. Supernatants were decanted and radioactivity in the pellets was measured with a Wallac Wizard 1470 γ-counter (Perkin Elmer, Woodbridge, Canada). To evaluate the effect of the extracts on β-cell proliferation, β cells were treated with vehicle control and

respective plant treatments overnight. The next day, 100 μL of water-soluble tetrazolium salt reagent (WST-1) was added to each well per the manufacturer's instructions and allowed to incubate for 1 h. Colorimetric analysis of formazan content was made by measuring the absorbance at excitation and emission wavelengths of 420 nm and 460 nm in a Wallac Victor 2 plate reader (Perkin-Elmer, Saint-Laurent, Canada).

Adipocyte differentiation assay

The objective of this assay was to screen for possible peroxisome proliferator-activated receptor gamma (PPARγ) stimulating activity. Activation of PPARγ in adipocytes by members of the thiazolidinediones class of antidiabetic medications, which includes rosiglitazone, results in accelerated adipogenesis that can be measured as increased accumulation of intracellular triglycerides early in the differentiation process (Gregoire 2001; Norisada et al. 2004; Tontonoz et al. 1995). As described above, differentiation was initiated in one-day post-confluent 3T3-L1 preadipocytes in the presence of vehicle, extract, or positive control (rosiglitazone 10 μmol/L; Alexis Biochemicals, Hornby, Canada). Differentiation was allowed to proceed for approximately 5 days, until triglyceride droplets could be visualized by phase-contrast microscopy in a small percentage of cells treated with vehicle. At this time, approximately 5- to 10-fold more cells contain triglyceride droplets in rosiglitazone-treated wells. Treatment was continuous throughout this differentiation period and medium containing the various conditions was replaced every day. Cells treated with vehicle in proliferation medium for the same duration served as a negative control. Intracellular triglyceride content was measured in live cells by AdipoRed fluorescent dye (Cambrex Bio Science, Walkersville, USA), a derivative of Nile red, according to the manufacturer's instructions. Briefly, the cells were washed twice with warm PBS (phosphate-buffered saline, in mmol/L: 8.1 Na_2HPO_4, 1.47 KH_2PO_4, pH 7.4, 137 NaCl, and 2.68 KCl), and then 2 mL of PBS were added to each well, followed by 60 μL of AdipoRed reagent. The reagent was mixed by gentle trituration and allowed to incorporate into cells for 15 min at 37 °C. Fluorescence was then measured in a Wallac Victor 2 plate reader at an excitation wavelength of 485 nm and an emission wavelength of 572 nm. Three separate experiments, each with 4 replicates, were conducted. For each experiment, the mean fluorescence value obtained from the negative control condition was considered as background and subtracted from all other readings.

Protection of PC12-AC cells from glucose toxicity and glucose deprivation

To evaluate the cytoprotective potential of extracts on neuronal precursor cells under conditions of hyperglycemia typical of unmanaged diabetes or of hypoglycemia typical of insulin shock, extracts were tested in cell-viability models of chronic glucose toxicity and of chronic glucose deprivation, as previously described (Harris et al. 2007*a*, 2007*b*; Spoor et al. 2006). PC12-AC cells were seeded in 96-well plates at a density of 6.25×10^3 cells/well and cultured with complete medium at 37 °C for 24 h. Medium was then replaced with serum-free medium supplemented with 0.025% BSA and with glucose concentration adjusted to 1.1 mmol/L (glucose deprivation) or 150 mmol/L glucose (glucose toxicity) plus extract or vehicle. Cells were maintained under these conditions for 96 h, over which time approximately 45% and 40% of vehicle-treated cells typically die under the glucose-toxicity and glucose-deprivation paradigms, respectively. The toxicity observed with high concentrations of glucose is not the result of osmotic stress, since replacing D-glucose by L-glucose abolishes toxicity (Koshimura et al. 2002). Cell viability at the end of the experimental period was assessed by WST-1 proliferation assay (cell proliferation reagent, Roche, Laval, Canada), in which 10 μL of WST-1 was added to each well and incubated for 45 min. The cells were then subjected to colorimetric analysis of resulting formazan content, and absorbance was measured at excitation and emission wavelengths of 420 nm and 620 nm. Absorbance was converted to number of viable cells using a standard curve generated in parallel with each experiment. Replicate experiments were conducted, each with 5 replicates for each condition. All extracts were tested at concentrations of 6.25, 12.5, and 25 μg/mL.

Evaluation of DPPH free radical scavenging activity

Antioxidant potential was evaluated by measuring reduction of the stable free radical DPPH (TCI America, Portland, USA) (Cotelle et al. 1996; McCune and Johns 2002). In each microplate well, 250 μL of 100 μmol/L DPPH (solubilized in methanol) was added to 40 μL of various concentrations of plant extract (range 5.0–100 μg/mL). After a 10-minute reaction at room temperature, the absorbance was read at 517 nm, with methanol used as a blank. Results were expressed as inhibitory concentration at 50% (IC_{50}) using the linear portion of a standard curve obtained with ascorbic acid, which was used as a reference antioxidant. The common polyphenols catechin, epicatechin, and quercetin were also used as reference antioxidants, and an extract of iceberg lettuce leaf (*Lactuca sativa*) was used as a negative control. Results were expressed as means ± SE from at least 3 independent experiments performed in duplicate.

Statistical analysis

Data were analyzed by one-way analysis of variance and Fisher PLSD (protected least significant difference) post hoc test using StatView software (SAS Institute, Cary, USA). Results are reported as means ± SE. The number of separate experiments conducted and the number of replicates per experiment are reported in the results section for each assay. Statistical significance was set at $p \leq 0.05$.

Results

Total phenolic content and phytochemical markers

A 20-fold range in phenolic content was observed across the different extracts. Extract yield, total phenolic content, and identified phytochemical markers are summarized in Table 3.

Effect of extracts on glucose uptake in myotubes and adipocytes

Plant extracts were tested for insulin-like or insulin-sensitizing properties by measuring effects on glucose transport in the presence and absence of insulin in 2 insulin-respon-

Table 3. Phytochemical characterization of extracts.

Sample	Source	% yield[a]	Total phenolic content[b]	Identified phenolic marker compounds
G. hispidula leaves	Mistissini	33.5	132.8±4.7	Chlorogenic acid; catechins; taxifolin; myricetin; quercetin glycosides
J. communis berries	Whapmagoostui	46.4	98.5±1.7	Catechins; kaempferol; quercetin glycosides
K. angustifolia leaves	Mistissini	41.8	268.5±5.7	Catechins; A-type procyanidin; quercetin glycosides
L. clavatum stems, shoots, and leaves	Mistissini	18.8	14.0±1.9	Ferulic acid derivatives; apigenin derivatives
P. glauca needles	Whapmagoostui	30.2	109.1±3.1	Hydroxystilbenes; kaempferol; quercetin and rhamnetin glycosides
P. balsamifera bark	Mistissini	26.5	94.5±7.7	Salicin; salicortins; salireposide; populosides
R. tomentosum leaves	Whapmagoostui	31.2	153.5±6.4	Chlorogenic acid; catechins; taxifolin; quercetin glycosides
S. planifolia bark	Whapmagoostui	35.0	240.3±6.5	Salicin; isosalireposide derivatives; tremulacin
V. vitis-idaea berries	Mistissini	74.5	59.9±4.2	*p*-Coumaric acid derivatives; catechins; cyanidin and quercetin glycosides

[a]Yield = (mass of recovered extract / mass dry plant material) × 100%.
[b]Total phenolics are quercetin equivalents (μg/mg of extract).

Fig. 1. Effect of extracts on basal and insulin-stimulated ^{3}H-deoxyglucose uptake in differentiated C2C12 skeletal muscle cells. Cells were treated with vehicle, extract (concentrations indicated in Table 2), or positive control for either 1 h (A) or 18 h (B) and then for 30 min in the absence or presence of insulin, as described in Materials and methods. Results are means ± SE relative to the vehicle-treated basal condition for 3 or 4 separate experiments each with 3 or 4 replicates. *, significantly increased uptake at $p \leq 0.05$ compared with vehicle without insulin, by ANOVA and post hoc Fisher LSD.

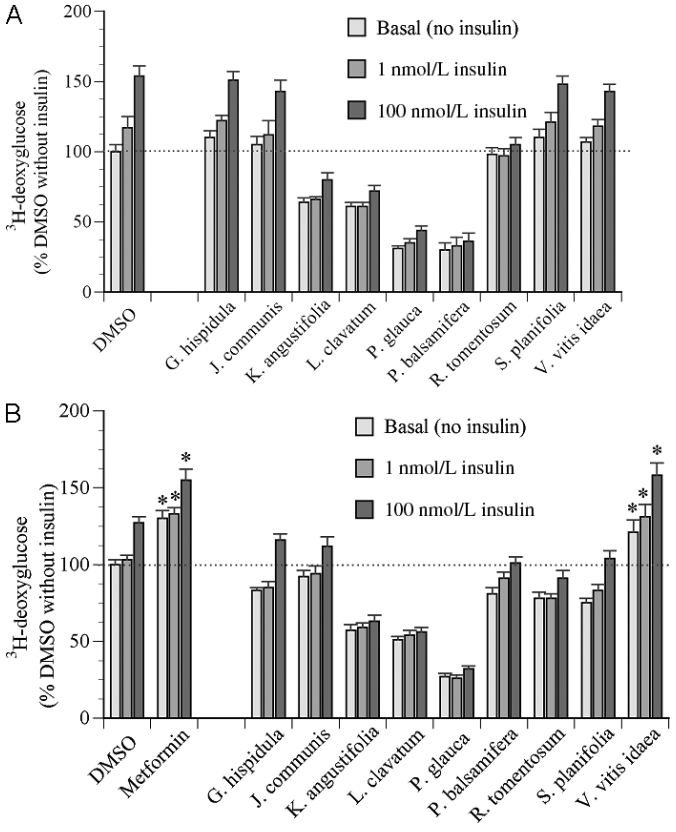
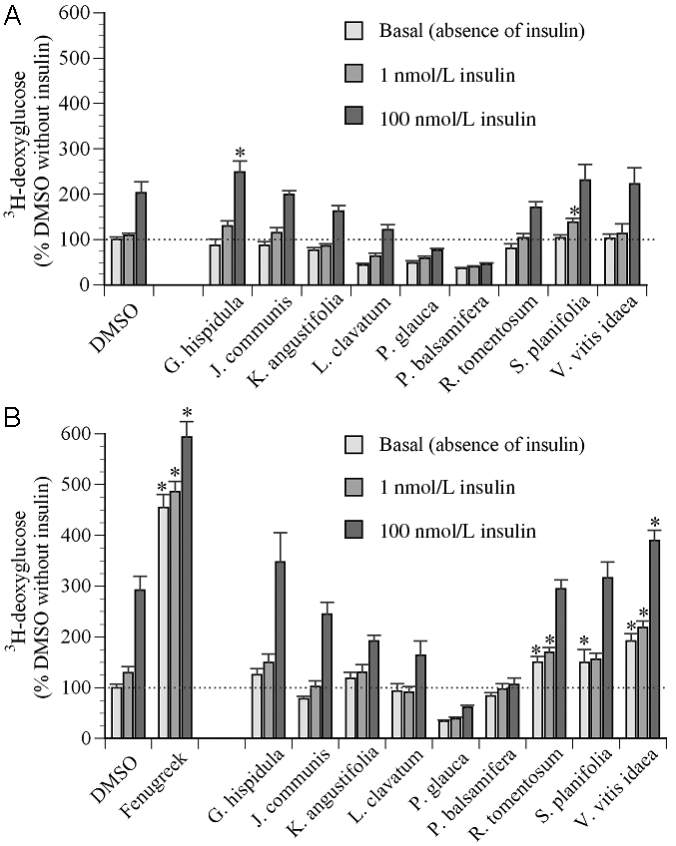


Fig. 2. Effect of extracts on basal and insulin-stimulated ^{3}H-deoxyglucose uptake in differentiated 3T3-L1 adipocytes. Cells were treated with vehicle, extract (concentrations indicated in Table 2), or positive control for either 1 h (A) or 18 h (B) and then for 30 min in the absence or presence of insulin, as described in Materials and methods. Results are means ± SE relative to the vehicle-treated basal condition for 3 or 4 separate experiments each with 3 or 4 replicates. *, significantly increased uptake at $p \leq 0.05$ compared with vehicle without insulin, by ANOVA and post hoc Fisher LSD.



sive and GLUT-4-containing cell lines, namely, C2C12 myoblasts (Fig. 1) and 3T3-L1 adipocytes (Fig. 2). Cells were treated with the maximum nontoxic concentration of each of the plant extracts either for 1 h (Figs. 1A and 2A) or 18 h (Figs. 1B and 2B) before the glucose uptake assay. In C2C12 cells, only *V. vitis-idaea* enhanced basal (21% more than that of vehicle alone) and insulin-stimulated glucose uptake (31% more than that of vehicle plus 1 nmol/L insulin) after 18 h incubation (Fig. 1B). In 3T3-L1 cells, *V. vitis-idaea* had a similar pattern of effects albeit of greater amplitude. Basal uptake was increased by 92% more than vehicle alone, and insulin-stimulated uptake was increased by 118% more than vehicle plus 1 nmol/L insulin. In addition 3 other extracts, *G. hispidula*, *R. tomentosum*, and *S. planifolia*, exhibited adipocyte-specific effects. *R. tomentosum* elicited effects only after 18 h of treatment. *G. hispidula* effects were statistically significant only after 1 h of treatment, but the strong tendency to increase glucose uptake in the presence of 100 nmol/L of insulin after 18 h of treatment failed to reach statistical significance. *S. planifolia* insulin-sensitizing effects were seen after 1 h whereas 18 h of treatment enhanced basal uptake. Differences in effect onset or duration may reflect differences in mechanism of action or in lipophilicity and metabolism of active molecules.

Effect of extracts on GSIS and total insulin content in pancreatic β cells

Basal (Figs. 3A, 3B, 3C), glucose-stimulated (Figs. 3A, 3B, 3C), and potassium-stimulated insulin secretion (Fig. 3D), as well as insulin content (Fig. 3E), were measured in βTC after treatment with extract or vehicle for 18 h. None of the 9 extracts potentiated secretion (Figs. 3A, 3B, 3C) or insulin content (Fig. 3E). Treatment with extracts did not significantly affect cell number (Fig. 3F).

Acceleration of adipogenesis in differentiating 3T3-L1 cells

Extracts were tested for adipogenesis-accelerating activity in 3T3-L1 preadipocytes treated continuously throughout a 5-day differentiation period. Intracellular content of triglycerides was assessed fluorescently in live cells at the end of the treatment. At that time, only a few vehicle-treated cells contained visible fat droplets, whereas a large number of rosiglitazone-treated cells contained such droplets, as assessed by phase-contrast microscopy. Triglyceride content was significantly increased by 3- to 4-fold by treatment with *K. angustifolia*, *L. clavatum*, *R. tomentosum*, and *V. vitis-idaea* extract (Fig. 4). These levels were statistically comparable with levels induced by treatment with 10 μmol/L rosiglitazone.

Cytoprotection of PC12-AC cells against glucose toxicity

The cytoprotective activity of the extracts under conditions of chronic glucose toxicity was assessed in PC12-AC cells cultured in serum-free medium containing 150 mmol/L glucose (14-fold more than normal) for 96 h (Fig. 5A). This model elicited a 45% decrease in viability of vehicle-treated cells by the end of the 96 h. Cells were treated continuously throughout this period with 6.25, 12.5, and 25 μg/mL of each extract. Five extracts were found to significantly inhibit glucose toxicity: *G. hispidula*, *L. clavatum*, *P. glauca*, *P. balsamifera*, and *R. tomentosum*. In the case of *P. glauca*, a dose–response effect was observed over the 4-fold range of concentrations tested. At 25 μg/mL, cytoprotection was almost complete (94% viability). The other 3 extracts exhibited maximum activity at lower concentrations (6.25 or 12.5 μg/mL). Nevertheless, *P. balsamifera* and *R. tomentosum* restored viable cell number to greater than 80% that of cells in optimal glucose conditions (Fig. 5).

Cytoprotection of PC12-AC cells against glucose deprivation

The cytoprotective activity of the extracts under conditions of chronic glucose deprivation was assessed in PC12-AC cells cultured in serum-free medium containing 1.1 mmol/L (10-fold less than normal) glucose for 96 h (Fig. 5B). This model elicited a 40% decrease in viability of vehicle-treated cells by the end of the 96 h. As before, cells were treated during this period with 6.25, 12.5, and 25 μg/mL of each extract. Four extracts significantly increased viability: *G. hispidula*, *P. glauca*, *R. tomentosum*, and *S. planifolia*. Two of these were also efficacious in the glucose toxicity paradigm above, and *P. glauca* again had the greatest cytoprotective effect. Overall, extracts were more effective at inhibiting glucose toxicity (Fig. 5A) then protecting cells from glucose deprivation (Fig. 5B) given the magnitude of cell response in the both paradigms.

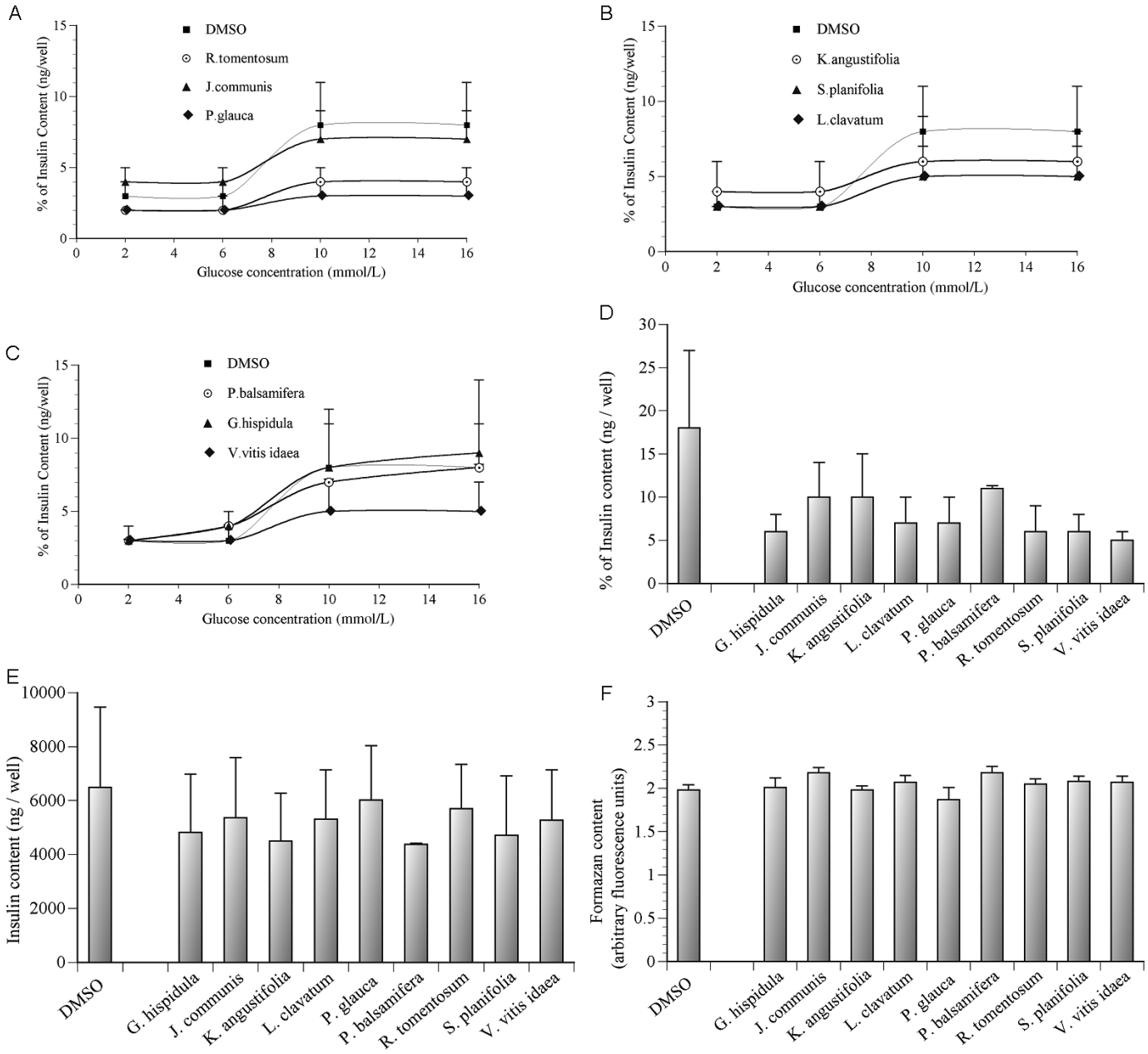
DPPH assay of free radical scavenging activity

The free radical scavenging activity of the 9 plant extracts was tested biochemically using the DPPH assay, with ascorbic acid as reference antioxidant and *Lactuca sativa* (iceberg lettuce) as negative control. Five species, *G. hispidula*, *K. angustifolia*, *P. glauca*, *R. tomentosum*, and *S. planifolia*, exhibited remarkable antioxidant activity statistically comparable with ascorbic acid (Fig. 6A). As expected, and as observed in the previous study, a significant positive correlation (Fig. 6B) was found between phenolic content (Table 3) and antioxidant activity measured with the DPPH assay.

Discussion

Type 2 diabetes has reached epidemic proportions among aboriginal populations of the world, such as the CEI of the James Bay area of northern Quebec in Canada. Among the CEI, an alarmingly high prevalence of diabetes is also accompanied by significantly poorer-than-average prognosis due in large part to inadequate compliance to conventional drugs and improperly managed hyperglycemia. Indeed, a need to establish complementary treatments was identified in a report on adherence to medicines in the CEI community of Mistissini, which concluded that cultural sensitivity is essential to the efficacy of prescribed treatments (Rideout and Menzies 1994). Our team therefore set out to evaluate the antidiabetic potential of CEI traditional medicines in close collaboration with healers and elders of CEI communities in the hope of identifying novel treatments for diabetes or complications of diabetes that would be more culturally acceptable to this population. Using a novel ethnobotanical approach, we identified several plant species of the CEI pharmacopoeia that are used traditionally to treat symptoms

Fig. 3. Effect of extracts on insulin secretion in pancreatic β cells (βTC). Cells were treated with vehicle or extract (concentrations indicated in Table 2) for 18 h and insulin secretion was then assessed over a 2 h period in response to 2, 6, 10, or 16 mmol/L glucose (panels A, B, and C), or 45 mmol/L potassium (D) by measuring insulin content of media at the end of this time. Insulin content was also assessed in cells from the 2 mmol/L glucose condition (E). Effect of extracts on cell proliferation was also assessed by looking at the WST-1 formazan content (F). Results are means ± SE normalized to insulin content for 3 or 4 replicates for each experimental condition. No significant increases in secretion, insulin content, or cell proliferation were observed. *, significantly decreased insulin content at $p \leq 0.05$ compared with vehicle-treated cells, by ANOVA and post hoc Fisher LSD.



associated with type 2 diabetes (Leduc et al. 2006). A first screening study successfully demonstrated the biological activity of 8 plant species that ranked highly in our ethnobotanical survey of 34 healers and elders of Mistissini (Spoor et al. 2006). Since this study, we have performed a second ethnobotanical survey of 31 healers and elders in Whapmagoostui, Quebec, a CEI community geographically distinct from Mistissini (results of this study are yet to be published). The combined ethnobotanical data indicate that there are 17 species relevant to the treatment of symptoms of diabetes in these 2 communities (Table 1). These have all been previously documented as medicinal plant species used by several aboriginal populations of Canada (Arnason et al. 1981; Black 1980; Leighton 1985; Marles and Farnsworth 1995; Marles et al. 2000; Moerman 1998; Siegfried 1994; Zieba 1992). Since we have already screened 8 of these 17 for potential antidiabetic activity, the present study was designed to assess the antidiabetic potential of the remaining 9 species using identical methodology as that in our first screening study.

Fig. 4. Effect of extracts on rate of adipogenesis, as assessed by triglyceride content at the end of a 5-day differentiation period with continuous treatment with vehicle, extract (concentrations indicated in Table 2), or positive control (10 μmol/L rosiglitazone). Results are means ± SE for 3 separate experiments each with 4 replicates each, normalized to the vehicle-treated condition. *, significantly increased intracellular triglyceride content at $p \leq 0.05$ compared with vehicle in differentiation medium, by ANOVA and post hoc Fisher LSD.

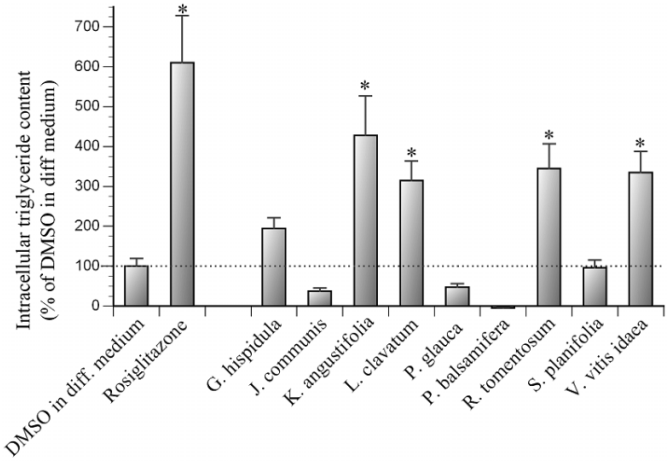
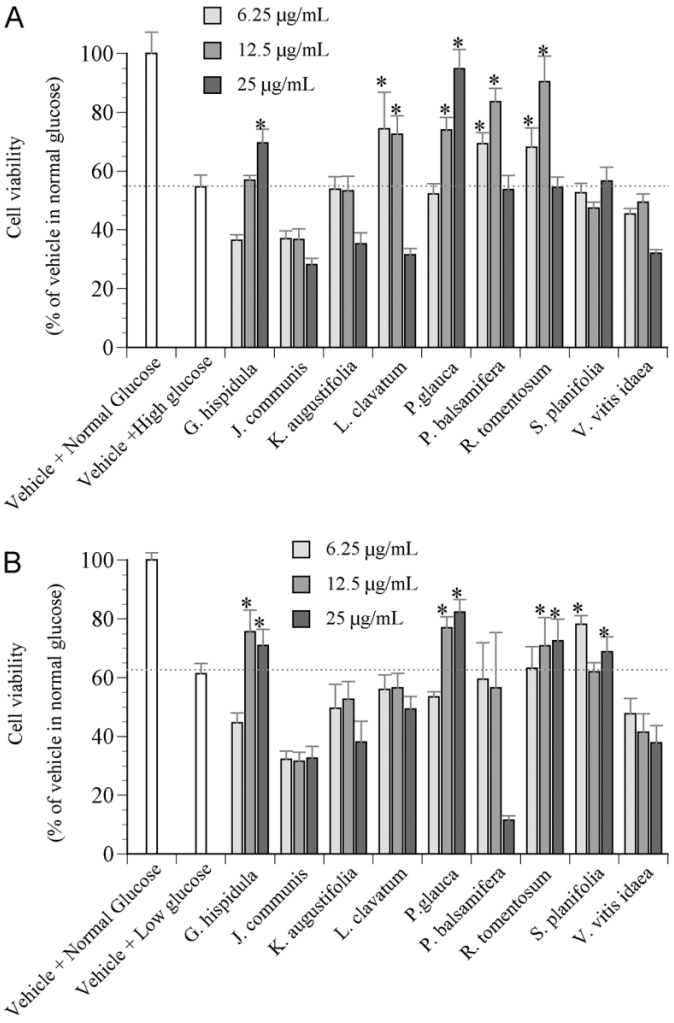


Fig. 5. Cytoprotective effects of extracts in PC12-AC cells under conditions of glucose toxicity (A) and glucose deprivation (B). Cells were treated with vehicle or 3 concentrations of extract for 96 h in 150 mmol/L or 1.1 mmol/L glucose. Number of viable cells was assessed by WST-1 viability–proliferation assay standardized to known cell number. Results are means ± SE ($n = 10$ for extracts, and $n = 64$ for pooled controls). *, significantly increased cytoprotection at $p \leq 0.05$ compared with vehicle-treated cells in high glucose (A) or low glucose (B), by ANOVA and post hoc Fisher LSD.

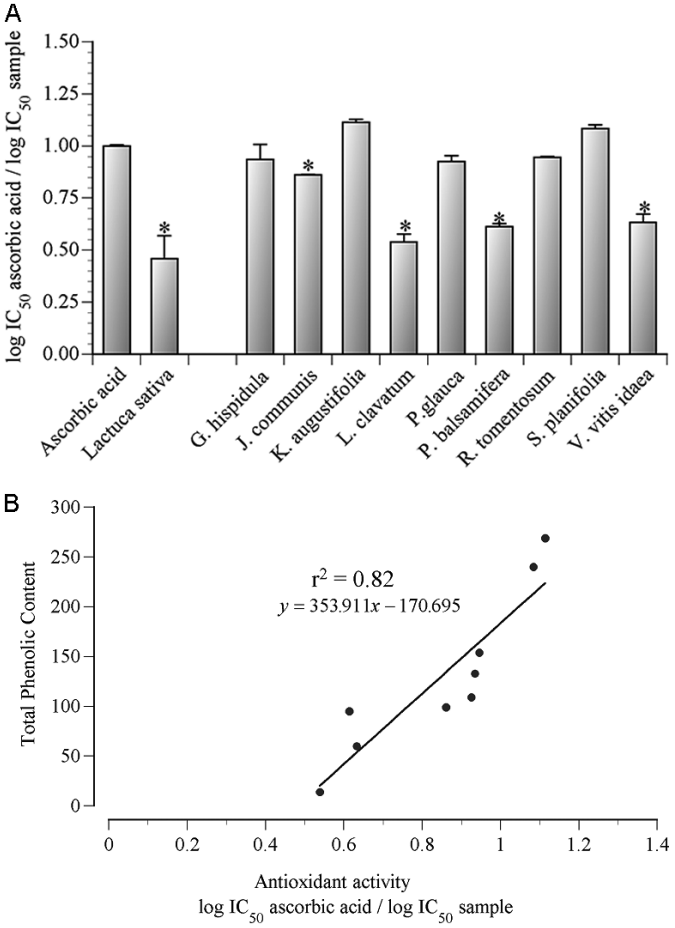


As before, plant material used in this study was collected as recommended by CEI healers and elders, and, for each species, the study focused on the specific plant organ used in traditional medicine. However, extracts were not prepared according to traditional methods such as hot water infusion; instead, plant material was extracted in 80% ethanol at room temperature, a well-established phytochemical approach that efficiently solubilizes the majority of polyphenolic molecules. Ethanolic extracts were then reconstituted in DMSO and applied directly to the various cell types, typically at the maximally tolerated concentration, to efficiently screen the 9 extracts using a series of integrative cell-based bioassays. This deviation from traditional methods of preparation was chosen to standardize the testing of the different species (hence allowing direct comparison to results from first screening study) and because detailed information concerning traditional methods was withheld during the ethnobotanical surveys to protect traditional knowledge. Currently CEI healers are using mostly single plant preparations, but it is possible that using multiple plants to treat symptoms of diabetes will give synergistic effects. For the purpose of this screening study, single plant preparations were used to clearly evaluate the effectiveness of individual plant species in the various bioassays performed.

The species studied here are already well-documented medicinal plants and at least 5 of the species evaluated in the present study belong to families that have been previously studied for antioxidant activity and other potentially antidiabetic activities (Cignarella et al. 1996; Liu et al. 2004; Marles and Farnsworth 1995; Pieroni et al. 2002; Schonlau and Rohdewald 2001; Virgili et al. 1998). However, the present study represents a first systematic screening of the antidiabetic potential of these species using complex bioassays.

Our bioassays were designed to address major components of the multifaceted etiology of type 2 diabetes. Hence, βTC were used to assess the ability of plant extracts to enhance glucose-stimulated insulin secretion. As was the case with our previous study (Spoor et al. 2006), none of the plants tested enhanced GSIS in our experimental conditions. However, other plants that we have studied using the same conditions had demonstrable effects on GSIS (Benhaddou-Andaloussi et al. 2008; Martineau et al. 2006). Nevertheless, it is possible that longer incubation periods may be necessary to rule out an action of the plant species on βTC. Glucose uptake in 2 insulin-sensitive tissues was selected to assess the ability of plant extracts to induce insulin-like effects or to potentiate the action of insulin. This bioassay identified 4 species with insulin-like effects. Similarly to our previous study (Spoor et al. 2006) and others by our laboratory (Benhaddou-Andaloussi et al. 2008; Martineau et al.

Fig. 6. (A) DPPH free radical scavenging activity of extracts. Ascorbic acid was used as the reference antioxidant, and *Lactuca sativa* (lettuce) extract was used as a negative control. Results are means ± SE for 3 experiments each with 4 replicates and are concentrations corresponding to ascorbic acid IC_{50}. *, significantly lower activity at $p \leq 0.05$ compared with ascorbic acid, by ANOVA and post hoc Fisher LSD. (B) Correlation between extract DPPH free radical scavenging activity and total phenolic content. Results were analyzed by linear regression and the equation is $y = 353.91x - 170.70$.



2006; Vuong et al. 2007), glucose uptake was typically increased in the absence of insulin, and this effect was additive to that of insulin rather than potentiating it. These findings suggest activation of an insulin-independent signaling pathway such as the AMP-activated protein kinase (AMPk) pathway. The latter is the pathway through which metformin increases glucose uptake in skeletal muscle and inhibits glucose production in liver while also restoring insulin sensitivity in these tissues (Klip and Paquet 1990; Musi et al. 2002). AMPk is also present in adipocytes and could similarly intervene in the action of the plant species. However, given the adipocyte-specific effects of some of the present and past (Spoor et al. 2006) Cree plant species, other adipocyte-specific mechanisms may also come in to play (Gual et al. 2003). It is unclear from the present study why 3 of the 4 species positive in these tests exhibit cell-type specificity; this may simply be the result of differences in onset or duration of action that are not captured by the 2 treatment durations chosen for screening. However, such cell-type differences may be advantageous for the design of more tissue-specific treatments.

An assay for accelerated adipogenesis, a hallmark of the thiazolidinedione class of antidiabetic medications, was used as a rapid screen of PPARγ activation. Clearly this test is prone to false positives because adipogenesis can be modulated by several other mechanisms; positive results therefore require confirmation with a specific reporter gene assay. Nevertheless, the finding that 4 extracts greatly accelerated triglyceride accumulation is a surprising and positive one. Interestingly, 2 of the species that enhanced adipogenesis also enhanced glucose uptake in mature adipocytes. Because activation of AMPk has been shown to increase adipogenesis as well as uptake (Krook et al. 2004; Misra and Chakrabarti 2007; Musi and Goodyear 2003), this may support this insulin-independent pathway as a mechanistic basis for some of the observed activities.

Antidiabetic activity can take the form of antihyperglycemic activity or of attenuation of complications secondary to hyperglycemia. Such complications occur in the pancreas, kidney, microvasculature, and perhaps most importantly, in the peripheral nervous system. Neuronal cells and their peripheral precursor cells are vulnerable to chronically elevated glycemia as well as to hypoglycemia, and either condition can induce apoptosis and inhibit peripheral nerve regeneration (Honma et al. 2003; Vincent et al. 2005). Five extracts protected PC12-AC cells from glucose toxicity. Four extracts inhibited toxicity associated with glucose deprivation. It is noteworthy that 3 extracts, *G. hispidula*, *P. glauca*, and *R. tomentosum*, exerted cytoprotective effects under both conditions of glucose toxicity and glucose deprivation and that *P. glauca* was the most efficacious in both cases. Interestingly, an absence of correlation between antioxidant activity and cytoprotection suggests that more complex mechanisms are mediating these effects. In support of this hypothesis, we have previously shown that discrete phenolics, many of which may represent active ingredients of the plant extracts tested in this study, exhibit novel mitogenic, antiapoptotic, and cytoprotective activities including direct inhibition of caspase-12 associated with endoplasmic reticulum stress (Harris et al. 2007*a*, 2007*b*). Thus, direct induction of antiapoptotic signaling pathways may in part underlie the observed protection against glucose toxicity and deprivation. Clearly, this hypothesis will require further validation once active compounds within these complex extracts are identified.

Finally, a measure of antioxidant activity, a property important to the pathophysiology of diabetic complications (Haidara et al. 2006; Triggiani et al. 2006), revealed that 5 extracts exhibited free radical scavenging activity comparable to vitamin C. As expected, antioxidant activity was well correlated to total phenolic content.

Because of the complexity of diabetes, numerous assays are required to confidently assess the antidiabetic potential of novel products. Although the assays used here test for classic antihyperglycemic and cytoprotective activities, it is clear that antidiabetic activity may be exerted at other sites not tested. For example, interfering with digestion, absorption, or nutrient sensing in the gut may represent other antihyperglycemic mechanisms, whereas the inhibition of

protein glycation may represent another form of cytoprotective activity. Further studies will be necessary to provide an exhaustive assessment of antidiabetic activity of the 17 boreal plant species of interest.

It is interesting that when screening results for all 17 plant species of interest are pooled (present study with that of Spoor et al. 2006), we find that 11 species stimulated glucose uptake, 8 accelerated adipogenesis, 8 protected against glucose toxicity, 7 protected against glucose deprivation, 9 exhibited high antioxidant activity, whereas none enhanced insulin secretion. Taken together, these findings are encouraging in light of the ultimate goal of this project, namely, developing culturally adapted treatments for diabetes. It is noteworthy that the bioassay that produced the highest number of positive results is the glucose uptake assay and it is tempting to speculate that a common mechanism may be involved. As a whole, the findings support the power of the ethnobotanical approach used to select species. It should be noted that the apparent absence of a correlation between measured activity and ethnobotanical ranking within our dataset is probably the result of our using the ranking primarily to eliminate species not likely relevant to the treatment of diabetes; the predictive power of the ethnobotanical approach may have been more apparent had all 35 cited species been tested.

The results of the present study clearly demonstrate that several species of this second set of medicinal plants also possess promising antidiabetic activity, thereby further confirming the soundness of our ethnobotanical approach and the potential of identifying efficacious treatments complementary to modern medication, such as biguanides, sulfonylureas, and thiazolidinedione. Further validation, notably using in vivo models of diabetes and human clinical trials, will constitute the next steps of this project. Studies will also be required to address the elucidation of active principles and their mechanisms of action. Such information will allow standardization of treatments based on content of active ingredients or on biological activity, rather than on content of nonspecific phytochemical markers.

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