Medicinal plants of Cree communities (Québec, Canada): antioxidant activity of plants used to treat type 2 diabetes symptoms[1]

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Abstract: Traditional medicines (TM) used to treat symptoms of diabetes by two Cree communities were assessed for their free radical scavenging activity using the stable 1,1-diphenyl-2-picrylhydrazyl radical, and their ability to protect human low-density lipoprotein from Cu^{2+}-mediated oxidation by measuring lag time before the appearance of conjugated dienes and formation of thiobarbituric acid-reactive substances. Water-soluble phenolic content was also measured. A total of 20 medicinal plants from Whapmagoostui and 16 from Mistissini were compared with 16 extracts of plants that were not used medicinally. Medicinal plant extracts, particularly those from *Larix laricina*, displayed high antioxidant activity, comparable with ascorbic acid, Trolox, and the known antioxidant flavonoids quercetin, epicatechin, catechin. Extracts of Pinaceae and Ericaceae contained the highest levels of phenolics. Factors such as season and area of plant collection, as well as the plant part from which the extract was derived, affected antioxidant activity. Positive correlations were found between the established traditional knowledge of Cree Elders and Healers and the antioxidant activity for medicinal plants used in Mistissini ($r = 0.3134$; $p = 0.058$) and in Whapmagoostui ($r = 0.5165$; $p = 0.001$). Significant correlations between phenolic content were also seen with the existing ethnobotanical data ($r = 0.5015$; $p = 0.003$) and bioassays ($r = 0.4178$; $p = 0.003$). These results indicate that a clear majority of plants used by the Cree are excellent sources of antioxidants.

Key words: antioxidants, phenols, Cree, diabetes, traditional medicine.

Résumé : La médicine traditionnelle utilisée par deux communautés cries du Québec pour traiter un ensemble de symptômes liés au diabète a été évaluée pour ses propriétés antioxydantes. Trois bio-essais ont été utilisés à cet effet, soit en recourant au radical 1,1-diphényl-2-picrylhydrazyl (DPPH), soit en mesurant le temps avant que n'apparaissent les dièmes conjuguées (CD) ou les substances réactives à l'acide thiobarbiturique (TBARS) alors que les lipoprotéines humaines de faible densité sont soumises à l'oxydation au Cu^{2+}. De plus, le contenu total en phénols hydrosolubles a été mesuré. Un groupe de 20 plantes médicinales de Whapmagoostui et de 16 autres provenant de Mistissini a été comparées à 16 extraits issus de plantes qui ne sont pas utilisées par les Cris de façon médicinale. Les plantes médicinales, et particulièrement le *Larix laricina*, ont montré une forte activité antioxydante comparable à celle des contrôles positifs (acide ascorbique, Trolox et certains flavonoïdes antioxydants tels que quercétine, épicatéchine et catéchine). Les Pinaceae et Ericaceae contiennent les taux de phénols les plus élevés. Plusieurs facteurs jouent sur l'activité antioxydante des plantes. Parmi ceux-ci, on peut énumérer la saison et le site de récolte ainsi que la partie ou organe utilisé. Des corrélations positives entre le savoir traditionnel des Aînés et guérisseurs cris, d'une part, et l'activité antioxydante des plantes médicinales utilisées à Mistissini ($r = 0{,}3134$; $p = 0{,}058$) et à Whapmagoostui ($r = 0{,}5165$; $p = 0{,}001$), d'autre part, ont été observées. Également, des corrélations significatives ont pu être observées entre le contenu en phénols et les données ethnobotaniques ($r = 0{,}5015$; $p = 0{,}003$), ainsi que les bio-essais ($r = 0{,}4178$; $p = 0{,}003$). Ces résultats indiquent clairement que la majorité des plantes sélectionnées par les Cris forment une excellente source d'antioxydants.

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Mots-clés : activité antioxydante, phénols, Cri, diabète, plantes médicinales.

Introduction

Type 2 diabetes (T2D) is increasing worldwide and now affects approximately 171 million people (World Health Organization 2006). Canadian indigenous people are especially at risk (Young et al. 1985) owing to changes in traditional lifestyle and the effects of industrialization on both environmental and sociocultural norms (Berkes and Farkas 1978). The Crees of Eeyou Istchee are particularly affected by this disease (Robinson 1988; Robinson et al. 1995; Thouez et al. 1990), with a doubling of T2D prevalence over the past 15 years from 7% to 14% (Brassard et al. 1993; CBHSSJB 2001).

T2D is a chronic disease involving insulin resistance and impaired glucose metabolism (World Health Organization 2006), characterized by chronic hyperglycemia and development of micro- and macrovascular complications. Among T2D complications, atherosclerosis (Piconi et al. 2003) is a major cause of death (Scheffer et al. 2005). Other T2D symptoms have been listed by Leduc et al. (2006) and McCune and Johns (2002). The vascular complications of T2D have been attributed, among other factors, to the accumulation of advanced glycation end products (AGE) formed by the non-enzymatic modification of tissue proteins by physiological sugars in vivo (Brownlee 1995). These promote the production of reactive oxygen species (ROS) (Opara 2002; Piconi et al. 2003), which cause cell injury. Therefore, the glycoxidation and lipoxidation of cellular components in the diabetic state suggest a potentially beneficial role for dietary antioxidants to counter the oxidative stress associated with the initiation and progression of T2D complications (Giugliano et al. 1996; Baynes and Thorpe 1999).

Low-density lipoprotein (LDL) aids in the transport of cholesterol in the blood to peripheral cells (Fruchart 1992). Modification of native LDL via glycation or oxidation leads to the retention of LDL in the vascular walls and the enhanced migration and uptake of oxidized LDL (oxLDL) by circulating macrophages, resulting in the formation of foam cells and eventually plaque (Baynes and Thorpe 2000; Scheffer et al. 2005). This process, which implicates oxLDL, provides a possible explanation for the increased risk of atherosclerosis among T2D patients (Baynes and Thorpe 2000; Levy et al. 2000).

Antioxidants, owing to their ability to inhibit the negative effects of ROS (Salonen et al. 1995; Halliwell 1997), are potential therapeutic aids for numerous diseases, including the cardiovascular complications associated with late-stage T2D. Antioxidants, in particular flavonoids and related phenolic compounds, vitamins C and E, are present in fruits, vegetables, spices, and medicinal plants (Chipault et al. 1952; Arnason et al. 1981; Larson 1988; Proteggente et al. 2003). The consumption of antioxidants has been shown to improve certain pathologies in T2D patients (Farvid et al. 2005) and even mediate metabolic oxidative status (Douillet et al. 1998). Likewise, ascorbic acid and α-tocopherol, when supplemented to human subjects (Rifici and Khachadurian 1993), inhibit in vitro Cu^{2+}-mediated lipid peroxidation of LDL as assessed by reduced levels of malondialdehyde (MDA) and prolongation of lag time before the appearance of conjugated dienes (CD) (Esterbauer et al. 1989, 1991). Thus antioxidant supplementation may be a plausible treatment for T2D and its complications (Opara 2002).

Traditional medicine (TM) continues to be used worldwide by many indigenous people (West 1974). To date, over 1200 antidiabetic plant species used around the world have been identified for the treatment of T2D symptoms (Marles and Farnsworth 1995), with more than 80% of 295 species reviewed showing strong activity in hypoglycaemic analyses. McCune and Johns (2002) assessed 35 medicinal plants from boreal areas that were traditionally used to treat T2D symptoms by First Nations people in Canada. Compared with some commercially available vegetables, these showed significantly higher antioxidant activity. Among the Cree Nation of Québec, a community-based program was established to assess the antidiabetic potential of TM in Mistissini (Leduc et al. 2006; Spoor et al. 2006) and Whapmagoostui (Fraser et al. in press). Given that TMs are currently used in both regions for the treatment of T2D symptoms, it is important to assess their bioactivity or toxicity to support or discourage their use in any possible public health care strategies targeting T2D.

The current report evaluates the antioxidant activity and LDL-protective effects of Cree medicinal plant extracts identified in the ethnobotanical surveys conducted by Leduc et al. (2006) and Fraser et al. (in press). Extracts were ranked according to their activity and compared with the Syndromic Importance Value (SIV), an index of relative antidiabetic importance calculated for each species that took into account frequency of mention, number of symptoms treated, importance of the symptom, and the number of persons interviewed (Leduc et al. 2006; Fraser et al., in press). In addition, the phenolic content of the extracts was calculated to determine whether these were the responsible antioxidant phytochemicals in our experiments.

Materials and methods

Plant extracts

Ethnobotanical studies were carried out in two Cree communities in Northern Québec in the summers of 2003 and 2004 (Leduc et al. 2006; Fraser et al. in press). Elders and Healers were interviewed concerning the treatment of 15 symptoms associated with T2D. A total of 5 collections of fresh material of specific plant tissues, for example berries, needles, or inner bark, were gathered in different plant populations or individuals to account for genotypic and phenotypic variation within a species. Plant species with no medicinal use by the Cree were chosen randomly from Whapmagoostui as negative controls. Voucher specimens were deposited at the Herbier Marie-Victorin (MT), the

Table 1. Characteristics of plants used (medicinal) or not used (non-medicinal) by the Crees in treatment of type 2 diabetes symptoms, with antioxidant ranking according to the combined ranks of 3 assays.

Scientific name	Cree name(s)	Family	Plant part[a]	Area[b]	Year	Extract yield, %	Voucher No.	Rank[c]
Medicinal plants								
Abies balsamea (L.) P. Mill.	Iyaasiht	Pinaceae	Ca	M	2003		2003–01	27
Alnus incana (L.) Moench subsp. *rugosa* (Du Roi) Clausen	Atushpi	Betulaceae	Ca	M	2003		2003–04	33
Cladonia rangiferina (L.) Weber *ex* F.H. Wigg.	Waapskamihkw	Cladoniaceae	A	W	2004	5.7	2004–51	49
Empetrum nigrum L.	Ishchiminh	Empetraceae	Be	W	2004	45.7	2004–05	48
Gaultheria hispidula (L.) Muhl. *ex* Bigelow[d]	Pieuminaan	Ericaceae	Be	M	2003		2003–07	36
Juniperus communis L.	Kaakaachiminh	Cupressaceae	Be	W	2004	46.7	2004–06	28
			N	W	2004	28.1	2004–06	29
			R	W	2004	5.07	2004–06	2
Kalmia angustifolia L.	Uishichipukw	Ericaceae	L	M	2004	32.8	2003–13	25
Larix laricina (Ru Roi) K. Koch.	Waachinaakan	Pinaceae	Ba	M	2003			
			Ba	M	2004	4.9		3
			Ca	W	2004	37.9	2004–11	1
Leymus mollis (Trin.) Pilger	Wiinpaakw miskuusiuh	Poaceae	A	W	2004	10.2	2004–08	39
Lycopodium clavatum L.	Pashtnahoagin	Lycopodiaceae	A	M	2004	15.3	2003–31	40
Picea glauca (Moench) Voss	Minihikw	Pinaceae	Co	W	2004	49.8	2004–12	8
			G	W	2004	87.0	2004–12	20
			N	W	2004	24.5	2004–12	7
Picea mariana (Mill.) B.S.P.			Co	M	2003		2003–15	31
			G	W	2004	91.3		22
Pinus banksiana Lamb.			Co	M	2003		2003–14	23
			Co	W	2004	6.8	2004–03	18
Populus balsamifera L.			Ca	M	2004	13.9	2003–49	32
Rhododendron groenlandicum (Oeder) Kron & Judd	Wiisichipukh	Ericaceae	L	M	2003		2003–02	
			L	M	2004	16.8		19
			L	W	2004	24.2	2004–15	17
Rhododendron tomentosum (Stokes) Harmaja subsp. *subarcticum* (Harmaja) G. Wallace	Wiisichipukh	Ericaeae	L	W	2004	17.35	2004–33	15
Salix planifolia Pursh subsp. *planifolia*	Waaskaayaapaatukh	Salicaceae	Ba	W	2004	26.0	2004–37	16
Sarracenia purpurea L.	Ayigadash	Sarraceniaceae	L, R	M	2004	4.52		24
			L, R	M	2003		2003–05	
Sorbus decora (Sarg.) Schneid.	Miskuumischi	Rosaceae	Ba	M	2004	4.5	2004–14	12
			Ba	M	2003		2003–10	5
			L	W	2004	25.7	2004–14	46
			S	W	2004	12.4	2004–14	9
Sphagnum fuscum (Schimp.) Klinggr.	Awasistche	Sphagnaceae	A	W	2004	0.9	2004–77	37
Vaccinium vitis-idaea L. var. *minus* (Lodd.) Hultén	Wiisichiminh	Ericaceae	Be	W	2004	48.4	2004–21	34
Vaccinium uliginosum L.	Iiyiyiminh	Ericaceae	R	W	2004	10.5	2004–20	6
Non-medicinal plants[e]								
Achillea millefolium L.	Miskogotuck, mishishstock, wabish	Asteraceae	A		2004	1.8	2004–29	38
Armeria maritima (P. Mill.) Willd. subsp. *sibirica* (Turcz. *ex* Boiss.) Nyman	Sheesheeminshounn	Plumbaginaceae	A		2004	3.8	2004–09	41
Betula glandulosa Michx.		Betulaceae	L		2004	29.4	2004–02	21
Campanula rotundifolia L.	Anstinnigansh	Campanulaceae	A		2004	26.4	2004–07	30
Chamerion angustifolium (L.) Holub	Michskishoe, nischiikhan, wobagun	Onagraceae	A		2004	15.8	2004–17	13
Equisetum arvense L.	Neeskann, miskouchoe	Equisetaceae	A		2004	8.4	2004–32	45
Hippuris vulgaris L.	Astchebaog	Hippuridaceae	A		2004	15.5	2004–23	10
Honckenya peploides (L.) Ehrh.	Weneboug	Crassulaceae	A		2004	8.0	2004–59	47
Loiseleuria procumbens (L.) Desv.	Itchkaseeboaukish	Ericaceae	A		2004	27.5	2004–75	4
Myrica gale L.	Estiminatuck	Myricaceae	L, S		2004	4.8	2004–36	14
Rhytidium rugosum (Hedw.) Kindb.	Beastaskugg, beastaskumuk	Sphagnaceae	A		2004	2.1	2004–38	42
Rubus chamaemorus L.	Shicoudaw	Rosaceae	A		2004	33.1	2004–58	26
Salix arctophila Cock. *ex* Heller	Waaskaayaapaatukh	Salicaceae	S, Ba		2004	25.7	2004–55	35

Table 1 (*concluded*).

Scientific name	Cree name(s)	Family	Plant part[a]	Area[b]	Year	Extract yield, %	Voucher No.	Rank[c]
Sphagnum russowii Warnst.	Awasistche, awashesstees, misheskosha, mistoushann	Sphagnaceae	A		2004	1.6	2004–34	11
Stereocaulon paschale (L.) Hoffm.	Wapi-jeshkumuk	Stereocaulaceae	A		2004	7.9	2004–76	44
Tanacetum bipinnatum (L.) Schultz-Bip. subsp. *huronense* (Nutt.) Breitung	Neebeshe, miskishaw	Asteraceae	A		2004	11.9	2004–19	43

[a]Abbreviations of part used: A, all; Ba, bark; Be, berries; Ca, cambium; Co, cone; G, gum; L, leaf; N, needle; S, stem; R, root.

[b]Fresh material was collected from 2 Cree communities in Northern Québec: M, Mistissini; W, Whapmagoostui.

[c]The ranking method measured the individual rank of antioxidant activity of plant species, which was the mean of normalized values of each plant obtained among bioassays, where 1 is the strongest in terms of antioxidant activity and 49 is the weakest.

[d]Only leaves were collected as fresh material.

[e]Non-medicinal plants were only collected in Whapmagoostui.

Cree Cultural Institute at Oujé-Bougoumou, and in schools of both Mistissini and Whapmagoostui. Fresh plant material, stored at –4 °C, was lyophilised, finely ground using a Wiley mill with a 2 mm mesh, and extracted with 80% ethanol using the Tecator Soxtec Extraction System HT2. Ethanol was evaporated under vacuum (Büchi 461 rotovapor, Switzerland), freeze-dried, and stored at –20 °C in amber vials with Teflon-lined caps.

DPPH free radical scavenging activity

The free radical scavenging activity of DPPH (1,1-diphenyl-2-picrylhydrazyl) (TCI America, Portland, Ore.) was measured according to the procedure described by Blois (1958) and Cotelle et al. (1996), with subsequent modifications by McCune and Johns (2002) and Owen and Johns (2002). Different concentrations of extract were dissolved in 0.5 mL ethanol, to which 3 mL of 100 μmol·L^{-1} DPPH was added. The reaction was allowed to stand for 10 min at room temperature. Absorbance at 517 nm was measured using a Beckman DU 640 spectrophotometer. An inhibitory concentration at 50% (IC_{50}) was calculated from the linear portion of the dose–response curve obtained from ascorbic acid. Based on the work of Yokozawa et al. (1998), an approximate scale was used to determine the scavenging activity of the plant extracts: below an IC_{50} of 25 ppm, plants were considered very strong scavengers; 25–50 ppm as strong; 50–100 ppm as moderate; and finally no activity for >100 ppm. Catechin, epicatechin, and quercetin were used as positive controls.

Low-density lipoprotein determination

Human LDL (Intracell, Frederick, Md.) dissolved in 1 mmol·L^{-1} PBS (0.175 g Na_2HPO_4 and 1.05 g $Na_2HPO_4{\cdot}H_20$ in 1000 g millipore water, pH 7.4) was passed through a Sephadex PD-10 (Sigma Chemical Co.) column to remove EDTA and NaCl. Lipoprotein concentration was estimated with a Sigma Diagnostics protein assay kit (Sigma Chemical Co.) using bovine serum albumin as a standard. The final LDL concentration obtained was 100 μg·mL^{-1}.

Conjugated dienes formation

Determination of lag time before the appearance of conjugated dienes on oxLDL was carried out according to the methods of Esterbauer et al. (1989). In a 96-well UV-transparent microtiter plate, 5 ppm plant extract, 15 μmol·L^{-1} $CuSO_4$ dissolved in phosphate-buffered saline (PBS), and 100 μg·mL^{-1} LDL were mixed to a final volume of 200 μL and absorbance continuously monitored at 234 nm for up to 15 h using a μQuant universal microplate spectrophotometer (Bio-Tek Instruments, Inc.). Lag time was calculated as the intersection with the propagation phase, described by Esterbauer et al. (1989, 1990). Ascorbic acid (28.39 μmol·L^{-1}) and Trolox (19.98 μmol·L^{-1}) were used as positive controls.

Thiobarbituric acid-reactive substances assay

Thiobarbituric acid-reactive substances (TBARS) were measured following the methods of Sobal et al. (2000). Plants extract at 5 ppm and 100 μg·mL^{-1} LDL dissolved in 1 mmol·L^{-1} PBS was incubated for 1 h at room temperature. The oxidation of LDL was initiated with 15 μmol·L^{-1} $CuSO_4$ and 120 μL of solution mixture (total volume 500 μL) removed immediately and again after 90, 180, and 240 min. At each end point, 10 μL of 400 μmol·L^{-1} EDTA dissolved in 1 mol·L^{-1} NaOH and 10 μL 10 μmol·L^{-1} BHT were added and the solution put on ice to halt oxidation. Thereafter, 50 μL of 50% *w*/*v* TCA and 75 μL of 1.3% *w*/*v* TBA dissolved in 0.05 mol·L^{-1} NaOH were added. The solution was incubated at 60 °C for 40 min, put on ice, and centrifuged at 2000*g* for 5 min. Then, 200 μL of supernatant was removed and transferred to a 96-well microtiter plate. Fluorescence (excitation wavelength 510 nm, emission wavelength 553 nm) was measured using a FlexStation II fluorometer (Molecular Devices Corp., Sunnyvale, Calif.). The amount of TBARS released was determined from a standard curve of 1,1,3,3-tetraethoxypropane and expressed as nanomoles of MDA equivalents per milligram LDL. Ascorbic acid (28.39 μmol·L^{-1}) and Trolox (19.98 μmol·L^{-1}) were used as positive controls.

Water-soluble phenolics

The amount of water-soluble phenolics was determined with the Folin-Ciocalteau colorimetric assay (Singleton and Rossi 1965) with the following modifications. Five millilitres of Folin-Ciocalteau reagent (Sigma Chemical Co.), 1:10 with distilled water, was added to 1 mL aliquots of the diluted sample, mixed well, and left at room temperature for 3–8 min. Subsequently, 4 mL of 7.5% Na_2CO_3 was added and the mixture incubated for 2 h at room temperature and in darkness. Phenol concentration was determined spectrophotometrically at 725 nm from a tannic acid standard curve

Table 2. DPPH radical scavenging activity and inhibition of Cu^{2+}-mediated oxidation of LDL by Cree medicinal and non-medicinal plant extracts from Mistissini and Whapmagoostui.

			TBARS, nmol MDA eq/mg LDL			
	DPPH, IC_{50}, ppm	CD, lag time, min	0 min	90 min	180 min	240 min
Native LDL			3.010±1.23[a]	4.43±1.91	2.65±1.42	1.57±1.01
Oxidized LDL		102.61±2.28[j,kl]	2.61±1.01[r,s,t]	47.82±9.15	158.29±16.52	201.92±12.39
Trolox		130.59±9.84[f,g,h]	0.75±0.27[a,b,c,d,e]	12.20±1.05	37.52±4.95	48.33±4.05
Ascorbic acid	3.84±0.01[a]	108.26±3.13[i,j]	4.04±3.48[s,u]	42.89±3.48	166.17±19.84	204.32±10.91
Quercetin	4.42±0.23[a,b]					
Catechin	5.56±0.37[a,b,c]					
Epicatechin	5.94±0.18[a,b,c]					
Medicinal plants						
A. balsamea	28.72±1.63[a,b,c,d,e]	117.45±10.27[f,g,h,i]	6.34±2.58[g,k,l,m,n,o]	0.41±0.80	0.82±1.043	33.83±3.84
A. incana subsp. *rugosa*	9.30±0.48[a,b,c]	53.09±8.26[t,u,v,w]	2.14±1.49[a]	2.71±1.87	6.34±2.58	0.41±0.46
C. rangiferina	636.47±23.54[n]	146.83±10.72[c,d,e,f]	5.54±0.18[o,q,r,t]	43.33±3.13	120.97±13.80	167.79±10.94
E. nigrum	86.32±1.80[f,g]	108.42±2.12[i,j,k]	5.08±0.86[u]	58.46±3.65	215.60±16.34	266.30±15.04
G. hispidula	25.44±1.24[a,b,c,d,e]	52.08±6.03[u,v,w]	2.15±0.29[a]	11.29±2.22	11.55±2.61	7.16±2.14
J. communis	(1) 30.31±1.45[a,b,c,d,e]	84.64±1.85[m,n,o,p,q,r]	6.02±2.64[a,b,c,d,e]	9.39±2.52	27.39±11.08	39.48±14.97
	(2) 30.56±0.61[b,c,d,e]	71.51±9.02[q,r,s,t,u]	4.99±1.05[a]	9.28±1.27	6.50±0.98	6.13±0.23
	(3) 15.15±0.36[a,b,c,d]	167.76±12.97[a,b,c]	3.11±2.30[a]	7.41±2.98	5.91±1.86	5.19±0.91
K. angustifolia	14.22±0.60[a,b,c]	70.78±8.15[q,r,s,t,u,v]	3.76±1.87[a]	4.85±1.54	5.24±0.93	5.15±2.34
L. laricina	(1) 31.52±0.37[b,c,d,e]					
	(2) 41.54±0.96[c,d,e]	187.65±2.63[a]	6.38±2.14[a,b,c,d,e]	22.44±2.52	62.84±17.80	48.33±2.68
	(3) 29.95±1.27[b,c,d,e]	182.42±5.34[a,b]	4.97±1.73[a]	8.92±2.02	11.80±2.90	6.91±1.15
L. mollis	316.28±20.99[k]	158.89±2.87[c,d,e]	4.73±0.87[n,q,r,t]	52.08±4.31	136.28±18.40	161.26±22.71
L. clavatum	698.78±74.28[o]	180.96±10.69[a,b]	4.76±1.45[d,f,g,h,i,j]	35.15±2.41	62.76±4.64	79.85±3.08
P. glauca	(1) 18.93±0.69[a,b,c,d,e]	115.26±2.83[h,i,j]	5.35±3.56[a]	8.45±1.25	7.19±0.92	5.39±0.74
	(2) 124.35±6.53[i]		5.83±0.86[a,b,c]	21.24±1.61	30.97±11.29	29.56±1.54
	(3) 23.67±1.06[a,b,c,d,e]	133.36±2.87[f,g,h]	1.64±1.66[a,b]	20.81±4.20	11.10±2.67	6.19±1.64
P. mariana	(1) 8.68±0.19[a,b,c]	60.66±7.83[s,t,u,v,w]	2.15±0.24[a]	6.86±2.45	4.49±0.10	3.45±0.81
	(2) 136.55±3.94[i]		6.64±1.87 [a,b,c]	19.33±2.93	29.33±7.64	24.11±4.04
P. banksiana	(1) 7.44±0.29[a,b,c]	74.22±0.79[q,r,s,t]	0.68±0.96[a]	4.62±1.96	2.39±0.80	–0.57±0.13
	(2) 14.98±0.46[a,b,c,d]	84.48±2.98[n,o,p,q,r]	3.51±1.15[a]	9.39±2.15	5.84±0.31	3.76±0.34
P. balsamifera	120.24±5.00[j]	102.65±2.91[j,k,l,m,n]	4.42±2.07[b,f,g,h,i,j]	23.46±7.83	50.10±38.80	65.66±55.06
R. groenlandicum	(1) 14.85±0.93[a,b,c,d]	80.95±9.37[o,p,q,r,s]	6.54±1.53[a]	7.57±2.78	5.53±1.52	4.52±1.70
	(2) 15.77±1.37[a,b,c,d]					
	(3) 19.55±0.69[a,b,c,d,e]	87.74±10.77[k,l,m,n,o,p,q]	4.69±1.02[a]	12.86±4.43	6.38±0.00	6.85±1.02
R. tomentosum subsp. *subarcticum*	20.63±0.65[a,b,c,d,e]	89.74±2.14[k,l,m,n,o,p,q]	4.76±1.63[a]	7.31±0.00	8.70±1.00	5.28±1.85
S. planifolia subsp. *planifolia*	11.47±0.37[a,b,c]	86.45±2.45[l,m,n,o,p,q]	7.61±3.88[a]	9.61±1.79	12.83±2.92	3.85±0.00
S. purpurea	(1) 27.65±0.77[a,b,c,d,e]	77.22±7.88[p,q,r,s]	9.24±5.05[a]	6.63±1.02	5.56±2.25	6.72±2.34
	(2) 38.88±1.19[c,d,e]					
S. decora	(1) 19.63±0.57[a,b,c,d,e]	101.68±2.08[j,k,l,m,o]	4.28±1.53[a]	7.83±1.94	8.86±1.61	9.02±3.18
	(2) 48.95±2.49[d,e]	145.62±9.27 [d,e,f]	5.51±2.69 [a,b]	14.57±5.58	24.16±4.07	25.89±8.73
	(3) 52.39±0.57[d,e]	38.85±0.88[w]	5.07±1.41[f,k,l,m,o]	25.75±8.56	77.50±43.19	110.60±61.84
	(4) 21.75±0.31[a,b,c,d,e]	108.97±4.41[i,j,k]	4.70±0.74[a]	8.28±1.09	4.83±0.24	4.36±0.44
S. fuscum	104.09±4.05[g,h,i]	112.49±3.52[h,i,j]	2.24±1.96[i,n,o,p]	27.10±3.48	87.23±15.40	127.77±17.67
V. vitis-idaea var. *minus*	140.06±6.82[i]	107.34±3.30[i,j,k,l]	3.29±0.37[e,f,g,h,u,j]	31.26±4.52	59.43±0.00	88.91±13.80
V. uliginosum var. *alpinum*	17.93±0.37[a,b,c,d,e]	128.59±5.92[f,g,h,i]	7.85±3.45[a]	10.00±1.12	8.66±1.03	4.67±0.39
Non-medicinal plants						
A. millefolium	109.99±3.73[g,h,i]	106.24±10.96[j,k,l]	0.17±0.42[i,k,l,m,n,o]	27.57±3.06	76.36±18.23	114.22±16.98
A. maritima subsp. *sibirica*	77.72±2.63[e,f,g]	105.57±9.02[j,k,l,m,n]	2.86±2.63[s,u]	45.3±5.03	146.75±4.29	194.31±12.41
B. glandulosa	22.02±1.06[a,b,c,d,e]	79.60±7.54[p,q,r,s]	0.45±0.27[a]	3.46±0.29	6.97±1.53	3.14±0.30
C. rotundifolia	118.59±4.20[h,i]	137.86±3.18[e,f,g]	1.09±1.80[k,p,q]	29.51±3.86	90.84±22.52	140.41±17.03
C. angustifolium	9.11±0.35[a,b,c]	96.12±2.25[j,k,l,m,n,o,p]	0.48±0.85[a]	2.57±0.53	5.75±2.34	2.07±0.44
E. arvense	356.77±13.02[l]	116.89±4.25[g,h,i,j]	2.80±2.11[m,p,q,r]	39.03±4.99	107.14±4.62	156.46±13.46
H. vulgaris	67.96±0.71[e,f]	130.84±9.26[f,g,h]	0.50±0.87[f,a]	10.15±3.06	38.15±22.83	51.23±35.50
H. peploides	206.34±3.20[j]	106.08±2.68[j,k,l,m]	2.22±1.23[t,u]	59.15±6.54	174.44±16.94	209.51±16.04
L. procumbens	30.29±0.89[a,b,c,d,e]	164.57±5.32[b,c,d,e]	1.76±0.00[a]	8.04±0.56	16.62±7.76	21.31±7.62
M. gale	17.60±0.34[a,b,c,d]	98.56±1.03[j,k,l,,o,p]	4.46±2.30[a]	13.32±7.44	15.02±4.08	12.39±4.66
R. rugosum	320.95±6.17[k]	133.60±5.72[f,g,h]	0.08±0.88[p,s,t]	39.92±4.37	130.29±6.34	177.09±13.19
R. chamaemorus	28.93±0.60[c,d,e]	78.62±2.61[p,q,r,s]	6.40±5.10[a]	15.71±4.15	17.70±6.13	8.34±1.62
S. arctophila	19.10±0.56[a,b,c,d,e]	49.72±1.59[v,w]	2.27±1.06[a]	4.95±0.67	6.50±1.31	3.67±2.00
S. russowii	70.06±2.19[e,f]	139.98±1.90[e,f]	0.09±0.88[c,f,g,h,i,j]	20.32±4.70	56.46±15.12	73.23±19.47
S. paschale	495.51±34.94[m]	141.16±3.63[e,f]	0.21±0.79[l,p,q]	24.95±1.02	87.10±18.05	142.59±4.84

Table 2 (*concluded*).

	DPPH, IC_{50}, ppm	CD, lag time, min	TBARS, nmol MDA eq/mg LDL			
			0 min	90 min	180 min	240 min
T. bipinnatum var. *huronense*	$77.33\pm1.53^{e,f,g}$	$64.66\pm10.80^{r,s,t,u,v}$	$1.19\pm0.00^{h,k,l,m,n,o}$	23.18±4.96	71.73±19.23	115.59±27.34

Note: DPPH, 1,1-diphenyl-2-picrylhydrazyl; CD, conjugated dienes; TBARS, thiobarbituric acid-reactive substances; MDA, malondialdehyde; LDL, low-density lipoprotein. LDL protective effects of plants were measured as lag time before the appearance of CD and production of TBARS up to 4 h incubation with 5 ppm plant extract or standard. Results are means ± SE of duplicate samples performed in at least $n = 3$ independent trials. Means with the same letters within an assay are not significantly different among species as determined by Tukey's multiple comparison ($p < 0.05$).

and results expressed as micrograms of tannic acid equivalents (TAE) per milligram extract.

Statistical analysis

Results are expressed as means ± SE of at least $n = 3$ independent experiments performed in duplicate. A one-tailed analysis of variance (ANOVA) with post hoc Tukey's was used to test significant differences between samples for DPPH, CD, and phenolic content and an ANOVA with repeated measures was used for TBARS results. Significance was set at $p < 0.05$.

From the data obtained, the following comparisons in respect to antioxidant activity and phenolic content were made using independent or paired Student's *t* tests: (*i*) activity from medicinal plants versus plants not used medicinally; (*ii*) activity from plant species recollected in subsequent years; (*iii*) activity of the same species from two distinct habitats, Mistissini (a more southern community) versus Whapmagoostui; (*iv*) activity from different plant parts of the same species, since Cree elders and healers sometimes used different parts of the same plant.

Each plant species was ranked according to their antioxidant activity for each of the three assays and averaged to obtain a final activity score. Spearman's correlation was used to test associations between the activity score, phenolic content, and the SIV. Before calculating the mean, values were centered reduced to be comparable. SAS 9.0 was used to perform these analyses.

Results

Antioxidant activity

Table 1 lists each plant or sample studied, including medicinal plant species mentioned during the ethnobotanical surveys and plant species not used medicinally. It includes a total of 20 plant families, within which 20 medicinal plants come from Whapmagoostui and 16 from Mistissini, as well as 16 plants not used medicinally (also from Whapmagoostui). Ericaceae and Pinaceae were the most represented families.

Among the medicinal plants of Mistissini and Whapmagoostui, respectively, 62.5% and 55% of extracts had DPPH scavenging activity (Table 2) statistically similar to the known antioxidants ascorbic acid (3.84 ± 0.01 ppm), quercetin (4.42 ± 0.23 ppm), catechin (5.56 ± 0.37 ppm), and epicatechin (5.94 ± 0.18 ppm). Of the plants not used medicinally, 37.5% displayed significant activity. Species that displayed the strongest activity were *Pinus banksiana* cone (waschiski; 7.44 ± 0.29 ppm), *Salix planifolia* bark (waaskaayaapaatukh; 11.47 ± 0.37 ppm), and *Chamerion angustifolium* (michskishoe, nischiikhan, or wobagun; 9.11 ± 0.35 ppm). Of all the plant species tested, 59.6% had an IC_{50} below 25 ppm, classifying them as effective radical scavengers. The majority of medicinal plants in both communities (except *Lycopodium clavatum* (pashtnahoagin), *Cladonia rangiferina* (waapskamihkw), *Leymus mollis* (wiinpaakw miskuusiuh), *Vaccinium vitis-idaea* (wiisichiminh), *Populus balsamifera* (mash-mitush), and gums of both *Picea*) displayed better scavenging activity than plants not used medicinally ($p < 0.001$). Indeed, the IC_{50} of many negative controls was greater than 50 ppm.

Forty-nine plant extracts were studied for their ability to prolong lag time until the appearance of conjugated dienes resulting from LDL peroxidation (Table 2). Because of solubility problems, the gums of *Picea glauca* (minihikw) and *Picea mariana* (iyaatuk) were excluded from analysis. Of the studied plants, 29.78% and 44.68% had longer lag time than Trolox and ascorbic acid, respectively, and therefore greater antioxidant potential. More than half of the samples had a lag time statistically similar to both Trolox and ascorbic acid. *Larix laricina* (waachinaakan) (187.65 ± 12.63 min Mist. and 182.42 ± 5.34 min Whap.) and *Loiseleuria procumbens* (itchkaseeboaukish) (164.57 ± 5.32 min; a plant not used medicinally) displayed the highest potential to prolong LDL oxidation.

The majority of the 49 plant samples analysed were able to decrease TBARS production (Table 2). In comparison with Trolox, more than 59.18% had higher antioxidant activity and 73.47% were statistically similar. The strongest antioxidant activity was observed in *P. banksiana* cone (Mistissini), *C. angustifolium* (a plant not used medicinally), and *Juniperus communis* root (*kaakaachiminh*; Whapmagoostui).

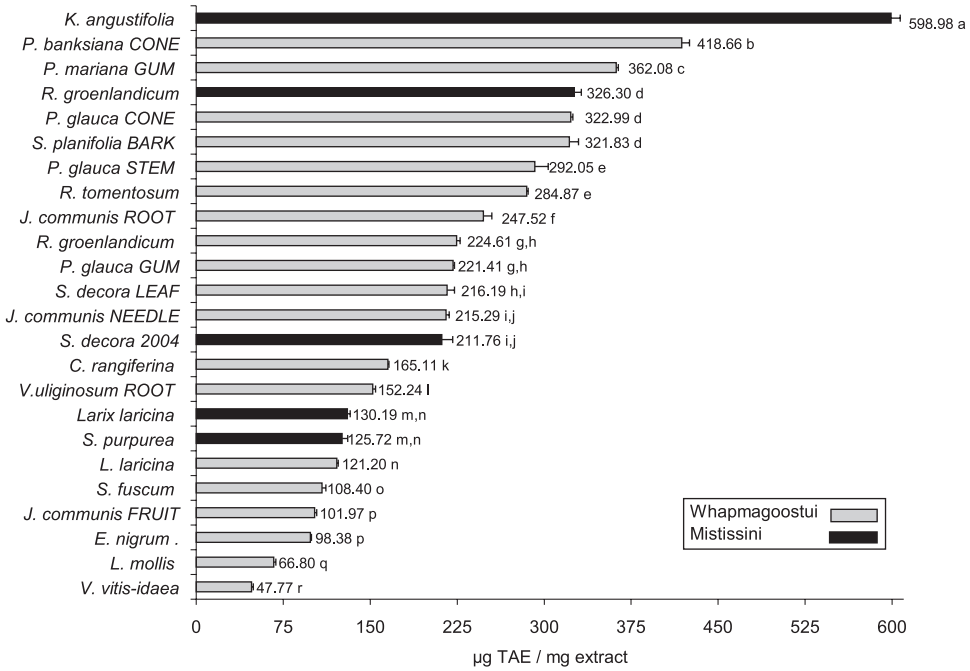
Water-soluble phenolics

Figure 1 illustrates the water-soluble phenolic content of the medicinal plant extracts. Of the 24 medicinal plants studied, *Kalmia angustifolia* (uishichipukw) (599.0 ± 8.1 μg phenol/mg extracts) contained the highest phenolic levels and *V. vitis-idaea* (47.7 ± 1.5 μg TAE/mg extract) had the lowest.

Comparisons

The 3 groups of plants (medicinal and plants not used medicinally from Whapmagoostui and medicinal plants from Mistissini) show statistical differences according to their antioxidant capacity. First, medicinal plants from Whapmagoostui had higher antioxidant potential than plants not used medicinally in the TBARS assay ($p = 0.0001$), although this was not observed between the DPPH and CD assays. Second, TM used in Whapmagoostui had higher antioxidant potential in comparison with medicinal plants used in Mistissini based on the TBARS ($p = 0.0001$) and CD ($p = 0.034$) results.

Fig. 1. Water-soluble phenol content of 24 medicinal plant species used in Mistissini and Whapmagoostui. TAE, tannic acid equivalents. Bars are means ± SE, $n = 5$ trials. Different letters after the means represent significant differences between species ($p < 0.05$).



Figures 2, 3, and 4 illustrate some of the results obtained from the DPPH, CD, and TBARS assays. In Fig. 2*a*, medicinal and non-medicinal species from the same family (Ericaceae and Salicaceae) were compared. With the exception of *V. vitis-idaea*, all medicinal species showed higher antioxidant activity than non-medicinal ones. However, for the TBARS and CD assays (Figs. 3*a* and 4*a*) *Salix arctophila* (waaskaayaapaatukh), a willow not used by the Cree, had higher activity compared with other species. After 90 min, TBARS concentration was below 15 nmol MDA eq/mg LDL for most extracts, although an overall better pattern of antioxidant activity was observed with longer time points for medicinal plant extracts compared with non-medicinal ones. Species comparisons within the Sphagnaceae and Cladoniaceae/Stereocaulaceae (lichen families) did not produce any statistical differences (results not shown).

With regard to using different parts from the same plant, *J. communis* (Figs. 2*b*, 3*c*, and 4*b*) demonstrated the potency of the root system over the berries and needles. At the end of 2 h, berries offered little antioxidant protection from LDL oxidation compared with the other parts. Similar comparisons were performed between *P. glauca* needles, cones, and gum and between *Sorbus decora* (miskuumischi) stems and leaves. Results showed that the gum and leaves possessed weaker antioxidant activity, respectively, compared with the other parts (results not shown).

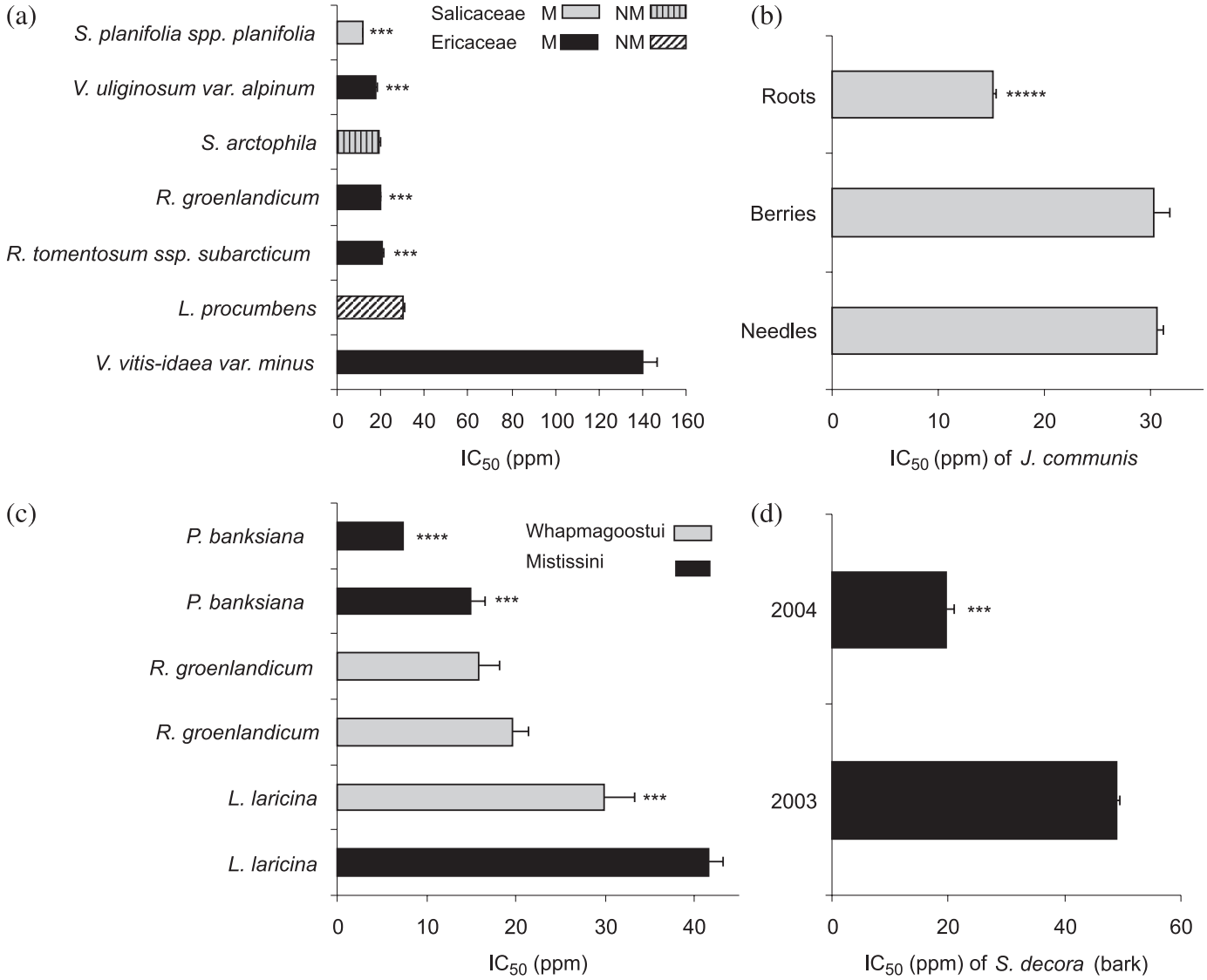
There were significant differences between the same species collected from the different communities such that *P. banksiana* and *Rhododendron groenlandicum* (wiisichipukh) from Mistissini displayed better DPPH scavenging activity (Fig. 2*c*), yet weaker LDL protective ability (Fig. 3*b*), compared with those from Whapmagoostui. The reverse pattern was observed for *L. laricina*. Interestingly, there were no differences in TBARS levels between species except *L. laricina* from Mistissini, which displayed particularly weak activity (Fig. 4*c*).

A clear pattern emerges when one compares the samples of *S. decora* collected in different years. Figures 2*d* and 4*c* clearly illustrate that more recent collections display stronger antioxidant activity.

Ranking

There is general agreement between the free radical scavenging activity of an extract and its ability to protect LDL from oxidation ($r = 0.7811$; $p = 0.000$). Table 1 gives the position of all plant samples according to the analysis ranking derived from the mean ranking score of the three assays. *Larix laricina* from Whapmagoostui, *L. laricina* from Mistissini, and *L. procumbens* (a plant not used medicinally) ranked 1, 3, and 4, respectively. Medicinal plants from both communities had stronger antioxidant potential than non-medicinal plants: 38.78% of medicinal extracts were found among the 25 highest-ranked samples in comparison with 12.24% for non-medicinal extracts. Also, 14 of the 25 most effective extracts in terms of antioxidant activity can be found within the Ericaceae and Pinaceae. Figure 5 illustrates the positive correlations between the antioxidant analysis ranking and the syndromic importance value derived from ethnobotanical surveys conducted in both Whapmagoostui ($r = 0.5165$; $p = 0.001$) and Mistissini ($r = 0.3134$; $p = 0.058$). In general, plants most often mentioned by interviewees and used to treat more T2D symptoms were the ones that had stronger antioxidant activity. In Figs. 6*a* and 6*b*, positive correlations between phenolic content and TBARS ($r = 0.3681$; $p = 0.002$) and DPPH ($r = 5968$; $p = 0.00015$) results are shown. Extract phenol concentration was also associated with the Whapmagoostui SIV index ($r = 0.5015$; $p = 0.003$; Fig. 6*c*) and the rank order of overall

Fig. 2. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of ethanolic plant extracts represented as inhibitory concentration at 50% (IC_{50}) from the linear portion of an ascorbic acid standard curve. Bars are means ± SE of at least 3 independent assays performed in duplicate. (*a*) Activity of medicinal (M) and non-medicinal (NM) extracts; (*b*) activity of different parts of *Juniperus communis*; (*c*) activity of the same species collected at different sites (Whapmagoostui and Mistissini); (*d*) activity of *Sorbus decora* extract collected in different years. ***, $p < 0.01$; ****, $p < 0.001$; *****, $p < 0.0001$.



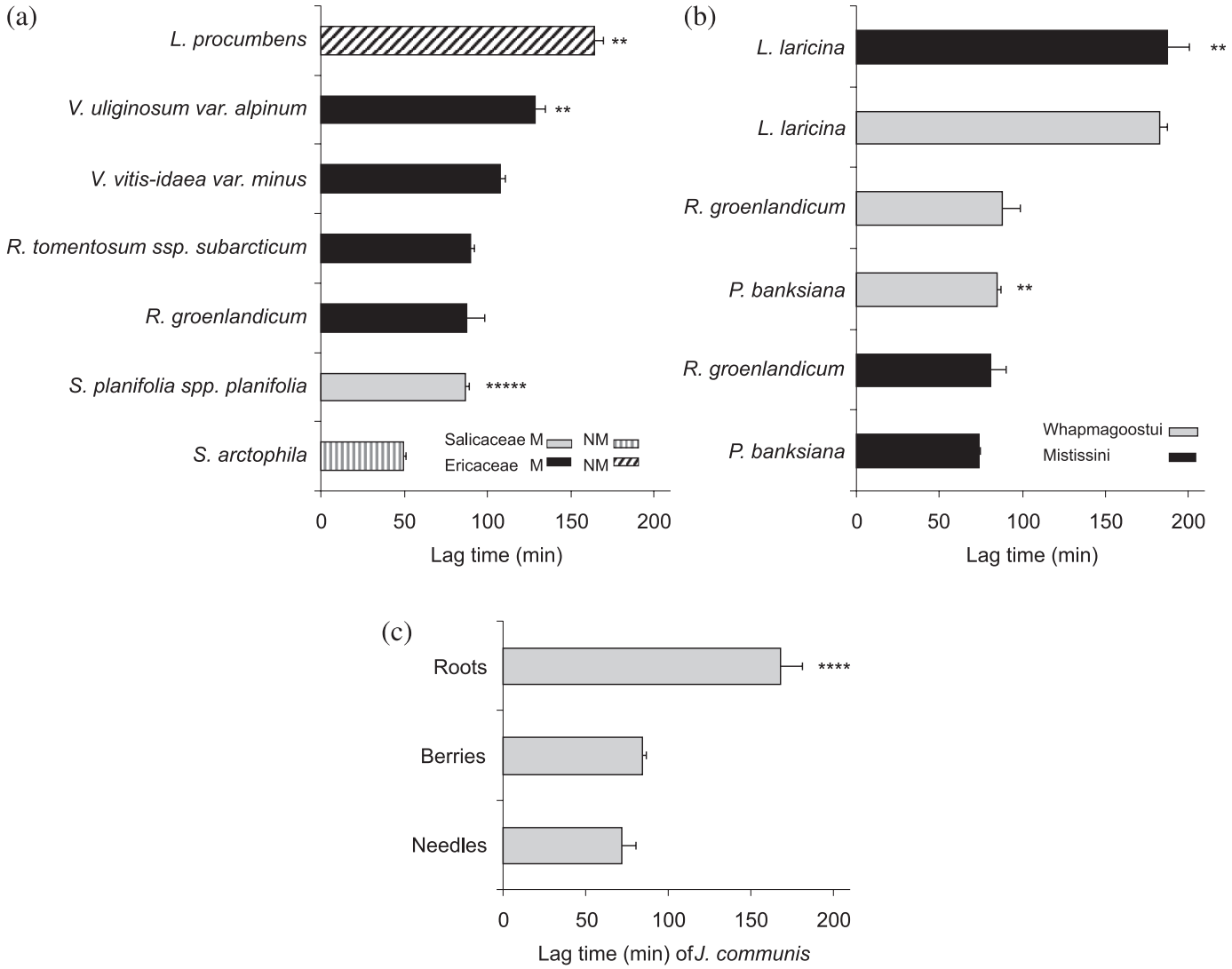
antioxidant activity ($r = 0.4178$; $p = 0.003$; Fig. 6*d*), indicating that phenols were the likely bioactive agents.

Discussion

Our results demonstrate that plants used by the Québec Cree to treat symptoms of T2D are often high in phenolic antioxidants, a finding corroborated by the work of McCune and Johns (2002). Over half (52%) of the medicinal plants identified by traditional healers had statistically similar or better free radical scavenging activity than ascorbic acid, and were comparatively better at protecting LDL from copper-induced peroxidation. Roughly 64% and 96% of the tested extracts were similar or superior to ascorbic acid in protecting LDL using lag time and TBARS production as endpoints, respectively. Vitamin E is a potent antioxidant endogenous to the lipid membranes of LDL particles (Pratt 1992). When compared with Trolox, a water-soluble analog of the vitamin, 40% and 73% of the tested extracts were comparable or superior in prolonging lag time and reducing TBARS levels, respectively. This is a higher success rate than that reported by McCune and Johns (2002), who found that 23% and 60% of their boreal plant extracts fared as well as Trolox using comparable analyses.

Most of the extracts investigated in the present study (72%) were effective DPPH scavengers, and IC_{50} values agree well with previous reports for certain species, particularly *K. angustifolia*, *J. communis*, *R. groenlandicum*, and *Abies balsamea* (iyaasiht) (Choi et al. 2002; McCune and Johns 2002). The two extracts with the highest water-soluble phenol content, *P. banksiana* and *C. angustifolium*, were also the most potent radical scavengers, suggesting that the bioactive components were phenolic compounds. Previous reports have observed the strong antioxidant potential of

Fig. 3. Lag time (min) before the appearance of conjugated diene formation on Cu^{2+}-mediated oxidation of LDL incubated with 5 ppm plant extracts. Plants with stronger antioxidant activity prolonged lag time. Bars are means ± SE of 3 independent experiments performed in duplicate. (*a*) Lag time of medicinal (M) and non-medicinal (NM) extracts belonging to the families Salicaceae and Ericaceae; (*b*) lag time of the same species collected at different sites (Whapmagoostui and Mistissini); (*c*) lag time of different parts of *Juniperus communis*. **, $p < 0.05$; ****, $p < 0.001$; *****, $p < 0.0001$.

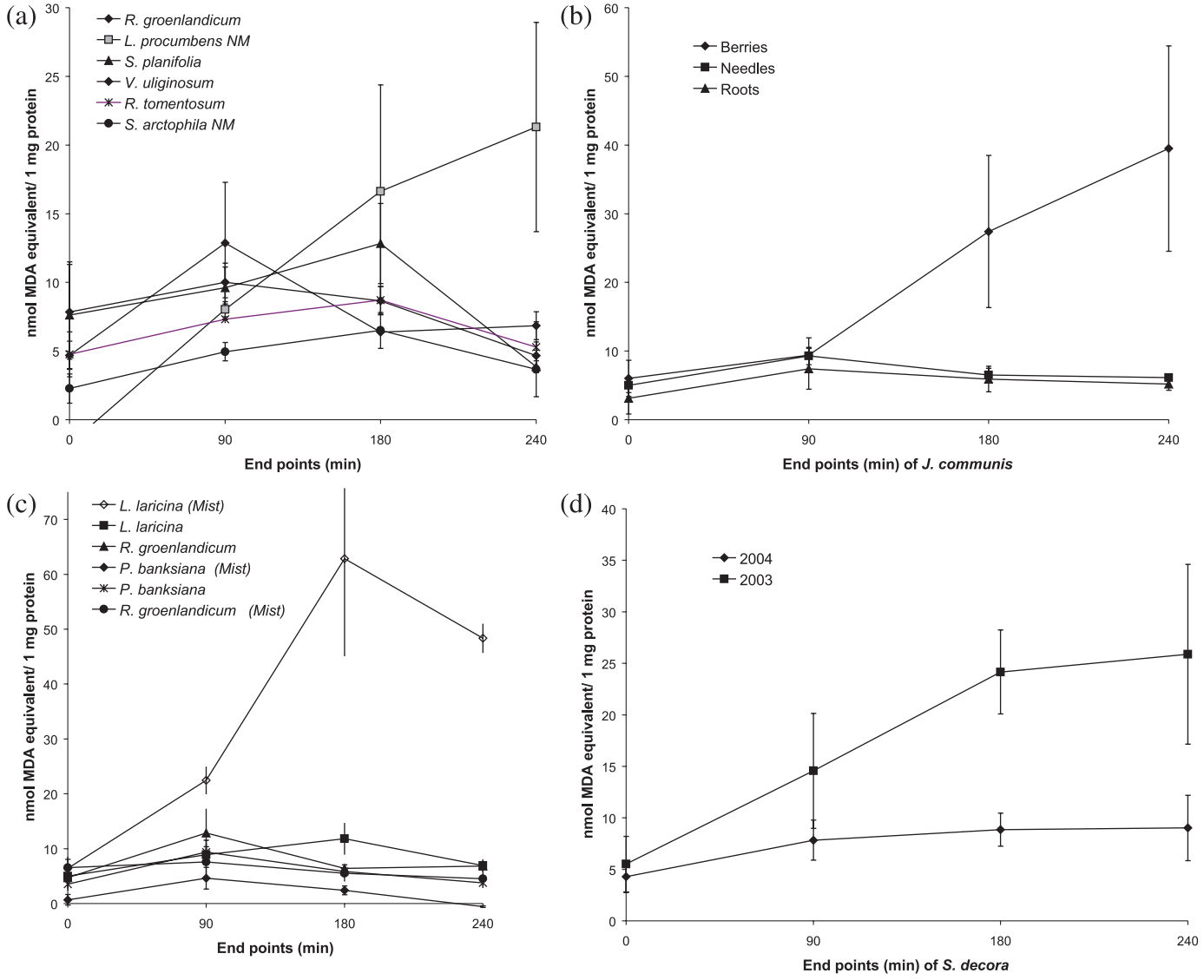


these species (Zulaica-Villagomez et al. 2005; Myagmar and Aniya 2000). In regard to their ability to protect LDL from metal ion-mediated oxidation, both *P. banksiana* and *C. angustifolium* were able to effectively inhibit the formation of TBARS for up to 4 h, but had little effect on prolonging lag time before the appearance of conjugated dienes.

Such discrepancies between the two commonly measured endpoints of LDL oxidation have been observed previously (Chajès et al. 1996; Diwadkar et al. 1999). Some researchers suggest that TBARS production and formation of conjugated dienes be considered independent parameters of oxidation because of experimental conditions. Katsube et al. (2004) suggested that difference could be a consequence of the affinity of phenolic antioxidants to LDL as chelating agents. A number of compounds occur in crude extracts that could interfere with reagents, with colorimetric readings, or with temperature changes over time. Sugars, for example, often inhibit the antioxidant properties of flavonoids. Esterbauer et al. (1987) has outlined the limitations of the TBARS assay; however, despite this it remains one of the most popularly used measures of oxidation. One critique is that TBARS reacts with more compounds than the aldehydes derived from lipid peroxidation and can lead to overestimation of antioxidant activity (Petlevski et al. 2003). Nevertheless, our TBARS results correlated strongly with those of the DPPH scavenging activity assay, as well as with the phenolic content of the extracts, and are considered reliable measures of antioxidant activity.

This study supports previous findings that antioxidant vitamins prolong lag time and retard oxidation during the propagation phase of LDL modification (Yen and Hsieh 2002; Mathiesen et al. 1996*a*, 1996*b*; Vaya et al. 1997). Ascorbic acid and Trolox had lag times of 108.26 ± 3.13 min and 130.59 ± 9.84 min, respectively; however, the former was not significantly different from the control, oxLDL. Ascorbic acid, as well as several other plant compounds in-

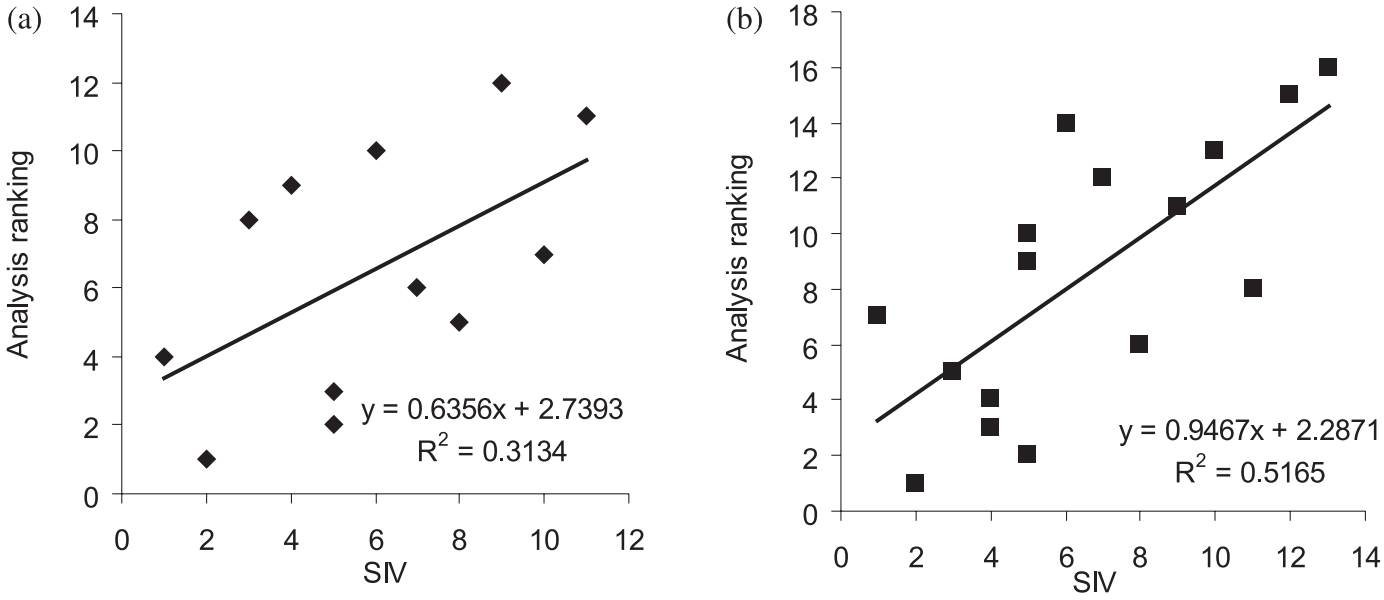
Fig. 4. Thiobarbituric acid-reactive substance (TBARS) production over time from Cu^{2+}-mediated oxidation of LDL incubated with 5 ppm plant extracts. Plants with stronger antioxidant activity were able to inhibit or minimize TBARS production for up to 4 h. Points are means ± SE of at least 3 independent experiments performed in duplicate. (*a*) Medicinal (blank) or non-medicinal (NM) plant extracts from the same families; (*b*) different parts of *Juniperus communis;* (*c*) the same species collected at different sites (Whapmagoostui (blank) and Mistissini (Mist)); (*d*) extracts of *Sorbus decora* collected in different years ($p = 0.01$).



cluding flavonoids, can display pro-oxidant activity depending on experimental conditions, particularly in the presence of transitional metal ions or at relatively high concentrations (Otero et al. 1997; Owen 2000; Patel et al. 1997). This may explain the significant pro-oxidant activity observed for *E. nigrum* (Table 2.). Interestingly, this species also had a low SIV score in both Cree communities, suggesting that the plant was not considered an important remedy for T2D (Fraser et al. in press).

Conversely, one of the more popular antidiabetic traditional medicinal plants according to SIV scores, *L. laricina*, exhibited the most potent free radical scavenging and LDL protective effects. This supports previous findings reported by McCune and Johns (2002). The cambium of tamarack, as a structure generated continuously from meristematic cells, may contain a higher concentration of tannins and other secondary metabolites than other tissues (Owen 2000). Surprisingly, the tannin concentration (a water soluble polyphenol) of our *L. laricina* extract was lower than that reported by Owen and Johns (1999). This may be due to different extraction methods (Cowan 1999) but may also be due to the environmental conditions in which the collected individuals grew, since exposure to sunlight (Czeczuga 1994), high altitude or latitude and stress from herbivory can affect tannin levels (Ernst et al. 1991). Indeed, the extract of *L. laricina* collected in Whapmagoostui displayed significantly stronger LDL protective effects than that collected in Mistissini. This is not surprising, since Whapmagoostui is located further north and contains some arctic flora. The colder temperatures, stronger winds, or shorter growing season may have affected the phytochemical content and thus the antioxidant activity of plant species that

Fig. 5. Spearman's correlation between antioxidant analysis ranking derived from the mean ranking scores of the 3 antioxidant assays and the syndromic importance value (SIV) of each plant species as determined from ethnobotanical surveys conducted in (*a*) Mistissini (r = 0.3134; p = 0.058) and (*b*) Whapmagoostui (r = 0.5165; p = 0.001). Positive correlations indicate that plants most often used to treat T2D symptoms are usually higher in antioxidants.



occur in both communities (Connor et al. 2002). This pattern, however, was not observed for the extracts of *R. groenlandicum* and *P. banksiana*, where those collected from Mistissini exhibited stronger free radical scavenging activity than their more northern counterparts. This was likely due to differences in harvesting periods, since collections in Misitissini took place in 2003 and those in Whapmagoostui were in 2004.

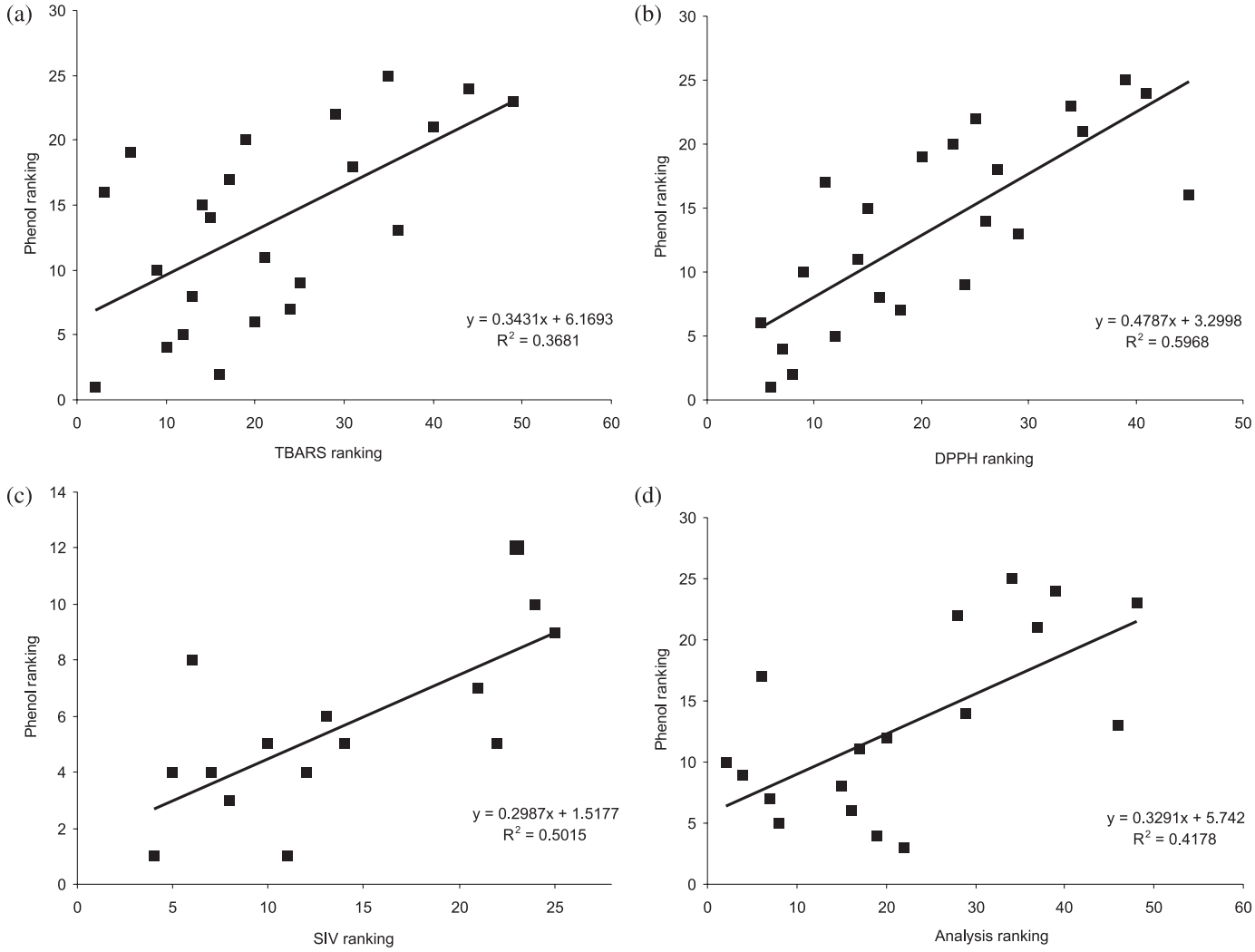
Time and place of plant collection is an important consideration that Cree healers recognize as effectors of potency and effectiveness for their medicines. For example, mature fruits of *V. vitis-idaea* collected in spring were considered optimal for their medicinal properties. In this study, berries of *V. vitis-idaea* were collected in late summer (August) and this may have explained their relatively low phenolic content, as well as the weak antioxidant activity observed in our experiments. A number of phenolic compounds occur in the berries that would have otherwise provided stronger activity (Kähkönen et al. 1999; Rimando et al. 2004). It would be of interest for future studies to investigate variations in phytochemical content and bioactivity of Cree plants as a function of season and growing conditions.

In addition to the time of plant harvest, extraction method and storage conditions are other important factors that influence bioactivity. *Sorbus decora* was collected twice from the same community, but in different years and then extracted using different methods. Those collected in 2003 were extracted by continuously shaking dried ground plant material in 80% ethanol (1:10 *w*/*v*) for up to 48 h (Spoor et al. 2006), whereas those collected in 2004 were extracted under heat and vacuum using the Soxtec extraction system. Although the latter method can destroy heat-sensitive compounds, there is usually a higher extraction yield. Also, antioxidant activity can deteriorate over time, especially compounds that are light or temperature sensitive. Not surprisingly, the *S. decora* extract collected in 2004 had significantly stronger antioxidant activity in all assays.

According to Cree specialists, every plant part has a particular use for a particular disease. This is reflected in the phytochemical distribution within a species (Fediuk et al. 2002; Angioni et al. 2003; Zulaica-Villagomez et al. 2005). For example, the higher concentration of water-soluble phenolic compounds in the roots of *Vaccinium uliginosum* (*iyiyiminh*) (152.2 µg TAE / mg extract) relative to the berries (28.7 mg TAE/g extracts) (Kähkönen et al. 1999) supports the biocultural view that berries are eaten, whereas roots are used for medicinal purposes. Anthocyanins and polyphenolic compounds are widespread among blueberries and are considered the bioactive components (Lee et al. 2004). Zulaica-Villagomez et al. (2005) showed that better activity was encountered in young pine cones in comparison with old ones. In Whapmagoostui, medicinal use of different plant tissues from *P. glauca* (gum, needles, cone), *J. communis* (berries, root, needles), and *S. decora* (stem, leaves) (Fraser et al. in press) closely parallels their phenolic content. Activity tends to be similar for needles and berries of *J. communis* (Figs. 2*b* and 3*c*), but roots showed stronger antioxidant activity. Shahmir et al. (2003) have reported the presence of 41 and 27 phytochemicals in the oil of juniper needles and berries, respectively, several of which that were non-phenolic in nature and could have contributed to the observed activity in the present study.

Antidiabetic plant species identified by the Cree showed overall higher antioxidant potential than the majority of the plants not used medicinally that were sampled. Extracts of the non-medicinal plants *Stereocaulon paschale* (wapi-jeshkumuk), *Armeria maritima* (sheesheeminshounn), *Equisetum arvense* (neeskann, miskouchoe), and *Rhytidium rugosum* (beastaskugg, beastaskumuk) ranked lower in our assay than medicinal plant extracts (Table 1). Hoffman et al. (1967) re-

Fig. 6. Spearman's correlation indicates positive associations between the ranking of water-soluble phenol content of a plant extract and the ranking of (*a*) thiobarbituric acid-reactive substances (TBARS) ($r = 0.3681$; $p = 0.002$); (*b*) DPPH scavenging activity ($r = 0.5968$; $p = 0.00015$); (*c*) the syndromic importance value (SIV) of plant species as determined from ethnobotanical surveys conducted at Whapmagoostui ($r = 0.5015$; $p = 0.003$) (Fraser et al. in press); (*d*) the antioxidant analysis ranking derived from the mean ranking scores of the 3 antioxidant assays ($r = 0.4178$; $p = 0.003$).



ported that eastern Arctic plants, both medicinal and non-medicinal, such as *L. mollis, Empetrum nigrum* (ishchiminh), *C. angustifolium*, *Rubus chamaemorus* (shicoudaw), *S. planifolia*, *S. arctophila*, and *V. vitis-idaea,* had a high content of either ascorbic acid, β-carotene, or both. In this study, some plants not used medicinally, notably *C. angustifolium*, *Myrica gale* (estiminatuck), *Betula glandulosa*, *L. procumbens*, and *S. arctophila* showed greater antioxidant potential than many of the medicinal plants. However, *C. angustifolium* is a traditional tea (Kiss et al. 2004), *S. arctophila* (Kuhnlein and Turner 1991) is an edible plant, and *L. laricina* (Powell and Raffa 1999*a*, 1999*b*), *L. procumbems* (Cuendet et al. 2000), and *M. gale* (Mathiesen et al. 1996*a*; 1996*b*) are known for their antioxidant compounds. Also, the antioxidant activity and phenolic content of *L. procumbens* (256.7 ± 3.9 μg TAE/mg extract) is typical of Ericaceae as exemplified by *R. groenlandicum*, *R. tomentosum*, *V. uliginosum*, and *V. vitis-idaea.* Many of these extracts have documented hypoglycaemic activity (Marles and Farnsworth 1995), suggesting that a plant need not be an antioxidant to be useful against T2D. These cases notwithstanding, our evidence supports the hypothesis that overall, Cree elders and healers purposefully select plants that have medicinal properties that can be attributed to their antioxidant activity.

Since small procumbent plants like *L. procumbens* are usually not selected as medicines among the Cree, it can be argued that the antioxidant potential or healing properties of this plant has not been recognized. Among the plants not used medicinally that fared well in the antioxidant assays, some might prove to be toxic or viewed as such by the elders and healers. Others might simply be inconspicuous plants or have a poor distribution. Uncommon or rare plants may not represent good options for medicinal purposes when time is a factor, especially if more commonly occurring plants possess similar healing powers.

Since elders and healers prepared medicinal plants by decoction, water-soluble methods were chosen for phenolic de-

termination to render results comparable with ethnobotanical data. Extractions were based on an organic solvent (EtOH) to maximize extract yield. Indeed, the water-soluble phenolic content of the extracts correlated strongly with their syndromic importance value calculated from ethnobotanical surveys ($r = 0.5015$; $p = 0.003$) and the biochemical assays ($r = 0.4178$; $p = 0.003$). It should be noted that antioxidants need not be phenolic, since *L. laricina*, *S. purpurea*, and *S. decora*, as well as a few others that attained a high SIV score, had a low-ranking score according to phenol concentration. The opposite was observed for the extracts of *K. angustifolia* and *P. banksiana.* With a more inland distribution, it may be that these species were less accessible and therefore used with less frequency by the Cree of Whapmagoostui despite their antioxidant potential (Fraser et al. in press). Known toxic diterpenes (andromedotoxin) (Burke et al. 1989; Lewis and Elvin-Lewis 2003) and toxic alkaloids (Mancini and Edwards 1979) in *K. angustifolia* may also account for its rarity as a medicine. The high phenolic and tannin (Marles 2001) content of *K. angustifolia* could contribute to its toxicity (Mallik 2003).

Conclusions

Most of the TM used by the Cree Nation of Eeyou Istchee to treat T2D symptoms showed antioxidant activity greater than that of plants not used medicinally and standard antioxidants, thus supporting the beneficial effects of Cree TM in the prevention of T2D and its complications. Results from our antioxidant assays also correlated strongly with the phenolic content of plant extracts and their ethnobotanical use when ranked according to activity. Strong candidates for further in vitro and in vivo studies (Spoor et al. 2006) would be *J. communis*, *K. angustifolia*, *L. laricina*, *P. glauca*, *S. decora*, *R. groenlandicum*, and *R. tomentosum.* These plants are already well known to the Cree and can be a culturally acceptable form of complementary medicine used to treat T2D. Incorporation of traditional medicines into indigenous public health care systems can contribute to disease alleviation and warrants further rigorous investigation to assure efficacy and reduce toxicity.

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