PHYTOTHERAPY RESEARCH
Phytother. Res. **24**: 741–747 (2010)
Published online 19 November 2009 in Wiley InterScience
(www.interscience.wiley.com) **DOI**: 10.1002/ptr.3025

Inhibitory Effect of the Cree Traditional Medicine Wiishichimanaanh (*Vaccinium vitis-idaea*) on Advanced Glycation Endproduct Formation: Identification of Active Principles

Louis-Philippe Beaulieu[1,2#], Cory S. Harris[1,2,3#], Ammar Saleem[1,2], Alain Cuerrier[1,4], Pierre S. Haddad[1,5], Louis C. Martineau[1,5], Steffany A.L. Bennett[1,3], John T. Arnason[1,2]*

[1]Canadian Institute of Health Research Team in Aboriginal Anti-diabetic Medicines, Department of Pharmacology, Université de Montréal, QC, H3C 3J7, Canada
[2]Centre for Research in Biopharmaceuticals and Biotechnology, Department of Biology, University of Ottawa, Ottawa, ON, K1N 6N5, Canada
[3]Neural Regeneration Laboratory, Ottawa Institute of Systems Biology, Department of Biochemistry, Microbiology, and Immunology, University of Ottawa, Ottawa, ON, K1H 8M5, Canada
[4]Jardin botanique de Montréal, Institut de recherche en biologie végétale, Université de Montréal, Montréal, QC H9X 3V9, Canada
[5]Department of Pharmacology, Université de Montréal and Montreal Diabetes Research Center. Montréal, QC, H3C 3J7, Canada

Like many aboriginal populations, First Nations communities such as the Cree of Eeyou Istchee are facing continuously increasing rates of diabetes and related complications. Advanced glycation endproducts (AGEs), which readily form and accumulate with sustained hyperglycemia, contribute to the development of diabetic complications and, as such, are considered a potential therapeutic target. In the present study, the inhibition of AGE formation by ethanolic extracts of the Cree medicinal plant *Vaccinium vitis-idaea* L. was assessed by fluorometric detection of fluorescent AGEs and immunodetection of N^{ε}-(carboxymethyl)lysine adducts of albumin. Extracts from *V. vitis-idaea* berries demonstrated a concentration-dependent inhibition of AGE formation in both measures. High performance liquid chromatography mass spectrometry (HPLC/MS) identified nine main phenolic constituents. Four were selected for further testing, of which catechin, quercetin-3-*O*-galactoside and cyanidin-3-*O*-glucoside but not *para*-coumaric acid displayed antiglycation activities. These results demonstrate that the flavonoid components of the berry extract are potent antiglycation agents and provide pharmacological validation for the traditional use of *V. vitis-idaea* as an antidiabetic remedy.

Keywords: Type 2 diabetes; diabetic complications; N^{ε}-(Carboxymethyl)lysine; medicinal plants; flavonoids; mountain cranberry; lingonberry.

INTRODUCTION

Recent global estimates suggest that the number of people with diabetes (over 180 million in 2000) will double by 2030 (Wild *et al.*, 2004). As with many aboriginal populations worldwide, the situation in Canada's First Nations communities is particularly devastating with prevalence 3.6 and 5.3 times higher (men and women, respectively) than in Canadians not of First Nations ancestry. Because compliance with modern pharmaceuticals may be low in aboriginal populations, sustained hyperglycemia results in increased incidence and severity of diabetic complications (Young *et al.*, 2000; Yu and Zinman, 2007). This disparity has been attributed, in part, to genetic susceptibility and environmental factors such as reduced physical activity and dietary acculturation in aboriginal communities (Hegele, 2001; Yu and Zinman, 2007). The traditional use of wild foods and plant medicines by aboriginal people of North America has generally been reduced in favor of a more 'Westernized' diet (Baynes and Thorpe, 1999; McCune and Johns, 2002). These traditional plants contain biologically active compounds including natural antioxidants and represent a culturally suitable adjuvant treatment that may assist in providing protection from the development of diabetic complications.

Based on ethnobotanical research conducted in collaboration with the Cree healers of Eeyou Istchee (CEI) of northern Quebec, a prioritized list of plant species used for the treatment of diabetic symptoms has been compiled (Leduc *et al.*, 2006; Fraser *et al.*, 2007). One of the identified plants, *Vaccinium vitis-idaea* L. (commonly referred to as mountain cranberry or lingonberry in English, and *wiishichimanaanh* (inland) or *wiisichiminh* (coastal) to the CEI) is a member of the Ericaceae family. Various members of the *Vaccinium* genus, including lowbush blueberry (*V. angustifolium*), American cranberry (*v. macrocarpon*), and European bilberry (*V. myrtillus*), have been used as traditional medicines for treating symptoms of diabetes (Jellin *et al.*, 2005; Leduc *et al.*, 2006) and possess reputed antidiabetic activity (Blumenthal, 1998; Chambers and Camire, 2003; Martineau *et al.*, 2006). *V. vitis-idaea* berries are traditionally used by the Cree to treat frequent

* Correspondence to: Dr John T. Arnason, Centre for Research in Biopharmaceuticals and Biotechnology, Department of Biology, University of Ottawa, 30 Marie Curie, Ottawa, ON, K1N 6N5, Canada.
E-mail: John.Arnason@uOttawa.ca
#Authors contributed equally.

Received 28 April 2009
Revised 12 August 2009
Accepted 26 August 2009

urination, abscesses, sore eyes, toothache, snow blindness and thrush (Leduc *et al.*, 2006). It is also used medicinally by Alaskan natives to treat colds, coughs and sore throats (Kari, 1985).

With high flavonoid content, *V. vitis-idaea* berry extracts demonstrate strong antioxidant capacity (Kahkonen *et al.*, 2001). Certain phenolic compounds, including flavonoids, are not only potent antioxidants but also possess additional properties relevant to diabetic complications, including the ability to inhibit the formation of advanced glycation endproducts (AGEs) (Wu and Yen, 2005). AGEs are a diverse class of biomolecules resulting from non-enzymatic reactions between lipid, nucleic acid, or protein substrates and reducing sugars such as glucose and fructose. In conditions of hyperglycemia, glycation leads to cellular damage by impairing the function of intracellular and extracellular proteins (Sheetz and King, 2002). This often affects the half-life of the biomolecule and, in cases such as insulin, decreases the molecule's bioactivity (Unoki and Yamagishi, 2008). Some AGEs, such as N^{ε}-(carboxymethyl)lysine (CML), interact with AGE receptor (RAGE), which elicits activation of NF-κB, participates in the initiation and progression of atherosclerosis, and contributes to inflammatory events that contribute to macro- and microvascular complications of diabetes (Ramasamy, 2006). During the conjugation of reducing sugars to biomolecules, several oxidative transformations occur resulting in oxidative stress (Thornalley *et al.*, 1999). Dietary antioxidants, such as flavonoids, therefore offer at least two means of protection, by inhibiting oxidative formation of AGEs and by scavenging reactive oxygen species (ROS) (Wu and Yen, 2005). Current strategies targeting AGE production have met with limited success, notably due to side-effects associated with synthetic inhibitors (Peyroux and Sternberg, 2006). A nutritional approach based on foods with high flavonoid content represents an alternative approach to AGE inhibition with a reduced risk of adverse effects.

The aim of the present study was to investigate the *in vitro* antiglycation activity of *V. vitis-idaea* and, in the process, bring forth evidence that use of this traditional food and medicine may provide protection from diabetic complications. Combining fluorometric and immunochemical methods for the detection of AGEs, *V. vitis-idaea* berry extract and its major phenolic metabolites were assessed for inhibitory effects on AGE formation. High-performance liquid chromatography mass spectrometry (HPLC/MS) was used to identify and quantify the phenolic constituents of the extract.

MATERIALS AND METHODS

Chemicals and materials. Bovine serum albumin (BSA) and quercetin were obtained from Sigma (St Louis, MO, USA). D-Glucose and D-Fructose were purchased from BDH Chemicals Ltd (Toronto, ON, CAN). HPLC-grade reference standards cyanidin-3-galactoside and quercetin-3-galactoside were obtained from Extrasynthèse (Genay, France), whereas (+)-catechin was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Para-coumaric acid was obtained from J.T. Baker Chemical Co. (Phillipsburg, NJ, USA). Sodium phosphate, monobasic, monohydrate was purchased from EM Science (Gibbstown, NJ, USA). The primary mouse monoclonal antibody targeted against CML-BSA adducts (Clone 318003), the secondary goat antimouse IgG polyclonal antibody conjugated with horseradish peroxidase and the luminol-based chemiluminescent reagent were purchased from R & D Systems (Minneapolis, MN, USA).

Plant materials and extractions. Fresh *V. vitis-idaea* berries were collected in August 2005 near Mistissini, Quebec, immediately frozen then transported to the University of Ottawa where an 80% ethanolic extract was prepared as previously reported for other Cree plants (Spoor *et al.*, 2006). Botanical identity was confirmed by Montreal Botanical Garden taxonomist Dr Alain Cuerrier and voucher specimens were deposited at the University of Ottawa herbarium. The dried berry extract was stored in darkness at 4ºC and reconstituted in ethanol/water (80%: 20%, v/v) for experimental use. Stock solutions of 40 and 50 mg extract/mL were filtered through a 0.2 µm nylon membrane filter (Chromatographic Specialties Inc., Brockville, ON, CAN) and subsequently diluted.

Fluorescence-based assay of the inhibition of AGE formation. The method was performed as previously described (Suzuki *et al.*, 2003; Farsi *et al.*, 2007) with additional modifications. Stock solutions of glucose (200 mM)/fructose (200 mM) and BSA (2 mg/mL) were prepared in 100 mM sodium phosphate monobasic monohydrate buffer (pH 7.4), each of which sterilized by vacuum filtration using Nalgene cellulose nitrate membrane filters (Fisher Scientific Ltd, Nepean, ON, CAN) prior to use. Incubation media containing BSA (1.0 mg/mL), glucose (100 mM), fructose (100 mM) and either *V. vitis-idaea* berry extract, standard chemical compounds or vehicle (80% EtOH: 20% H_2O, v/v) in 100 mM sodium phosphate buffer were prepared. Ten concentrations of the berry extract (0.39–200 µg/mL; prepared by serial dilution of the 40 mg/mL stock extract) and of the standard chemical compound solutions (0.05–50 µg/mL; prepared by serial dilution of a 1 mg/mL stock standard solution) were included to determine concentration-dependent responses. Since BSA is autofluorescent, two additional controls were incorporated in each assay: a BSA blank (1 mg/mL in 100 mM sodium phosphate buffer) and a vehicle control (100 mM sodium phosphate buffer). To determine whether the berry extract or pure compounds interfered with fluorescence, an extract blank containing glucose (100 mM) and fructose (100 mM) in 100 mM phosphate buffer was prepared for each dilution. The negative control for this fluorescence-based assay contained glucose (100 mM), fructose (100 mM), BSA (1 mg/mL) and vehicle in 100 mM phosphate buffer. The positive control was quercetin (2.5 µg/mL), a flavonoid with established antiglycation activity (Wu and Yen, 2005). Once prepared, replicate volumes of 200 µL of each treatment were delivered into four wells of sterile opaque 96-well plates with clear bottoms (Corning Inc., New York, NY, USA), with a BSA blank, a phosphate buffer blank, and negative and positive controls included on every plate. The plates were then sealed with parafilm and incubated at 37ºC in darkness with shaking in a Series 25 Incubator Shaker (New Brunswick Scientific

DOI: 10.1002/ptr

Co., Inc., Edison, NJ, USA). After 7 days of incubation, the formation of fluorescent AGEs was quantitatively assessed using a SpectraMax Gemini XS microplate fluorometer (Molecular Devices, Sunnyvale, CA, USA) at excitation and emission wavelengths of 355 nm and 460 nm, respectively. The fluorescence readings for the experimental treatment (containing BSA, sugar and either berry extract or pure standard) and the negative control were blanked against BSA, phosphate buffer, and the appropriate extract blanks to exclude baseline fluorescence. The corrected fluorescence readings (F) for the negative control ($F_{\text{negative control}}$) and for the experimental treatments ($F_{\text{experimental corrected}}$) were used to determine the percent inhibition of AGE formation by the following formula:

$$\text{Percent inhibition} = \frac{(F_{\text{negative control}} - F_{\text{experimentalcorrected}})}{F_{\text{negative control}}} \times 100\%$$

Immunochemical detection of CML-BSA adducts. After incubation and fluorescence measurements, 500 µL of selected treatments (experimental treatments with berry extract at the following concentrations: 6.25, 25, 50, 100 and 200 µg/mL; experimental treatment with 2.50 µg/mL quercetin; negative control and BSA blank) were concentrated using Ultracel YM-10 membrane centrifugal filters (Millipore, Billerica, MA, USA). The purified protein samples were quantified using a BioRad DC Protein assay Kit (BioRad Laboratories (Canada) Ltd., Mississauga, ON, CAN) according to supplier protocol. Two simultaneous SDS-PAGE separations were performed under reducing conditions on acrylamide/bisacrylamide separating gel with 1.9 µg of protein loaded in each well. After the electrophoresis step, one of the gels was stained with Coomassie Blue to show equal loading. To ensure consistent loading between gels, reversible Ponceau staining was performed on the second gel prior to immunochemical analysis. Western blot analysis employed a primary mouse monoclonal antibody targeted against CML-BSA adducts. A secondary goat antimouse IgG polyclonal antibody conjugated with horseradish peroxidase and a luminol-based chemiluminescent reagent were used to visualize the CML-BSA adducts.

Identification of phenolic constituents from *V. vitis-idaea* berry extract. HPLC-MS analyses were performed on an Agilent 1100 series LC-MSD system (Agilent, Palo Alto, CA, USA) consisting of an autosampler, a quaternary pump, a column thermostat, a photodiode array detector (DAD), and an online mass spectrometric system consisting of an atmospheric pressure chemical ionization (APCI) ion source. A Synergi Polar-RP column (150 mm × 3 mm; 4 µm particle size, Phenomenex, Torrence, CA USA) was maintained at 45ºC at a flow rate of 0.3 mL/min and a 1–3 µL sample injection. All solvents for LC-MS analysis were of HPLC grade purchased from Fisher Scientific (Ottawa, ON, Canada). The mobile phase system consisted of methanol (solvent A), acetonitrile (solvent B) and aqueous trifluoroacetic acid (0.05% V/V, solvent C). The gradient elution conditions were optimized to a 40 min program: initial conditions 6:6:88 (A:B:C), changing to 25:25:50 in 25 min, then to 50:50:0 in 3 min, then isocratic elution with 50:50:0 for 2 min, finally returning to initial conditions in 4 min, which were held for 6 min to re-equilibrate the column. The separation profiles were monitored at 325 nm for detecting UV absorbing phenolics and 520 nm for detecting anthocyanins, while the DAD and MS detection was performed in positive ionization mode. Mass spectrometer conditions were: APCI source temperature at 200˚C, with the vaporizer at 450˚C, nebulizer pressure of 40 psi, nitrogen (drying gas) flow rate of 4.0 L/min, fragmentation voltage ramping from 40 V (100 mass units) to 100 V (1000 mass units), the capillary voltage of 3000 V and corona current of 5.0 µA. The MS data were collected in scan mode for ions from 100 to 1000 mass units to obtain total ion chromatograms. The phytochemical constituents of the extract were initially identified by comparing the UV spectrum of unknown peaks to a ChemStation library of spectra from known phenolic compounds, as previously described (Harris *et al.*, 2007). Using pure standards, identifications were confirmed based on three requirements: retention time, UV spectrum obtained from the DAD and fragmentation pattern recorded by APCI/MS. Quantification of the following standards in the plant extract was based on a linear four-point calibration curve of different concentrations: quercetin-3-galactoside (100–300 µg/mL), cyanidin-3-galactoside (100–500 µg/mL), (+)-catechin (100–300 µg/mL) and para-coumaric acid (50–150 µg/mL).

Statistical analysis. Comparison of the IC_{50} data for the fluorescence-based assay of the inhibition of AGE formation by standard molecules was processed by the S-Plus software using a one-way ANOVA with Tukey *post-hoc* analysis.

RESULTS AND DISCUSSION

The *V. vitis-idaea* ethanolic berry extract was evaluated in the fluorescence-based assay of AGE formation in quadruplicate experiments revealing a log-correlation between inhibition of fluorescent AGE crosslink formation and the concentration of the berry extract (Fig. 1). The IC_{50} of the ethanolic extract was calculated as

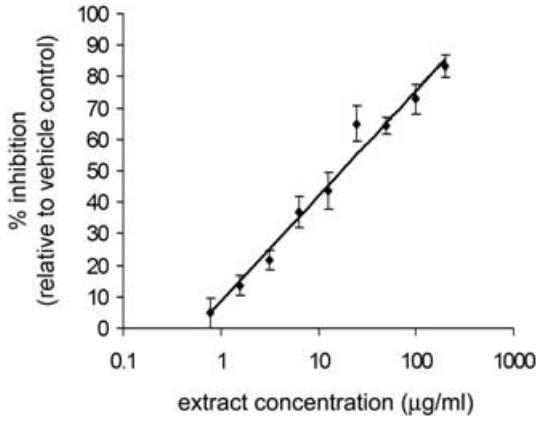

Figure 1. Inhibition of the formation of fluorescent AGE crosslinks by ethanol extract of *V. vitis-idaea* berry. Results are expressed as the percent inhibition relative to negative control versus plant extract concentration (means ± SEM for n = 4). A logarithmic regression was fitted to the data: $R^2 = 0.98$.

DOI: 10.1002/ptr

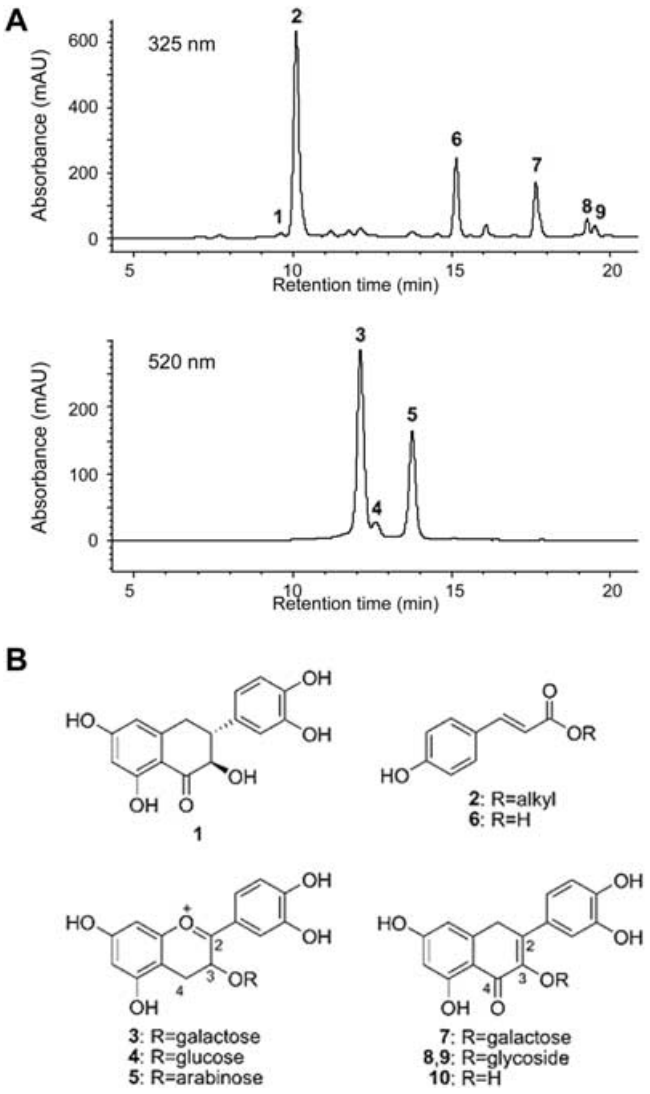

Figure 2. (A) HPLC spectra of *V. vitis-idaea* berry ethanolic extract. Data represent absorbance at 325 and 520 nm (in milliabsorbance units, mAU) plotted against retention time (in minutes). The HPLC method consisted of injecting 3 μL of the 50 mg/mL 80% ethanolic berry extract into a Synergi Polar-RP column. The mobile phase followed a linear gradient from 6%MeOH: 6%MeCN: 88% of 0.05% aqueous TFA to 25% MeOH: 25% MeCN: 50% of 0.05% aqueous TFA in 25 min. (B) Chemical structure of quercetin (10) and of members of the four classes of phenolic molecules found in *Vaccinium vitis-idaea* berries: catechin (1), *p*-coumaric aid (6) along with its ester analog (2), cyanidin glycosides (3, 4, 5) and quercetin glycosides (7, 8, 9).

13.5 μg/mL. Given that many of the constituents of the *V. vitis-idaea* berry extract may account for its inhibition of AGE formation, phytochemical characterization of the berry extract was undertaken using HPLC-MS (Fig. 2A). The identified constituents of the *V. vitis-idaea* berry extract matched those of previous investigations (Zheng and Wang, 2003; Ek *et al.*, 2006), revealing a variety of phenolic metabolites, some of which were quantified relative to reference standards (Fig. 2B, Table 1). The IC_{50} values for the positive control quercetin (**10**) and identified phenolic constituents from the berry extract, for which pure standards were available (quercetin-3-galactoside (**3**), cyanidin-3-galactoside (**7**) and (+)-catechin (**1**)), were determined and are reported in Table 1. The observed IC_{50} values for quercetin-3-galactoside, cyanidin-3-galactoside and quercetin were not statistically different, while the IC_{50} of (+)-catechin was significantly higher ($p < 0.001$). Only *para*-coumaric acid (**6**) failed to effectively inhibit formation of fluorescent AGEs. Thus, quercetin-3-galactoside and cyanidin-3-galactoside had a potency similar to that of quercetin, while (+)-catechin was about five times less potent. Given their demonstrated antiglycation activity and relatively high abundance in *V. vitis-idaea* berries, cyanidin-3-glycosides (anthocyanins) and quercetin-glycosides (flavonols) likely represent the main active principles within the *V. vitis-idaea* berry extract. (+)-Catechin, whose quantity in berries was similar to those of quercetin-3-glycosides, was significantly less potent and is therefore expected to contribute less to the antiglycation properties of the berry extract.

The relative activities of the identified phenolics (Table 1, Fig. 2) are consistent with the relative antioxidant capacities of each compound. As radical intermediates play an important role in the chemistry of AGE formation (Rizzi, 2003), phenolic antioxidants (PhOH) from dietary sources serve as potent inhibitors of AGE formation through their radical-scavenging effects (Matsuda *et al.*, 2003; Wu and Yen, 2005). The ensuing phenoxy radicals (PhO) are stabilized by resonance delocalization and may react with other radical species to form non-radical products (Wright *et al.*, 2001). The antioxidant activities of the identified phenolics in *V. vitis-idaea* berries have been well studied and demonstrate that flavonoids generally possess greater activity than phenolic acids such as *para*-coumaric acid (Rosch *et al.*, 2003). Among flavonoids, quercetin and cyanidin glycosides display slightly less potent antioxidant activity than their aglycones but stronger activity than (+)-catechin, a flavan-3-ol (Rice-Evans *et al.*, 1996). Reported relative ranking of antioxidant ability among phenolic antioxidants was therefore identical to the observed antiglycation effects in this study (Table 1). Several structural aspects of the phenolic molecules account for individual variability and thus their differing antioxidant potential. For instance, (+)-catechin has the same number of hydroxyl groups in the same positions as quercetin, but lacks the 4-oxo function and the 2,3-double bond, which contributes to electron delocalization, stabilizes the phenoxy radical and results in quercetin's higher relative antioxidant potential (Rice-Evans *et al.*, 1996).

Because the CEI use berry preparations in their treatment of diabetic symptoms, we sought to further validate the inhibitory effect of the *V. vitis-idaea* berry extract on AGE formation. Immunochemical characterization was performed using a monoclonal antibody targeting CML-BSA adducts. Detection of CML-BSA adducts in the proteins purified from the incubation media showed a concentration-dependent decrease in CML-BSA formation with increasing extract concentration (Fig. 3A). The large band labelled 66 kDa corresponds to a monomeric BSA containing CML while the two upper bands represent adducts consisting of two and three cross-linked BSA molecules. As expected, no CML formation occurred in the absence of sugar (Fig. 3, lane 3). Quercetin was used as a positive control, revealing that, at a concentration of 8 μM, CML formation was partially inhibited (Fig. 4).

Together, these complementary assays provide converging evidence for the anti-AGE effects of *V. vitis-idaea* extracts and active phenolic constituents. Interestingly, the inhibitory effects of *V. vitis-idaea*

DOI: 10.1002/ptr

Table 1. Phytochemical constituents of *V. vitis-idaea* ethanolic extract, determined by HPLC-MS and their antiglycation activities.

Compound	Quantitation[a] (mg compound/g fresh berries)	IC_{50}[b] (μg/mL)	IC_{50}[b] (μM)
1 – Catechin	1.62 ± 0.02	8.35 ± 0.43	28.75 ± 1.48[c]
2 – *p*-coumaric acid derivative	1.71 ± 0.01[e]	–[d]	–
3 – Cyanidin-3-*O*- galactoside	2.04 ± 0.06	3.10 ± 0.18	6.40 ± 0.36
5 – Cyanidin-3-*O*-arabinoside	3.49 ± 0.18[f]	–	–
6 – *p*-coumaric acid	0.53 ± 0.00	N/D	N/D
7 – Quercetin-3-*O*-galactoside	1.59 ± 0.00	2.86 ± 0.53	6.16 ± 1.15
10 – Quercetin	N/D[g]	1.62 ± 0.24	4.78 ± 0.72

N/D, not detected.

[a]Quantification results expressed as milligrams of the standard molecule per gram of fresh berries. Data presented as means ± SEM, for n = 3.

[b]IC_{50} was defined as the concentration producing 50% AGE inhibition. Data presented as means ± SEM, for n = 3.

[c]The IC_{50} for catechin, expressed in μM, was statistically different from the IC_{50}'s of compounds 3, 7 and 10 ($p < 0.001$, ANOVA with *post hoc* Tukey's test).

[d]Hyphen denotes that the compound was not assessed in glycation assays because a pure standard was unavailable.

[e]Para-coumaric acid derivative quantified using the calibration curve of para-coumaric acid.

[f]Cyanidin-3-arabinoside quantified using the calibration curve of cyanidine-3-galactoside.

[g]Pure quercetin was used as a positive control.

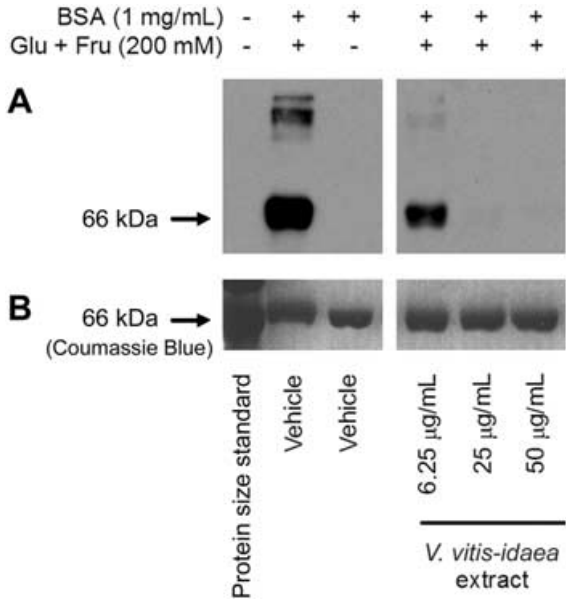

Figure 3. Western blot of glycated-albumin resulting from a 7-day incubation at 37°C of BSA with glucose/fructose and increasing concentrations of *V. vitis-idaea* extract or vehicle (0.4% ethanol). Two simultaneous SDS-PAGE separations were performed, in which 1.9 μg of protein was loaded into the wells. The first gel was used for Western blotting with a primary monoclonal antiCML-BSA antibody (A) and the second was used to verify equal protein loading with Coomassie Blue staining (B).

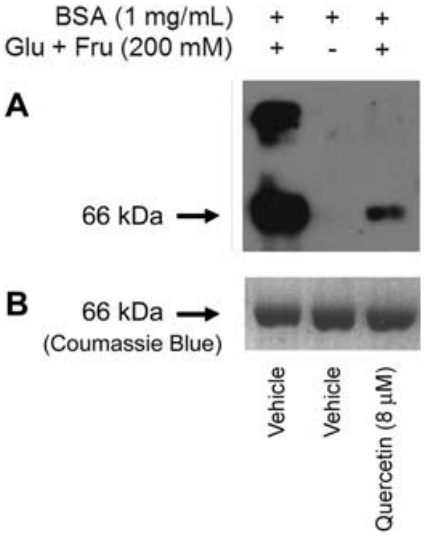

Figure 4. Western blot of glycated-albumin resulting from a 7-day incubation at 37°C of BSA with glucose/fructose and quercetin standard or vehicle (0.4% ethanol). Two simultaneous SDS-PAGE separations were performed, in which 1.9 μg of protein was loaded into the wells. The first gel was used for Western blotting with a primary monoclonal antiCML-BSA antibody (A) and the second was used to verify equal protein loading with Coomassie Blue staining (B).

extract were similar as determined by both CML-immunodetection and fluorometric AGE assays. Indeed, CML-adduct formation was completely prevented and fluorescent AGEs were reduced by nearly 70% in the presence of 25 μg/mL berry extract (Figs 1 and 3). Efficient inhibition of CML formation demonstrates that the antiglycation actions of *V. vitis* and flavonoids are not limited to fluorescent AGEs, which account for only a small fraction of physiologically occurring cross-linking structures (Dyer *et al.*, 1991), but extend to non-fluorescent AGEs such as CML. The latter can accumulate in large quantities in diabetic patients and have been correlated with the development of diabetic complications (Schleicher *et al.*, 1997; Wautier *et al.*, 2003). Recently, Yamabe *et al.* (2007) determined that the renoprotective effects of *Cornus officinalis officinalis* Sieb. & Zucc. on streptozotocin-treated diabetic rats was due in part to inhibited formation of AGEs, namely CML, and reduced downstream signaling through targets such as RAGE. Our data indicate that *V. vitis-idaea* may confer similar antidiabetic effects that will require future *in vivo* validation.

When compared to other berries and fruits, *V. vitis-idaea* berries possess high levels of anthocyanins, flavonols and flavan-3-ols and superior *in vitro* antioxidant activity (Kahkonen *et al.*, 2001; Lichtenthaler and Marx, 2005). The berry extract also reduces the oxidation of

DOI: 10.1002/ptr

Table 2. Medicinal uses of wiisichiminh among the Cree of Mistissini and Whapmagoostui. Additional traditioanal uses by Cree groups in Canada have been reported and are also listed.

Symptom or illness	Part used	Community (Source)
Uses by the Cree of Eeyou Istchee (CEI)		
Sore eyes	Berry, juice of	Whapmagoostui
Abscesses	Berry, juice of	Whapmagoostui
Snow blindness	Berry, juice of	Whapmagoostui
Toothache (baby)	Branches and leaves	Whapmagoostui
Thrush[†]	Berry, juice of	Whapmagoostui
Urinary problems	Boiled berries and drink	Whapmagoostui
Increased urination	Berry, eat raw or make jam	Mistissini (Leduc *et al.*, 2006)
Mentions of medicinal uses in the Cree Literature		
Snow blindness	Berry	Waskaganish (Marshall *et al.*, 1989)
Stomach (cleaning the)	Berry	Northern Manitoba (Marles *et al.*, 2000)
Fever	Berry	Northern Manitoba (Marles *et al.*, 2000)
Bladder problem	Berry	Northern Manitoba (Marles *et al.*, 2000)
Post-partum application	Root	Northern Quebec (Zieba, 1992)

[†] Adopted from Inuits (Cuerrier, 2005)

low-density lipoproteins (LDLs) (Fraser, 2007). Oxidized and glycated LDLs are retained in vascular walls and lead to macrophage recruitment and the eventual formation of plaques associated with atherosclerosis (Baynes and Thorpe, 1999). By scavenging ROS and reducing both glycation and oxidation, *V. vitis-idaea* extracts offer multiple means of protecting the vascular system from diabetes-related damage. Since consumption of fresh *V. vitis-idaea* berries has been shown to elevate plasma concentrations of quercetin (Erlund *et al.*, 2006), the observed *in vitro* activities are likely relevant *in vivo.* Moreover, A-type procyanidins, which are responsible for the uroprotective actions of American cranberries (*V. macrocarpon*) (Foo *et al.*, 2000), have been reported in *V. vitis-idaea* berries and possess antiviral properties (Cheng *et al.*, 2005). These diverse biological activities are reflected in the varied traditional uses of the plant. When New World (*V. vitis idaea* ssp. *minus*) and Eurasian (*V. vitis-idaea* ssp. *vitis-idaea*) subspecies are combined, *V. vitis-idaea* has a circumpolar distribution and a correspondingly rich history of traditional and modern uses as food and medicine. Traditionally, the plant has been used among native peoples of North America and Northern Eurasia (Tunon *et al.*, 1995; Moerman, 1998). The Cree nations within Canada have been using the plant for numerous illnesses, notably for bladder infection, snow blindness, stomach problems, fever and post-partum problems (Table 2). Although the berries are mainly used, the root system or the whole plant has also been used traditionally. Within the Inuit communities of Nunavik, the berries are actively sought to treat mouth problems such as baby thrush, a usage adopted by the Cree from their Inuit neighbors in Whapmagoostui (Cuerrier, 2005). Presently, the berries are widely harvested, processed and sold commercially as food and beverage products around the world.

As the incidence of diabetes continues to rise worldwide, the study of natural products for the treatment and prevention of diabetes and associated complications is a promising opportunity for complementary interventions that may be more acceptable to high-risk populations in search of non-pharmaceutical alternatives. Our results demonstrate that *V. vitis-idaea* berries, particularly the flavonoids, are potent inhibitors of *in vitro* AGE formation. *V. vitis-idaea* is a traditional remedy against diabetic symptoms that, with further *in vivo* validation, may provide a culturally compatible treatment option for the Cree as well as other native communities. Such validation of CEI traditional knowledge will also support the continued two-way exchange between traditional and Western knowledge systems for the benefit of public health. The described assays, which detect both fluorescent and non-fluorescent AGE species, offer a straightforward and robust method for assessing the antiglycation activity of other plants from the Cree pharmacopoeia.

Acknowledgements

This work was supported by a Canadian Institutes of Heath Research (CIHR) Team Grant (CTP-79855) to PSH, JTA, AC, SALB and LM. Very special thanks are due to Elizabeth Coon Come, Mable Gunner, Charlotte Husky Swallow, Johnny Husky Swallow, Ronny Loon and Girty Loon from the Cree Nation of Mistissini as well as to the 59 other Cree Elders from the Nations of Mistissini and Whapmagoostui who kindly agreed to be interviewed. They made this paper possible by allowing us to use, for the purposes of this research, their knowledge relating to medicinal plants, transmitted to them by their elders. Their trust has also enabled a useful exchange between indigenous knowledge and Western science. PSH is a National Research Scholar of the Fond de la Recherche en Santé du Québec. SALB is a CIHR New Investigator and an Ontario Mental Health Intermediate Investigator. LPBB was funded by a Natural Sciences and Engineering Research Council of Canada Undergraduate Student Research Award and CSH by a Canadian Graduate Scholarship.

REFERENCES

Baynes JW, Thorpe SR. 1999. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes* **48**: 1–9.

Blumenthal M. 1998. *The complete German commision E monographs – Therapeutic guide to herbal medicines.* American Botanical Council: Austin, TX.

Chambers BK, Camire ME. 2003. Can cranberry supplementation benefit adults with type 2 diabetes? *Diabetes Care* **26**: 2695–2696.

Cheng H-Y, Lin T-C, Yang C-M, Shieh D-E, Lin C-C. 2005. In vitro anti-HSV-2 activity and mechanism of action of proanthocyanidin A-1 from *Vaccinium vitis-idaea*. *J Sci Food Agric* **85**: 10–15.

Cuerrier A, Aînés de Kangirsujuq, Q, 2005. *Le savoir botanique des Inuits de Kangirsujuaq, Nunavik 4e éd. Étude effectuée pour l'Administration régionale Kativik*. Institut culturel Avataq & Institut de recherche en biologie végétale: Montreal; 67.

Dyer DG, Blackledge JA, Katz BM, Hull CJ, Adkisson HD, Thorpe SR, Lyons TJ, Baynes JW. 1991. The Maillard reaction in vivo. *Z Ernahrungswiss* **30**: 29–45.

Ek S, Kartimo H, Mattila S, Tolonen A. 2006. Characterization of phenolic compounds from lingonberry (Vaccinium vitis-idaea). *J Agric Food Chem* **54**: 9834–9842.

Erlund I, Freese R, Marniemi J, Hakala P, Alfthan G. 2006. Bioavailability of quercetin from berries and the diet. *Nutr Cancer* **54**: 13–17.

Farsi DA, Harris CS, Reid L, Bennett SA, Haddad PS, Martineau LC, Arnason JT. 2007. Inhibition of non-enzymatic glycation by silk extracts from a Mexican land race and modern inbred lines of maize (Zea mays). *Phytother Res* **22**: 108–112.

Foo LY, Lu Y, Howell AB, Vorsa N. 2000. A-Type proanthocyanidin trimers from cranberry that inhibit adherence of uropathogenic P-fimbriated Escherichia coli. *J Nat Prod* **63**: 1225–1228.

Fraser M-H, Cuerrier A, Haddad P, Arnason JT, Owen P, Johns T. 2007. Medicinal plants of Cree communities (Québec, Canada): Antioxidant activity of plants used to treat Type 2 Diabetes symptoms. *Canadian Journal of Physiology and Pharmacology* **85**: 1200–1214.

Harris CS, Burt AJ, Saleem A, Le PM, Martineau LC, Haddad PS, Bennett SA, Arnason JT. 2007. A single HPLC-PAD-APCI/MS method for the quantitative comparison of phenolic compounds found in leaf, stem, root and fruit extracts of *Vaccinium angustifolium*. *Phytochem Anal* **18**: 161–169.

Hegele RA. 2001. Genes, environment and diabetes in Canadian aboriginal communities. *Adv Exp Med Biol* **498**: 11–20.

Jellin J, Gregory PJ, Batz F, Hitchens K. 2005. Natural medicines comprehensive database. Therapeutic Research Faculty: Stockton.

Kahkonen MP, Hopia AI, Heinonen M. 2001. Berry phenolics and their antioxidant activity. *J Agric Food Chem* **49**: 4076–4082.

Kari PR. 1985. Upper Tanana Ethnobotany. Alaska Historical Commission, Anchorage.

Leduc C, Coonishish J, Haddad P, Cuerrier A. 2006. Plants used by the Cree Nation of Eeyou Istchee (Quebec, Canada) for the treatment of diabetes: A novel approach in quantitative ethnobotany. *J Ethnopharmacol* **105**: 55–63.

Lichtenthaler R, Marx F. 2005. Total oxidant scavenging capacities of common European fruit and vegetable juices. *J Agric Food Chem* **53**: 103–110.

Marles RJ, Clavelle C, Monteleone L, Tays N, Burns D. 2000. *Aboriginal plant use in Canada's northwest Boreal Forest*. UBC Press: Vancouver.

Marshall S, Diamond L, Blackned S. 1989. Healing ourselves, helping ourselves: The medicinal use of plants and animals by the people of Waskaganish, Cree Regional Authority, Val d'Or, Quebec.

Martineau LC, Couture A, Spoor D, Benhaddou-Andaloussi A, Harris C, Meddah B, Leduc C, Burt A, Vuong T, Mai Le P, Prentki M, Bennett SA, Arnason JT, Haddad PS. 2006. Anti-diabetic properties of the Canadian lowbush blueberry *Vaccinium angustifolium* Ait. *Phytomedicine* **13**: 412–423.

Matsuda H, Wang T, Managi H, Yoshikawa M. 2003. Structural requirements of flavonoids for inhibition of protein glycation and radical scavenging activities. *Bioorg Med Chem* **11**: 5317–5323.

McCune LM, Johns T. 2002. Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the indigenous peoples of the North American boreal forest. *J Ethnopharmacol* **82**: 197–205.

Moerman D. 1998. *Native American Ethnobotany.* Timber Press: Portland.

Peyroux J, Sternberg M. 2006. Advanced glycation endproducts (AGEs): Pharmacological inhibition in diabetes. *Pathol Biol (Paris)* **54**: 405–419.

Ramasamy R. 2006. Glycation and RAGE: Common links in the pathogenesis of microvascular and macrovascular complications of diabetes. *Canadian Journal of Diabetes* **30**: 422–429.

Rice-Evans CA, Miller NJ, Paganga G. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med* **20**: 933–956.

Rizzi G. 2003. Free radicals in the Maillard Reaction. *Food Reviews International* **19**: 375–395.

Rosch D, Bergmann M, Knorr D, Kroh LW. 2003. Structure-antioxidant efficiency relationships of phenolic compounds and their contribution to the antioxidant activity of sea buckthorn juice. *J Agric Food Chem* **51**: 4233–4239.

Schleicher ED, Wagner E, Nerlich AG. 1997. Increased accumulation of the glycoxidation product N(epsilon)-(carboxymethyl)lysine in human tissues in diabetes and aging. *J Clin Invest* **99**: 457–468.

Sheetz MJ, King GL. 2002. Molecular understanding of hyperglycemia's adverse effects for diabetic complications. *Jama* **288**: 2579–2588.

Spoor DC, Martineau LC, Leduc C, Benhaddou-Andaloussi A, Meddah B, Harris C, Burt A, Fraser MH, Coonishish J, Joly E, Cuerrier A, Bennett SA, Johns T, Prentki M, Arnason JT, Haddad PS. 2006. Selected plant species from the Cree pharmacopoeia of northern Quebec possess anti-diabetic potential. *Can J Physiol Pharmacol* **84**: 847–858.

Suzuki R, Okada Y, Okuyama T. 2003. Two flavone C-glycosides from the style of Zea mays with glycation inhibitory activity. *J Nat Prod* **66**: 564–565.

Thornalley PJ, Langborg A, Minhas HS. 1999. Formation of glyoxal, methylglyoxal and 3-deoxyglucosone in the glycation of proteins by glucose. *Biochem J* **344**(1): 109–116.

Tunon H, Olavsdotter C, Bohlin L. 1995. Evaluation of anti-inflammatory activity of some Swedish medicinal plants. Inhibition of prostaglandin biosynthesis and PAF-induced exocytosis. *J Ethnopharmacol* **48**: 61–76.

Unoki H, Yamagishi S. 2008. Advanced glycation end products and insulin resistance. *Curr Pharm Des* **14**: 987–989.

Wautier MP, Massin P, Guillausseau PJ, Huijberts M, Levy B, Boulanger E, Laloi-Michelin M, Wautier JL. 2003. N(carboxymethyl)lysine as a biomarker for microvascular complications in type 2 diabetic patients. *Diabetes Metab* **29**: 44–52.

Wild S, Roglic G, Green A, Sicree R, King H. 2004. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* **27**: 1047–1053.

Wright JS, Johnson ER, DiLabio GA. 2001. Predicting the activity of phenolic antioxidants: theoretical method, analysis of substituent effects, and application to major families of antioxidants. *J Am Chem Soc* **123**: 1173–1183.

Wu CH, Yen GC. 2005. Inhibitory effect of naturally occurring flavonoids on the formation of advanced glycation endproducts. *J Agric Food Chem* **53**: 3167–3173.

Yamabe N, Kang KS, Goto E, Tanaka T, Yokozawa T. 2007. Beneficial effect of *Corni fructus*, a constituent of Hachimijio-gan, on advanced glycation end-product-mediated renal injury in Streptozotocin-treated diabetic rats. *Biol Pharm Bull* **30**: 520–526.

Young TK, Reading J, Elias B, O'Neil JD. 2000. Type 2 diabetes mellitus in Canada's first nations: status of an epidemic in progress. *CMAJ* **163**: 561–566.

Yu CH, Zinman B. 2007. Type 2 diabetes and impaired glucose tolerance in aboriginal populations: A global perspective. *Diabetes Res Clin Pract* **78**: 159–170.

Zheng W, Wang SY. 2003. Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. *J Agric Food Chem* **51**: 502–509.

Zieba RA. 1992. *Healing and healers among the northern Cree*. National Library of Canada: Ottawa; 197.