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A combination of (+)-catechin and (−)-epicatechin underlies the *in vitro* adipogenic action of Labrador tea (*Rhododendron groenlandicum*), an antidiabetic medicinal plant of the Eastern James Bay Cree pharmacopeia

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ABSTRACT

Ethnopharmacological relevance: *Rhododendron groenlandicum* (Oeder) Kron & Judd (Labrador tea) was identified as an antidiabetic plant through an ethnobotanical study carried out with the close collaboration of Cree nations of northern Quebec in Canada.
Objectives: In a previous study the plant showed glitazone-like activity in a 3T3-L1 adipogenesis bioassay. The current study sought to identify the active compounds responsible for this potential antidiabetic activity using bioassay guided fractionation based upon an *in vitro* assay that measures the increase of triglycerides content in 3T3-L1 adipocyte.
Materials and methods: Isolation and identification of the crude extract's active constituents was carried out. The 80% ethanol extract was fractionated using silica gel column chromatography. Preparative HPLC was then used to isolate the constituents. The identity of the isolated compounds was confirmed by UV and mass spectrometry.
Results: Nine chemically distinct fractions were obtained and the adipogenic activity was found in fraction 5 (RGE-5). Quercetins, (+)-catechin and (−)-epicatechin were detected and isolated from this fraction. While (+)-catechin and (−)-epicatechin stimulated adipogenesis (238 ± 26% and 187 ± 21% relative to vehicle control respectively) at concentrations equivalent to their concentrations in the active fraction RGE-5, none afforded biological activity similar to RGE-5 or the plant's crude extract when used alone. When cells were incubated with a mixture of the two compounds, the adipogenic activity was close to that of the crude extract (280.7 ± 27.8 vs 311 ± 30%).
Conclusion: Results demonstrate that the mixture of (+)-catechin and (−)-epicatechin is responsible for the adipogenic activity of Labrador tea. This brings further evidence for the antidiabetic potential of *R. groenlandicum* and provides new opportunities to profile active principles in biological fluids or in traditional preparations.

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1. Introduction

The incidence of metabolic diseases, such as type 2 diabetes mellitus (T2DM), is increasing at an alarming rate and therefore represents a major worldwide health concern. Over 2.4 million Canadians are currently living with diabetes (Lage et al., 2008; Ruderman et al., 2003). The prevalence of this disease is three to five times higher among indigenous people than in the general Canadian population (Douglas, 2013; Hegele, 2001). Our team conducted an ethnobotanical study with the close collaboration of the Cree of Eeyou Istchee (James Bay area of northern Quebec) and identified 17 potential antidiabetic medicinal plants used by Cree healers to treat symptoms of diabetes (Fraser et al., 2007; Harbilas

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et al., 2009; Leduc et al., 2006; Spoor et al., 2006). In the course of screening these plants using several cell-based bioassays, a number of extracts were found to stimulate the differentiation of 3T3-L1 preadipocytes into mature adipocytes (adipogenesis) capable of synthesizing and accumulating triglycerides (TG) as lipid droplets (Spoor et al., 2006).

While obesity is a major risk factor for T2DM, an interesting hypothesis suggests that the latter may result in part from the inability of new adipocytes to be recruited (through differentiation), thus preventing the expansion of adipose tissue to accommodate excess calories (Freedland, 2004; Kletzien et al., 1992). In fact, thiazolidinediones (TZDs), a class of oral hypoglycemic drugs, exert their antidiabetic effect in part by inducing adipocyte differentiation through activation of PPARγ (Lehmann et al., 1995; Spiegelman, 1998).

Rhododendron groenlandicum (Oeder) Kron & Judd (commonly known as Labrador tea) is a shrub of the Ericaceae family that grows in the tundra, bogs and forests of North America (Chartier et al., 2005). The leaves are used to make a popular beverage tea that was one of the most highly recommended plant-products by Cree healers for the treatment of diabetic symptoms (Leduc et al., 2006). In an adipogenesis assay, an 80% ethanol extract of *R. groenlandicum* was able to stimulate adipogenesis to a similar extent as rosiglitazone, a representative TZD oral hypoglycemic (Spoor et al., 2006). Such adipogenic activity can lead to a reduction in circulating free fatty acids and hence reduce their accumulation in liver and muscle; steatosis in these insulin sensitive tissues being involved in insulin resistance and type 2 diabetes (Lewis et al., 2002). In a previous study, we reported that Labrador tea exerted antidiabetic activity by reducing hyperglycemia and improving insulin resistance as well as hepatic steatosis in a mouse model of diet-induced obesity and insulin resistance (Ouchfoun et al., 2015). Moreover, it acted as a PPAR- γ partial agonist in a gene reporter assay using the HEK 293-T cell line. (Ouchfoun et al., 2015). Therefore, the objective of the current study was to use the adipogenesis assay as a tool to guide the fractionation of the crude ethanol extract of *R. groenlandicum* (RGE) in order to identify the active compounds responsible for this putative antidiabetic activity.

2. Materials and methods

2.1. Cell lines, chemicals and biochemicals

Pre-adipocyte 3T3-L1 cell line was purchased from the American Type Culture Collection (ATCC; Manassas, VA). Dexamethasone), bovine pancreatic insulin, 3-isobutyl-1-methylxanthine (IBMX), dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (Oakville, ON). Rosiglitazone from was obtained Alexis Biochemicals (Hornby, ON). Dubelcco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS) and bovine calf serum (BCS) were from Wisent Inc (St-Bruno, QC). AdipoRed reagent was purchased from Combrex Bio Science Walkersville Inc (Walkersville, MD). All solvents (Chromsolv Plus grade) used for plant extraction and isolation, as well as reference compounds (+)-catechin, (−)-epicatechin and quercetin, silica gel 60 (70–230 mesh) and 60 F254 silica gel thin layer chromatography plates (20.0 × 20.0 cm) were obtained from Sigma-Aldrich (Oakville, ON).

2.2. Plant material

Fresh leaves of Rhododendron groenlandicum (Oeder) Kron & Judd (Ericaceae) were collected during August 2007 in Eeyou Istchee (Eastern James Bay area of northern Quebec, Canada) with the help of Cree elders. Species were identified by a plant taxonomist, Dr. Alain Cuerrier (Montreal Botanical Garden), and a voucher specimen (MIS 03-9) was deposited at Marie Victorin Herbarium of the Montreal Botanical Garden (Quebec, Canada).

2.3. Extraction and isolation

The dried and shredded leaves (1.6 kg) were extracted with 15 L ethanol (80% in MilliQ water) with 24 h continuous stirring at room temperature using a Caframo® mixer (Ultra Torque Model BDC1850, Ontario, ON). The pellet was then extracted with 7 L of 80% ethanol during 24 h. The supernatants were combined and evaporated to dryness *in vacuo* using a Yamato rotatory evaporator (Model RE300, Yamato America Inc., Santa Clara, CA) at 40 °C to yield 290 g of a dark green crude extract (yield 18.1%).

The dried leaf extract (100 g) was chromatographed on an open glass column packed with 1.6 kg of silica gel. The elution was carried out sequentially with a hexanes: ethyl acetate gradient (1:0→0:1) and an ethyl acetate: methanol (1:0→0:1) gradient to yield one hundred and ten (110) eluates of 250 ml each. Chemically similar eluates were pooled on the basis of thin layer chromatographic profiles performed on silica gel plates visualized with UV (λ 254 nm) and developed with ceric sulfate solution (5% of H_2SO_4). This procedure yielded nine primary fractions identified as RGE-1 though RGE-9 (Fig. 1). Biological activity was assessed using the adipogenesis assay (described below) in order to select the active fraction(s) to be subjected to further fractionation. The crude extract and pooled fractions were tested at 75 μg/mL, the concentration found to be optimal for the crude extract in previous studies (Spoor et al., 2006). The most active fraction (RGE-5) was further purified by preparative-HPLC as described in the next section.

2.4. Preparative-HPLC

Compound purification was carried out with preparative scale HPLC using a reverse phase Gemini Axia 250 mm × 21.2 mm column, particle size 10 μm (Phenomenex Inc., Torrance, CA, USA). The Agilent 1200 series preparative HPLC-DAD system (Agilent Technologies, Montreal, QC, Canada) consisted of a binary pump, an auto-sampler with a 2 ml loop, a diode array detector and a fraction collector. Separation was achieved using a gradient of acetonitrile in water + 0.1% TFA at a flow rate of 31.5 mL/min, with a monitoring wavelength of 210 nm, band width 4, reference off. Isolate purity was verified on an Agilent 1100 series analytical HPLC-DAD system using methods described below.

2.5. HPLC-DAD-MS analyses

The identification of the compounds in the crude extract and in the active fraction, RGE-5 (1.2 g; eluted with hexanes-ethyl acetate (1:9)), was carried out by chromatographic analyses performed on an Agilent 1100 series HPLC-DAD-APCI/MSD system (Agilent Technologies Inc., Palo Alto, CA). Co-chromatography and authentication were determined by automated UV library matching and mass spectrometry using a method previously described in Saleem et al., 2010 (Saleem et al., 2010). Briefly, the compounds were identified at the monitoring wavelengths of 280 nm (band width 4 nm, reference off) for (+)-catechin and (−)-epicatechin and of 325 nm (band width 4 nm, reference off) for quercetin. Compounds were quantified using a five point serial dilution calibration curve of UV absorbance versus concentration for each authenticated standard. The identification of the compounds was corroborated by comparing the fragments of the compounds with the authentic standards using atmospheric pressure chemical ionization-mass selective detector (APCI-MSD) in positive scan mode within a mass range of 100–500 amu.

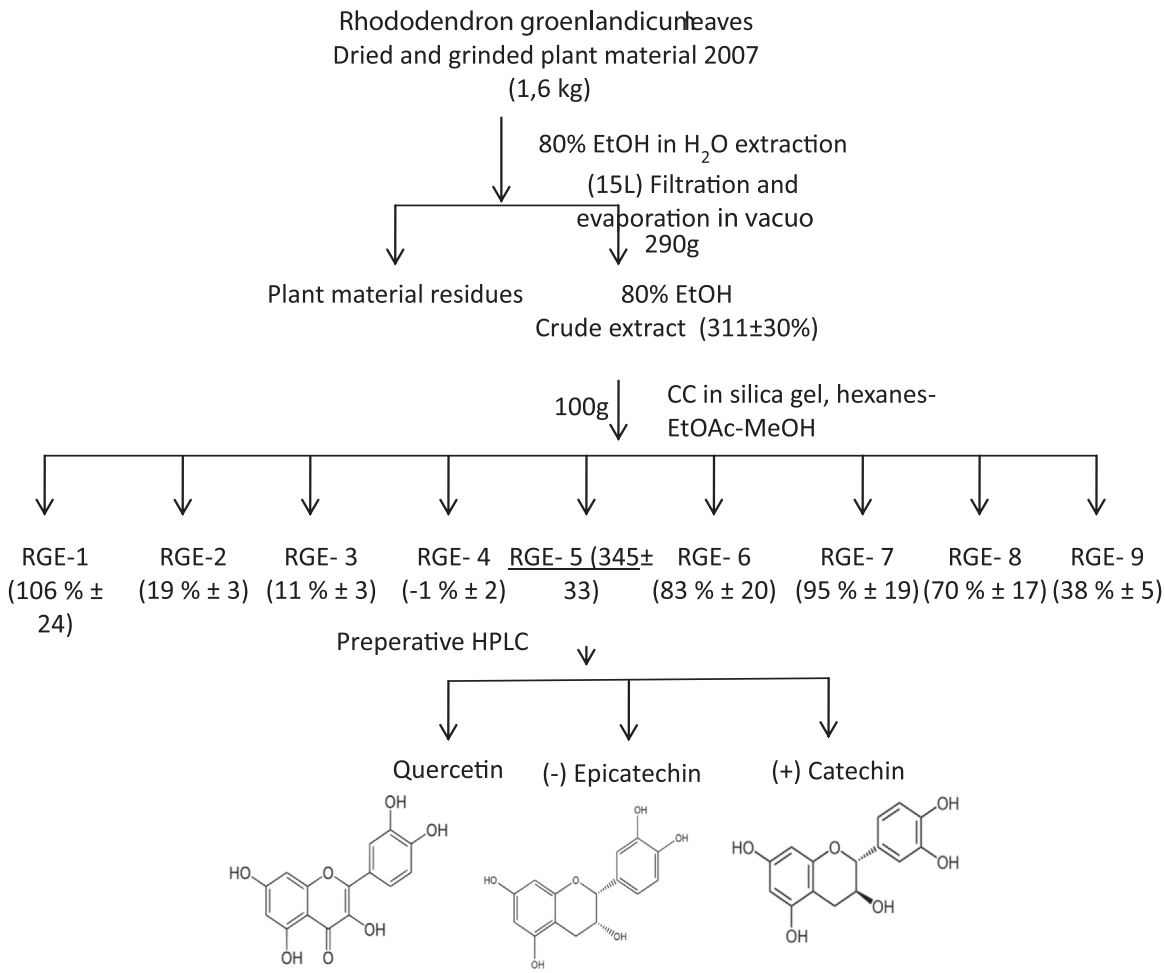

Fig. 1. Phytochemical fractionation of the crude ethanol extract of *Rhododendron groenlandicum* (RGE-CEE) guided by an *in vitro* assay measuring the increase of triglycerides content in the 3T3-L1 adipocyte model cell line. The crude extract and the fractions were tested simultaneously at 75 μg/ml. Values in brackets represent activity expressed as percentage change in the intracellular triglyceride content relative to the vehicle control (0.1% DMSO). Fractionation of the crude extract resulted in the isolation and identification of (+)-catechin, (−)-epicatechin and quercetin as the respective active principles.

2.6. Adipogenesis

Pre-adipocyte 3T3-L1 cells were grown to confluence in DMEM containing 10% FBS (proliferative medium). 24 h post-confluence (day 0), cell were induced to differentiate with DMEM supplemented with 10% FBS, 1 μM dexamethasone, 250 μM IBMX and 500 nM insulin. After 48 h, the media was replaced with DMEM containing 10% FBS and 500 nM insulin. Cells were differentiated for a total of 7 days with media change every 2 days. Plant extracts and fractions (75 μg/ml), (+)-catechin and (−)-epicatechin (12.5, 25, 50, 100 and 200 μM), and quercetin (5, 10, 20, 40 and 80 μM) as well as Rosiglitazone (10 μM; inducer of differentiation) were dissolved in DMSO and added to the cells, being present continuously since day 0 of differentiation (DMSO set at 0.1% final concentration throughout). The concentration of plant extract (75 μg/ml) was selected on the basis of our previous studies that established it as the maximal non-toxic concentration for 3T3-L1 adipocytes (Spoor et al., 2006). The concentration of the standard (10 μM rosiglitazone) was chosen based on previous experiments from the literature and from our own studies (Spoor et al., 2006; Ouchfoun et al., 2015).

Once differentiated, adipocytes accumulate triglycerides (TG). We measured intracellular TG content at day 7 of differentiation by using the AdipoRed reagent according to the manufacturer's instructions, as previously described (Harbilas et al., 2009). Briefly, after washing each well twice with PBS, 2 ml of PBS containing 60 μl of AdipoRed reagent were added to each well, mixed by gentle trituration and incubated for 15 min at room temperature. AdipoRed becomes fluorescent when partitioned in a hydrophobic compartment. The fluorescence of each well was measured with a Wallac Victor2 fluorimeter (Perkin-Elmer, St-Laurent, QC) at an excitation wavelength of 485 nm and an emission wavelength of 572 nm. The results were reported as percentage of the vehicle control (0.1% DMSO).

To test for potential interaction between the three isolated compounds on adipogenesis, a 2^3 full factorial design with four replicates was used in this study. The three compounds represent the three independent factors, each at two levels: present (+) or absent (−). The eight conditions representing all the possible combination between the three factors are presented in Table 2. The three compounds were tested at concentrations calculated from the HPLC quantification of each compound in the RGE-5 fraction (49.3 μM (+) catechin, 37.3 μM epicatechin and 5 μM quercetin) during the differentiation period as described above.

2.7. Statistical analysis

Intracellular TG content results are presented as the mean ± SEM of 3 independent experiments, each performed in triplicate. The SigmaStat software (Jandel Scientific, San Rafael, CA) was used to perform the statistical analysis. Differences between group means were analyzed by one-way analysis of variance (ANOVA).

The EC_{50} and IC_{50} values were calculated using GraphPad Prism (version 6, GraphPad Software Inc, La Jolla, CA). The results from two-level factorial design were analyzed with JMP software (version 8.0.2, SAS Institute Inc., Cary, NC). P values < 0.05 were considered statistically significant.

3. Results

3.1. Phytochemical characterization and adipogenic activity of R. groenlandicum extract

The phytochemical characterization of Labrador tea crude ethanolic extract has been reported previously (Saleem et al., 2010; Spoor et al., 2006). In the present study, the following compounds were identified by HPLC: (+)-catechin, chologenic acid, (−)-epicatechin, procyanidin B2, procyanidin A1, and several quercetin glycosides. Our previous screening studies (Spoor et al., 2006) revealed that *R. groenlandicum* strongly stimulates the differentiation of 3T3-L1 cells (adipogenesis). Therefore, a classical bioassay-guided fractionation approach was used to isolate the constituents of *R. groenlandicum* with adipogenic activity (Fig. 1).

3.2. Bioassay guided fractionation, isolation, and identification of active principles

When tested on the 7th day of differentiation and at the previously determined optimal concentration of 75 μg/ml (Spoor et al., 2006), *R. groenlandicum* crude extract increased the intracellular TG content of 3T3-L1 cells by 311 ± 30% as compared to vehicle control (0.1% DMSO) (Fig. 1; $p < 0.05$). Next, the crude extract (100 g) was subjected to silica gel column chromatography to yield nine primary fractions (RGE-1 to RGE-9). Fraction RGE-5 was the most active of the nine fractions with respect to adipogenesis (354 ± 32%; $p < 0.05$; Fig. 1); its activity being comparable to that of the crude extract and somewhat below that of the positive control rosiglitazone (507 ± 38%, $n=3$; results not illustrated). RGE-5 also showed a clear dose-response relationship (Fig. 2) with an EC-50 of 20.21.

Preparative reversed-phase HPLC purification of fraction RGE-5 yielded three major compounds: (+)-catechin, (−)-epicatechin and quercetin (Table 1). The RP-HPLC-DAD chromatographic conditions employed led to the separation and confirmation of eight

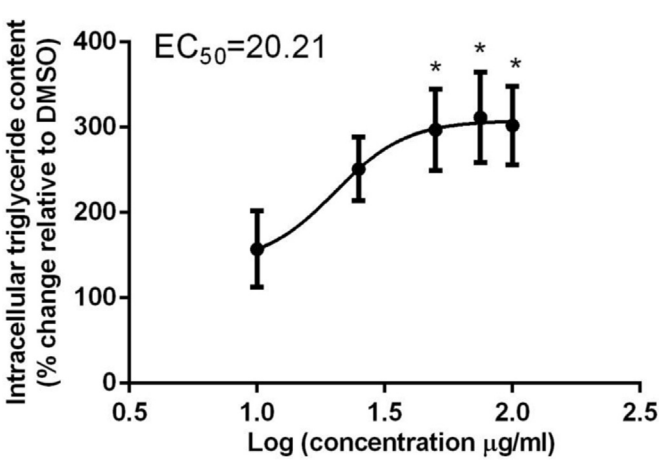

Fig. 2. Dose-response analysis of the adipogenic action of fraction RGE-5. Data are represented as mean ± SEM and are expressed as % change in the rate of basal adipogenesis relative to the vehicle control (0.1% DMSO). * indicates a p-value < 0.05 significantly different from control treated with 0.1% DMSO.

Table 1
Yield of *R. groenlandicum* constituents in the crude extract and fraction RGE-5 resulted from bioassay-guided fractionation.

Compound	Crude extract (mg/g)	RGE-5 (mg/g)
(+)-Catechin	12.1 ± 0.1	192.0 ± 3.2
(−)-Epicatechin	1.1 ± 0.1	146.5 ± 2.2
Quercetin	7.09 ± 0.8	22.3 + 2.0
Total	2.1 ± 0.1%	36.1 ± 0.3%

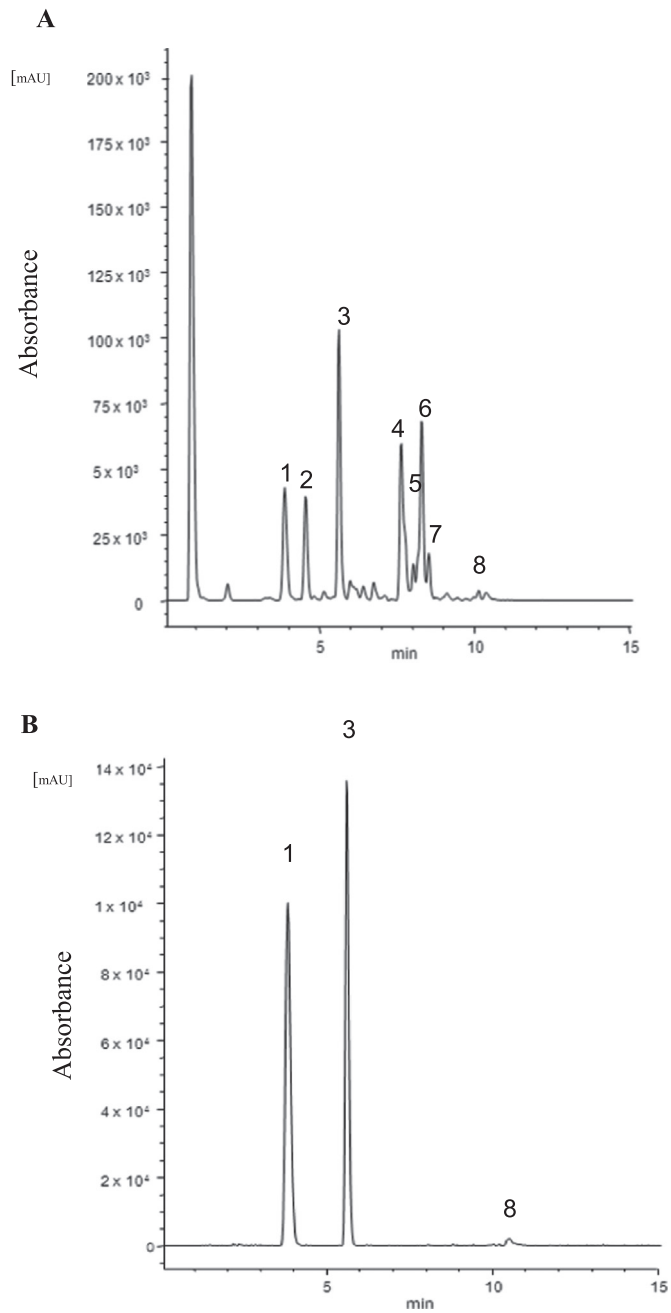

Fig. 3. HPLC chromatograms of *R. groenlandicum* crude ethanol extract (A) and of fraction RGE-5 (B) identifying the three major compounds listed in Table 1. Absorbance at 280 nm is plotted against retention time (in min). Constituent compounds of crude extract were identified previously (Saleem et al., 2010). 1: (+)-catechin, 2: chlorogenic acid, 3: (−)-epicatechin, 4: quercetin-3-O-galactoside, 5: quercetin-3-O-glucoside, 6: quercetin-glycoside, 7: quercetin-3-O-arabinoside, 8: quercetin.

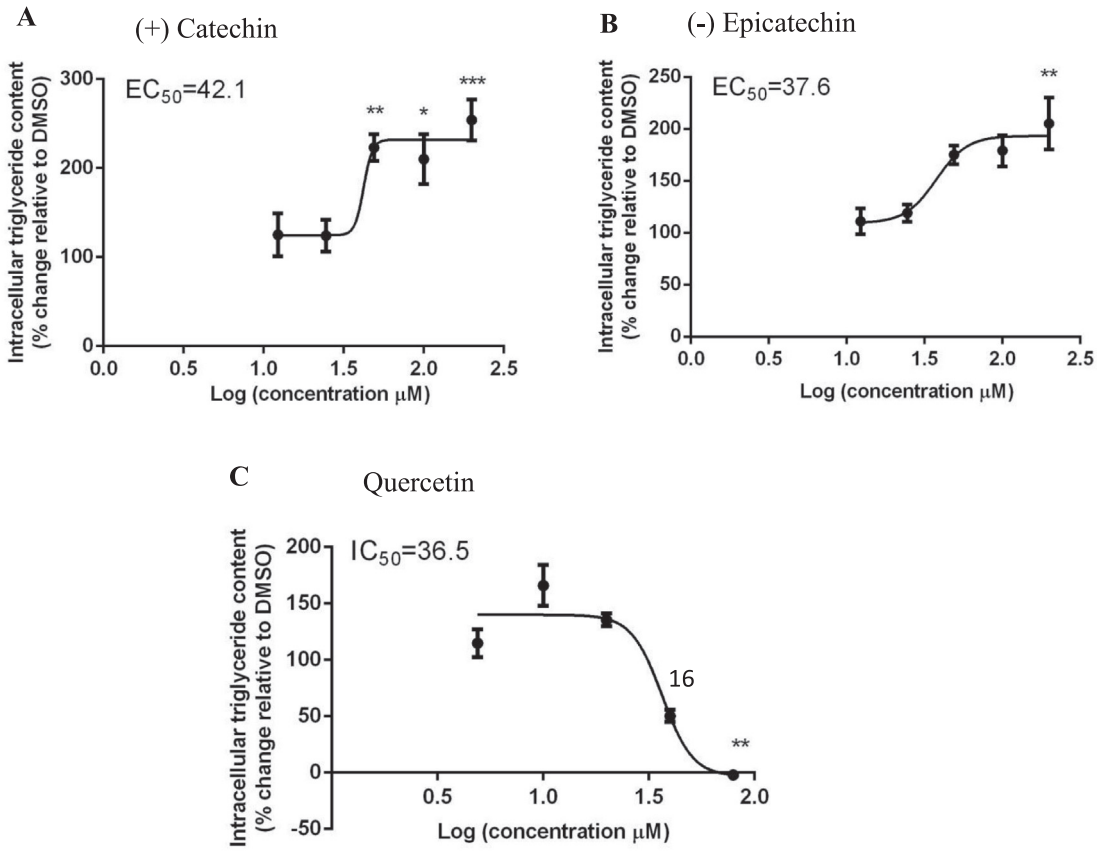

Fig. 4. Dose-response analysis of the adipogenic action of compounds found in fraction RGE-5. (+)-Catechin (A), (−)-epicatechin (B) and quercetin (C). (+) Catechin and (−) epicatechin were tested at the following concentrations; 12.5, 25, 50, 100 and 200 μM and quercetin was tested at concentrations: 5, 10, 20, 40 and 80 μM. Values are represented as mean ± SEM and are expressed as % change in the rate of basal adipogenesis relative to the vehicle control (0.1% DMSO). * indicates adipogenic activity significantly different from vehicle control treated with 0.1% DMSO.with a p-value <0.05 and ** a p-value <0.01.

previously identified compounds in the crude extract (Fig. 3A) in addition to the three aforementioned compounds in the active fraction RGE-5 (Fig. 3B). Quantification of the three compounds revealed that they are major components of this fraction (Table 1). Together, the three compounds account for 2.1 ± 0.1% and 36.1 ± 0.3% of the dry weight of the crude extract and fraction RGE-5, respectively (Table 1).

3.3. The adipogenic activities of the active compounds

Next, the adipogenic activity of each of the three compounds was assessed at concentrations of 12.5, 25, 50, 100 and 200 μM for (+)-catechin and (−)-epicatechin and of 5, 10, 20, 40 and 80 μM for quercetin (Fig. 4). At low doses, quercetin did not significantly stimulate adipogenesis (115 ± 12%, 166 ± 18 and 135.4 ± 5% at 5, 10 and 20 μM respectively, N.S., Fig. 4C) as compared to vehicle control. This activity profile shifted steeply toward inhibition of adipogenesis at higher concentration, yielding a calculated EC_{50} of 36.5 μM. Interestingly, the adipogenesis was completely blocked by the highest tested dose (80 μM) (Fig. 4C; $p<0.001$). In contrast, (+)-catechin significantly stimulated adipogenesis between 50 and 200 μM (Fig. 4A; $p<0.01$, $p<0.05$ and $p<0.001$ respectively); while the stimulation of adipogenesis by (−)-epicatechin was only statistically significant at 200 μM (Fig. 4B; $p<0.01$). However, there was a strong tendency to stimulate adipogenesis at 50 and 100 μM (−)-epicatechin (Fig. 4B; $p=0.07$ and 0.056 respectively). The calculated EC_{50} for the adipogenic activity of (+)-catechin and (−)-epicatechin were 42.1 and 37.6 μM respectively.

3.4. Combined effects of (+) catechin, (−) epicatechin and quercetin on 3T3-L1 adipogenesis

The effects of the different combination of the three isolated compounds on adipogenesis in 3T3-L1 adipocytes were examined using multifactorial analysis (Table 2). As presented in Fig. 5, individually, the rank order of adipogenic potential of the three compounds was (+)-catechin, (−)-epicatechin and quercetin. When quercetin was added to (+)-catechin or (−)-epicatechin, results appeared to be additive as illustrated by the lack of significance in the statistical interaction parameter. In contrast, when (+)-catechin and (−)-epicatechin were combined, the adipogenic activity was inferior to the sum of activities for each compound,

Table 2
Full 2^3 factorial experimental design for quercetin, epicatechin and catechin interactions.

Condition	Factor		
	Quercetin	Epicatechin	Catechin
1	−	−	−
2	+	−	−
3	−	+	−
4	−	−	+
5	+	+	−
6	+	−	+
7	−	+	+
8	+	+	+

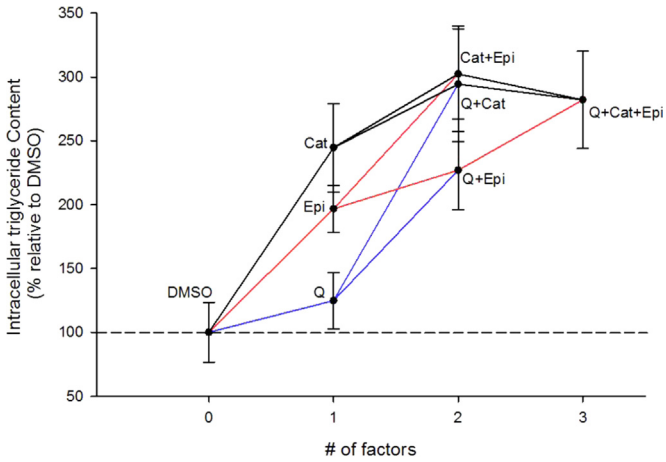

Fig. 5. Adipogenic profile of *R. groenlandicum*'s isolated compounds (+) catechin (black line), (−)epicatechin (red line) and quercetin (blue line) and their combinations. Compounds were tested at their concentrations in RGE-5 as determined by HPLC analysis. Data are represented as mean ± SEM and are expressed as % change in the rate of basal adipogenesis relative to the vehicle control (0.1% DMSO). cat: (+) catechin; epi: (−) epicatechin; Q: quercetin. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

even though this was the combination that yielded the highest adipogenic potential. Indeed, statistical analysis indicated that a statistically significant interaction existed between (+) catechin and (−) epicatechin (p=0.0387). Combining all three compounds together did not further enhance biological activity.

4. Discussion

The Cree of Eeyou Istchee, like many indigenous populations in Canada and elsewhere, suffer from an alarmingly high incidence of type 2 diabetes (Health Canada, 2006). Moreover, they suffer greater diabetic complications, partly because of poor compliance to modern pharmaceuticals that are considered culturally inadequate (Hegele, 2001). With this in mind, our research team has worked for several years in close collaboration with Cree Elders and healers, as well as local and regional Cree health organizations, to explore the potential of Cree traditional medicine to help mitigate the devastating impact of type 2 diabetes. The present work contributes to this effort by determining the active principles found in one of the most promising antidiabetic medicinal plants identified from the Cree traditional pharmacopeia, in part by virtue of its stimulation of adipogenesis in 3T3-L1 cells.

Indeed, it is well established that stimulators of adipogenesis offer a promising therapeutic approach for type 2 diabetes treatment (Freedland, 2004; Kletzien et al., 1992). For instance, TZDs are widely used oral hypoglycemic drugs that induce PPARγ activity and consequently promote adipogenesis (Lehmann et al., 1995; Spiegelman, 1998). In a previous study from our laboratory (Spoor et al., 2006), RGE was found to induce adipocyte differentiation similar to rosiglitazone, a reference TZD. A classical bioassay-guided fractionation approach, combined with standard analytical chemistry tools, was thus used in the present study to isolate and identify the active compounds responsible for the adipogenic activity of this plant extract.

Using silica gel column chromatography, nine distinct fractions were obtained and only one of these, RGE-5, exhibited adipogenic activity higher than the crude ethanol extract. RGE-5 ad was therefore further fractionated using preparative reversed phase HPLC. Three phenolic compounds were found to be part of this active fraction of Labrador tea: (+)-catechin, (−)-epicatechin and quercetin. These belong to the flavonoid family, which are ubiquitously found in plants, fruits and beverages derived thereof. Flavonoids possess several important and beneficial effects on human health such as anti-inflammatory, antioxidant (Harmon and Harp, 2001), antigenotoxic (Pinent et al., 2005) and anti-atherogenic activities, several of these playing favorable roles to mitigate type 2 diabetes and related complications (Pinent et al., 2005). Furthermore, various flavonoids stimulate the differentiation of several cell lines, including adipocytes (Hu et al., 2011; Satue et al., 2013), whereas other compounds of the same family inhibit such differentiation (Harmon and Harp, 2001; Kim et al., 2012).

(+)-Catechin is one of the major flavonoids present in green tea that has shown mixed effects on adipogenesis. The results of the present study showed that, when tested alone, (+)-catechin stimulated adipogenesis at concentrations equal to or above 50 μM. In a recent study, Shin et al. (2009) reported that (−)-catechin (the enantiomer of the compound found in RGE-5) and (−)-epicatechin (the epimer of (+) catechin) strongly stimulated the differentiation of human bone marrow mesenchymal stem cells into adipocytes, while (+)-catechin did not affect adipogenesis (Shin et al., 2009). On the other hand, Mochizuki et al. (Mochizuki and Hasegawa, 2004) showed that 70 μM of (+)-catechin stimulated adipogenesis, while the same concentration of (−)-catechin significantly inhibited it. Therefore, it seems that the two isomers can have opposing effects at different concentrations. In our case, *R. groenlandicum* was found to contain only (+)-catechin (Saleem et al., 2010; Spoor et al., 2006), which at concentrations of 50-100 μM were found to stimulate adipogenesis.

For (−)-epicatechin, our results showed that its effect on adipogenesis was significant at concentrations of 200 μM and there was a strong tendency to stimulate adipogenesis at 50 and 100 μM. Although there are conflicting data regarding the effect of (−)-epigallocatechin and (−)-epicatechin gallate (two closely related flavonoids) on adipogenesis (Furuyashiki et al., 2004; Shin et al., 2009), little has been published on the effect of (−)-epicatechin on adipogenesis. Consistent with our findings, Furuyashiki et al. (Furuyashiki et al., 2004) failed to see any effect of 5 μM (−)-epicatechin on adipogenesis. On the other hand, Shin et al. (2009) as mentioned above showed that a 10 μM dose of this compound stimulated the differentiation of human bone marrow mesenchymal stem cells into adipocytes (Shin et al., 2009). Therefore, it is possible that bone marrow mesenchymal cells are more sensitive to (−)-epicatechin than 3T3-L1 cells.

Finally, the third flavonoid identified in our active fraction, quercetin, has been more consistently shown to inhibit adipogenesis in 3T3-L1 preadipocytes and human bone marrow mesenchymal cells in a dose-dependent manner (Chien, 2005). We observed similar dose-dependent inhibition of adipogenesis at quercetin concentration levels greater than 20 μM (IC50=36.9 μM). In fact, Chien et al. showed that 25 μM of quercetin inhibited adipogenesis (Chien, 2005). Of note, quercetin concentration in fraction RGE-5, calculated from the HPLC quantification of the compound (Table 1), is about 5 μM, a concentration that does not interfere with adipogenesis.

We then applied a factorial experimental design to test all possible combinations of the three compounds. Quercetin appeared to be "permissive" in that its weak adipogenic activity was added to that of (+) catechin and (−) epicatechin, when combined with the latter. Unexpectedly, the combined effect of (+) catechin and (−) epicatechin was lower than the sum of each compound given alone at the indicated doses. These results indicate that the two enantiomers significantly interact with each other's biological activity and that this interaction lowers the adipogenic activity of their mixture.

5. Conclusions

In summary, we have identified the combination of (+)-catechin and (−)-epicatechin as the active compounds responsible for the adipogenic activity of Labrador tea crude extract by applying a classical bioassay guided isolation approach. Quercetin, a known inhibitor of adipogenesis, was also present in the crude extract and the active fraction of *R. groenlandicum*, but at concentration that did not interfere with adipogenesis. Overall, the present study provide further validation of the traditional medical knowledge of the Eastern James Bay Cree and suggests that Labrador tea can represent a valuable, culturally relevant therapeutic approach to help mitigate the devastating effects of type 2 diabetes and related complications. Moreover, the identification of the plant's active compounds paves the way to their use for pharmacokinetic studies (measurement in biological fluids) and in the development of new therapeutic agents for the treatment of metabolic diseases.

Author contributions

H.M.E carried out the adipogenesis assays, the HPLC analysis of the crude extract and the active fraction RGE-5, the statistical data analysis and contributed to drafting and revising the manuscript. M.O. performed the adipogenesis assays and the statistical data analysis and wrote the first draft of the manuscript. A.S. developed the method for HPLC analysis. J.A.G carried out the fractionation of the crude extract and the active fraction RGE-5. L.M. and L.C.M helped coordinate the studies. B.W.R. contributed to the HPLC analysis. M.R. and A.C helped with the statistical analysis. J.T.A. collaborated with our team by providing plant material and precious professional advice for the experimental protocols involving the plant extracts, as well as revising the manuscript. P.S.H is the principal investigator and the corresponding author.

Conflict of interest

The authors declare that there is no conflict of interest.

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