Antidiabetic Compounds from *Sarracenia purpurea* Used Traditionally by the Eeyou Istchee Cree First Nation

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ⓢ *Supporting Information*

ABSTRACT: Through ethnobotanical surveys, the CIHR Team in Aboriginal Antidiabetic Medicines identified 17 boreal forest plants stemming from the pharmacopeia of the Cree First Nations of Eeyou Istchee (James Bay region of Northern Quebec) that were used traditionally against diabetes symptoms. The leaves of *Sarracenia purpurea* (pitcher plant), one of the identified Cree plants, exhibited marked antidiabetic activity in vitro by stimulating glucose uptake in C2C12 mouse muscle cells and by reducing glucose production in H4IIE rat liver cells. Fractionation guided by glucose uptake in C2C12 cells resulted in the isolation of 11 compounds from this plant extract, including a new phenolic glycoside, flavonoid glycosides, and iridoids. Compounds **6** (isorhamnetin-3-*O*-glucoside), **8** [kaempferol-3-*O*-(6″-caffeoylglucoside], and **11** (quercetin-3-*O*-galactoside) potentiated glucose uptake in vitro, which suggests they represent active principles of *S. purpurea* (EC_{50} values of 18.5, 13.8, and 60.5 μM, respectively). This is the first report of potentiation of glucose uptake by compounds **6** and **8**, while compound **11** (isolated from *Vaccinium vitis*) was previously shown to enhance glucose uptake. Treatment of H4IIE liver cells with the new compound **1**, 6′-*O*-caffeoylgoodyeroside, decreased hepatic glucose production by reducing glucose-6-phosphatase enzymatic activity (IC_{50} = 13.6 μM), which would contribute to lowering glycemia and to the antidiabetic potential of *S. purpurea*.

6′-*O*-caffeoyl-goodyeroside **1**
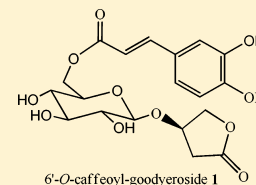
The prevalence of diabetes is increasing worldwide at a staggering pace, with Aboriginal populations being most affected. Adults (over 20 years) of the Cree First Nations of Eeyou Istchee (James Bay region of Northern Quebec, Canada) exhibited an age-adjusted prevalence of 29% for this disease in 2009. This situation is exacerbated by the cultural disconnect of modern pharmacological treatments offered to such Aboriginal populations. The CIHR Team in Aboriginal Antidiabetic Medicines is a multidisciplinary team aimed at alleviating the impact of this disease by using a culturally adapted approach: identification of medicinal plants with potential antidiabetic properties stemming from the Cree traditional pharmacopeia. Based on 15 symptoms of type 2 diabetes, our team interviewed Cree elders and healers to identify several potential antidiabetic plants, 17 of which were found to be most promising. These plants were ranked according to their syndromic importance value for the treatment of diabetes using quantitative ethnobotanical methods.[1] *Sarracenia purpurea* L., commonly known as the purple pitcher plant, was among the top ranked species. It is a carnivorous plant from the family Sarraceniaceae, which is widely distributed across North America. These plants adapt to their nitrogen-poor habitats (such as bogs and peatlands) by consuming nitrogen from insects trapped within their pitchers (fused leaves). *S. purpurea* extract exhibited interesting antidiabetic potential in an initial screening study.[2] In particular, *S. purpurea* extract significantly increased glucose uptake in C2C12 mouse muscle cells under basal and insulin-stimulated conditions. This plant extract also decreased hepatic glucose output by reducing the activity of glucose-6-

phosphatase, a key enzyme in gluconeogenesis and by increasing glucose storage through an increase in glycogen synthase activity (Nachar et al., unpublished observation). In addition, the extract also protected PC12 neuronal cells against cell death caused by hyper- or hypoglycemic conditions. Therefore, this plant exhibits both primary (glycemia-lowering) and secondary (protection against diabetes complications) antidiabetic activities. Given these interesting antidiabetic effects, the objective of this study was to elucidate the active principles of *S. purpurea* responsible for stimulating glucose uptake and hepatic glucose homeostasis using a bioassay-guided fractionation approach.

RESULTS AND DISCUSSION

Glucose Uptake Activity of *S. purpurea* Fractions and Compounds. The leaves of *S. purpurea* were extracted with 80% aqueous EtOH and fractionated using polyamide 6 chromatography. Separation of the EtOH extract resulted in eight fractions (SPE-1−SPE-8). The crude extract and fractions SPE-3 to SPE-7 (100 μg/mL) significantly increased glucose uptake in C2C12 mouse muscle cells between 30% and 60% as compared to a 0.1% DMSO vehicle control (Figure 1).

Further separation of the active fractions by preparative recycling HPLC resulted in the identification of the new 6′-*O*-caffeoylgoodyeroside (**1**) and 10 known compounds (Figure 2): taxifolin-3-*O*-glucoside (**2**),[3] 7α-*O*-methylmorroniside (**3**),[4]
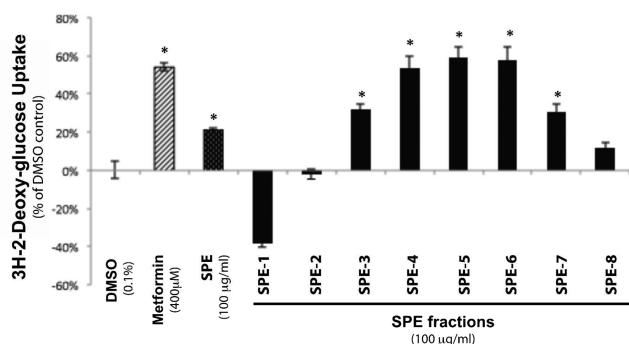
Figure 1. Effect of the crude extract (SPE; 100 μg/mL) and active fractions (SPE-1 to SPE-8; 100 μg/mL) of *S. purpurea* on glucose uptake in C2C12 mouse muscle cells. Results are represented as mean $\pm$ SEM ($n = 4$ wells). * denotes significantly different as compared to DMSO 0.1% control ($p < 0.05$) using one-way ANOVA analysis followed by Dunnett's t test.

7β-O-methylmorroniside (**4**),[4] rutin (**5**),[5] isorhamnetin-3-O-glucoside (**6**),[6] kaempferol-3-O-rutinoside (**7**),[7] kaempferol-3-O-(6″-caffeoylglucoside) (**8**),[8] morroniside (**9**),[9] goodyeroside (**10**),[10] and quercetin-3-O-galactoside (**11**).[11] New compound identification was conducted by analysis of ^{1}H, ^{13}C, and 2D NMR spectroscopic and mass spectrometry data. The known compounds were identified by comparison of their spectroscopic data with literature data.

We initially tested the compounds at 50 μM to determine their effect on glucose uptake in C2C12 muscle cells. Most isolates (except isolates **1**−**3** and **5**) showed significant potentiation of glucose uptake (ranging between 25% and 101%) as compared to 0.1% DMSO control ($p \leq 0.05$; Figure 3). We then conducted a dose−response curve for each of the seven promising isolates at concentrations ranging from 6.25 to 100 μM in order to determine their EC_{50} value (Table 2). Compounds **6** (isorhamnetin-3-O-glucoside), **8** [kaempferol-3-O-(6″-caffeoylglucoside)], and **11** (quercetin-3-O-galactoside) exhibited significant potency and the highest maximal efficacy on glucose uptake. This suggests that compounds **6**, **8**, and **11** are most likely the active principles responsible for *S. purpurea*'s ability to potentiate glucose uptake in these cells (Table 2, Figure 4). It is noteworthy that **11** (quercetin-3-O-galactoside), which significantly potentiated glucose uptake in C2C12 muscle cells in this study ($EC_{50} = 60.5$ μM; max = 211%; Table 2, Figure 4), has previously been identified as one of the active principles of *Vaccinium vitis* berries.[12] Kaempferol and its derivatives, on the other hand, were reported to have mixed effects on glucose uptake. Indeed, kaempferol[13] and kaempferol-3-neohesperidoside[14] have been shown to stimulate glucose uptake in adipocytes and muscle cells, respectively. On the other hand, kaempferitine inhibited glucose uptake in adipocytes. Kaempferol-3-O-(6″-caffeoylglucoside), identified herein as compound **8**, is a potent stimulator of glucose uptake, with an EC_{50} value of 13.8 μM and maximal efficacy of 188% as compared to DMSO control. Finally, **6** (isorhamnetin-3-O-glucoside) also stimulated glucose uptake in C2C12 muscle cells ($EC_{50} = 18.5$ μM; max = 150% of DMSO; Table 2, Figure 4), thereby providing the first report of a potential direct glycemia-lowering effect.

Identification of Compound 1. Compound **1** was obtained as a light brown solid, mp 210−212 °C, $[\alpha]_D$ +64.5. The molecular formula of **1** was assigned as $C_{19}H_{22}O_{11}$ by HRESIMS, which showed an m/z 426.1162. The major bands

in the IR spectrum exhibited absorption at 3450 (hydroxy groups), 1770 (five-membered lactone), and 1640 (an α,β-unsaturated ester) cm^{-1}. The ^{1}H and ^{13}C NMR spectra showed signals and coupling patterns similar to those of goodyeroside, except for the caffeoyl moeity. The ^{13}C NMR spectrum revealed 19 signals (see Table 1). By using DEPT experiments, the presence of three methylene, 11 methine, and five quaternary carbons was uncovered. An anomeric proton signal at δ 4.39 (1H, d, $J = 8.0$ Hz, 1′-H) in the ^{1}H NMR spectrum was assigned as a glucosyl anomeric proton, suggesting that the glucosidic bond had a β-linkage.[15,16] The most upfield methylene carbon appeared at δ 37.01 (C-2), which was correlated to protons in the ^{1}H NMR spectrum at δ 2.69 (1H, d, $J = 18.0$ Hz, H-2$_{ax}$) and 2.80 (1H, dd, $J = 6.0$, 18.0 Hz, H-2$_{eq}$) by using the HMQC spectrum. These proton signals correlated in the COSY spectrum with two other spin systems at δ 4.62 (1H, m, 3-H) and 4.40 (2H, m, 4-H) and their respective carbons at δ 76.5 and 75.2. The two spin systems correlated in the HMBC spectrum with a downfield carbon signal at δ 178.7. HMBC correlations between the anomeric signal (1′-H, δ 4.39, HMQC δ 103.6) and C-3 (δ 76.5) and vice versa confirmed the position of the sugar attachment to the butanolide moiety.

The ^{1}H NMR spectrum exhibits downfield signals due to an AB system at δ 6.27 (1H, d, $J = 16.0$ Hz, 2″-H) and 7.56 (1H, d, $J = 16.0$ Hz, 3″-H), the large coupling constant being due to the *trans* double bond. The other downfield signals at δ 6.75 (1H, d, $J = 8.0$ Hz, 8″-H), 6.94 (1H, d, $J = 8.0$ Hz, 9″-H), and 7.02 (1H, s, 5″-H) were found to be similar to signals for caffeic acid.[17] The position of the caffeoyl moiety was determined by using ^{13}C NMR (see Table 1) data, which showed a downfield shift of C-6′ by 2.0 ppm and upfield shift of C-5′ by 2.0 ppm.[16] The position of the caffeoyl moiety was further confirmed by long-range correlation of H′-6$_{ax}$ and H′-6$_{eq}$ with C-1″ (δ 169.0) in the HMBC spectrum. These spectroscopic analyses led to the assignment of structure **1** for the new compound as 6′-O-caffeoylgoodyeroside (Table 1, Figure 1).

Biological Activity of 6′-O-Caffeoylgoodyeroside. As mentioned above, the new compound **1** had no effect on glucose uptake in C2C12 muscle cells. Noteworthy, parent compound **10** (goodyeroside) has been shown to exert a hepatoprotective effect.[18,19] Therefore, we tested both 6′-O-caffeoylgoodyeroside (**1**) and goodyeroside (**10**) on glucose-6-phosphatase (G6Pase) activity, the rate-limiting step in hepatic glucose production, in H4IIE rat liver cells. Insulin (100 nM) decreased the activity of this enzyme by 65.1 $\pm$ 2.0% as compared to DMSO ($p \leq 0.001$; Figure 5) after 18 h of treatment, which leads to decreased hepatic glucose production. In comparison, the crude extract SPE (25 μg/mL) diminished G6Pase activity by 23.7 $\pm$ 1.6% ($p \leq 0.001$ vs DMSO; Figure 5). Interestingly, compound **1** (50 μM) inhibited G6Pase activity by 44.0 $\pm$ 1.8% ($p \leq 0.001$ vs DMSO; Figure 5), while compound **10** decreased its activity by 19.6 $\pm$ 1.0% ($p \leq 0.001$ vs DMSO; Figure 5). In fact, 6′-O-caffeoylgoodyeroside (**1**) exhibited more potent inhibitory effect on G6Pase activity ($IC_{50} = 13.9$ μM) than goodyeroside alone (compound 10, $IC_{50} = 391$ μM). It is worthy to note that although insulin decreases G6Pase activity by reducing the expression of this enzyme, the *S. purpurea* crude extract and compounds **1** and **10** might exert a more direct effect on the enzyme. However, the extract and its compounds produced their inhibitory effect only after 18 h of cell treatment, similar to insulin. This suggests that the observed decrease in G6Pase activity might also be due to decreased expression of the enzyme. Further investigation of
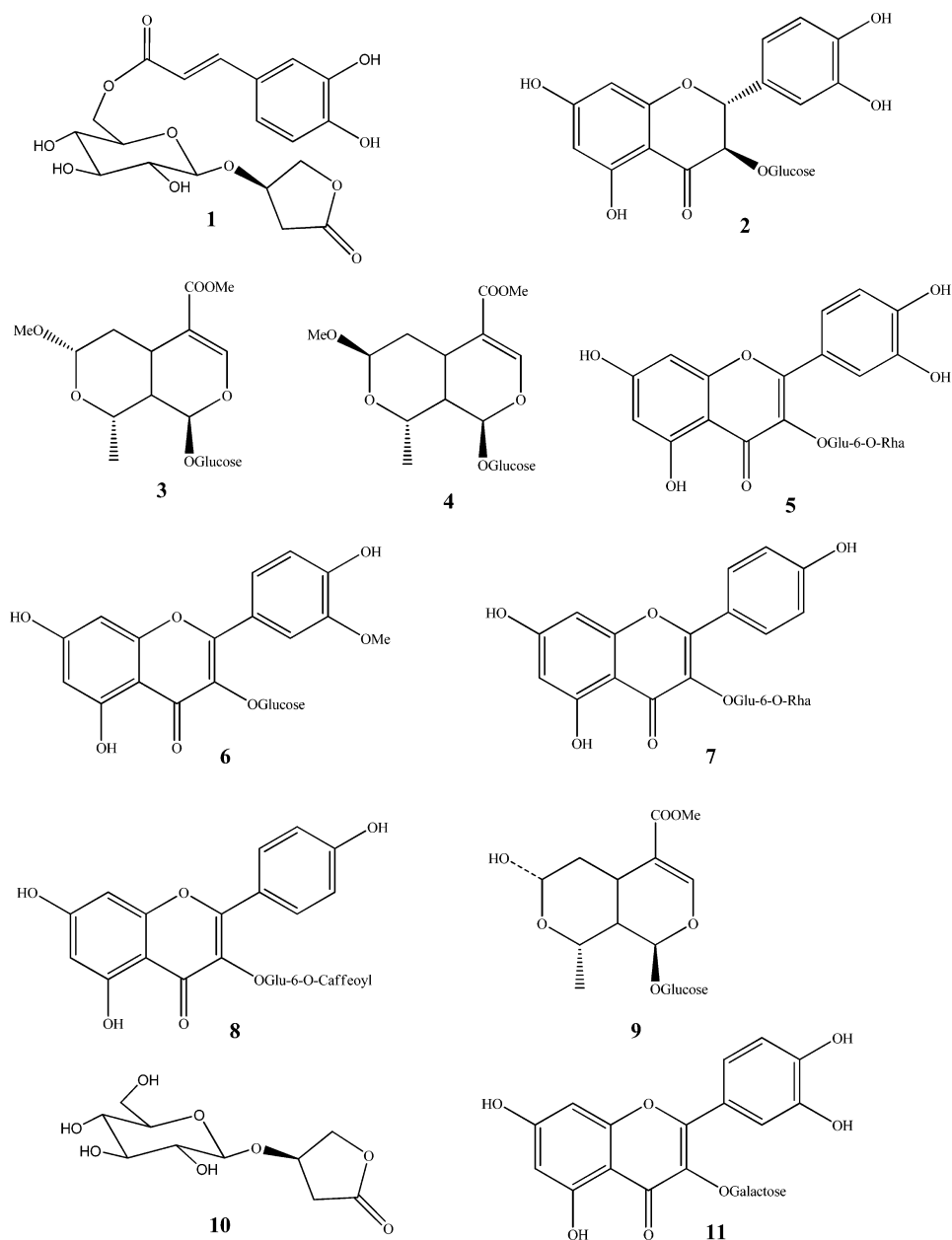
Figure 2.

the mechanisms of *S. purpurea* extract as well as of compounds **1** and **10** is warranted.

Finally, it is interesting to observe that fraction SPE-1 significantly inhibited glucose uptake by 40% (Figure 1). This suggests that *S. purpurea* extract contains both inhibitory and stimulatory active principles of glucose uptake. However, when present simultaneously in the crude extract, the overall effect is potentiation of glucose uptake.

These in vitro results suggest that *S. purpurea* L. exerts its antidiabetic effects through several active compounds, which act on different cellular targets in the liver and the muscle. These multiple modes of action increase the interest in this Cree medicinal plant's use as a therapeutic intervention for diabetes. In vivo studies in the appropriate animal models are the next step in validating the antidiabetic potential of this medicinal plant.

■ EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Melting points were determined by using a Thomas-Hoover Capillary melting point apparatus. ESIMS data were obtained using a Micromass Quattro LC instrument. IR spectra were recorded on a Shimadzu 8400-S FT/IR spectrometer. NMR spectra were obtained in methanol-d_4 or DMSO-d_6 on Bruker Avance 400 and AMX-500 NMR spectrometers. Solvents for extractions and chromatographic purifications were routinely distilled before use. Column chromatographic purifications were performed with polyamide 6. Preparative HPLC work was performed on a LC-908 JAIGEL recycling HPLC equipped with a fixed-wavelength UV detector (254 nm) and a 15 mm reversed-phase column (ODS-S-343—-15). HPLC grade MeCN (Omnisolv) was filtered through Millipore filters before use. Water for HPLC work was obtained from a Millipore filtration system.

Collection, Extraction, and Isolation Procedures. *S. purpurea* L. leaves were collected in a bog in Mistissini, Quebec, Canada, in collaboration with Cree healers. A gift of tobacco was given in
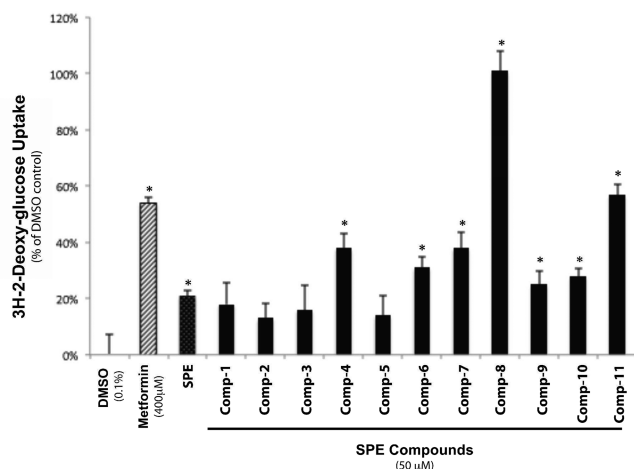
Figure 3. Effect of the crude extract (SPE; 100 μg/mL) and isolated compounds (**1** to **11**; 50 μM) of *S. purpurea* on glucose uptake in C2C12 mouse muscle cells. Results are presented as mean ± SEM (*n* = 4 wells) for each group. * denotes significantly different as compared to DMSO 0.1% control (*p* < 0.05) using one-way ANOVA analysis followed by Dunnett's *t* test.

Table 1. NMR Data of Compound 1 in Methanol-d_4

C/H no.	δ_C	δ_H (*J* in Hz)	COSY	HMBC
1	178.7			
2_{ax}	37.01	2.69 d (18.0)	3	1, 3
2_{eq}		2.80 dd (6.0, 18.0)	3	1
3	76.5	4.62 m	2, 3	1, 2, 4
4_{ax}	75.2	4.40 m	3	3, 1
4_{eq}		4.40 m	3	
1′	103.6	4.39 d (8.0)	2′	3, 2′
2′	74.8	3.18 t (8.0)	1′, 3′	1′, 3′
3′	77.8	3.33 t (7.6)	2′, 4′	2′, 4′
4′	71.6	3.32 m	3′, 5′	3′, 5′
5′	75.6	3.51 m	4′, 6′	4′, 6′
$6'_{ax}$	64.5	4.29 dd (6.4, 12.0)	5′	5′, 1″
$6'_{eq}$		4.50 d (12.0)		
1″	169.0			
2″	114.8	6.27 d (16.0)	3″	1″, 3″
3″	147.3	7.56 d (16.0)	2″	2″, 4″
4″	127.7			
5″	115.2	7.02 s		4″, 6″
6″	146.8			
7″	149.7			
8″	116.5	6.75 d (8.0)	9″	7″, 9″
9″	123.0	6.94 d (8.0)	8″	8″, 4″

exchange for the healing plant. Voucher numbers have been reported previously.[1] The leaves were dried and ground into 20 mm mesh size using a Wiley mill. The ground leaves (450 g) were extracted three times with 80% aqueous EtOH at room temperature, for 24 h each time. The combined extracts were evaporated using a rotary evaporator, yielding a gummy, brownish solid (150 g).

The crude extract was washed with hexanes, and the nonsoluble fraction (EtOH soluble) was subjected to polyamide 6 chromatography. The column was eluted using a gradual gradient of 0 to 100% EtOAc/MeOH: the eluents represent fractions SPE-1 through SPE-8. Compound separation was done using a recycling HPLC equipped with a preparative reversed-phase column, eluted at a rate of 5 mL·min^{-1} with 60% H_2O in MeCN.

Compound 1: yield 0.0017%; mp 210−212.°C; $[\alpha]^{25}_D$ +64.5 (*c* 0.005, MeOH); IR 3450, 1770, 1640 cm^{-1}; ESIMS *m/z* 426 [MH]$^+$

Table 2. Effects of Compounds 4 and 6−11 on Glucose Uptake in C2C12 Mouse Muscle Cells[a]

SPE compound	EC_{50} (μM)	maximal efficacy (% of DMSO)
4	25.9	120%
6	18.5	150%
7	NE[b]	NE
8	13.8	188%
9	NE	NE
10	25.6	124%
11	60.5	211%

[a]C2C12 mouse muscle cells were treated with varying concentrations (6.25−100 μM) of seven of the compounds identified from *S. purpurea* bioassay fractionation in order to obtain a dose−response curve. EC_{50} values were estimated using the nonlinear regression GraphPad Prism version 5.02 software (San Diego, CA, USA). [b]NE (value not estimated due to absence of dose-response profile).
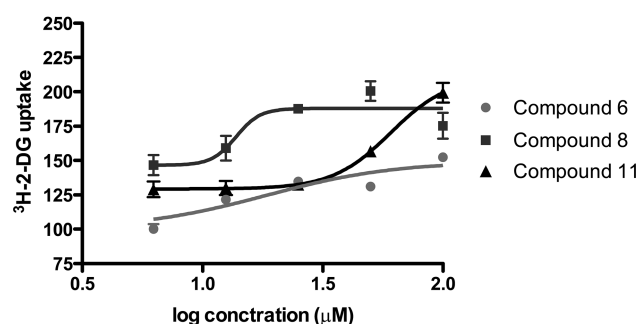

Figure 4. Dose−response curve of isorhamnetin-3-*O*-glucoside, kaempferol-3-*O*-(6″-caffeoylglucoside), and quercetin-3-*O*-galactoside on glucose uptake. C2C12 mouse muscle cells were treated with varying concentrations (6.26−100 μM) of isorhamnetin-3-*O*-glucoside (compound **6**), kaempferol-3-*O*-(6″-caffeoylglucoside) (compound **8**), and quercetin-3-*O*-galactoside (compound **11**) for 18 h. Then glucose uptake was determined as described in the Experimental Section. Results are presented as mean ± SEM (*n* = 3 wells) for each group.

(100); HRESIMS *m/z* 426.1162 [MH]$^+$ (calcd for $C_{19}H_{22}O_{11}$, 426.1164).

Glucose UptakeAassay. 2-Deoxy-D-glucose uptake assay was performed as described previously with a few modifications.[7] Confluent and differentiated C2C12 myotubes (in 12-well plates) were incubated for 18 h in differentiation medium (DMEM with 2% horse serum) containing different concentrations of the crude extract, the fractions (100 μg/mL), or the pure compounds (6.25, 12.5, 25, 50, and 100 μM) of *Sarracenia purpurea*. DMSO (0.1%) and metformin (400 μM) were used as negative and positive controls, respectively. After 18 h, cells were washed twice with prewarmed (37 °C) Krebs phosphate buffer, pH 7.4 (136 mM NaCl, 20 mM HEPES, 4.7 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 4.05 mM Na_2HPO_4, 0.95 mM NaH_2PO_4), containing glucose (5 mM) and then incubated in the same buffer for 30 min at 37 °C. After 30 min, cells were rinsed three times with Krebs phosphate buffer, pH 7.4 (37 °C), with no glucose. Glucose uptake was initiated by the addition of 500 μL (in each well) of Krebs phosphate buffer (37 °C) containing 10 μM 2-deoxy-D-glucose and 1 μCi/mL of [^{3}H-]-2-deoxy-D-glucose. After 10 min incubation at 37 °C, cells were rinsed three times with cold Krebs phosphate buffer (4 °C) containing glucose (5 mM), then lysed by incubating the samples with 500 μL of 0.1 M NaOH for 30 min at room temperature. Lysates were added to 4 mL of liquid scintillation cocktail (Ready-gel, Beckman Coulter Inc.), and radioactivity from [^{3}H]-2-deoxy-D-glucose incorporated into cells was measured in a scintillation counter.
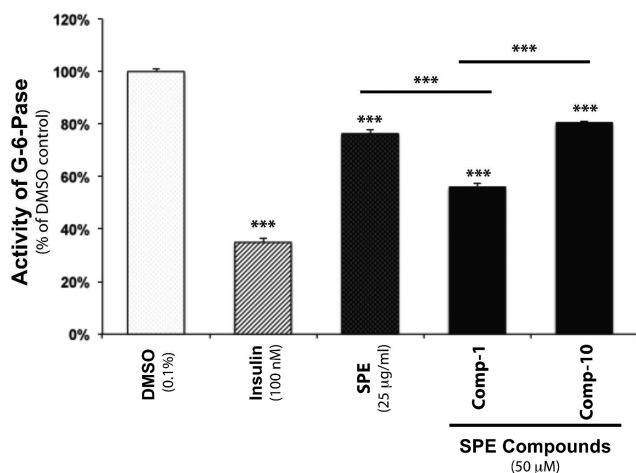
Figure 5. Effect of *S. purpurea* crude extract, 6′-*O*-caffeoylgoodyeroside, or goodyeroside on glucose-6-phosphatase activity. H4IIE rat liver cells were treated with *S. purpurea* crude extract (SPE; 25 μg/mL), 6′-*O*-caffeoylgoodyeroside (**1**), or goodyeroside (**10**) at 50 μM. Insulin (100 nM) was used as a positive control, and DMSO 0.1% was the negative control. Results are presented as % activity of DMSO (mean ± SEM; *n* = 3 wells for each group). ***$p < 0.001$ significantly different as compared to control group (DMSO, 0.1%) using one-way ANOVA analysis followed by Dunnett's *t* test.

Hepatic Glucose Production. Glucose-6-phosphatase activity was assessed in the H4IIE rat hepatoma cell line. Briefly, cells (90% confluent in 12 wells/plate) were treated for 18 h with insulin (100 nM), *S. purpurea* (25 μg/mL; higher concentration is toxic to H4IIE cells), and compounds **1** and **10** (concentration ranging from 1 to 100 μM). After 18 h, cells are washed and then lysed in 15 mM phosphate buffer containing 0.05% Triton and 1.3 mM phenol (pH = 6.5). Cell lysates were incubated in glucose-6-phosphate-containing buffer (200 mM) for 40 min at 37 °C, where the G6P serves as a substrate for endogenous glucose-6-phosphatase to yield glucose. Quantification of the glucose generated in this reaction was measured using Wako AutoKit Glucose (Wako Chemicals USA Inc., Richmond, VA, USA), according to the manufacturer's recommendations. This is a colorimetric assay based on the glucose oxidase method, where samples are incubated for 5 min at 37 °C with Wako kit color reagent (composed of mutarotase, glucose oxidase, peroxidase, 4-aminoantipyrine, and ascorbate oxidase); then absorbance is recorded at 505 nm. Protein content was determined using the BSA method. Results were presented as percentage of vehicle control activity (DMSO 0.1%).

ASSOCIATED CONTENT

Ⓢ Supporting Information

¹H, ¹³C, and 2D NMR spectra of compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

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