Tropical Journal of Pharmaceutical Research August 2013; 12 (4): 549-552
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v12i4.16

Original Research Article

Acylated Flavonoid from *Vaccinium Corymbosum (Ericaceae)* Flowers with Yeast α-Glucosidase Inhibitory Activity

Chunpeng Wan[1]* and Shouran Zhou[2]
[1]Jiangxi Key Laboratory for Postharvest Technology and Nondestructive Testing of Fruits & Vegetables, College of Agronomy, Jiangxi Agricultural University, Nanchang 330045, [2]Jiangxi University of Traditional Chinese Medicine, Nanchang 330006, China

For correspondence: lemonwan@126.com; **Tel: +86 791 83813185; **Fax:** +86 791 83813185*

Received: 21 September 2012 *Revised accepted:5 June 2013*

Abstract

Purpose: *To isolate and identify chemical constituents with **yeast** α-glucosidase inhibitory activity from the methanol extract of V. myrtillus (blueberry) flowers.*
Methods: *The active fraction (ethyl acetate extract) were chromatographed on C18 MPLC column, Sephadex LH-20 column and semi-preparative HPLC column. The isolated compounds were identified by the extensive ^{1}H-nuclear magnetic resonance spectroscopy (NMR), ^{13}C-NMR, 2D-NMR and high resolution mass spectral (HR-MS) analyses.*
Results: *Two phenolic compounds, an acylated flavonoid and a coumaric acid derivative, were isolated and identified as isorhamnetin-3-O-(6''-O-coumaroyl)-β-D-glucoside (**1**) and* cis-cinnamic acid methyl *(**2**). Compound **1** showed powerful α-glucosidase inhibitory activity and in this regard, was superior to the positive drug, acarbose.*
Conclusion: *Compounds **1** and **2** were isolated for the first time from this species and the genus of Vaccinium. This is the first report on characterization of these phenolic compounds and the possible utilization of blueberry flowers for nutraceutical and functional food applications.*

Keywords: *Vaccinium corymbosum, Blueberry, Acylated flavonoid, **Yeast** α-Glucosidase, Inhibitory activity, Nutraceuticals*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Postprandial hyperglycemia is recognized as characteristic of type **2** diabetes mellitus and plays an important role in the development of some chronic complications including circulatory disease, stroke, hypertension, blindness, kidney failure, uremia and gangrene of the lower limbs [1]. It is well known that inhibition of carbohydrate hydrolysis enzymes linked to diabetes is considered an effective approach to treat or prevent type **2** diabetes mellitus. Therefore, α-glucosidase and α-amylase are frequently used to screen therapeutic agents derived from the natural plants and isolated compounds for control of postprandial hyperglycemia [2]. *Vaccinium corymbosum*, also called the northern highbush blueberry, is a species of blueberry native to eastern North America. It is a deciduous shrub growing to 6 – 12 feet tall and wide.

There are a great number of interests worldwide in blueberry because of its high content of beneficial constituents (such as anthocyanins, flavonols, tannins, stilbenoids and phenolic acids) and potential health benefits. Among

those constituents, anthocyanins are considered to be one of the dominant blueberry polyphenols. It has been reported that blueberry extracts and its phytochemicals exhibit antioxidant [3], α-amylase and α-glucosidase inhibitory activity [4], anti-proliferation [5], lipid-lowering activity [6] and cardioprotective effect [7].

Recently blueberry fruits extracts have been shown to exhibit carbohydrate enzyme inhibitory activities but the active compounds were not identified [4]. The purpose of this study was to isolate and investigate the hypoglycemic activity compounds against inhibition of yeast α-glucosidase *in vitro*.

EXPERIMENTAL

Plant material

The flowers of high bush blueberry were collected from Morgan Farms (North Kingstown, RI, USA) in May 2009 and authenticated by Mr J Peter Morgan (a senior gardener of University of Rhode Island). A voucher specimen (16JPM51-VCJ51309FL) was deposited in the Heber-Youngken Garden and Greenhouse at the College of Pharmacy, University of Rhode Island (Kingston, RI, USA).

Equipment and chemicals

^{1}H and ^{13}C-NMR data were recorded on a Varian 500 MHz instrument with tetramethylsilane (TMS) as internal standard. High solution electrospray ionization mass spectral (HR-ESI-MS) data were acquired on a Q-Star Elite (Applied Biosystems MDS, USA) mass spectrometer. Medium pressure liquid chromatography (MPLC) separations were carried out on a C18 column connected to a DLC-10/11 isocratic liquid chromatography pump (D-Star Instruments, Manassas, VA, USA). High performance liquid chromatography (HPLC) was performed on a Hitachi Elite LaChrom system consisting of a L-2130 pump, L-2200 autosampler, and a L-2455 diode array detector was operated by EZChrom Elite software. All solvents, either ACS or HPLC grade, were purchased from Wilkem Scientific (Pawtucket, RI, USA). α-glucosidase (yeast, EC 3.2.1.20) powder and 4-nitrophenyl-α-D-glucopyranoside (pNPG) were purchased from Sigma–Aldrich (St. Louis, MO, USA).

Extraction and chromatography

The flowers (1.3 kg, fresh weight) were extracted exhaustively with MeOH (3 × 4.3 L) at room temperature to yield a dried MeOH extract (138 g). A portion of the extract (133 g) was re-suspended in H_2O (750 mL) and partitioned with EtOAc (3 × 750 mL) and *n*-butanol to yield a dried EtOAc extract (33 g) and *n*-butanol soluble fractions, respectively.

Isolation of active compounds

The EtOAc fraction (A) (32 g) was chromatographed on a C18 MPLC column (4 × 37 cm) eluting with a gradient system of MeOH/H_2O (1:9 to 7:3, v/v) to afford 6 sub-fractions (A_1-A_6) which were combined based on analytical HPLC analyses. Fraction A_1 (18 g) was chromatographed over a column of Sephadex LH-20 (3.5 × 120 cm) eluted with MeOH to give 5 sub-fractions (B_1 - B_5). Fraction B_5 was chromatographed on a C18 MPLC column (2 × 15 cm) eluting with a gradient system of MeOH/H_2O (2:8 to 7:3, v/v) to afford 9 sub-fractions (C_1 - C_9). Fraction C_8 was separated by semi-preparative HPLC eluted with MeOH:H_2O (61:39, v/v; 3.0 mL/min) to yield compound **1** (1.5 mg). Fraction A_5 was separated by semi-preparative HPLC eluted with MeOH:H_2O (from 4:6 to 6.5:3.5, v/v, for a period of 25 min, at a rate of 3.0 mL/min) to yield compound **2** (1.2 mg).

Yeast α-Glucosidase inhibitory assay

Yeast α-Glucosidase inhibitory activity was determined as described in the literature [10]. Briefly, a mixture of 50 µL of different concentrations of each of the samples (ethyl acetate fraction, *n*-buthanol fraction or two pure compounds) and 100 µL of 0.1 M phosphate buffer (pH 6.9) containing yeast α-glucosidase solution (1.0 U/ml) was incubated in 96 well plates at 25 °C for 10 min. After this pre-incubation period, 50 µL of 5 mM pNPG solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at predetermined intervals. The reaction mixtures were incubated at 25 °C for 5 min. Absorbance was recorded at 405 nm before and after incubation with a micro-plate reader (SpectraMax M2) and compared to that of the control which had 50 µL buffer solutions instead of test samples. α-Glucosidase inhibitory activity, expressed as inhibition (%),was calculated as as in Eq 1.

Inhibition (%) = {(Ac – As)/Ac}100 ---------- (1)

where Ac is the difference between the absorbance values of the control at 5 znd 0 min, and As is the difference between the absorbance values of the sample at 0 and 5 min.

Statistical analysis

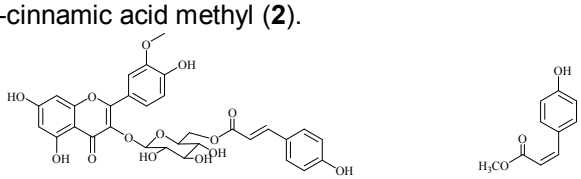
All experiments were performed in triplicate. Statistical analysis of data was by Microsoft Excel XP and the results were given as mean ± standard deviation (SD). $p < 0.05$ was considered statistically significant difference.

RESULTS

Structure elucidation of compounds 1 and 2 by NMR and HR-ESI-MS analyses

Compound 1 obtained as yellowish amorphous powder; UV-vis (MeOH) λ_{max} = 316, 257 nm; HR-ESI-MS *m/z*: 623.0118 $[M-H]^-$(calculated for molecular formula $C_{31}H_{27}O_{14}$), 662.9734$[M+K]^+$; ^{1}H-NMR (500 MHz, CD_3OD) *δ*: 7.87 (1H, brs, H-2'), 7.56 (1H, brd, *J* = 8.5 Hz, H-6'), 6.86 (1H, d, *J* = 8.4 Hz, H-5'), 6.31 (1H, d, *J* = 2.0 Hz, H-8) , 6.14 (1H, d, *J* = 2.0 Hz, H-6), 7.38 (1H, d, *J* = 15.9 Hz, H-7'"), 7.31 (2H, brd, *J* = 8.2 Hz, H-2'", 6'"), 6.81 (2H, brd, *J* = 8.2 Hz, H-3'", 5'"), 6.06 (1H, dd, *J* = 15.9, 1.2 Hz, H-8'"), 5.35 (1H, d, *J* = 7.4 Hz, H-1"), 4.28 (1H, d, *J* = 11.5 Hz, H-6"a), 4.20 (1H, dd, *J* = 11.6, 6.7 Hz, H-6"b), 3.51 – 3.45 (3H, m, H-2", 3", 4"). ^{13}C-NMR (125 MHz, CD_3OD) *δ*: 178.0(C-4), 164.3(C-7), 161.6(C-5), 157.4(C-2), 157.0(C-9), 149.4(C-4'), 146.8(C-3'), 134.5(C-3), 122.4(C-6'), 121.3(C-1'), 114.5(C-5'), 112.8(C-2'), 103.6(C-10), 98.5(C-6), 93.3(C-8), 167.2(C-9'"), 159.7(C-4'"), 145.1(C-7'"), 129.8(C-2'", 6'"), 125.5(C-1'"), 115.3(C-3'", 5'"), 113.1(C-8'"), 102.3(C-1"), 76.5(C-3"), 74.4(C-5"), 74.3(C-2"), 70.3(C-4"), 62.7(C-6"), 55.2(OCH_3). The NMR data were consistent with the literature [8]. Compound 1 was identified as isorhamnetin-3-O-(6"-O-coumaroyl)-β-D-glucoside (**1**).

Compound 2 obtained as yellowish amorphous powder; UV-vis (MeOH) λ_{max} = 309, 228 nm; (+) HR-ESI-MS, *m/z*: 201.0480 $[M+Na]^+$(calcd for molecular formula $C_{10}H_{10}O_3Na$). ^{1}H-NMR (500 MHz, CD_3OD) *δ*: 7.61 (2H, d, *J* = 8.3 Hz, H-2, 6), 6.86 (1H, d, *J* = 12.8 Hz, H-7), 6.75 (2H, d, *J* = 8.3 Hz, H-3, 5), 5.77 (1H, d, *J* = 12.8 Hz, H-8), 3.70 (3H, s, OCH_3). ^{13}C-NMR (125 MHz, CD_3OD) *δ*: 167.3(C-9), 158.6(C-4), 143.7(C-7), 132.2 (C-2, 6), 126.2(C-1), 114.4(C-3, 5), 114.8(C-8), 50.3(OCH_3). The NMR data were consistent with the literature [9]. Compound 2 was identified as *cis*-cinnamic acid methyl (**2**).



Isorhamnetin-3-O-(6"-O-coumaroyl)-β-D-glucoside (**1**) cis-cinnamic acid methyl (**2**)

Figure 1: Chemical structures of the compounds (**1** and **2)** isolated from blueberry flowers.

Two phenolic compounds were isolated from the ethyl acetate fraction and identified by the NMR and HR-ESI-MS analyses. The ^{1}H-NMR spectrum of compound **1** exhibited typical signals for an isorhamnetin moiety [*δ*: 7.87(1H, brs, H-2'), 7.56(1H, brd, *J* = 8.5 Hz, H-6'), 6.86(1H, d, *J* = 8.4 Hz, H-5'), 6.31 (1H, d, *J* = 2.0 Hz, H-8) , 6.14 (1H, d, *J* = 2.0 Hz, H-6)] and a *trans*-coumaroyl moiety [*δ*: 7.38 (1H, d, *J* = 15.9 Hz, H-7'"), 7.31 (2H, brd, *J* = 8.2 Hz, H-2'", 6'"), 6.81(2H, brd, *J* = 8.2 Hz, H-3'", 5'"), 6.06 (1H, dd, *J* = 15.9, 1.2 Hz, H-8'")] and signals for an anomeric proton of a sugar at *δ*: 5.35 (1H, d, *J* = 7.4 Hz, H-1"). The coupling constant of *J* = 7.4 Hz indicated a *β*-configuration for the glucose moiety. The *trans*-coumaroyl moiety was attached to the C-6 of the glucose moiety for the H-6" proton signals of the glucose moiety was shifted downfield at *δ*: 4.28 (1H, d, *J* = 11.8 Hz) and 4.20 (1H, dd, *J* = 11.6, 6.7 Hz), respectively. It was further confirmed by the HMBC spectrum, which showed the correlations between H-6" proton and the ester carbonyl (C-7'"). The structure of compound **1** was confirmed as isorhamnetin-3-O-(6"-O-coumaroyl)-β-D-Glucoside by comparison with published NMR data [8]. ^{1}H-NMR and ^{13}C-NMR spectrum of compound **2** exhibited similar signals to the coumaroyl moiety mentioned above. The coupling constant of *J* = 12.8 Hz indicated a *cis*-configuration. The structure of compound **2** was confirmed as *cis*-cinnamic acid methyl by comparison with published NMR data [9].

The extracts of blueberry flowers showed good α-glucosidase inhibitory activity and hence, two phenolic compounds (Figures 1 and 2) were isolated from the ethyl acetate extract. Also isorhamnetin-3-O-(6"-O-coumaroyl)-β-D-glucoside (**1**) was identified as an active constituent in the ethyl acetate extract of blueberry flowers.

α-Glucosidase inhibitory activity

Table 1 shows the α-glucosidase inhibitory activity of the crude extracts and two isolated compounds from blueberry flowers. The ethyl acetate and *n*-buthanol soluble fractions showed promising α-glucosidase inhibitory activity compared with the positive control drug, acarbose. The IC_{50} values of ethyl acetate and *n*-buthanol fractions, and acarbose were 132.7 µg/mL, 32.3 µg/mL and 129.6 µg/mL (or 200.7 µM), respectively. Although the *n*-buthanol fraction showed better activity than ethyl acetate fraction, HPLC chromatography (data not shown) showed that the ethyl acetate extract exhibited more detectable peaks. Therefore, further isolation was conducted on the ethyl acetate fraction.

The α-glucosidase inhibitory activity of the two isolated compounds was tested at the original concentration of 2 mg/mL. Compound **1** showed > 50 % inhibition activity ($p < 0.05$), while compound **2** showed < 50 % ($p > 0.05$). Hence, compound **1** was further tested and the IC_{50} was calculated (Table 1).

Table 1: Yeast α-glucosidase inhibitory activity (mean ± SD, n = 3) of crude extracts and isolated compounds of *Vaccinium corymbosum*

Sample	IC_{50} (μg/mL) [b]	Isolate	IC_{50} (μM) [c]
Ethyl acetate extract	132.7 ± 2.2A	Compound **1**	80.9 ± 11.3A
n-Buthanol extract	32.3 ± 0.3B	Compound **2**	–
Acarbose [a]	129.6 ± 12.7A	Acarbose [a]	200.7 ± 19.7B

[a] Positive control; [b] Crude extract IC_{50} values are expressed as μg/mL; [c] IC_{50} values of isolated pure compounds expressed as μM; data shown as mean ± SD (n = 3); different letters within the same column indicate significant difference at $p < 0.05$ by Duncan's test.

DISCUSSION

Compound **1** showed better yeast α-glucosidase inhibitory activity than the positive drug acarbose (Table 1), which was in agreement with previous reports that many flavonoids from plants have been reported as α-glucosidase inhibitors [11]. The present study also suggests that coumaroylated moiety could increase the activity of the flavonoid glycosides, which was in agreement with those concluded from the studies of acylated anthocyanins and flavonol monorhamnosides against α-glucosidase [12,13].

CONCLUSION

Isorhamnetin-3-O-(6''-O-coumaroyl)-β-D-glucoside (**1**) is a promising α-glucosidase inhibitor. Therefore, blueberry flower is potentially a good source of α-glucosidase inhibitors for hyperglycemic therapy. Further studies are needed to continue to isolate other the chemical constituents of the active fractions and to determine the α-glucosidase inhibitory mechanism of the active compound (Compound **1**).

ACKNOWLEDGMENT

The authors would like to thank Drs Navindra P Seeram and Emmanouil Apostolidis (University of Rhode Island) for assistance with plant collection and α-glucosidase inhibitory assay, respectively

REFERENCES

1. *Israili ZH. Advances in the treatment of type 2 diabetes mellitus. Am J Ther 2011; 18: 117-152*
2. *Wang SOY, Camp MJ, Ehlenfeldt, MK. Antioxidant capacity and α-glucosidase inhibitory activity in peel and flesh of blueberry (*Vaccinium *spp.) cultivars. Food Chem 2012; 132: 1759-1768.*
3. *Faria A, Oliveira J, Neves P, Gameiro P, Santos-Buelga C, de Freitas V, Mateus N. Antioxidant Properties of Prepared Blueberry (Vaccinium myrtillus) extracts. J Agric Food Chem 2005; 53: 6896-6902.*
4. *Johnson MH, Lucius A, Meyer T, de Mejia EG. Cultivar evaluation and effect of fermentation on antioxidant capacity and in vitro inhibition of α-amylase and α-glucosidase by highbush blueberry (Vaccinium corymbosum). J Agric Food Chem 2011; 59: 8923-8930.*
5. *Adams LS, Phung S, Yee N, Seeram NP, Li LY, Chen, SA. Blueberry phytochemicals inhibit growth and metastatic potential of MDA-MB-231 breast cancer cells through modulation of the phosphatidylinositol 3-kinase pathway. Cancer Res 2010; 70: 3594-3605.*
6. *Kim H, Bartley GE, Rimando AM, Yokoyama W. Hepatic Gene Expression Related to Lower Plasma Cholesterol in Hamsters Fed High-Fat Diets Supplemented with Blueberry Peels and Peel Extract. J Agric Food Chem 2010; 58: 3984-3991.*
7. *Xie C, Kang J, Chen JR, Nagarajan S, Badger TM, Wu X. Phenolic Acids Are in Vivo Atheroprotective Compounds Appearing in the Serum of Rats after Blueberry Consumption. J Agric Food Chem 2011; 59: 10381-10387.*
8. *Karioti A, Skaltsa H, Heilmann J, Sticher O. Acylated flavonoid and phenylethanoid glycosides from Marrubium velutinum. Phytochemistry 2003; 64: 655-660.*
9. *Jou SJ, Chen CH, Guh JH, Lee CN, Lee SS.* Flavonol glycosides *and cytotoxic triterpenoids from Alphitonia philippenensis. J Chin Chem Soc 2004; 51: 827-834.*
10. *Apostolidis E, Lee CM. In vitro potential of Ascophyllum nodosum phenolic antioxidant-mediated α-glucosidase and α-amylase inhibition. J Food Sci 2010; 75: H97-H102.*
11. *Kim JS, Kwon YS, Sa YJ, Kim MJ. Isolation and Identification of Sea Buckthorn (Hippophae rhamnoides) Phenolics with Antioxidant Activity and α-Glucosidase Inhibitory Effect. J Agric Food Chem 2011; 59: 138-144.*
12. *Lee SS, Lin HC, Chen CK. Acylated flavonol monorhamnosides, α-glucosidase inhibitors, from Machilus philippinensis. Phytochemistry 2008; 69: 2347-2353.*
13. *Matsui T, Ueda T, Oki T, Sugita K, Terahara N, Matsumoto, K. α-Glucosidase Inhibitory Action of Natural Acylated Anthocyanins.* ***2****. α-Glucosidase Inhibition by Isolated Acylated Anthocyanins. J Agric Food Chem 2001; 49: 1952-1956.*