

PHENOLIC CONSTITUENTS FROM *SARCOPYRAMIS NEPALENSIS* AND THEIR α -GLUCOSIDASE INHIBITORY ACTIVITY

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Abstract

Background: This study was carried out to fully investigate the phenolic chemical constituents of *Sarcopyramis nepalensis* and determine their α -glucosidase inhibitory activity.

Materials and Methods: *S. nepalensis* was extracted using the ultrasonic assistant extraction (UAE) method and further fractionated with petroleum ether (PE), chloroform (CHCl₃), ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH), respectively. The active fraction was chromatographed on AB-8 macroporous resin column, silica gel column, Sephadex LH-20 column, RP-ODS column and semi-preparative HPLC column. The isolated phenolic constituents were identified by ¹H-Nuclear Magnetic Resonance (NMR), ¹³C-NMR and mass spectral (MS) analyses and detected their α -glucosidase inhibitory activity by micro-plate.

Results: Ten phenolic constituents were isolated and identified from the active fraction of *S. nepalensis*. They were identified as isorhamnetin(**1**), quercetin(**2**), isorhamnetin-3-O- β -D-glucopyranoside(**3**), isoquercetin (**4**), astragalin(**5**), isorhamnetin-3-O-(6"-*p*-coumaroyl)- β -D-glucopyranoside(**6**), isorhamnetin-3-O-(6"-caffeoyle)- β -D-glucopyranoside(**7**), isoferulic acid(**8**) Caffeic acid(**9**) and ellagic acid(**10**). All of the phenolic compounds were assayed for their hypoglycemic activity against α -glucosidase *in vitro*. Compound **4**, **6** and **7** showed promising α -glucosidase inhibitory activity with the IC₅₀ values of 0.69 mg/ml, 0.56 mg/ml, 0.45 mg/ml, respectively.

Conclusion: Compounds **5-8** were isolated for the first time from *S. nepalensis*. This is the first report on the characterization of phenolic compounds and possible utilization of *S. nepalensis* for therapeutic intervention in type 2 diabetes.

Key words: α -glucosidase inhibitory, Phenolic constituents, *Sarcopyramis nepalensis*

Introduction

Sarcopyramis nepalensis belongs to the genus of *Sarcopyramis*, which is comprised of 4 species and 2 varieties in china (Chen 1999). Most species are medicinal plants used in folk medicine to treat liver and other inflammatory diseases. *S. nepalensis* showed good hepatoprotective activities for lowering aminotransferase and curing choleplania and hepatoma (Guo et al. 2012). Phytochemistry studies of the plant revealed that phenolic acid and flavonoids were (the) major constituents in the plant (Lan 2010; Zhang et al. 2011; Huang et al. 2013; Wei et al. 2014). It is well known that phenolic acid and flavonoids have showed good hypoglycemic activity *in vivo* and *in vitro* (Ranilla et al. 2010; Sharma et al. 2008). However, the hypoglycemic activity of *S. nepalensis* was not fully illuminated.

α -glucosidase is a key enzyme hydrolysing dietary carbohydrate into glucose leading to high blood glucose. So the α -glucosidase inhibitory model was frequently used to screen the therapeutic agents for the control of postprandial hyperglycemia arising from use of natural medicinal plants and isolated compounds(Wan et al. 2012ab). The purpose of this study is to fully elaborate the yeast α -glucosidase inhibitory phenolic constituents from *S. nepalensis*.

Materials and methods

Plant material

The specimen of *S. nepalensis* was collected from Sanming city, Fujian Province, P.R. China, in May 2012. A voucher specimen (2012R06) was deposited at the pharmacy of The First College of Clinical Medical Science, China Three Gorges University. The whole parts of *S. nepalensis* were dried at 40 °C in an air oven for 48 h and finely powdered.

General experimental procedures

¹H and ¹³C- Nuclear Magnetic Resonance (NMR) data were recorded on a Bruker Avance-600 FT NMR spectrometer with Tetramethylsilane (TMS) as internal standard. Mass spectral (ESI-MS) data were acquired on a Q-Star Elite (Applied Biosystems MDS, USA) mass spectrometer. High performance liquid chromatography (HPLC) was performed on a Shimadzu LC-20AT system. Column chromatography was carried with silica gel (200-300 mesh) was obtained from Qingdao Marine Chemistry Co. Ltd., Qingdao, People's Republic of China. ODS (50 μm) and Sephadex LH-20 (18-110 μm) were obtained from Pharmacia Co (USA). α-glucosidase (yeast, EC 3.2.1.20) powder and 4-nitrophenyl-α-D-glucopyranoside (pNPG) were purchased from Sigma-Aldrich (USA).

Extraction and fractionation with organic solvent

The air-dried plant material (4 kg) was ground and extracted exhaustively by UAE with 75% ethanol. The extracts were pooled and concentrated. A total extract (580 g) were fractionated with petroleum ether, CHCl₃, EtOAc and *n*-BuOH, respectively. Those solvent extracts were tested for their α-glucosidase inhibitory activity in order to choose the active fraction. The EtOAc fraction showed the best α-glucosidase inhibitory activity and further to isolate the active constituents.

Isolation of the active constituents

The EtOAc extract (78 g) was suspended in hot water (1 L) and subjected to AB-8 macroporous adsorption resin column, eluted with different concentration of ethanol (from 0%, 30%, 50%, 70% to 100%) to obtain 5 fractions. Fraction 4 (4.76 g, eluted with 70% ethanol) was subjected to a Sephadex LH-20 column (4 × 120 cm) eluted with methanol to give compound **1** (105 mg) and **2** (780 mg). Fraction 3 (13.8 g, eluted with 50% ethanol) was subjected to Sephadex LH-20 column (5 × 150 cm) to give 6 sub-fractions(Fr.3A-3F); Fr. 3A (2.2 g) was repeatedly chromatographed on Silica gel (mesh 200-300) and RP-ODS column gradient eluted with MeOH-H₂O to give compound **3** (43 mg) and **5** (18 mg); Fr. 3B (1.9 g) was repeatedly chromatographed on Silica gel (mesh 200-300), RP-ODS column and semi-preparation HPLC to give compound **4** (21 mg), **6** (19 mg) and **7** (13.5 mg). Fraction 2 (31 g, eluted with 30% ethanol) was chromatographed on Sephadex LH-20 column (5 × 150 cm) and repeatedly RP-ODS column to give compound **8** (18 mg), **9** (69 mg) and **10** (190 mg).

α-glucosidase inhibitory assay

α-glucosidase inhibitory activity was determined by method of literature (Wan et al. 2013). Briefly, a mixture of 50 μL of different concentrations of each isolates 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. Then 50 μL of 5 mM pNPG solution (dissolve in 0.1 M phosphate buffer) was added to each well. Absorbance was recorded immediately at 405 nm. The reaction mixtures were incubated at 25 °C for 5 min and the Absorbance was recorded immediately at the same wavelength. 50 μL buffer solutions instead of test isolates were used as control. α-glucosidase inhibitory activity, expressed as inhibition (%), was calculated as in Eq 1.

$$\text{inhibition(\%)} = \left(\frac{\Delta\text{Abs}_{\text{control}} - \Delta\text{Abs}_{\text{sample}}}{\Delta\text{Abs}_{\text{control}}} \right) 100 \dots\dots\dots(1)$$

Where $\Delta\text{Abs}_{\text{control}}$ is the difference value of the control (absorbance value of control in 5 minutes minus the absorbance value in 0 minute) and

$\Delta\text{Abs}_{\text{sample}}$ is the difference value of the samples (absorbance value of sample in 5 minutes minus the absorbance value in 0 minute).

Statistical analysis

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

Results and Discussion

Identification of the isolates (Fig. 1) by NMR and ESI-MS

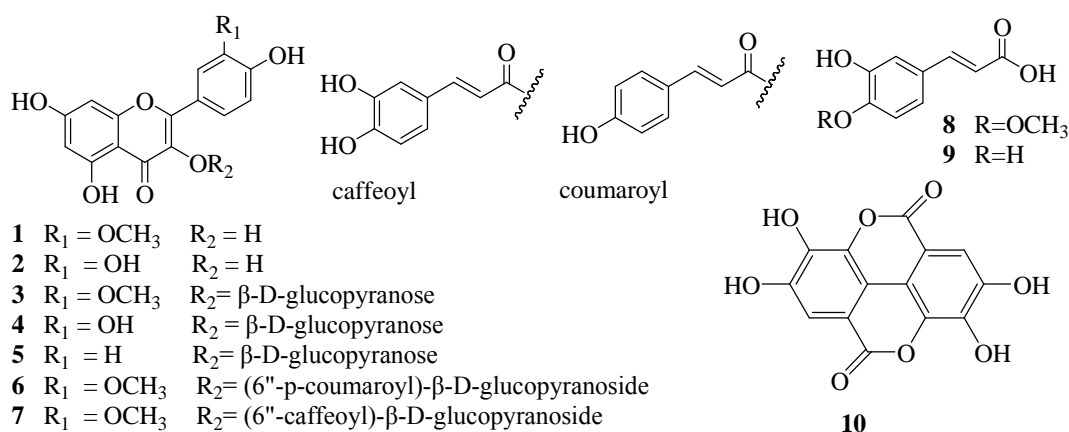


Figure. 1: Phenolic compounds isolated from *S. nepalensis*

isorhamnetin(1) obtained as yellow amorphous powder; UV(MeOH) λ_{max} :371, 255 nm; ESI-MS m/z : 315[M-H]⁻; ¹H-NMR (DMSO-*d*₆, 600 MHz) δ : 7.74 (1H, d, $J = 2.0$ Hz, H-2'), 7.69(1H, dd, $J = 2.0, 8.4$ Hz, H-6'), 6.94(1H, d, $J = 8.4$ Hz, H-5'), 6.48(1H, d, $J = 2.0$ Hz, H-8), 6.19(1H, d, $J = 2.0$ Hz, H-6), 3.84(3H, s, OCH₃); ¹³C-NMR(DMSO-*d*₆, 150 MHz) δ : 147.3(C-2), 135.6(C-3), 175.9(C-4), 156.3(C-5), 98.4(C-6), 163.7(C-7), 93.6(C-8), 160.6(C-9), 103.2(C-10), 121.6(C-1'), 115.4(C-2'), 146.5(C-3'), 148.7(C-4'), 111.3(C-5'), 121.4(C-6'), 55.6(OCH₃). The NMR data were consistent with the literature (Liu et al. 2008).

quercetin(2) obtained as yellow amorphous powder; UV(MeOH) λ_{max} :370, 256 nm; ESI-MS m/z : 301[M-H]⁻; ¹H-NMR (DMSO-*d*₆, 600 MHz) δ : 7.68 (1H,d, $J = 2.2$ Hz, H-2'), 7.54(1H, dd, $J = 2.2, 8.4$ Hz, H-6'), 6.88(1H, d, $J = 8.4$ Hz, H-5'), 6.40(1H, d, $J = 2.0$ Hz, H-8), 6.18(1H, d, $J = 2.0$ Hz, H-6). The NMR data were consistent with the literature (Yang N et al. 2006).

isorhamnetin-3-O- β -D-glucopyranoside(3) obtained as yellowish amorphous powder; UV(MeOH) λ_{max} :354, 256 nm; ESI-MS m/z : 477[M-H]⁻; ¹H-NMR(DMSO-*d*₆, 600 MHz) δ : 7.95 (1H, d, $J = 2.0$ Hz, H-2'), 7.50(1H, dd, $J = 2.0, 8.4$ Hz, H-6'), 6.92(1H, d, $J = 8.4$ Hz, H-5'), 6.44(1H, d, $J = 2.0$ Hz, H-8), 6.21(1H, d, $J = 2.0$ Hz, H-6), 5.57(1H, d, $J = 7.4$ Hz, H-1''), 3.84(3H, s, OCH₃); ¹³C-NMR (DMSO-*d*₆, 150 MHz) δ : 156.3(C-2), 133.4(C-3), 177.2(C-4), 160.8(C-5), 98.3(C-6), 163.8(C-7), 93.6(C-8), 156.6(C-9), 103.3(C-10), 121.5(C-1'), 115.2(C-2'), 146.5(C-3'), 149.2(C-4'), 111.3(C-5'), 121.8(C-6'), 55.7(OCH₃), 100.6(C-1''), 74.3(C-2''), 77.5(C-3''), 70.2(C-4''), 76.3(C-5''), 60.8(C-6''). The NMR data were consistent with the literature (Hong et al. 2000).

isoquercetin (4) obtained as yellowish amorphous powder; UV(MeOH) λ_{max} : 356, 258 nm; ESI-MS m/z : 463[M-H]⁻; ¹H-NMR(DMSO-*d*₆, 600 MHz) δ : 7.58(1H, dd, $J = 2.2, 9.0$ Hz, H-6'), 7.57 (1H, d, $J = 2.2$ Hz, H-2'), 6.84(1H, d, $J = 9.0$ Hz, H-5'),6.40(1H, d, $J = 2.1$ Hz, H-8), 6.19(1H, d, $J = 2.1$ Hz, H-6), 5.47(1H, d, $J = 7.3$ Hz, H-1''); ¹³C-NMR(DMSO-*d*₆, 150 MHz) δ : 156.3(C-2), 133.2(C-3), 177.3(C-4), 161.1(C-5), 98.7(C-6), 163.9(C-7), 93.5(C-8), 156.1(C-9), 103.8(C-10), 121.2(C-1'), 115.2(C-2'), 145.3(C-3'), 148.6(C-4'), 115.3(C-5'), 121.4(C-6'), 100.7(C-1''), 74.2(C-2''), 77.5(C-3''), 69.8(C-4''), 76.3(C-5''), 60.9(C-6''). The NMR data were consistent with the literature (Wan et al. 2012a).

astragalins(5) obtained as yellowish amorphous powder; UV (MeOH) λ_{\max} : 346, 265 nm; ESI-MS m/z : 447[M-H]⁻; ¹H-NMR(DMSO-*d*₆, 600 MHz) δ : 12.62 (1H, s, OH-5), 10.88 (1H, s, OH-7), 8.04 (2H, d, J = 8.8 Hz, H-2',6'), 6.89 (2H, d, J = 8.8 Hz, H-3',5'), 6.44 (1H, d, J = 1.6 Hz, H-8), 6.21 (1H, d, J = 1.6 Hz, H-6), 5.47 (1H, d, J = 7.6 Hz, glu-H-1"). The NMR data were consistent with the literature (Wan et al. 2011).

isorhamnetin-3-O-(6''-*p*-coumaroyl)- β -D-glucopyranoside(6) obtained as yellowish amorphous powder; UV(MeOH) λ_{\max} : 356, 257 nm; ESI-MS m/z : 623[M-H]⁻; ¹H-NMR(DMSO-*d*₆, 600 MHz) δ :12.58(1H, s, OH-5), 7.87(1H, d, J = 2.0 Hz, H-2'), 7.52(1H, dd, J = 2.0, 8.4 Hz, H-6'), 7.36(1H, d, J = 8.6 Hz, H-2''', H-6''), 7.34(1H, d, J = 8.6 Hz, H-7'''), 6.90(1H, d, J = 8.4 Hz, H-5'), 6.78(2H, d, J = 14.5 Hz, H-3'', H-5''), 6.39 (1H, d, J = 2.0 Hz, H-8), 6.16 (1H, d, J = 2.0 Hz, H-6), 6.10(1H, dd, J = 2.1, 15.9 Hz, H-8''), 3.84(3H, s, OCH₃). The NMR data were consistent with the literature (Karioti et al. 2003).

isorhamnetin-3-O-(6''-caffeoyl)- β -D-glucopyranoside(7) obtained as yellowish amorphous powder; UV(MeOH) λ_{\max} :356, 257 nm; ESI-MS m/z : 639 [M-H]⁻; ¹H-NMR(DMSO-*d*₆, 600 MHz) δ : 12.58(1H, s, OH-5), 7.85(1H, d, J = 2.0 Hz, H-2'), 7.52(1H, dd, J = 2.0, 8.4 Hz, H-6'), 7.28(1H, d, J = 8.6 Hz, H-7'''), 6.96(1H, s, H-2''), 6.89(1H, d, J = 8.4 Hz, H-6''), 6.78(2H, d, J = 14.5 Hz, H-5''), 6.75(2H, d, J = 14.5 Hz, H-5'), 6.36(1H, s, J = 2.0 Hz, H-8), 6.14 (1H, d, J = 2.0 Hz, H-6), 5.98 (1H, d, J = 15.9 Hz, H-8''), 5.51(1H, d, J = 7.02 Hz, H-1''), 3.83(3H, s, OCH₃). The NMR data were consistent with the literature (Karioti et al. 2003).

isoferulic acid(8) obtained as white amorphous powder; UV(MeOH) λ_{\max} :326 nm; ESI-MS m/z : 193[M-H]⁻; ¹H-NMR(DMSO-*d*₆, 600 MHz) δ : 7.56 (1H, d, J = 15.9 Hz, H-7), 6.27(1H, J = 15.9 Hz, H-8); 6.80(1H, d, J = 8.2 Hz, H-5), 6.96(1H, dd, J = 1.8, 8.2 Hz, H-6), 7.06(1H, d, J = 1.8 Hz, H-2). The NMR data were consistent with the literature (Lan 2010).

Caffeic acid(9) obtained as white amorphous powder; UV(MeOH) λ_{\max} :325 nm; ESI-MS m/z : 179[M-H]⁻; ¹H-NMR(DMSO-*d*₆, 600 MHz) δ : 6.17(1H, d, J = 15.9 Hz, H-8), 7.42(1H, d, J = 15.9 Hz, H-7), 6.76(1H, d, J = 8.1 Hz, H-5), 6.96(1H, dd, J = 8.1 Hz, H-6), 7.03(1H, s, H-2), 9.15, 9.55(each 1H, s, OH), 12.08(1H, s, COOH). The NMR data were consistent with the literature (Lan 2010).

Ellagic acid(10) obtained as white amorphous powder; UV(MeOH) λ_{\max} :368, 253 nm; ESI-MS m/z : 301[M-H]⁻; ¹H-NMR(DMSO-*d*₆, 600 MHz) δ : 7.47(s); ¹³C-NMR (DMSO-*d*₆, 150 MHz) δ : 159.1(C=O), 148.1(C-3, 3'), 139.6(C-5, 5'), 136.3 (C-4, 4'), 112.3(C-6, 6'), 110.1(C-1, 1'), 107.5(C-2, 2'). The NMR data were consistent with the literature (Lan 2010).

α -glucosidase inhibitory activity

Table 1 shows the α -glucosidase inhibitory activity of the isolates from *S. nepalensis*. The ethyl acetate soluble fractions showed best α -glucosidase inhibitory activity compared with other solvent extracts and the positive control drug, acarbose. Therefore, further isolation was conducted on the ethyl acetate fraction. The α -glucosidase inhibitory activity of the ten isolates was tested at the original concentration of 2.5 mg/mL. Compound **8** and **9** showed < 50 % inhibition activity (p < 0.05), while others showed > 50 %. Hence, all of the compounds except **8** and **9** were further tested and the IC₅₀ was calculated (Table 1).

Table 1: Yeast α -glucosidase inhibitory activity (mean \pm SD, n = 3) of isolates of *S. nepalensis*

isolates	IC ₅₀ ^b (mg/mL)	isolates	IC ₅₀ (mg/mL)
1	2.09 \pm 0.11	6	0.56 \pm 0.08
2	1.59 \pm 0.23	7	0.45 \pm 0.06
3	1.28 \pm 0.15	8	>2.5
4	0.69 \pm 0.09	9	>2.5
5	1.42 \pm 0.24	10	2.19 \pm 0.31
Acarbose ^a	1.23 \pm 0.12		

^a Positive control; ^b Isolates showed mg/mL as the unit of IC₅₀.

It was well known that the phenolic constituents maybe responsible for the α -glucosidase inhibitory activity. However, the active constituents were unknown. An activity-guided active compounds isolation method was used to study the α -glucosidase inhibitory active compounds in *G. medica* previously(Tan et al. 2013). In continuation of our studies on α -glucosidase inhibitory phenolic constituents from natural medicinal plants,

we make a fully investigate on the α -glucosidase inhibitory constituents from *Sarcopyramis nepalensis*. Ethyl acetate extract of *S. nepalensis* showed good activity and further isolation was conducted on the ethyl acetate extract. Ten phenolic constituents that include seven flavonols and three phenolic acids were isolated. Flavonol and its glycosides (**1-7**) showed good activity, which was in good agreement with our previous reports that many flavonoids from plants have been reported as α -glucosidase inhibitors (Tan et al. 2013).

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