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PHENOLIC GLUCOSIDES FROM THE LEAVES OF *DESMODIUM GANGETICUM* (L.) DC

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Abstract

From the water-soluble extract of the leaves of *Desmodium gangeticum*, three phenolic glucosides were isolated. By means of spectroscopic methods they were identified as methyl salicylate β-D-glucopyranoside (**1**), leonuriside A (**2**) and syringaresinol-4'-O-β-D-glucopyranoside (**3**). These compounds were isolated for the first time from the genus *Desmodium*. Compound **1** significantly inhibited α-glucosidase in comparison with diabetic drug acarbose.

Keywords. *Desmodium gangeticum*, methyl salicylate β-D-glucopyranoside, leonuriside A, syringaresinol-4'-O-β-D-glucopyranoside, α-glucosidase

1. INTRODUCTION

Desmodium gangeticum (L.) DC. (Fabaceae) is a slightly woody perennial herb and widely distributed in South East Asia, India and Africa. In Vietnam, *D. gangeticum* has been used for various purposes such as hemostatic, antiseptic, urinary discharges, detoxication, anti-ophidic, anti-oedema [1]. In India, *D. gangeticum* has a considerable reputation as a bitter tonic, febrifuge, digestive, anti-emetic, antipyretic and anticatarrhal, in inflammatory conditions of chest and in various other inflammatory conditions. It is also widely used in aryuveda for the treatment of neurological disorders. This plant is an accepted source of Shaliparni as per Ayurvedic Pharmacopoeia of India [2]. *D. gangeticum* is known to be rich in flavonoids, alkaloids, sterols and glycolipids with antioxidant, antibacterial, antidiabetic, antiulcer activities [2]. Most recently, Yadav et al. isolated a new aliphatic enone and a new bisindole alkaloid that inhibited TNF-α and IL-6 in an LPS-stimulated macrophages model [3]. This might explain the anti-inflammatory activity of *D. gangeticum* in animal model [4]. In Vietnam, very few studies on the chemical constituents of *D. gangeticum* have been reported except for the qualitative identification of coumarin and flavonoid [5, 6]. In our continuing research on the chemical composition of *D. gangeticum*, we reported the presence of quercetin 3-O-β-D-apiofuranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-galactopyranoside from leaves of this plant [7]. The present paper deals with the isolation and structural identification of methyl salicylate β-D-glucopyranoside (**1**), leonuriside A (**2**) and syringaresinol-4'-O-β-D-glucopyranoside (Fig. 1) from the water-soluble extract of the leaves of *D. gangeticum.*

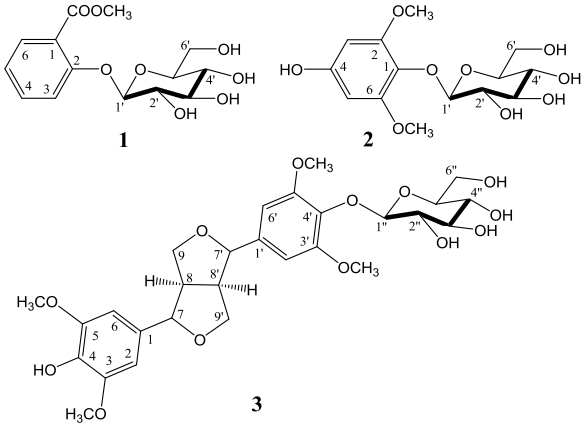

Fig. 1: Chemical structure of compounds **1-3** isolated from the leaves of *D. gangeticum*

2. EXPERIMENTAL

2.1. General procedures

Column chromatography was performed on silica gel 230-400 mesh (0.040-0.063 mm, Merck), YMC RP-18 (30-50 μm, Fujisilisa Chemical Ltd.), sephadex LH-20 and diaion HP-20 (Sigma-Aldrich).

Thin layer chromatography was performed on DC-Alufolien 60 F_{254} or RP_{18} F_{254} plates (Merck). Compound traces were visualized by UV light at 254 and 366 nm, and by spraying with aqueous 10 % H_2SO_4 under heating for 5 minutes. Optical rotation was measured on a JASCO P-2000 polarimeter. NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer using TMS as an internal standard. ESI-MS spectra were recorded on an Agilent 1260 series LC-MS single quadrupole.

2.2. Plant materials

The leaves of *D. gangeticum* were collected in Me Linh, Vinh Phuc province in April 2012, and identified by Prof. Tran Huy Thai, Institute of Ecology and Biological Resources (IEBR). Voucher specimens have been deposited at the herbarium of the IEBR.

2.3. Extraction and isolation

The air-dried and powdered leaves of *D. gangeticum* (5 kg) were extracted with MeOH at room temperature (15 L x 3 times). The methanol extract was evaporated to give a methanol residue (300 g) that was suspended in water and then partitioned successively with *n*-hexane and ethyl acetate to obtain *n*-hexane, ethyl acetate and water extracts, respectively. The water residue was passed through a diaion HP-20 column eluted by 0, 50 and 100 % methanol in water. The 100 % methanol-eluted fraction was chromatographed on a silica gel column using a gradient of 1-100 % methanol in chloroform to afford seven fractions B1→B7. The B2 was fractionated on a sephadex LH-20 column to give two fractions B2.1 and B2.2. Fraction B2.1 was chromatographed on a silica gel column using chloroform-ethyl acetate-methanol (5:1:0.5 v/v) to obtain **3** (9 mg). Compound **1** (15 mg) was purified from B2.2 fraction by a silica gel column chromatography using mobile phase chloroform-methanol-water (6:1:0.05 v/v). Fraction B3 was chromatographed on a YMC RP18 column eluted by methanol-water 1:1 to obtain **2** (12 mg).

Methyl salicylate 2-*O*-*β*-*D*-glucopyranoside (1): white powder. ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H 7.63 (1H, dd, *J* = 1.5, 8.0 Hz, H-3), 7.53 (1H, dd, *J* = 1.5, 8.5, Hz, H-5), 7.25 (1H, d, *J* = 8.5 Hz, H-6), 7.10 (1H, t, *J* = 8.0 Hz, H-4), 4.90 (1H, d, *J* = 7.5 Hz, H-1'), 4.57 (1H, t, *J* = 5.5 Hz, H-5'), 4.10 (1H, dd, *J* = 5.5, 10.5 Hz, H-6'a), 3.79 (3H, br s, OMe), 3.69 (1H, dd, *J* = 5.5, 10.5 Hz, H-6'b), 3.47 (1H, m, H-2'), 3.27 (2H, m, H-3',4'). ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C 156.1 (C-1), 121.2 (C-2), 130.2 (C-3), 121.6 (C-4), 133.2 (C-5), 116.3 (C-6), 100.9 (C-1'), 72.3 (C-2'), 76.4 (C-3'), 69.9 (C-4'), 77.1 (C-5'), 60.9 (C-6'), 51.9 (OMe). ESI-MS (positive): *m/z* 315 $[M+H]^+$.

Leonuriside A (**2**): white powder. ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 6.05 (2H, br s, H-3,5), 4.64 (1H, d, *J* = 7.0 Hz, H-1'), 4.26 (1H, t, *J* = 5.5 Hz, H-5'), 3.67 (6H, br s, OMe), 3.59 (1H, m, H-6'a), 3.41 (1H, m, H-6'b), 2.99-3.16 (m, H-2', 3', 4'). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 127.4 (C-1), 153.1 (C-2, C-6), 93.8 (C-3, C-5), 153.9 (C-4), 103.3 (C-1'), 74.4 (C-2'), 76.4 (C-3'), 70.0 (C-4'), 77.0 (C-5'), 61.0 (C-6'). ESI-MS (positive): *m/z* 333.2 $[M+H]^+$.

Syringaresinol-4'-O-β-D-glucopyranoside (3): colorless oil, $[\alpha]_D^{25}$ = –12,0 (*c* 0.01, MeOH); ^{1}H-NMR (500 MHz, CD_3OD): δ_H 6.73 (2H, s, H-2', 6'), 6.67 (2H, s, H-2, 6), 4.87 (1H, d, *J* = 7.5 Hz, H-1"), 4.77 (1H, d, *J* = 4.0 Hz, H-7), 4.72 (1H, d, *J* = 4.5 Hz, H-7'), 4.28 (2H, dd, *J* = 15.0, 9.0 Hz, H-9b, H-9'b), 3.91 (2H, dd, *J* = 9.0, 3.0 Hz, H-9a, H-9'a), 3.86 (6H, br s, 3',5'-OCH_3), 3.85 (6H, br s, 3,5-OCH_3), 3.79 (1H, dd, *J* = 12.0, 2.5 Hz, H-6"a), 3.67 (1H, dd, *J* = 12.0, 5.5 Hz, H-6"b), 3.49 (1H, m, H-2"), 3.43 (2H, m, H-3", H-4"), 3.21 (1H, m, H-5"), 3.12 (2H, m, H-8, 8'). ^{13}C-NMR (125 MHz, CD_3OD): see Table 1.

2.4. Assay for α-glucosidase inhibition

The α-glucosidase (G0660-750UN, Sigma) enzyme inhibition assay was performed according to the previously described method [8]. Briefly, the sample solution (2 µl dissolved in DMSO) and 0.5 U/ml α-glucosidase (40 µl) were mixed in 120 µl of 0.1 M phosphate buffer (pH 7.0). After 5 min pre-incubation, 5 mM *p*-nitrophenyl-α-D-glucopyranoside solution (40 µl) was added and the solution was incubated at 37 °C for 30 min. The absorbance of released 4-nitrophenol was measured at 405 nm by using a microplate reader (Molecular Devices, CA). Acarbose was used as positive control.

3. RESULTS AND DISCUSSION

Compound **1** was isolated as an amorphous powder. The ESI-MS of **1** revealed the ion peak at *m/z* 315.5 $[M+H]^+$. The ^{1}H NMR showed four protons belonging to a 1,2-disubstituted benzene ring at δ 7.63 (1H, dd, *J* = 1.5, 8.5 Hz, H-3), 7.10 (1H, t, *J* = 8.5 Hz, H-4), 7.53 (1H, dd, *J* = 1.5, 8.5

Hz, H-5) and 7.25 (1H, d, *J* = 8.5 Hz, H-6). A β-linkage sugar moiety was also recognized based on the anomeric proton signal at δ 4.90 (1H, d, *J* = 7.5 Hz, H-1'). The ^{13}C-NMR and DEPT spectra of **1** indicated the presence of 14 cacbon signals including a carboxylic at δ 166.4 (C-7), a methoxy at δ 51.9 (C-8), and six signals of a glucose at δ 100.9 (C-1′), 72,3 (2′), 76,4 (C-3′), 69,9 (C-4′), 77,1 (C-5′), 60,9 (C-6′). By comparing these data with those reported, **1** was identified to be methyl salicylate β-D-glucopyranoside [9].

Compound **2** was obtained as an amorphous powder and possesses molecular mass of 332 based on the ion peak at *m/z* 333.2 $[M + H]^+$ in the ESI-MS spectrum. The ^{1}H-NMR spectrum of **2** exhibited a two-proton singlet at δ 6.05 (2H, br s, H-3,5), which is characteristic of the symmetric tetra-substituted benzene ring. In addition, two methoxy groups at δ_H 3.67 (6H, br s, OMe) and a β-glycosidic anomeric proton at δ 4.64 (1H, d, *J* = 7.0 Hz, H-1') were observed. The ^{13}C-NMR and DEPT spectra of **2** confirmed the presence of the symmetric tetra-substituted benzene ring at δ 127.4 (C-1), 153.1 (C-2, 6), 93.8 (C-3, 5), 153.9 (C-4) together with a β-D-glucopyranoside moiety at δ 103.3 (C-1'), 74.4 (C-2'), 76.4 (C-3'), 70.0 (C-4'), 77.0 (C-5'), 61.0 (C-6'). Thus **2** was determined as leonuriside A [10].

Table 1: ^{13}C-NMR data of compound **3**

Carbon	**3**	Syringaresinol-4'-O-β-D-glucopyranoside [11]
1	139.7	139.5
2, 6	105.0	104.8
3, 5	154.6	154.4
4	135.8	135.6
7	87.8	87.1
8	55.9	55.7
9	73.1	72.8
1'	133.2	133.0
2', 6'	104.7	104.5
3', 5'	149.5	149.3
4'	136.3	136.2
7'	87.3	87.5
8'	55.9	55.4
9'	73.1	72.9
3, 5-OCH_3	57.2	57.0
3', 5'-OCH_3	57.0	56.8
1''	105.5	105.3
2''	75.9	75.7
3''	78.0	77.8
4''	71.5	71.3
5''	78.5	78.3
6''	62.7	62.5

Compound **3** was a colorless oil and its ESI-MS spectrum revealed the pick at *m/z* 581 $[M+H]^+$. The ^{1}H NMR spectrum of **3** showed signals representative for two aromatic symmetrical protons at δ_H 6.73 (2H, s, H-2', 6'), 6.67 (2H, s, H-2, 6), four methoxy groups at δ_H 3.86 (6H, br s, 3',5'-OCH_3), 3.85 (6H, br s, 3,5-OCH_3) and a β-glycosidic anomeric proton at δ_H 4.87 (1H, d, *J* = 7.5 Hz, H-1''). The ^{13}C-NMR and DEPT spectra of **3** revealed the presence of a lignan with two assymetric aromatic rings together with two oxymethines (δ_C 87.5 and 87.1), two oxymethylenes (δ_C 72.9 and 72.8), two methines (δ_C 55.7 and 55.9), and four methoxy groups. In addition, a glucose moiety was recognized at δ_C 105.5 (C-1''), 75.9 (C-2''), 78.0 (C-3''), 71.5 (C-4''), 78.5 (C-5''), 62.7 (C-6''). From these data, compound **3** was identified as syringaresinol-4'-O-β-D-glucopyranoside (tortoside A), a lignan widely found in plants [11].

All the isolated compounds were tested for the α-glucosidase inhibition. Compound **1** showed significant inhibitory effect (38 %) in comparison with antidiabetic agent acarbose (18 %) at the concentration 100 μg/ml. Compound **2** showed weak effect while **3** was inactive (Fig. 2). It is known that methyl salicylate β-D-glucopyranoside (**1**) is a storage form of a defense signal against pathogens, releasing free salicylic acid (SA) to meet the requirements in plants for their pathogenic resistance [12]. This compound also exhibited a promising treatment option for prevention or treatment of diabetes by lowering glucose levels [13]. Consistently, our result showed that methyl salicylate β-D-glucopyranoside inhibited α-glucosidase, an enzyme involved in the hyperglycemia.

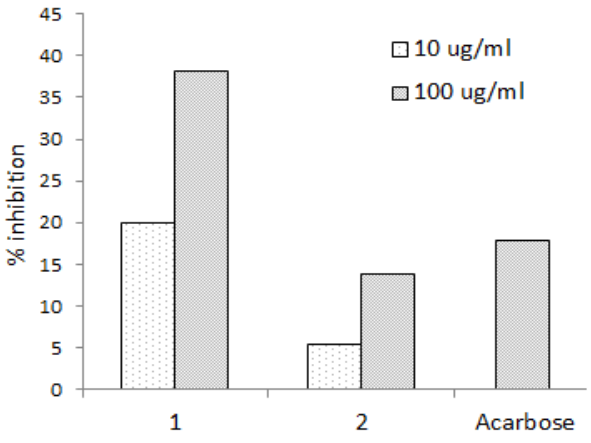

Fig. 2: α-glucosidase inhibitory effects of compounds **1** and **2**

4. CONCLUSION

A phytochemical investigation of the water-soluble extract of the leaves of *Desmodium gangeticum* led to the isolation of three phenolic glucosides methyl salicylate β-D-glucopyranoside (**1**), leonuriside A (**2**) and syringaresinol-4'-O-β-D-glucopyranoside (**3**). These compounds were isolated for the first time from the genus *Desmodium*. Compound **1** showed inhibitory activity against α-glucosidase.

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REFERENCES

1. Vo Van Chi. *Dictionary of Vietnamese medicinal plants*, Medical Publishing House, 1157-1158 (1997).
2. Vaghela BD, Patel BR, Pandya PN. *A comparative pharmacognostical profile of Desmodium gangeticum DC. and Desmodium laxiflorum DC.* Ayu, **33**, 552-526 (2012).
3. Yadav AK, Agrawal J, Pal A, Gupta MM. *Novel anti-inflammatory phytoconstituents from Desmodium gangeticum*, Nat Prod Res, **27**, 1639-1645 (2013).
4. Govindarajan R, Vijayakumar M, Rao CV, Shirwaikar A, Kumar S, Rawat AK, Pushpangadan P. *Antiinflammatory and antioxidant activities of Desmodium gangeticum fractions in carrageenan-induced inflamed rats*. Phytother Res, **21**, 975-979, (2007).
5. Dinh Thi Lan Huong, Nguyen Thi Phuong, Le Thi Thanh Huong, Trinh Ngoc Hoang, Dau Ba Thin, Nguyen Nghia Thin. *Coumarin qualitification and evaluation of antibacterial property of several medicinal plant extracts: experience of the Muong ethnic people in Nho Quan, Ninh Binh province*, Journal of Science-Can Tho University, **24b**, 140-146, (2012).
6. Ngo Thi Nhung. *Study on the botanical characteristics and chemical composition of Desmodium gangeticum*, Undergraduate thesis in Pharmacy, Hanoi University of Pharmacy (2008).
7. Tran Thi Hong Hanh, Nguyen Thi Luyen, Nguyen Thanh Binh, Ha Manh Tuan, Nguyen Hai Dang, Nguyen Tien Dat. *A flavonoid trisaccharide from the leaves of Desmodium gangeticum* (L.) DC, Journal of Medicinal Materials, **18(5)**, 313-316 (2013).
8. Tran Thi Hong Hanh, Nguyen Minh Chau, Le Hoang Tram, Nguyen Thi Luyen, Pham Thanh Binh, Chau Van Minh, Nguyen Hoai Nam, Nguyen Tien Dat. *Inhibitors of α-glucosidase and α-amylase from Cyperus rotundus*, Pharm. Biol., **52**, 74-77 (2014).
9. Ushiyama M, Furuya T. *Glycosylation of phenolic compounds by roots culture of Panax ginseng*, Phytochemistry, **28**, 3009-3013 (1989).
10. Otsuka H., Takeuchi M., Inoshiri S., Sato T., Yamasaki K. *Phenolic compounds from Coix lachryma-jobi var. Ma-yuen*, Phytochemistry, **28**, 883-886 (1989).
11. Hoang Thanh Huong, Tran Hong Quang, Cam Thi Inh, Chau Van Minh, Phan Van Kiem, Joelle Quetin-Leclerc, Ivan Vander Heyden. *Isolation of three lignans from the leaves of Ficus religiosa* L., Journal of Chemistry, **47**, 758-762 (2009).
12. Kawano T, Tanaka S, Kadono T, Muto S. *Salicylic acid glucoside acts as a slow inducer of oxidative burst in tobacco suspension culture*, Z. Naturforsch C. **59**, 684-692 (2004).
13. Rumore MM, Kim KS. *Potential role of salicylates in type 2 diabetes*, Ann Pharmacother., **44**, 1207-1221 (2010).

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