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SOME GLYCOSIDES ISOLATED FROM *DESMODIUM GANGETICUM* (L.) DC. OF VIET NAM

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ABSTRACT

From the methanol extract of the leaves of *Desmodium gangeticum* collected in Me Linh, Ha Noi, we isolated 5 compounds. In which, there are four glycosides including (*6S,9R*)-roseoside (**1**), kaempferol-3-O-rutinoside (nicotiflorin) (**2**), quercetin-3-O-rutinoside (rutin) (**3**), β-sitosterol-3-O- β-D-glucopyranoside (**4**), and the other is protocatechuic acid (**5**). Kaempferol-3-O-rutinoside (**2**) was isolated from *Desmodium gangeticum* for the first time while (*6S,9R*)-roseoside (**1**) was isolated from the genus *Desmodium* for the first time. Their structures were determined by 1D and 2D NMR spectra.

Keywords: Desmodium gangeticum, (*6S,9R*)-roseoside, kaempferol-3-O-rutinoside, quercetin-3-O-rutinoside, β-sitosterol-3-O-β-D-glucopyranoside, protocatechuic acid.

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 and other countries, *D. gangeticum* has been used for various purposes such as wound ulcers, snake bites, diuretic, edema, asthma, stomatitis, arthritis, eczema, hair loss, neurological disorders, premature ejaculation and to make tonic. *D. gangeticum* is known to be rich in flavonoids, alkaloids, sterols and glycolipids with antioxidant, antibacterial, antidiabetic, antiulcer activities. To date, over 30 compounds were found from this plant in the world [1, 2]. However, in Vietnam, very few studies on chemical constituents of *D. gangeticum* have been reported. Only two publics of Nguyen Tiet Dat et al. isolated 4 phenolic glucosides from the leaves of *D. gangeticum* along with few other researches on qualitative identification of coumarin and flavonoid. [3,4,5,6] To

clearify chemical constituents of *D. gangeticum* Vietnam, in this paper we report the isolation and structural identification of four other glycosides such as (*6S,9R*)-roseoside (**1**), kaempferol-3-O-rutinoside (**2**), quercetin-3-O-rutinoside (**3**), β-sitosterol-3-O-β-D-glucopyranoside (**4**), and another compound protocatechuic acid (**5**) from methanol extract of the leaves of *D. gangeticum*.

2. EXPERIMENTAL

2.1. Plant materials

The leaves of *D. gangeticum* were collected at Melinh, Hanoi, Vietnam in June 2017. The scientific name was identified by Dr Bui Van Thanh, Institute of Ecology and Biological Resources, VAST. The voucher specimen no. TL-DG20062017 is preserved at Institute of Natural Product Chemistry, Vietnam Academy of Science and Technology.

2.2. General experimental procedures

The ^{1}H-NMR (500MHz) and ^{13}C-NMR (125MHz) spectra were recorded on a Bruker AM500 FT-NMR spectrometer and tetramethylsilane was used as an internal standard. Column chromatography (CC) was performed using a silica gel (0.040 – 0.063 mm) and YMC RP-18 resins (30 – 50 μm). Thin layer chromatography (TLC) used pre-coated silica gel 60 F_{254} and RP-18 F_{254S} plates. Compounds were visualized by UV light at 254 and 365 nm, and by spraying with the solution of 10 % H_2SO_4 in ethanol and heating for 1-3 minutes.

2.3. Extraction and isolation

The dried leaves of *D. gangeticum* (1.5 kg) were powdered and extracted in turn with n-hexane, ethyl acetate, methanol at 50^{o}C (3 times x 2 hours per time) on heated ultrasonic machine. Filtered extracts were combined and concentrated under low pressure to give n-hexane (24 g), ethyl acetate (52 g) and methanol (85 g) extracts. The methanol extract (50 g) was separated on a silica gel column eluted with ethyl acetate : methanol (100:1 → 1:100, v:v) to obtain 6 fractions (M1→M6). The fraction M1 was chromatographed on a silica gel column eluted with chloroform: methanol (15:1, v:v) to give **1** (9.0 mg). The M2 was fractioned on a silica gel column using ethyl acetate: methanol (25:1, v:v) to obtain 4 subfractions (M2.1→M2.4). The M2.1 was purified on an YMC RP-18 column eluted with methanol : water (2:1, v:v) to give **5** (12.0 mg). The M2.3 was passed through on a silica gel column using chloroform: methanol (10:1, v:v) to give **2** (10.5 mg). The fraction M3 was chromatographed on a silica gel column eluted with dichlomethane: methanol (15:1, v:v) to give **4** (16.5 mg). The fraction M5 was separated on a silica gel column and eluted with chloroform : methanol : water (8:1:0.05, v:v:v) to obtain 5 fractions (M5.1→M5.5). The M5.3 was purified on an YMC RP-18 column eluted with methanol : water (1:2, v:v) to obtain **3** (15.0 mg).

(*6S,9R*)-roseoside (1): Colorless resin. ^{1}H-NMR (500 MHz, MeOD), δ (ppm): 1.05 (3H, s, CH_3-12); 1.06 (3H, s, CH_3-11); 1.31 (3H, d, *J* = 6.0 Hz, CH_3-10); 1.94 (3H, brs, CH_3-13); 2.18 (1H, d, *J* = 17.0 Hz, $H_α$-2); 2.55 (1H, d, *J* = 17.0 Hz, $H_β$-2); 3.20 (1H, m, H-2'); 3.24 (1H, m, H-5'); 3.29 (1H, m, H-4'); 3.35 (1H, m, H-3'); 3.65 (1H, m, $H_α$-6'); 3.87 (1H, d, *J* = 2.0, 15.0 Hz, $H_β$-6'); 4.36 (1H, d, *J* = 7.5 Hz, H-1'); 4.44 (1H, m, H-9); 5.87 (1H, m, H-8); 5.88 (1H, brs, H-4); 5.89 (1H, m, H-7). ^{13}C-NMR (125 MHz, MeOD), δ (ppm): 201.36 (C-3); 167.34 (C-5); 135.26 (C-7); 131.55 (C-8); 127.18 (C-4); 102.72 (C-1'); 80.01 (C-6); 78.08 (C-3'); 78.00 (C-5'); 77.33 (C-9); 75.24 (C-2'); 71.63 (C-4'); 62.79 (C-6'); 50.69 (C-2); 42.43 (C-1); 24.67 (C-12); 23.42 (C-11); 21.19 (C-10); 19.57 (C-13).

kaempferol-3-O- rutinoside (2): Yellow powder. ^{1}H-NMR (500 MHz, MeOD), δ (ppm): 8.09

(2H, d, *J* = 8.0 Hz, H-2',6'); 6.92 (2H, d, *J* = 8.0 Hz, H-3',5'); 6.43 (1H, d, *J* = 2.5 Hz, H-8); 6.24 (1H, d, *J* = 2.0 Hz, H-6); 5.15 (1H, d, *J* = 7.5 Hz, H-1"); 4.54 (1H, brs, H-1"'); 3.40 – 3.90 (10H, rhamnose and glucose); 1.14 (3H, d, *J* = 6.5 Hz, H-6"'). ^{13}C-NMR (125 MHz, MeOD), δ (ppm): 179.40 (C-4); 166.71 (C-7); 162.98 (C-5); 161.60 (C-4'); 159.56 (C-9); 158.68 (C-2); 135.51 (C-3); 132.41 (C-2',6'); 122.08 (C-1'); 116.20 (C-3',5'); 104.72 (C-10); 102.48 (C-1", 1"'); 100.30 (C-6); 95.12 (C-8); 77.80 (C-3"); 77.20 (C-5"); 75.71 (C-2"); 73.91 (C-4"'); 72.30 (C-4"); 72.10 (C-2"'); 71.53 (C-3"'); 69.78 (C-5"'); 68.70 (C-6"); 17.90 (C-6"').

quercetin-3-O-rutinoside (3): Yellow powder. ^{1}H-NMR (500 MHz, MeOD), δ (ppm): 7.69 (1H, d, *J* = 2.5 Hz, H-2'); 7.65 (1H, dd, *J* = 8.5, 2.0 Hz, H-6'); 6.90 (1H, d, *J* = 8.5 Hz, H-5'); 6.42 (1H, d, *J* = 2.0 Hz, H-8); 6.23 (1H, d, *J* = 2.0 Hz, H-6); 5.13 (1H, d, *J* = 7.5 Hz, H-1"); 4.54 (1H, s, H-1"'); 3.40 – 3.90 (10H, rhamnose and glucose); 1.14 (3H, d, *J* = 6.0 Hz, H-6"'). ^{13}C-NMR (125 MHz, MeOD), δ (ppm): 179.42 (C-4); 166.01 (C-7); 162.97 (C-5); 159.34 (C-9); 158.51 (C-2); 149.80 (C-4'); 145.83 (C-3'); 135.63 (C-3); 123.56 (C-6'); 123.14 (C-1'); 117.71 (C-2'); 116.07 (C-5'); 105.64 (C-10); 104.71 (C-1"); 102.42 (C-1"'); 99.96 (C-6); 94.88 (C-8); 78.19 (C-3"); 77.22 (C-5"); 75.73 (C-2"); 73.95 (C-4"'); 72.26 (C-3"'); 72.10 (C-3"'); 71.41 (C-4"); 69.71 (C-5"'); 68.56 (C-6"); 17.87 (C-6"').

daucosterol (4): White crystals. ^{1}H -NMR (500MHz, DMSO-d_6): δ (ppm): 0.78 (3H, s, CH_3-18); 0.81 (3H, d, *J* =7.0 Hz, CH_3-27); 0.83 (3H, d, *J* = 7.0 Hz, CH_3-26); 0.84 (3H, t, *J* = 7.0 Hz, CH_3-29); 0.91 (3H, d, *J* = 6.5 Hz, CH_3-21); 0.97 (3H, s, CH_3-19); 3.00 (1H, m, H-2'); 3.04 (1H, m, H-4'); 3.08 (1H, m, H-5'); 3.14 (1H, m, H-3'); 3.43 (1H, m, H-6'a); 3,47 (1H, m, H-3); 3.67 (1H, m, H-6'b); 4.22 (1H, d, *J* = 7.0 Hz , H-1'); 5.33 (1H, br d, *J* = 5.0 Hz, H-6). ^{13}C-NMR (125MHz, DMSO-d_6): δ (ppm) 37.2 (C-1); 29.5 (C-2); 70.1 (C-3); 38.7 (C-4); 140.2 (C-5); 122.1 (C-6); 31.8 (C-7); 31.8 (C-8); 50.1 (C-9); 36.6 (C-10); 21.0 (C-11); 39.7 (C-12); 42.2 (C-13); 56.7 (C-14); 24.2 (C-15); 28.1 (C-16); 55.9 (C-17); 11.8 (C-18); 19.6 (C-19); 36.0 (C-20); 19.1 (C-21); 33.9 (C-22); 26.0 (C-23); 45.8 (C-24); 29.1 (C-25); 18.9 (C-26); 18.6 (C-27); 23.0 (C-28); 11.7 (C-29); 101.0 (C-1'); 75.6 (C-2'); 76.3 (C-3'); 73.4 (C-4'); 79.1 (C-5'); 61.8 (C-6').

protocatechuic acid (5): White crystals. ^{1}H-NMR (500 MHz, MeOD), δ (ppm): 6.79 (1H, d, *J* = 7.5 Hz, H-5); 7.43 (1H, d, *J* = 8.0, H-6); 7.47 (1H, s, H-2). ^{13}C-NMR (125 MHz, MeOD), δ (ppm): 168.01 (C-7); 150.45 (C-4); 145.73 (C-3); 123.62 (C-6); 123.60 (C-1); 117.87 (C-5); 115.54 (C-2).

3. RESULTS AND DISCUSSION

Compound **1** was obtained as colorless resin. The ^{13}C-NMR spectra of **1** showed 19 carbon signals. Six of them were assigned as glucose moiety with the anomeric carbon at δ_C 102.72. Four carbon signals at δ_C 167.34 (C-5); 135.26 (C-7); 131.55 (C-8); 127.18 (C-4) and three proton signals at 5.87-5.89 ppm suggested the present of two double bonds in its structure. In proportion to ^{13}C-NMR, the ^{1}H-NMR spectra of **1** displayed signals of four methyl groups and 6 protons of glucose moiety at δ_H 3.20-3.87 with anomeric proton at δ_H 4.36 (*J* = 7.5 Hz). The coupling constant at 7.5 Hz established glucose attached to aglycone by a β-D-glycoside linkage. From the above evidences and comparison with spectral data of (*6S,9R*)-roseoside in literature [6], compound **1** was idendifed as (*6S,9R*)-roseoside.

Compound **2** was obtained as yellow powder. The ^{1}H-NMR spectra of **2** displayed 6 aromatic protons including 2 protons at δ_H 8.09 (d, *J* = 8.0 Hz, H-2',6'), 2 protons at δ_H 6.92 (d, *J* = 8.0 Hz, H-3',5'), one proton at δ_H 6.43 (d, *J* = 2.5 Hz, H-8), and the other at δ_H 6.24 (d, *J* = 2.0 Hz, H-6) along with sugar signals from δ_H 3.40 to 3.90 suggested **2** is a flavonoid glycoside. In addition, the ^{13}C-NMR spectra of **2** showed 15 carbon signals belonging the flavonoid part

and 12 carbon signals belonging sugar moiety. The ^{1}H- and ^{13}C-NMR spectral data of **2** were the same as those of kaempferol-3-O-rutinoside [7]. Thus, compound **2** was identified as kaempferol-3-O- rutinoside (nicotiflorin).

Compound **3** was obtained as yellow powder. The ^{1}H- and ^{13}C-NMR spectral data of **3** were similar to those of **2** except the signals in B rings. Compound **3** has three dissymmetrical protons at δ_H 7.69 (1H, d, *J* = 2.5 Hz, H-2'); 7.65 (1H, dd, *J* = 8.5, 2.0 Hz, H-6'); 6.90 (1H, d, *J* = 8.5 Hz, H-5'). These signals of **3** were compatible with those of quercetin. In addition, the signals of sugar moiety of **3** were similar to those of **2** and identified as rutinose. From these spectral data comparion with a literature [8], compound **3** was determined as quercetin-3-O-rutinoside (rutin).

Compound **4** was obtained as white crystals. The ^{1}H-NMR, ^{13}C-NMR and DEPT data of **4** revealed 6 methyl groups in aglycone moiety CH_3-18, CH_3-19, CH_3-21, CH_3-26, CH_3-27, CH_3-29 at δ_H 0.78, 0.97, 0.91, 0.83, 0.81, 0.84 and δ_C 11.8, 19.6, 19.1, 18.9, 18.6, 11.7, a double bone (C-5, C-6) at δ_H 5.33 and δ_C 140.2, 122.1. These signals were appropriate to spectral data of a known alycone β-sitosterol. The remaining signals were identified as glucose moiety with the anomeric carbon at δ_C 101.0 and δ_H 4.22. From the above evidences and comparison with those reported in literature [9], compound **4** was deduced to be β-sitosterol-3-O-β-D-glucopyranoside.

Compound **5** was obtained as white crystals. The ^{1}H-NMR of **5** showed three aromatic proton signals. Two doublet signals at δ_H 7.43 and 6.79 with coupling constant 8.0 and 7.5 suggested they are ortho- protons. The other was a singlet signal at δ_H 7.47. According to ^{13}C-NMR of **5**, seven carbon signals from δ_C 115.54 to 168.01 were seen. By comparing these data with those reported [10], compound **5** was identified to be protocatechuic acid.

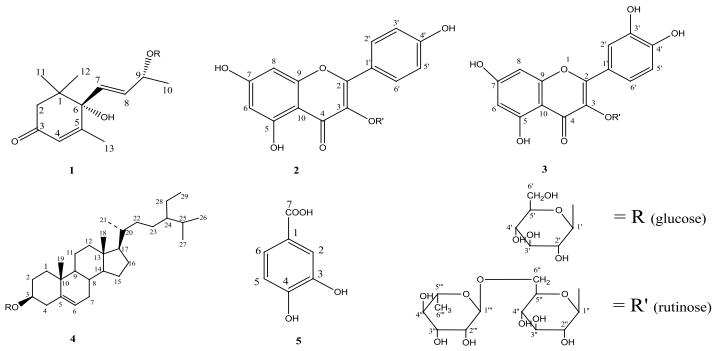

Figure 1. The structure of five isolated compounds from the leaves of *D. gangeticum.*

4. CONCLUSION

From the methanol extract of of the leaves of *D. gangeticum* (Fabaceae), four glycosides were isolated and indentified structures such as (*6S,9R*)-roseoside (**1**), kaempferol-3-O-rutinoside (nicotiflorin) (**2**), quercetin-3-O-rutinoside (rutin) (**3**), β-sitosterol-3-O- β-D-glucopyranoside (**4**) along with protocatechuic acid (**5**). This is the first time kaempferol-3-O-rutinoside (**2**) was isolated from *Desmodium gangeticum* and (*6S,9R*)-roseoside (**1**) was reported from the genus *Desmodium*. Both of them are typical flavonoids which have been reported to express many valuable bioactivities especially antioxidant, antidiabetic, anti-inflammatory, neuroprotective, hepatoprotective, vasoprotective properties, anticancer, etc. [11, 12]. Follow-up investigations of chemical constituents and biological properties of *D. gangeticum* are still continuing to carry out by us.

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REFERENCES

1. Do Tat Loi - Vietnamese medicinal plants and herbs, Medicinal Publishing House, 2004, pp. 144 (in Vietnamese).
2. Vaghela B.D., Patel B.R., Pandya P.N. - A comparative pharmacognostical profile of *Desmodium gangeticum* DC. and *Desmodium laxiflorum* DC. Ayu **33** (2012) 552-526.
3. Nguyen Tien Dat, Nguyen Thi Luyen, Nguyen Hai Dang - Phenolic glucosides from the leaves of *Desmodium gangeticum* (L.) DC., Vietnam Journal of Chemistry **53** (2e) (2015) 69-72 (in Vietnamese).
4. 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) (2013) 313-316 (in Vietnamese).
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** (2012) 140-146 (in Vietnamese).
6. Mamadalieva N.Z., Sharopov F., Girault J.-P., Wink M., Lafont R. - Phytochemical analysis and bioactivity of the aerial parts of *Abutilon theophrasti* (Malvaceae), a medicinal weed, Natural Product Research **28** (20) (2014) 1777–1779.
7. Shahat A.A., Abdel-Azim N.S., Pieters L., Vlietinck A.J - Flavonoids from *Cressa cretica*, Pharmaceutical Biology **42** (4–5) (2004) 349–352.
8. Phan Van Cu - Isolated flavonoids from butanol extract of *Houttuynia Cordata* Thunb in Thua Thien Hue province, Hue University Journal of Science **63** (2010) 27-32 (in Vietnamese).
9. Nguyen Thi Hong Van, Le Minh Ha, Ngo Thi Phuong, Luu Tuan Anh, Pham Cao Bach, Nguyen Quoc Binh, Trinh Anh Vien, Pham Quoc Long – Some naphthalene lactone relatives from *Eleutherine bulbosa* in Vietnam, Vietnam Journal of Chemistry **51** (2AB) (2013) 30-33 (in Vietnamese).
10. Liao CR, Kuo YH, Ho YL, Wang CY, Yang CS, Lin CW, Chang YS – Studies on Cytotoxic Constituents from the Leaves of *Elaeagnus oldhamii* Maxim. In Non-Small Cell Lung Cancer A549 Cells, Molecules **19** (2014) 9515-9534.
11. Aditya Ganeshpurkar, Ajay K. Saluja – The Pharmacological Potential of Rutin, Saudi Pharm J. **25** (2) (2017) 149-164.
12. Yu Wang, Chang yun, Tang Hao Zhang – Hepatoprotective effects of kaempferol 3-O-rutinoside and kaempferol 3-O-glucoside from *Carthamus tinctorius* L. on CCl4-induced oxidative liver injury in mice, Journal of Food and Drug Analysis **23**(2) (2015) 310-317.