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ACONTRIBUTION TO STUDY CHEMICAL CONSTITUENTS OF *DESMODIUM GANGETICUM* OF VIET NAM[#]

Le Minh Ha[1, *], Ngo Thi Phuong[1], Do Thi Thanh Huyen[1], Duong Bich Ngoc[1], Nguyen Minh Viet[2], Nguyen Tuan Anh[3], Nguyen Tien Đat[4], Pham Quoc Long[1]

[1]*Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Ha Noi*

[2]*Hanoi University of Industry, No. 298, Cau Dien street, Bac Tu Liem, Ha Noi*

[3]*Hanoi University of Science and Technology, No. 1, Dai Co Viet street, Ha Noi*

[4]*Center for Research and Technology Transfer, Vietnam Academy of Science and Technology, 18-Hoang Quoc Viet, Cau Giay, Ha Noi*

[*]Email: *halm2vn@gmail.com*

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Abstract. From the ethyl acetate extract of the leaves of *Desmodium gangeticum* collected in Me Linh, Ha Noi, we isolated five compounds including luteolin (**1**), luteolin tetramethyl ether (**2**), *N*,*N*-dimethyltetradecan-1-amine (**3**), (+)-pinitol (**4**) and stigmasterol (**5**). In which, luteolin (**1**) was isolated from *Desmodium gangeticum* for the first time, luteolin tetramethyl ether (**2**) and N,N-dimethyltetradecan-1-amine (**3**) were first isolated from genus of *Desmodium*. Their structures were determined by 1D and 2D NMR spectra.

Keywords: Desmodium gangeticum, luteolin, luteolin tetramethyl ether, N,N-dimethyltetradecan-1-amine, (+)-pinitol, stigmasterol.

Classification numbers: 1.1.1

1. INTRODUCTION

Desmodium gangeticum (L.) DC., a slightly woody perennial herb belonging to family Fabaceae, has Vietnamese name as Thoc Lep and wildly grows in moutainous areas [1]. This plant is also distributed in India, Africa and other countries of Southeast Asia. In traditional folk medicines, *D. gangeticum* has been used to treat a lot of diseases including wound ulcers, snake bites, diuretic, edema, asthma, stomatitis, arthritis, eczema, hair loss, neurological disorders and premature ejaculation [1, 2]. Some studies in the world showed that *D. gangeticum* is rich in flavonoids, alkaloids, sterols and phospholipids [3]. The plant is reported to possess

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antileishmanial, immunomodulatory, hepatoprotective, cardio-protective, anti-inflammatory, antinociceptive, wound healing, antidiabetic, antiamnesic and antiulcer activities [3, 4]. However, in Vietnam, very few studies on chemical constituents of *D. gangeticum* have been reported and almost no biological studies have seen [5, 6]. Therefore, it is necessary to clarify chemical constituents, as well as biological activities of *D. gangeticum* of Vietnam. In present paper, we continue to report the isolation and structural identification of five compounds including luteolin (**1**), luteolin tetramethyl ether (**2**), N,N-dimethyltetradecan-1-amine (**3**), (+)-pinitol (**4**) and stigmasterol (**5**) from the ethyl acetate extract of the leaves of *D. gangeticum*.

2. MATERIALS AND METHODS

2.1. Plant materials

The leaves of *Desmodium gangeticum* (L.) DC. were collected at Melinh, Hanoi, Vietnam in May 2017. The scientific name was identified by Dr Bui Van Thanh, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST). The voucher specimen (DG05/2017) is preserved at Institute of Natural Products Chemistry, VAST.

2.2. General experimental procedures

NMR 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 F254 and RP-18 F254S plates. Compounds were visualized by UV light at 254 and 365 nm, 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 hexane, ethyl acetate and methanol at 50 ^oC (3 times x 2 hours per time) on heated ultrasonic machine. Filtered extracts were combined and concentrated under low pressure to give hexane (24 g), ethyl acetate (52 g) and methanol (85 g) extracts. The ethyl acetate extract (50 g) was separated on a silica gel column, eluted with hexane:ethyl acetate (100:1 → 1:100, v/v) to obtain 6 fractions (E1→E6). The fraction E5 was chromatographed on a silica gel column, eluted with hexane:ethyl acetate (1:1, v/v) to get 4 subfractions (E5.1→E5.4). The subfraction E5.2 was continuously chromatographed on a silica gel column, eluted with chloroform: methanol (25:1, v/v) to give **1** (10.0 mg). The subfraction E5.4 was separated on a silica gel column using chloroform:methanol (10:1, v/v) as eluent to obtain 3 subfractions (E5.4.1→ E5.4.3). The subfraction E5.4.3 was then purified on an YMC RP-18 column, eluted with methanol:water (4:1, v/v) to give **3** (18.0 mg). The fraction E2 was chromatographed on a silica gel column, eluted with *n*-hexane:ethyl acetate (2:1, v/v) to get 3 subfractions (E2.1→E2.3). The subfraction E2.3 was continuously separated on a silica gel column using *n*-hexane:ethyl acetate (2:3, v/v) as eluent to obtain **2** (6.5 mg). The fraction E1 was chromatographed on a silica gel column, eluted with *n*-hexane:acetone (20:1, v/v) to give **5** (25 mg). The fraction E6 was chromatographed on a silica gel column, eluted with ethyl acetate:methanol (20:1, v/v) to give 3 subfractions (E6.1→E6.3). The subfraction E6.2 was then purified on an YMC RP-18 column, eluted with methanol:water (1:2, v/v) to give **4** (30.0 mg).

Luteolin (1): Yellow crystal; ^{1}H-NMR (500 MHz, Methanol-d_4) δ_H (ppm): 6.22 (1H, d, J = 1.5 Hz, H-6), 6.45 (1H, d, J = 1.5 Hz, H-8), 6.55 (1H, s, H-3), 6.92 (1H, d, J = 8.5 Hz, H-5′), 7.39 (1H, d, J = 2.0 Hz, H-2′), 7.40 (1H, dd, J = 7.0, 2.0 Hz, H-6′); ^{13}C-NMR (125 MHz, Methanol-d_4) δ_C (ppm): 166.12 (C-2), 103.88 (C-3), 183.87 (C-4), 163.21 (C-5), 100.17 (C-6), 166.37 (C-7), 95.03 (C-8), 159.43 (C-9), 105.30 (C-10), 123.72 (C-1′), 114.18 (C-2′), 147.05 (C-3′), 151.01 (C-4′), 116.80 (C-5′); 120.31 (C-6′).
Luteolin tetramethyl ether (2): White crystal; ^{1}H-NMR (500 MHz, Methanol-d_4) δ_H (ppm): 6.56 (1H, d, J = 2.0 Hz, H-6), 6.67 (1H, s, H-3), 6.85 (1H, d, J = 2.0 Hz, H-8), 7.14 (1H, d, J = 7.5 Hz, H-5′), 7.53 (1H, d, J = 2.0 Hz, H-2′), 7.64 (1H, dd, J = 7.5, 2.0 Hz, H-6′), 3.94 (6H, s, 3′,5-OCH_3), 3.97 (3H, s, 4′-OCH_3), 3.98 (3H, s, 7-OCH_3); ^{13}C-NMR (125 MHz, Methanol-d_4) δ_C (ppm): 180.07 (C-4), 166.49 (C-7), 163.46 (C-2), 162.09 (C-5), 161.37 (C-9), 153.82 (C-3′), 150.88 (C-4′), 124.83 (C-1′), 121.20 (C-6′), 112.81 (C-5′), 110.48 (C-2′), 107.62 (C-3), 97.43 (C-6), 94.27 (C-8), 56.76 (4′-OCH_3), 56.59 (3′,5-OCH_3), 56.54 (7-OCH_3).
N,N-dimethyltetradecan-1-amine (3): Pale yellow oil; ^{1}H-NMR (500 MHz, Methanol-d_4) δ_H (ppm): 0.92 (3H, t, J = 7.0 Hz, H-15), 2.26 (6H, s, H-16, H-17); 2.33 (2H, dd, J = 6.0, 10.0 Hz, H-2), 1.50 (2H, m, H-3), 1.33 (2H, m, H-14), 1.31 [20H, m, H-4 to H-13); ^{13}C-NMR (125 MHz, Methanol-d_4) δ_C (ppm): 14.41 (C-15), 45.34 (C-16,17), 23.71 (C-14), 28.24 (C-4), 28.56 (C-3), 30.44-30.57 (C-5 to C-12), 33.05 (C-13).
(+)-pinitol (4): White powder; ^{1}H-NMR (500 MHz, Methanol-d_4) δ_H (ppm): 3.91 (2H, dd, J = 6.0, 3.5 Hz, H-1, H-6), 3.77 (1H, dd, J = 10.0, 2.5 Hz, H-5), 3.72 (1H, dd, J = 10.0, 2.5 Hz, H-2), 3.63 (3H, s, 1-OCH_3), 3.61 (1H, t, J = 9.5 Hz, H-4), 3.29 (1H, t, J = 9.5 Hz, H-3); ^{13}C-NMR (125 MHz, Methanol-d_4) δ_C (ppm): 84.92 (C-1), 74.32 (C-5), 73.76 (C-3), 73.46 (C-6), 72.57 (C-2), 72.03 (C-4), 60.79 (1-OCH_3).
Stigmasterol (5): White crystal. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H (ppm): 0.71 (3H, s, H-28), 0.80 (3H, d, J = 6.5 Hz, H-27), 0.82 (3H, d, J = 6.5 Hz, H-26), 0.83 (3H, t, J = 7.0 Hz, H-24), 0.91 (3H, d, J = 6.5 Hz, H-19), 1.03 (3H, s, H-29), 3.51 (1H, m, H-3), 4.98 (1H, m, H-20), 5.14 (1H, m, H-21), 5.31 (1H, br d, J = 6.0 Hz, H-6); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C (ppm): 37.61 (C-1), 32.09 (C-2), 72.10 (C-3), 42.42 (C-4), 141.10 (C-5), 121.82 (C-6), 31.78 (C-7), 31.82 (C-8), 50.21 (C-9), 36.60 (C-10), 21.51 (C-11), 39.94 (C-12), 42.41 (C-13), 56.80 (C-14), 24.40 (C-15), 29.31 (C-16), 56.18 (C-17), 40.62 (C-18), 21.71 (C-19), 138.69 (C-20), 129.60 (C-21), 46.12 (C-22), 25.40 (C-23), 12.11 (C-24), 29.60 (C-25), 20.23 (C-26), 19.81 (C-27), 18.90 (C-28), 12.20 (C-29).

3. RESULTS AND DISCUSSION

Compound **1** was obtained as yellow crystal. The ^{1}H-NMR spectra of **1** showed five aromatic proton signals at δ_H 6.22 (1H, d, J = 1.5 Hz, H-6), 6.45 (1H, d, J = 1.5 Hz, H-8), 6.92 (1H, d, J = 8.5 Hz, H-5′), 7.39 (1H, d, J = 2.0 Hz, H-2′), 7.40 (1H, dd, J = 7.0, 2.0 Hz, H-6′) and a singlet signal at δ_H 6.55 (1H, s, H-3). The ^{13}C-NMR spectra of **1** showed fifteen carbons including a carbonyl carbon at δ_C 183.87 (C-4) and the others from δ_C 95.03 to 166.37. These data suggested **1** to be a tetrasubstituted flavone. By comparing these data with those reported [7], **1** was identified to be luteolin, which was isolated from *Desmodium gangeticum* for the first time. Luteolin has been reported to possess antioxidant, anti-inflammatory, antimicrobial, anticancer, cardio-vascular protective, antidiabetes, neuroprotective and antiallergic activities [8].

Compound **2** was obtained as white crystal. Similar to **1**, compound **2** also has five aromatic protons, a singlet signal at δ_H 6.67 (1H, s, H-3) and fifteen carbons of a tetrasubstituted

flavone which were observed from the ^{1}H- and ^{13}C-NMR spectra. Apart from these signals, the ^{1}H- and ^{13}C -NMR spectra of **2** showed four methoxy groups [δ_H 3.94 (6H, s), 3.97 (3H, s) and 3.98 (3H, s)/δ_C 56.76, 56.59 and 56.54]. The substitutional position of four methoxy groups was identified by using HMBC correlations. The HMBC correlations of methoxy protons (δ_H 3.94) to C-5 (δ_C 162.09) and C-3′ (δ_C 153.82), methoxy protons (δ_H 3.97) to C-4′ (δ_C 150.88), as well as methoxy protons (δ_H 3.98) to C7 (δ_C 166.49) established these methoxy groups attached to C-5, C-3′, C-4′ and C-7, respectively. From the above evidence and comparison with spectral data of those in literature [9], compound **2** was idendifed as luteolin tetramethyl ether. This is the first time luteolin tetramethyl ether was isolated from the genus *Desmodium*. This compound has been shown to have anti-fungal, chondroprotective, anti-inflammatory, antiallergic, anti-mycobacterial, and anti-malarial activities [10, 11].

Compound **3** was obtained as pale yellow oil and reacted with Dragendorff's reagent. Thus, this compound must contain nitrogen element in its molecule. The ^{1}H-NMR spectra of **3** showed a singlet signal of two methyl groups at δ_H 2.26 (6H, s, H-16, H-17), a triplet methyl group at δ_H 0.92 (3H, t, J = 7.0 Hz, H-15), together with long-chain aliphatic at δ_H 1.31 (20H, m). These data suggested that **3** must be a tertiary amine with substituents including two methyl groups and a long-chain aliphatic. On the other hand, the ^{13}C-NMR spectra of **3** showed 16 carbon atoms at δ_C 45.34 (C-16, C-17), 14.41 (C-15), 23.71 (C-14), 60.77 (C-2), 28.24 – 33.05 (C-3 to C-13). The HMBC correlations from H-16, H-17 (δ_H 2.26) to C-2 (δ_C 60.77), from H-15 (δ_H 0.93) to C-13 (δ_C 33.05) and C-14 (δ_C 23.71) confirmed the structure of **3** as figure 2. The NMR data of **3** were absolutely compatible with NMR predition of N,N-dimethyltetradecan-1-amine carried out in ChemBioDraw Ultra 11.0 software. Hence, compound **3** was deduced to be N,N-dimethyltetradecan-1-amine which isolated from the genus of *Desmodium* for the first time.

Compound **4** was obtained as white powder. The ^{1}H-NMR spectra of **4** revealed the signal of 9 protons from δ_H 3.29 to 3.91. In which, the singlet signal of three protons at δ_H 3.63 was assigned to be a methoxy group. In addition, the ^{13}C-NMR spectra of **4** showed the signal of 7 carbon atoms at δ_C 84.92 (C-1), 74.32 (C-5), 73.76 (C-3), 73.46 (C-6), 72.57 (C-2), 72.03 (C-4), 60.79 (-OCH_3). All above NMR data suggested that **4** may be a cyclohexitol. The HMBC correlation from three protons at δ_H 3.63 to C-1 (δ_C 84.92) suggested the methoxy group attached to C-1. Based on these evidences and comparison with the published data [12], compound **4** was identified as (+)-pinitol. (+)-Pinitol has well-known insulin-like effects. In addition, (+)-pinitol possess multifunctional properties, including feeding stimulant, anti-inflammatory, cardioprotective, anti-hyperlipidemic and creatine retention promotion properties [13].

Compound **5** was obtained as white crystals. The ^{1}H-NMR spectra of **5** revealed three olefinic proton signals at 5.31 (br d, J = 6.0 Hz, H-6), 4.98 (m, H-20), 5.14 (m, H-21), along with 6 methyl signals at δ_H 0.71 (3H, s, H-28), 0.80 (3H, d, J = 6.5 Hz, H-27), 0.82 (3H, d, J = 6,5 Hz, H-26), 0.83 (3H, t, J = 7.0 Hz, H-24), 0.91 (3H, d, J = 6.5 Hz, H-19) and 1.03 (3H, s, H-29), suggested the presence of a sterol skeleton. Moreover, the ^{13}C-NMR spectra of **5** showed 29 carbon signals. Four of which at δ_C 141.10 (C-5), 121.82 (C-6), 138.69 (C-20) and 129.60 (C-21) were assigned to two olefinic groups. These spectral data of **5** are fully consistent with those of stigmasterol reported in literature [14]. Thus **5** was identified as stigmasterol. Stigmasterol has been investigated for its pharmacological prospects such as antiosteoarthritic, antihypercholestrolemic, cytotoxicity, antitumor, hypoglycaemic, antimutagenic, antioxidant, anti-inflammatory and CNS effects [15].

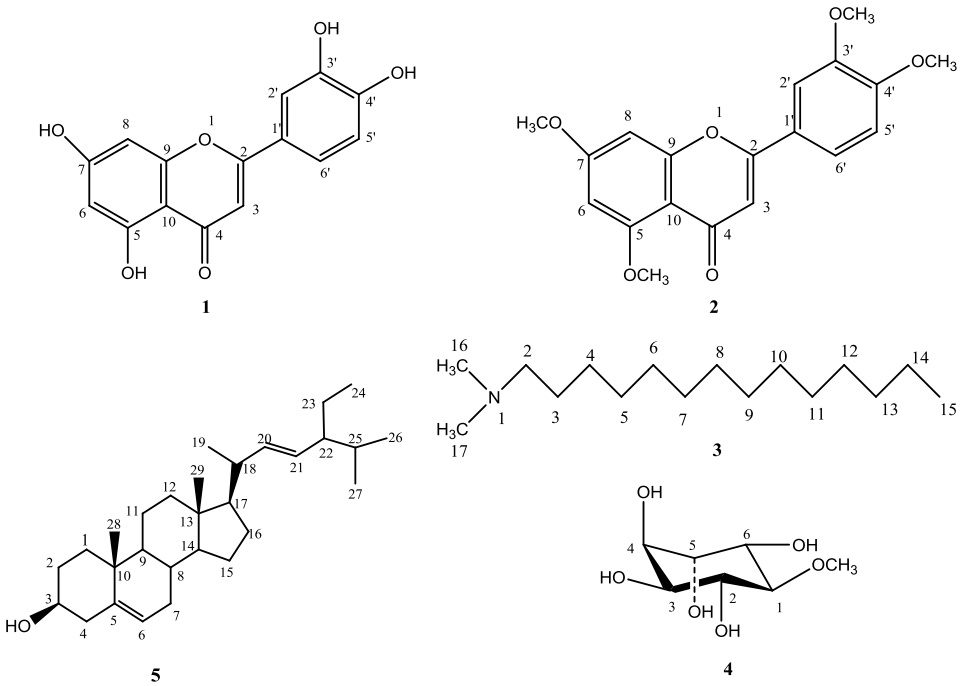

Figure 1. The structures of isolated compounds (**1**-**5**) from the leaves of *D. gangeticum.*

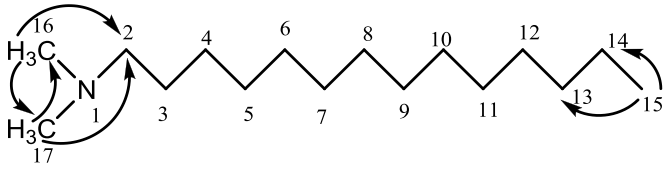

Figure 2. HMBC correlations of compound **3**.

4. CONCLUSIONS

From the ethyl acetate extract of the leaves of *Desmodium gangeticum* (Fabaceae), five compounds were isolated and indentified structures including luteolin (**1**), luteolin tetramethyl ether (**2**), N,N-dimethyltetradecan-1-amine (**3**), (+)-pinitol (**4**) and stigmasterol (**5**). In which, luteolin (**1**) was isolated from *Desmodium gangeticum* for the first time while luteolin tetramethyl ether (**2**) and N,N-dimethyltetradecan-1-amine (**3**) was first reported from the genus of *Desmodium*. This is the first time five compounds are reported from *Desmodium gangeticum* of Vietnam. 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. Ho P. H., Cay co Viet Nam, Tre Publishing House **1** (1999) 928 (in Vietnamese).
2. Suman S., Neha P., Bhupesh P. – A review on Shalparni (*Desmodium gangeticum* DC.) and Desmodium species (*Desmodium triflorum* DC. & Desmodium laxiflorumDC.)– Ethnomedicinal perspectives, Journal of Medicinal Plants Studies **3** (4) (2015) 38-43.
3. Subha R., Madan M. P., Ajay K. S. R. - Review: An ethnomedicinal, phytochemical and pharmacological profile of *Desmodium gangeticum* (L.) DC. and *Desmodium adscendens* (Sw.) DC., Journal of Ethnopharmacology **136** (2011) 283–296.
4. Yadav K A., Agrawal J., Pal A., Gupta M.M. - Novel anti-inflammatory phytoconstituents from *Desmodium gangeticum*, Natural Product Research **27** (18) (2013)1639-45.
5. Đat N. T., Luyen N. T., Dang N. H. - Phenolic glucosides from the leaves of *Desmodium gangeticum* (L.) DC., Vietnam Journal of Chemistry **53** (2e) (2015) 69-72 (in Vietnamese).
6. Ha L. M., Phuong N. T., Hung L. N., Ha V. T. H., Anh B. K., Long P. Q. - Some glycosides isolated from *Desmodium gangeticum* (L.) DC. of Vietnam, Vietnam Journal of Science and Technology **56** (2A) (2018) 99-103.
7. Lin L. C., Pai Y. F., Tsai T. H. - Isolation of Luteolin and Luteolin-7-O-glucoside from *Dendranthema morifolium* Ramat Tzvel and Their Pharmacokinetics in Rats, Journal of Agricultural and Food Chemistry **63** (35) (2015) 7700-7706.
8. López-Lázaro M. - Distribution and biological activities of the flavonoid luteolin, Mini-Reviews in Medicinal Chemistry **9** (1) (2009) 31-59.
9. Valdir A. Facundo, Selene M. Morais, Raimundo Braz Filho - Constituintes químicos de *Ottonia corcovadensis* Miq. da floresta amazônica – atribuicão dosdeslocamentos químicos dos átomos de hidrogênio e carbono, Quim. Nova **27** (1) (2004) 79-83.
10. Patel A. B. and Theoharides T. C. - Methoxyluteolin inhibits neuropeptide-stimulated proinflammatory mediator release via mTOR activation from human mast cells, Journal of Pharmacology and Experimental Therapeutics **361** (3) (2017) 462-471.
11. Wu L., Liu H., Li L., Xu D., Gao Y., Guan Y., Chen Q. - 5,7,3′,4′-Tetramethoxyflavone protects chondrocytes from ER stress-induced apoptosis through regulation of the IRE1α pathway, Connective Tissue Research **59**(2) (2018) 157-166.
12. Jawla S., Kumar Y., Khan M.S.Y. - Isolation of Antidiabetic Principle from *Bougainvillea spectabilis* Willd (Nyctaginaceae) Stem Bark, Tropical Journal of Pharmaceutical Research **12** (5) (2013) 761-765.
13. Anandakumar P., Vanitha M.K., Gizaw M., Dereje G. - A review on the diverse effects of D-pinitol, Adv J Pharm Life sci Res **6** (1) (2018) 1-7.
14. Chaturvedula V. S. P., Prakash I. - Isolation of Stigmasterol and β-Sitosterol from the dichloromethane extract of *Rubus suavissimus*, International Current Pharmaceutical Journal **1** (9) (2012) 239-242.
15. Navpreet K., Jasmine C., Akash J., Lalit K. – Stigmasterol: a comprehensive review, International Journal of Pharmaceutical Sciences and Research **2** (9) (2011) 2259-2265.