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Research Article

SOME TERPENOID, FLAVONOID, AND MONOCYCLIC AROMATIC COMPOUNDS FROM LEAVES OF *VITEX NEGUNDO*

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ABSTRACT

*Seven compounds including abietatriene (**1**), oleanolic acid (**2**), quercetagetin 3,7-dimethyl ether (**3**), luteolin (**4**), p-hydroxybenzoic acid (**5**), p-methylbenzoic acid (**6**), and protocatechuic acid (7) were isolated from the n-hexane: ethyl acetate (1:1) extract of Vitex negundo leaves collected in Binh Thuan Province. Their chemical structures were elucidated by comparing their spectroscopic data with reported data in the literature. Six compounds (exception of **4**) were known to be present in Vitex negundo.*

Keywords*: *flavonoid;* monocyclic aromatic compounds*; terpenoid; Vitex negundo

1. Introduction

Different parts of *Vitex negundo* are widely used in traditional medicine. In which, leaves are used the most popular to cure blood in urine, cloudy urine, rheumatism, malaria, poisoning, colds, stomach pain, urticaria, venomous snakebite, edema, epilepsy, vomiting, and diarrhea. Extracts and organic compounds isolated from leaves, roots, and stem were evaluated numerous bioactivities such as anti-bacterial, anti-fungi, anti-inflammatory, antioxidant, hepatoprotective, cytotoxicity against many cancer cell lines (Khokra et al. 2019, Rideout et al. 1999, Tandon et al. 2008, Alam et al. 2009, Díaz et al. 2003, Arulvasu

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et al. 2010, Kannikaparameswari et al. 2013, Xin et al. 2013). Phytochemical data of *V. negundo* reported the presence of numerous terpenoid, lignan, flavonoid glycoside, iridoid glycoside, and glycoside (Khokra et al. 2008, Malik et al. 2006, Zheng et al. 2011, Zheng et al. 2012, Gautam et al. 2008, Sharma et al. 2009, Shahnaz et al. 2017). This paper reported the isolations and structural elucidation of seven compounds from *V. negundo* leaves collected in Binh Thuan Province, Vietnam.

2. Experiment

2.1. General experimental procedures

The NMR spectra were recorded on a Bruker Avance spectrometer (500 MHz or 400 MHz for ^{1}H–NMR and 125 MHz or 100 MHz for ^{13}C–NMR) in acetone-d_6, chloroform-*d*, dimethylsulfoxide-d_6, or methanol-d_4. Thin-layer chromatography was carried out on silica gel 60 (Merck, 40-63 μm) and spots were visualized by spraying with 10% H_2SO_4 solution, followed by heating.

2.2. Plant material

Leaves of *Vitex negundo* (L.) were collected in Phu Quy Island, Binh Thuan Province in August 2019. The scientific name of the leaves was identified as *Vitex negundo* (L.) by Dr. Tran Cong Luan, Tay Do University, Vietnam. A voucher specimen (No UP-010) was deposited in the herbarium of the Department of Organic Chemistry, Faculty of Chemistry, Ho Chi Minh University of Education, Ho Chi Minh City, Vietnam.

2.3. Extraction and isolation

Dried leaves of *V. negundo* (12.1 kg) were extracted with ethanol (50Lx3) at room temperature. The filtrated solution was evaporated at reduced pressure to obtain a crude extract (1.7 kg). This extract was separated into *n*-hexane extract (370 g, **H**), *n*-hexane: EtOAc extract (88 g, **HEA**), EtOAc (594 g, **EA**), and remaining extract (561.7 g, **RE**) by liquid-liquid partition method. The **HEA** extract was applied to Sephadex LH-20 chromatography and eluted with methanol to afford four fractions (coded, HEA1-HEA4). The fraction HEA4 (21 g) was further subjected to a silica gel column chromatography, eluted with the solvent system of *n*-hexane:ethyl acetate:acetone (20:1:1, v/v/v) to give 7 subfractions (HEA4.1-HEA4.7). Next, subfraction HEA4.1 (3.1 g) was separated into three different polar fractions (coded, N1-N3) by a silica gel column chromatography, eluted with the solvent system of *n*-hexane:ethyl acetate:acetone:methanol (30:1:1:0.01, v/v/v/v). This procedure was applied to fraction N1 to obtain compounds **1** (3.5 mg) and **2** (23.5 mg). Whereas compounds **3** (12.1 mg), **5** (45.7 mg), **6** (13.7 mg), and **7** (31.2 mg) were obtained from N3 via a silica gel column chromatography, eluted with the solvent system of *n*-hexane: ethyl acetate: acetone (2:1:1, v/v/v). Subfraction HEA4.7 (1.9 g) was subjected to a silica gel column chromatography, using a solvent system of *n*-hexane: ethyl acetate: acetone

(10:1:1, v/v/v) to give three different fractions (coded, M1-M3). The same method was applied to the fraction M3 to afford compounds **3** (11.6 mg) and **4** (37.2 mg).

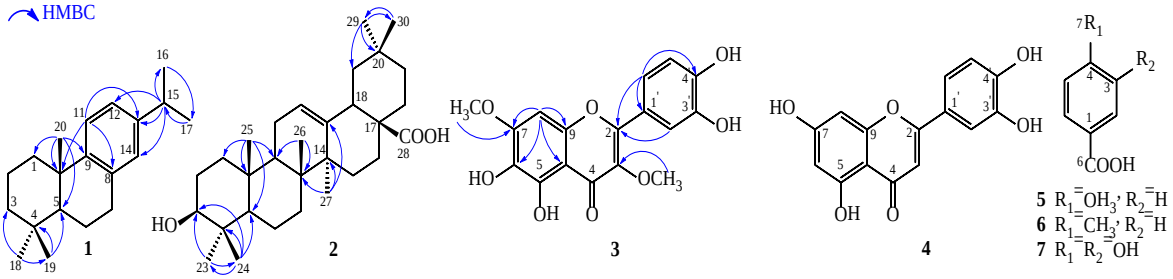

Figure 1. *Chemical structures and some HMBC correlations of isolated compounds* ***1-7***

- **Abietatriene** (**1**). White amorphous solid. ^{1}H-NMR (400 MHz, chloroform-*d*, δ ppm, *J* in Hertz): 7.11 (1H, *d*, 8.0, H-11), 6.92 (1H, *dd*, 8.0, 1.6, H-12), 6.82 (1H, *brs*, H-14), 2.75 (1H, septet, 7.2, H-15), 1.15 (6H, *d*, 7.2, H-16/H-17), 1.11 (3H, *s*, H-20), 0.87 (3H, *s*, H-18), 0.85 (3H, *s*, H-19). ^{13}C-NMR (125 MHz, chloroform-*d*, δ ppm): 38.9 (C-1), 19.4 (C-2), 41.8 (C-3), 33.6 (C-4), 50.5 (C-5), 19.2 (C-6), 30.6 (C-7), 135.1 (C-8), 147.7 (C-9), 37.6 (C-10), 124.4 (C-11), 123.9 (C-12), 145.5 (C-13), 126.9 (C-14), 33.6 (C-15), 24.1 (C-16), 24.1 (C-17), 33.5 (C-18), 21.7 (C-19), and 25.0 (C-20) (Tsujimura et al. 2019).
- **Oleanolic acid** (**2**). White amorphous solid. ^{1}H-NMR (500 MHz, acetone-d_6, δ ppm, *J* in Hertz): 3.14 (1H, *m*, H-3), 5.24 (1H, *t*, 3.0, H-12), 0.98 (3H, *s*, H-23), 0.80 (3H, *s*, H-24), 0.93 (3H, *s*, H-25), 0.78 (3H, *s*, H-26), 1.17 (3H, *s*, H-27), 0.91 (3H, *s*, H-29), 0.94 (3H, *s*, H-30). ^{13}C-NMR (125 MHz, acetone-d_6, δ ppm): 39.4 (C-1), 28.1 (C-2), 78.7 (C-3), 39.5 (C-4), 56.3 (C-5), 19.2 (C-6), 33.7 (C-7), 39.6 (C-8), 48.6 (C-9), 37.9 (C-10), 24.2 (C-11), 123.1 (C-12), 145.0 (C-13), 42.6 (C-14), 28.5 (C-15), 23.8 (C-16), 46.9 (C-17), 42.3 (C-18), 46.9 (C-19), 30.4 (C-20), 34.5 (C-21), 33.4 (C-22), 28.7 (C-23), 16.3 (C-24), 15.8 (C-25), 17.7 (C-26), 26.3 (C-27), 178.9 (C-28), 33.4 (C-29), and 23.9 (C-30) (Guvenalp et al. 2009).
- **Quercetagetin 3,7-dimethyl ether** (**3**). Yellow powder. ^{1}H-NMR (500 MHz, methanol-d_4, δ ppm, *J* in Hertz): 6.74 (1H, *s*, H-8), 7.65 (1H, *d*, 2.0, H-2'), 6.91 (1H, *d*, 8.5, H-5'), 7.55 (1H, *dd*, 8.5, 2.0, H-6'), 3.98 (3H, *s*, 3-OC<u>H</u>$_3$), 3.80 (3H, *s*, 7-OC<u>H</u>$_3$). ^{13}C-NMR (125 MHz, methanol-d_4, δ ppm): 146.5 (C-2), 139.4 (C-3), 180.2 (C-4), 146.8 (C-5), 130.9 (C-6), 155.7 (C-7), 91.6 (C-8), 151.2 (C-9), 107.1 (C-10), 123.1 (C-1'), 116.6 (C-2'), 158.4 (C-3'), 150.1 (C-4'), 116.4 (C-5'), 122.2 (C-6'), 60.3 (3-O<u>C</u>H$_3$), 57.0 (7-O<u>C</u>H$_3$) (Ulubelen et al. 1980; Grayer et al. 2010).
- **Luteolin** (**4**). Yellow powder. ^{1}H-NMR (500 MHz, dimethylsulfoxide-d_6, δ ppm, *J* in Hertz): 12.99 (1H, *brs*, 5-O<u>H</u>), 6.56 (1H, *s*, H-3), 6.09 (1H, *d*, 2.0, H-6), 6.34 (1H, *d*, 2.0, H-8), 6.79 (1H, *d*, 8.5, H-5'), 7.37 (1H, *dd*, 8.5, 2.0, H-6'), 7.34 (1H, *d*, 2.0, H-2') (Okamura et al. 1994).

- ***p*-Hydroxybenzoic acid** (**5**). White powder. ^{1}H-NMR (500 MHz, acetone-d_6, δ ppm, *J* in Hertz): 7.90 (2H, *d*, 8.5, H-2/H-6) and 6.90 (2H, *d*, 8.5, H-3/H-5). ^{13}C-NMR (125 MHz, acetone-d_6, δ ppm): 122.7 (C-1), 132.7 (C-2/C-6), 115.9 (C-3/C-5), 162.5 (C-4), and 167.4 (C-7) (Lin et al. 2014).
- ***p*-Methylbenzoic acid** (**6**). White powder. ^{1}H-NMR (400 MHz, chloroform-*d*, δ ppm, *J* in Hertz): 7.91 (2H, *d*, 8.0, H-2/H-6), 6.89 (2H, *d*, 8.0, H-3/H-5), and 2.56 (3H, *s*, H-7) (Wu et al. 2020).
- **Protocatechuic acid** (**7**). White powder. ^{1}H-NMR (500 MHz, acetone-d_6, δ ppm, *J* in Hertz): 7.52 (1H, *d*, 2.0, H-2), 6.88 (1H, *d*, 8.0, H-5), and 7.46 (1H, *dd*, 8.0, 2.0, H-6) (Lin et al. 2014).

3. Results and discussion

Compound **1** was obtained as a white amorphous solid. The ^{13}C-NMR spectrum of **1** showed signals of twenty carbons including six aromatic carbon signals resonated from 123.9 to 174.7 and fourteen aliphatic carbons from 19.2 to 50.5 ppm. This suggested that **1** could be a diterpenoid. The proton spectrum of **1** displayed a set of three signals at δ_H 7.11 (1H, *d*, 8.0, H-11), 6.92 (1H, *dd*, 8.0, 1.6, H-12), and 6.82 (1H, *brs*, H-14) characterized for a 1,2,4-trisubstituted benzene ring. The presence of a doublet proton signal, integrated six protons, at δ_H 1.15 (6H, *d*, 7.2, H-16/H-17) which had HMBC correlations to carbons at δ_C 33.6 (C-15), 24.1 (C-16), and 24.1 (C-17) as well as a methine proton H-15 appearing as a septet signal at 2.75 which possessed HMBC cross-peaks to carbons C-16, C-17, 123.9 (C-12), 145.5 (C-13), and 126.9 (C-14), suggested that there was an isopropyl group attaching to the benzene ring. Three remaining singlet methyl proton signals at 1.11 (3H, *s*, H-20), 0.87 (3H, *s*, H-18), and 0.85 (3H, *s*, H-19) showed HMBC correlations which fixed nicely to an abietane diterpenoid skeleton as shown in Figure 1. Based on the comparison of its NMR data with the published ones (Tsujimura et al. 2019), **1** was thus determined to be abietatriene.

Compound **2** was obtained as a white amorphous solid. The ^{13}C-NMR spectrum of **2** showed signals of thirty carbons, comprising of two olefinic carbon signals at δ_C 123.1 (C-12) and 145.0 (C-13) characterized for an oleanane skeleton triterpenoid. It corresponded to the observation of an olefinic methine proton signal at δ_H 5.24 (1H, *t*, 3.0, H-12) and seven singlet methyl proton signals from 0.77 to 1.67 ppm. A multiplet proton signal at 3.14 (1H, *m*) and a carbon signal at 78.7 were assigned to H-3 and C-3, respectively, through the HMBC cross-peaks of both singlet methyl proton signals H-23 and H-24 to carbons C-3, C-4, and C-5. The HMBC correlations of all methyl proton signals to adjacent carbons were used to determine their positions. It meant that a carboxylic carbon signal at 178.9 was suggested to be C-28 as usual. Additionally, the comparison of its NMR data with the reported ones (Guvenalp et al. 2009) showed good compatibility, **2** was then elucidated as oleanolic acid.

Compound **3** was obtained as yellow solid. The proton spectrum of **3** showed three proton signals characterized for a 1,2,4-trisubstituted benzene ring, including δ_H 7.65 (1H, *d*, 2.0, H-2'), 6.91 (1H, *d*, 8.5, H-5'), and 7.55 (1H, *dd*, 8.5, 2.0, H-6'). A singlet aromatic methine proton signal at δ_H 6.74 (1H, *s*, H-8) belonged to a pentasubstituted benzene ring. Two left singlet proton signals at 3.98 and 3.80, each integrated three-protons, demonstrated the presence of two methoxy groups. The ^{13}C-NMR spectra revealed seventeen carbon signals including two methoxy carbons at δ_C 57.0 and 60.3, one conjugated carbonyl carbon at δ_C 180.2, and fourteen carbons resonated from 91.6 to 158.4 ppm. An oxygenated carbon signal at δ_C 139.4 (C-3) was assigned to belong to C-3 of flavonol as usual. These suggested that **3** could be a flavonol containing two methoxy groups. The HMBC spectrum displayed the correlations of the sole singlet methine proton with three oxygenated carbons [δ_C 130.9 (C-6), 155.7 (C-7), and 151.2 (C-9)], and a quaternary carbon [δ_C 107.1 (C-10)], therefore this methine proton was assigned to be H-8 or H-6 of ring A. The positions of the two methoxy groups were determined at C-3 and C-7 via the HMBC correlations of the methoxy protons with carbons at δ_C 139.4 (C-3) and 155.7 (C-7), respectively. Based on the above analysis along with the good compatibility of its NMR data with those of quercetagetin 3,7-dimethyl ether possessing the methine proton H-8 (Ulubelen et al. 1980; Grayer et al. 2010), the chemical structure of **3** was identified to be quercetagetin 3,7-dimethyl ether.

Compound **4** was obtained as a yellow solid. The proton spectrum of **4** showed characteristic signals of a flavone skeleton including a singlet proton signal at δ_H 12.99 (1H, *brs*, 5-O<u>H</u>), two doublet proton signals with the small coupling constant [δ_H 6.09 (1H, *d*, 2.0, H-6) and 6.34 (1H, *d*, 2.0, H-8)] of ring A, three methine signals [δ_H 7.34 (1H, *d*, 2.0, H-2'), 6.79 (1H, *d*, 8.5, H-5'), and 7.37 (1H, *dd*, 8.5, 2.0, H-6')] of the 1,3,4-trisubstituted benzene ring of ring B. Moreover, the NMR data of **4** were consistent with those reported in the literature (Okamura et al. 1994), thus, **4** was determined as luteolin.

The NMR data analysis of compounds **5**, **6**, and **7** showed many similar characteristics of monocyclic aromatic compounds. The proton spectra of **5** showed two doublet proton signals with the large coupling constant of 8.5 Hz, each integrated two protons, at δ_H 7.90 and 6.90 ppm of a para-disubstituted benzene ring. It corresponded to the ^{13}C-NMR spectrum of **5** revealing five carbon signals comprising of two signals at δ_C 132.7 (C-2/C-6) and 115.9 (C-3/C-5) appearing in the double intensity of a symmetrical benzene ring. Two quaternary carbons resonating at δ_C 122.7 (C-1) and 162.5 (C-4) suggested that **5** possessed one hydroxy group. The last carbon signal at δ_C 167.4 (C-7) should belong to a conjugated carboxylic group. Additionally, the good compatibility of its NMR data with the previous ones (Lin et al. 2014) contributed to the chemical structure elucidation of **5** as *p*-hydroxybenzoic acid.

The proton spectrum of **6** was identical with those of **5**, except for the appearance of one more methyl group at C-4. The good compatibility between its NMR data and those in the literature (Wu et al. 2020) determined the structure of **6** to be *p*-methylbenzoic acid.

Whereas the proton spectrum of **7** showed signals of a 1,3,4-trisubstituted benzene ring at δ_H 7.52 (1H, *d*, 2.0, H-2), 6.88 (1H, *d*, 8.0, H-5), and 7.46 (1H, *dd*, 8.0, 2.0, H-6) which was identical signals of protocatechuic acid (Lin et al. 2014). **7** was thus determined as protocatechuic acid.

4. Conclusions

From the leaves of *V. negundo* collected in Binh Thuan Province, seven compounds, including two terpenoids [abietatriene (**1**) and oleanolic acid (**2**)], two flavonoids [quercetagetin 3,7-dimethyl ether (**3**) and luteolin (**4**)], and three monocyclic aromatic compounds [*p*-hydroxybenzoic acid (**5**), *p*-methylbenzoic acid (**6**), and protocatechuic acid (7)] were isolated. Their chemical structures were determined by using NMR spectroscopic method as well as comparison with the related literature. To the best of our knowledge, six compounds (exception of **4**) were isolated from *V. negundo* for the first time. Further studies on this species are in the progress.

❖ ***Conflict of Interest:*** *Authors have no conflict of interest to declare.*

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MỘT SỐ TERPENOID, FLAVONOID VÀ HỢP CHẤT ĐƠN VÒNG THƠM TỪ LÁ CÂY NGŨ TRẢO *VITEX NEGUNDO*

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TÓM TẮT

*Bảy hợp chất bao gồm abietatriene (**1**), oleanolic acid (**2**), quercetagetin 3,7-dimethyl ether (**3**), luteolin (**4**), p-hydroxybenzoic acid (**5**), p-methylbenzoic acid (**6**) và protocatechuic acid (7) được cô lập từ cao n-hexane:ethyl acetate (1:1) của lá cây ngũ trảo, thu hái tại Bình Thuận bằng các phương pháp sắc kí. Cấu trúc hóa học của các hợp chất được xác định bằng các phương pháp phổ nghiệm đồng thời so sánh với các dữ liệu phổ trong tài liệu tham khảo. Ngoại trừ hợp chất **4**, sáu hợp chất còn lại lần đầu tiên được biết có hiện diện trong cây ngũ trảo.*

Từ khóa: *flavonoid;* hợp chất đơn vòng thơm*; terpenoid; Vitex negundo*