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Chemical Constituents from Leaves Extract of Bouea Oppositifolia (Roxb.) Meisn. (Anacardiaceae)

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Abstract

Bouea oppositifolia also called Marian plum belongs to the family Anacardiaceae, is an edible fruit tree and well-known in Binh Minh district, Vinh Long province, Vietnam. Fresh Marian plum can be used like other fruits, and it is also used in traditional medicine to treat digestive diseases, and constipation. From the methanol extract of *B. oppositifolia* leaves, seven compounds were isolated including taraxerol (**1**), taraxerone (**2**), friedelin (**3**), 3,4-secofridelan-3-oic acid (**4**), gallic acid (**5**), methyl gallate (**6**), and naringenin (**7**). The structure of these compounds was elucidated by ESI-MS, NMR spectroscopic methods, and by comparison with the published data. Compound **1**-**6** are recorded for the first time from this species.

Keywords: Bouea oppositifolia, triterpenoid, phenolic compounds, flavanone

1. Introduction

Bouea is a genus of Anacardiaceae, comprising two common species of genus *Bouea* including *Bouea macrophylla* Griff. and *Bouea oppositifolia* (Roxb.) Meisn. due to the difference between the leaves and fruits of two species [1], [2]. Marian plum (*B. oppositifolia*) is endemic to the Southeast Asia region. This tree is widely grown in Indonesia, Malaysia, Thailand, Myanmar, Laos, Cambodia, Vietnam, China, and the Andaman Islands [2]. In Vietnam, the Marian plum is cultivated in some provinces in the Mekong Delta, especially in Vinh Long province. Fresh Marian plum can be used like other fruits and is sometimes made into preserves and jams [2], [3]. The Marian plum not only has economic value but also medicinal properties. The roots are used to treat fevers, the fruits are used to treat digestive diseases, and constipation, the outer layer containing essential oils is used to treat coughs, while the bitter-tasting leaves are

used for treating swelling, arthritis, and burns [3], [4]. Currently, there is a limited publication regarding the bioactivity and chemical composition of *B. oppositifolia*. The research of Ashraful Islam showed that the methanol extract of *B. oppositifolia* leaves possessed antidiarrheal and analgesic activities through *in vivo* studies in Swiss Albino mice [5]. In the phytochemical investigation, nine flavonoids were isolated from the twigs of *B. oppositifolia*, consisting of naringenin, eriodictyol, isoliquiritigenin, liquiritigenin, butin, taxifolin, fustin, garbanzol, and sulfuretin [6]. In our previous publication, the ethyl acetate fraction possessed the best antioxidant activity by using DPPH and phosphomolybdenum assays, and also exhibited antimicrobial activity against *E. coli*, *P. aeruginosa*, *B. subtillis*, and *A. niger* at the concentration of 200 - 400 mg/mL [7]. This paper presents the isolation and structural elucidation of seven compounds from the methanol extract of *B. oppositifolia* leaves including taraxerol (**1**), taraxerone (**2**), friedelin (**3**), 3,4-secofridelan-3-oic acid (**4**), gallic

acid (**5**), methyl gallate (**6**), and naringenin (**7**). Among those, there are six compounds (**1** to **6**) first described from this species.

2. Experimental

2.1. Instruments and chemicals

The solvents used in extraction and chromatographic procedures were p.a. grade including *n*-hexane, chloroform, ethyl acetate, acetone, methanol (Chemsol, Vietnam). The melting point (m.p.) was recorded on a non-adjustable melting apparatus (Electrothermal IA 9100-UK). Thin layer chromatography (TLC) was performed on Merck 60 F254 or RP-18 F254S silica gel plates. Flash chromatography (FC), and column chromatography (CC) was performed with normal phase adsorbents (Silica gel 60 particle size 0.04-0.063 mm, Merck) and with reverse phase adsorbents (LiChroprep RP-18 particle size, 0.04-0.063 mm, Merck). Electrospray ionization mass spectroscopy (ESI-MS) measurements were conducted on AGILENT 1200 LC-MSD Trap. Nuclear magnetic resonance (NMR) spectroscopy measurements were conducted using on NMR Bruker AVANCETM 500 MHz and NMR Bruker AVANCETM 600 MHz Spectrometer at the Institute of Chemistry, Vietnam Academy of Science and Technology.

2.2. Materials

Marian plum leaves (*Bouea oppositifolia* (Roxb.) Meisn.) were collected in May 2019 in Binh Minh district, Vinh Long province, Vietnam. The scientific name was identified by Assoc. Prof. Dang Minh Quan (Department of Biology, School of Education, Can Tho University). A voucher specimen, designated as Bou052019-VL(BM)046, was deposited in the Department of Chemistry at the College of Natural Sciences, Can Tho University, Vietnam.

2.3. Extraction and Isolation

Fresh Marian plum leaves (22 kg) were washed, dried and ground into a powder (yielding 10 kg). The dry powder was successively extracted by maceration with three 30-liter portions of methanol at room temperature for 24 hours each. The crude methanol extract (1.35 kg) was suspended in water and fractionated by liquid-liquid extraction with two solvents of increasing polarity (*n*-hexane and ethyl acetate) to obtain three fractions respectively: *n*-hexane fraction (210 g), ethyl acetate fraction (550 g), and aqueous fraction (500 g).

n-Hexane fraction (180 g) was separated using flash chromatography (FC) on silica gel, eluting with *n*-hexane-acetone (100:0 to 0:100, v/v) to yield ten subfractions (H1-H10). Subfraction H4 (8.55 g) was divided into 11 subfractions by silica gel CC, eluting with *n*-hexane-CHCl$_3$ (100:0 to 0:100, v/v). Subfraction H4.8 (478 mg) was further subjected to silica gel column chromatography (CC), eluted with *n*-hexane-CHCl$_3$ 50:50 (v/v) to obtain compound **3** (81 mg). Subfraction H5 (43 g) was separated by silica gel CC, eluted with *n*-hexane-acetone 70:30 (v/v) to afford compound **2** (14 mg), and compound **1** (40 mg), respectively. Subfraction H8 (10 g) was similarly fractionated on a silica gel CC, the elution solvent was *n*-hexane-ethyl acetate (100:0 to 0:100, v/v) to yield eleven subfractions (H8.1-H8.11). Subfraction H8.6 (360 mg) was purified by silica gel CC, eluted with *n*-hexane: ethyl acetate (80:20, v/v) to yield compound **4** (8 mg).

Similarly, ethyl acetate fraction (250 g) was subjected to FC on silica gel, eluting with EtOAc-CH$_3$OH (100:0 to 0:100, v/v) to yield ten subfractions (EA1-EA10). Subfraction EA2 (40 g, eluted with EtOAc 100%) was separated by silica gel CC to yield 05 subfractions (EA2.1-EA2.5). Subfraction EA2.2 (10 g) was purified by another silica gel CC, eluting with CHCl$_3$-CH$_3$OH (95:5, v/v) to yield compound **6** (1 g). Subfraction EA2.4 (2 g) was similarly subjected to silica gel CC, eluting with CHCl$_3$-AcOEt (100:0 to 0:100, v/v) to obtain 05 subfractions. Then subfraction EA2.4.4 (300 mg, eluted with CHCl$_3$-EtOAc 70:30) was purified by silica gel RP-C18 CC to afford compound **7** (8 mg, eluted with CH$_3$OH-H$_2$O 80:20). Subfraction EA4 (4 g) was subjected to silica gel CC using CHCl$_3$-CH$_3$OH (100:0 to 0:100, v/v) for elution to collect 12 subfractions (EA4.1–12). Subsequently, subfraction EA4.7 (300 mg) was repeatedly purified by silica gel RP-C18 CC to obtain compound **5** (14 mg, eluted with CH$_3$OH-H$_2$O 50:50 v/v).

Taraxerol (**1**): White powder, mp 278-280°C. (+) ESI-MS: m/z = 427.2 [M+H]$^+$ (molecular formula C$_{30}$H$_{50}$O). ^{1}H-NMR (500 MHz, CDCl$_3$, δ, ppm, J/Hz): 5.53 (1H, *dd*, J = 8.0, 3.0 Hz, H-15), 3.19 (1H, *dd*, J = 11.0, 4.0 Hz, H-3), 2.03 (1H, *dt*, J = 12.5, 3.3 Hz, H-7b), 1.92 (1H, dd, J = 14.5, 3.0 Hz, H-16b), 1.56 (1H, m, H-6a), 1.49 (1H, *m*, H-11a), 1.47 (1H, *m*, H-18), 1.43 (1H, *m*, H-7a), 1.38 (1H, *m*, H-22b), 1.36 (2H, *m*, H-19a), 1.32 (1H, *m*, H-12b), 1.26 (2H, *m*, H-21), 1.09 (3H, *s*, H-26), 1.03 (1H, *m*, H-12a), 1.00 (1H, *m*, H-22a), 0.99 (1H, *m*, H-1a), 0.98 (3H, *s*, H-23), 0.95 (3H, s, H-29), 0.93 (3H, *s*, H-25), 0.91 (6H, *s*, H-27, H-30), 0.82 (3H, *s*, H-28), 0.80 (3H, *s*, H-24). ^{13}C-NMR (125 MHz, CDCl$_3$, δ, ppm: 38.1 (C-1), 27.2 (C-2), 79.1 (C-3), 38.8 (C-4), 55.6 (C-5), 18.8 (C-6), 35.1 (C-7), 39.0 (C-8), 48.8 (C-9), 37.8 (C-10), 17.5 (C-11), 35.6 (C-12), 37.7 (C-13), 158.1 (C-14), 116.9 (C-15), 36.7 (C-16), 37.6 (C-17), 49.3 (C-18), 41.4 (C-19), 28.8 (C-

20), 33.7 (C-21), 33.1 (C-22), 28.0 (C-23), 15.4 (C-24), 15.5 (C-25), 29.9 (C-26), 25.9 (C-27), 29.8 (C-28), 33.4 (C-29), 21.3 (C-30) [8].

Taraxerone (**2**): White powder, mp 240-242°C. (+) ESI-MS: m/z = 425.2 [M+H]$^+$ (molecular formula $C_{30}H_{48}O$). ^{1}H-NMR (500 MHz, CDCl$_3$, δ, ppm, J/Hz): 5.56 (1H, dd, J = 8.3, 3.3 Hz, H-15), 2.57 (1H, m, H-2b), 2.33 (1H, ddd, J = 15.9, 6.4, 3.4 Hz, H-2a), 2.08 (1H, dt, J = 13.0, 3.3 Hz, H-7b), 1.92 (1H, dd, J = 14.5, 3.0 Hz, H-16b), 1.88 (1H, m, H-1b), 1.67 (1H, m, H-11b), 1.65 (1H, m, H-1a), 1.62 (1H, m, H-16a), 1.57 (2H, m, H-6b, H-7a), 1.54 (1H, m, H-11a), 1.52 (1H, m, H-18), 1.50 (1H, m, H-6a), 1.37 (1H, m, H-19a), 1.34 (1H, m, H-12b), 1.32 (1H, m, H-22b), 1.30 (1H, m, H-22a), 1.25 (2H, m, H-21), 1.14 (3H, s, H-25), 1.09 (3H, s, H-26), 1.08 (3H, s, H-23), 1.07 (3H, s, H-24), 0.96 (3H, s, H-29), 0.92 (3H, s, H-27), 0.91 (3H, s, H-30), 0.83 (3H, s, H-28). ^{13}C-NMR (125 MHz, CDCl$_3$, δ, ppm): 38.4 (C-1), 34.1 (C-2), 217.5 (C-3), 47.6 (C-4), 55.8 (C-5), 20.0 (C-6), 35.1 (C-7), 38.9 (C-8), 48.7 (C-9), 37.6 (C-10), 17.5 (C-11), 35.8 (C-12), 37.8 (C-13), 157.6 (C-14), 117.2 (C-15), 36.7 (C-16), 37.7 (C-17), 48.8 (C-18), 40.7 (C-19), 28.8 (C-20), 33.6 (C-21), 33.1 (C-22), 26.1 (C-23), 21.5 (C-24), 14.8 (C-25), 29.8 (C-26), 25.7 (C-27), 29.9 (C-28), 33.4 (C-29), 21.4 (C-30) [8].

Friedelin (**3**): White powder, mp 252-254°C. (+) ESI-MS: m/z = 427.2 [M+H]$^+$ (molecular formula $C_{30}H_{50}O$). ^{1}H-NMR (500 MHz, CDCl$_3$, δ, ppm, J/Hz): 2.39 (1H, ddd, J = 14.0, 5.0, 2.0 Hz, H-2b), 2.29 (1H, m, H-2a), 2.25 (1H, q, J = 7,0 Hz, H-4), 1.97 (1H, m, H-1b), 1.76 (1H, m, H-6b), 1.69 (1H, m, H-1a), 1.57 (1H, m, H-16b), 1.56 (1H, m, H-18), 1.55 (2H, m, H-10, H-16a), 1.51 (2H, m, H-15b, H-22b), 1.49 (1H, m, H-7b), 1.47 (1H, m, H-21b), 1.45 (1H, m, H-11b), 1.39 (2H, m, H-7a, H-8), 1.38 (1H, m, H-19b), 1.35 (2H, m, H-12), 1.30 (1H, m, H-15a), 1.29 (1H, m, H-6a), 1.28 (1H, m, H-21a), 1.26 (1H, m, H-11a), 1.22 (1H, m, H-19a), 1.18 (3H, s, H-28), 1.05 (3H, s, H-27), 1.01 (3H, s, H-26), 1.00 (3H, s, H-30), 0.96 (4H, m, H-22b, H-29), 0.88 (3H, d, J = 6.5 Hz, H-23), 0.87 (3H, s, H-25), 0.73 (3H, s, H-24). ^{13}C-NMR (125 MHz, CDCl$_3$, δ, ppm): 22.3 (C-1), 41.5 (C-2), 213.1 (C-3), 58.3 (C-4), 42.1 (C-5), 41.3 (C-6), 18.3 (C-7), 53.1 (C-8), 37.5 (C-9), 59.5 (C-10), 35.7 (C-11), 30.5 (C-12), 39.7 (C-13), 38.3 (C-14), 32.4 (C-15), 36.0 (C-16), 30.0 (C-17), 42.8 (C-18), 35.4 (C-19), 28.2 (C-20), 32.8 (C-21), 39.3 (C-22), 6.8 (C-23), 14.7 (C-24), 17.9 (C-25), 20.3 (C-26), 18.7 (C-27), 32.1 (C-28), 35.0 (C-29), 31.8 (C-30) [9].

3,4-secofriedelan-3-oic acid (**4**): White powder, mp 118-120°C. (+) ESI-MS: m/z = 444.9 [M+H]$^+$ (molecular formula $C_{30}H_{52}O_2$). ^{1}H-NMR (500 MHz, CDCl$_3$, δ, ppm, J/Hz): 2.38 (2H, dt, J = 2.0, 7.5 Hz, H-2), 1.55 (3H, m, H-1, H-16b), 1.52 (1H, m, H-18), 1.49 (1H, m, H-6b), 1.48 (1H, m, H-15b), 1.45 (1H,

m, H-21b), 1.42 (1H, m, H-22b), 1.39 (3H, m, H-7b, H-11), 1.36 (2H, m, H-7a, H-19b), 1.34 (2H, m, H-4b, H-16a), 1.30 (2H, m, H-12), 1.28 (1H, m, H-15a), 1.25 (1H, m, H-21a), 1.21 (1H, m, H-8), 1.19 (1H, m, H-19a), 1.17 (3H, s, H-28), 1.15 (1H, m, H-6a), 1.10 (1H, m, H-4a), 1.01 (3H, s, H-27), 0.99 (3H, s, H-30), 0.98 (3H, s, H-26), 0.95 (3H, s, H-29), 0.92 (1H, m, H-22a), 0.87 (3H, s, H-25), 0.82 (1H, t, J = 3.5 Hz), 0.79 (3H, s, H-24), 0.78 (3H, t, J = 7.5 Hz, H-23). ^{13}C-NMR (125 MHz, CDCl$_3$, δ, ppm, J/Hz): 21.1 (C-1), 37.3 (C-2), 178.6 (C-3), 36.1 (C-4), 37.8 (C-5), 38.9 (C-6), 18.1 (C-7), 53.0 (C-8), 39.1 (C-9), 59.8 (C-10), 35.2 (C-11), 30.2 (C-12), 39.6 (C-13), 38.3 (C-14), 32.3 (C-15), 36.1 (C-16), 30.0 (C-17), 42.8 (C-18), 35.3 (C-19), 28.2 (C-20), 32.9 (C-21), 39.3 (C-22), 7.6 (C-23), 19.4 (C-24), 17.9 (C-25), 20.1 (C-26), 18.7 (C-27), 32.1 (C-28), 35.0 (C-29), 31.8 (C-30) [10].

Gallic acid (**5**): White powder, mp 250-252°C. (+) ESI-MS: m/z = 170.8 [M]$^+$ (molecular formula $C_7H_6O_5$). ^{1}H-NMR (500 MHz, CD$_3$OD, δ, ppm): 7.06 (2H, s, H-2, H-6). ^{13}C-NMR (125 MHz, CD$_3$OD, δ, ppm): 122.1 (C-1), 110.4 (C-2, C-6), 146.4 (C-3, C-5), 139.6 (C-4), 170.4 (C-7) [11].

Methyl gallate (6): White powder, mp 200-202°C. (-) ESI-MS: m/z = 183.2 [M-H]$^-$ (molecular formula $C_7H_6O_5$). ^{1}H-NMR (500 MHz, CD$_3$OD, δ, ppm): 7.06 (2H, s, H-2, H-6), 3.83 (3H, s, H-8). ^{13}C-NMR (125 MHz, CD$_3$OD, δ, ppm): 121.5 (C-1), 110.1 (C-2, C-6), 146.5 (C-3, C-5), 139.8 (C-4), 169.0 (C-7), 52.2 (C-8) [12].

Naringenin (**7**): White powder, mp 250-252°C. (+) ESI-MS: m/z = 273.0 [M+H]$^+$ (molecular formula $C_{15}H_{12}O_5$). ^{1}H-NMR (600 MHz, acetone-d6, δ, ppm, J/Hz): 5.44 (1H, dd, J = 13.2, 3.0 Hz, H-2), 3.17 (1H, dd, J = 16.8, 12.6 Hz, H-3a), 2.72 (1H, dd, J = 17.4, 3.0 Hz, H-3b), 5.94 (1H, d, J = 2.4 Hz, H-6), 5.95 (1H, d, J = 2.4 Hz, H-8), 7.38 (2H, d, J = 8.4 Hz, H-2', H-6'), 6.89 (2H, d, J = 9.0 Hz, H-3', H-5'), 12.17 (1H, s, OH-5). ^{13}C-NMR (150 MHz, acetone-d6, δ, ppm): 79.9 (C-2), 43.4 (C-3), 197.2 (C-4), 167.5 (C-5), 96.8 (C-6), 164.3 (C-7), 95.8 (C-8), 165.2 (C-9), 103.1 (C-10), 130.7 (C-1'), 128.9 (C-2', C-6'), 116.2 (C-3', C-5'), 158.8 (C-4') [13].

3. Result and Discussion

Seven compounds were isolated from the methanol extract of *B. oppositifolia* leaves through repeated silica gel flash chromatography (FC) and column chromatography (CC) consisting of four triterpenoids (compound **1** to **4**), two phenolic compounds (**5** and **6**) and one flavanone (compound **7**). The structures of these metabolites (Fig. 1) were elucidated by analyzing 1D-NMR, 2D-NMR, and MS spectroscopic

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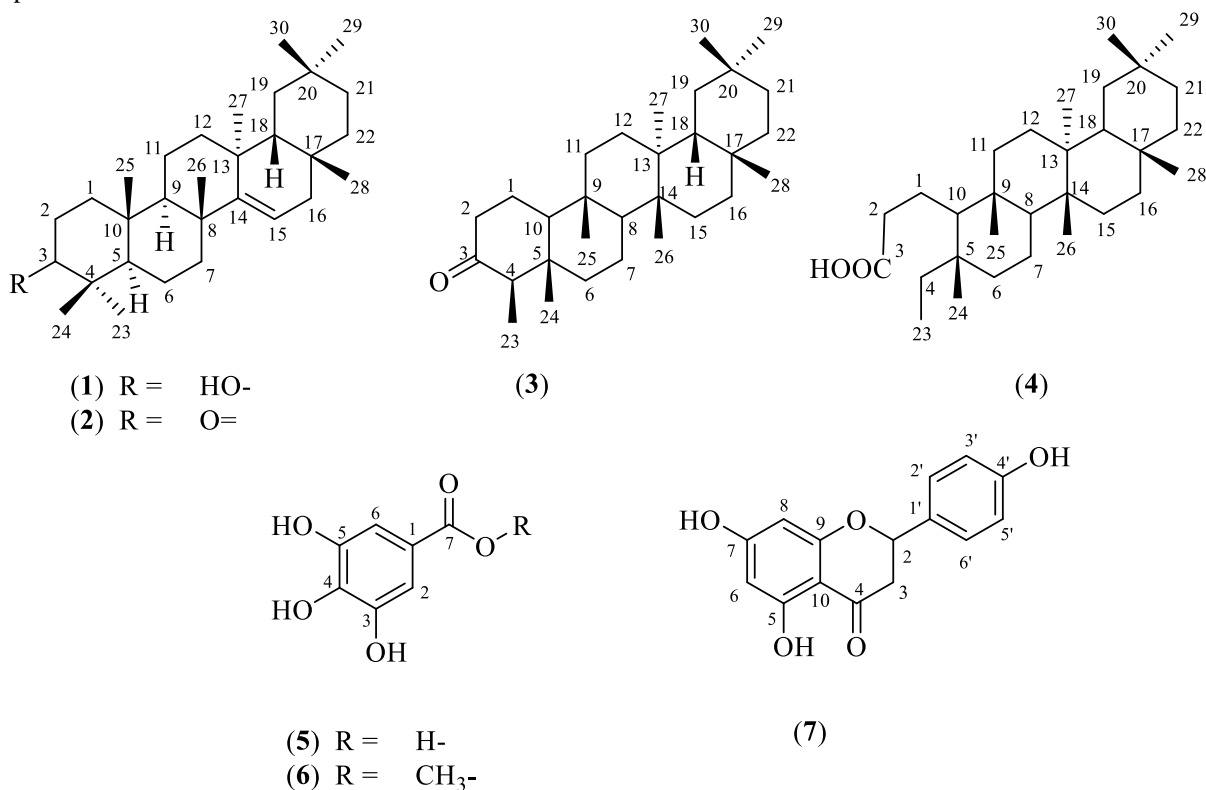data, and comparing them with previously reported spectral data.

Fig 1. Chemical structure of compounds 1-7 isolated from *B. oppositifolia* leaves

Compound **1** was obtained as white powder. The molecular formula of compound **1** was determined to be $C_{30}H_{50}O$ as indicated by the (+) ESI-MS molecular ion peak at *m/z* 427.2 [M+H]+. The ^{13}C-NMR and DEPT spectra of 1 showed 30 carbons including eight methyl carbons at the zone from 15 to 25 ppm, two olefinic carbons at δ_C 158.1 and 116.9 which were characterized for a double bond at C-14/C-15 in a taraxerane skeleton. Besides, there was one carbinol methine carbon at δ_C 79.1 corresponding to the presence of one hydroxyl group at position 3 of taraxerane skeleton. This corresponded to the observation of one carbinol methine proton at δ_H 3.19 (1H, dd, *J* = 4.3, 11.3 Hz) in the ^{1}H-NMR spectrum. Moreover, the presence of one olefinic proton at δ_H 5.53 (1H, *dd*, *J* = 3.0, 8.0 Hz) together with eight singlets of methyl groups at the zone from 0.80 to 1.10 ppm indicated **1** was a triterpenoid compound possessed the taraxerane skeleton. Additionally, the good compatibility of NMR data between compound **1** and those of taraxerol in the previously reported publication [8], the structure of **1** was elucidated as taraxerol.

Compound **2** was isolated as a white powder. The NMR data of **2** was similar to those of **1** as a triterpenoid with a taraxerane skeleton characterized

by the signals of two olefinic carbons at δ_C 157.6 and 117.2 of a double bond at C-14/C-15 in the ^{13}C-NMR

spectrum which corresponded to the presence of an olefinic proton at δ_H 5.56 (1H, *dd*, *J* = 3.3, 8.3 Hz) in the ^{1}H-NMR spectrum, and eight singlets of methyl groups at the zone from 0.80 to 1.20 ppm. However, the ^{13}C-NMR spectrum of **2** revealed a carbonyl carbon at δ_C 217.5 instead of a carbinol carbon at δ_C 79.1 those of **1** indicating that **2** was a ketone triterpenoid. The (+) ESI-MS spectrum of **2** was in agreement with NMR spectra data, in which the molecular formula of **2** was determined to be $C_{30}H_{48}O$ by the observation of an ion peak at m/z 425.2 [M+H]+. This corresponded to a decreasing 2 amu unit when compared to compound **1** and, therefore to a hydrogen molecule. Based on the above analysis and in comparison with the previous publication of taraxerone [8], compound **2** was elucidated as taraxerone.

Compound **3** was also purified as white powder. The (+) ESI-MS spectrum of **3** showed a molecular ion peak at *m/z* 427.2 [M+H]+ corresponding to the molecular formula of $C_{30}H_{50}O$. The ^{13}C-NMR and DEPT spectra of **3** showed 30 carbons including one carbonyl carbon at δ_C 213.1, and eight methyl carbons at the zone from 6.0 to 35.0 ppm indicating that **3** was

also a ketone triterpenoid. This corresponded to the signals of protons located at the upfield from 0.7 to 2.5 ppm in the ^{1}H-NMR spectrum. Besides, the presence of eight methyl groups was observed by one doublet at δ_H 0.88 (3H, d, J = 6.5 Hz), and seven singlets (three protons for each signal) at δ_H 0.7 – 1.2 ppm. Additionally, the ^{1}H-NMR spectrum also displayed a quartet of methine proton at δ_H 2.25 (1H, q, J = 7.0 Hz) indicated that this proton was linked to one methyl group. These signals were characteristics of a triterpenoid compound with a skeleton of friedelane. The HMBC correlations of protons H-2, H-4, and H-23 with carbonyl carbon at δ_C 213.1 confirmed the position 3 of carbonyl carbon in the friedelane skeleton. From this evidence combined with the comparison of spectra data of the reported publication [9], compound 3 was determined as friedelin.

Compound 4 was obtained as white powder. The (+) ESI-MS spectrum of 4 showed a molecular ion peak at m/z = 444.9 [M+H]$^+$ suggested a molecular formula of $C_{30}H_{52}O_2$. The ^{13}C-NMR and DEPT spectra of 4 also showed 30 carbons including eight methyl carbons at δ_C 7.0 – 35.0, one carboxyl carbon at δ_C 178.6. These features proved that compound 4 was a secotriterpene acid with the A-ring opened. The ^{1}H-NMR spectrum displayed the signals of protons at the upfield from 0.7 to 2.5 ppm, in which seven singlets of methyl group was observed at δ_H 0.7 – 1.2 ppm, one triplet of three protons presented at δ_H 0.78 (3H, t, J = 7.5 Hz) revealed this methyl group was adjacent a methylene group in the A-ring opened of secotriterpene acid. These features were confirmed by the observations of C-H correlations in HSQC and HMBC. The HMBC spectrum showed the cross peaks between methylene group CH$_2$-2 (δ_H 2.38; δ_C 37.3) and carboxyl carbon C-3 (δ_C 178.6), and with methylene carbon C-1 (δ_C 21.1). Furthermore, the triplet of methyl group at δ_H 0.78 linked to C-23 at δ_C 7.6 showed HMBC correlations with methylene carbon C-4 (δ_C 36.1) and quaternary carbon C-5 (δ_C 37.8) proved that the A-ring is opened. Base on the above analysis, and the good compatible NMR data of 4 with those reported in the previous publication [10], compound 4 was thus identified as 3,4-secofriedelan-3-oic acid.

Compound 5 was also purified as white powder. The ^{1}H-NMR spectrum only showed a singlet of two aromatic protons at δH 7.06, assignable to symmetrical protons of H-2 and H-6. The ^{13}C-NMR spectrum displayed four signals corresponding to six aromatic carbons at δC 122.1 (C-1), 110.4 (C-2, C-6), 146.4 (C-3, C-5) and 139.6 (C-4). In addition, the presence of one carboxyl carbon was observed at δC 170.4 (C-7). In agreement with NMR data, its molecular formula was established as $C_7H_6O_5$ based on the deprotonated ion peak at m/z = 170.8 [M]$^+$ in

the (+) ESI-MS spectrum. From the above analysis with the comparison to the literature values [11], compound 5 was elucidated as gallic acid.

Compound 6 was also isolated as white powder. The ^{1}H-NMR spectrum of 6 also showed a singlet of two aromatic protons at δH 7.06 as those in compound 5, and a singlet of three protons at δ_H 3.83 was corresponding to a methoxy group. The ^{13}C-NMR spectrum revealed the presence of eight carbons, in which there were six aromatic carbons at δ_C 121.5 (C-1), 110.1 (C-2, C-6), 146.5 (C-3, C-5), 139.8 (C-4). Besides, one carbonyl carbon at δ_C 169.0 (C-7) combined with one methoxy carbon at δ_C 52.2 (C-8) indicated the presence of methyl ester group in 6. Additionally, the (-) ESI-MS spectrum of 6 showed a deprotonated ion peak at m/z = 183.2 [M–H]$^-$ suggested a molecular formula of $C_8H_8O_5$. Thus, the good compatibility of its NMR data with those reported in the previous literature [12], the structure of 6 was determined as methyl gallate.

Compound 7 was obtained as white powder. The (+) ESI-MS spectrum of 7 showed a protonated ion peak at m/z = 273.0 [M+H]$^+$ suggested a molecular formula of $C_{15}H_{12}O_5$. The NMR data of 7 exhibited the characteristic signals of a flavanone. The ^{1}H-NMR spectrum displayed four aromatic protons of an AA'BB' system in the B-ring at δ_H 7.38 (2H, d, J = 8.4 Hz, H-2', H-6'), and 6.89 (2H, d, J = 9.0 Hz, H-3', H-5'), two doublets of an ABX system in the A-ring with a small coupling constant ^3J = 2.4 Hz at δ_H 5.94 (1H, d, J = 2.4 Hz, H-6) and 5.95 (1H, d, J = 2.4 Hz, H-8). Moreover, a doublet of doublets at δ_H 5.44 (1H, dd, J = 13.2, 3.0 Hz, H-2) was assigned to the olefinic proton at position 2 of flavanone skeleton. Besides, the methylene group at position 3 was observed by two doublets of doulets at δ_H 3.17 (1H, dd, J = 16.8, 12.6 Hz, H-3a), 2.72 (1H, dd, J = 17.4, 3.0 Hz, H-3b). The ^{13}C-NMR spectrum showed fifteen carbons charracteristing of flavanone consisting of one carbonyl carbon at δ_C 197.2 (C-4), six quaternary carbons, seven methine carbons and a methylene carbon at δ_C 43.4. From the spectroscopic data analysis and the comparison of NMR data of 7 with those reported in the previous publication [13], compound 7 was suggested as naringenin which was also isolated from the twigs of *B. oppositifolia* [6].

In summary, the crude methanol extract of *B. oppositifolia* leaves was fractionated by liquid-liquid extraction to obtain three fractions (n-hexane, ethyl acetate and aqueous fractions). Four compounds were isolated from the *n*-hexane fraction including taraxerol, taraxerone, friedelin and 3,4-secofridelan-3-oic acid. Besides, from the ethyl acetate fraction, three compounds were isolated including gallic acid, methyl gallate and naringenin. This research confirms the presence of phenolic and flavonoid compounds in the

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ethyl acetate fraction which may explain the best bioactivities of these fractions in previous literature [7].

4. Conclusions

From the methanol extract of *B. oppositifolia* leaves, seven compounds were isolated including four triterpenoids (taraxerol (**1**), taraxerone (**2**), friedelin (**3**) and 3,4-secofridelan-3-oic acid (**4**)), two phenolic derivatives (gallic acid (**5**) and methyl gallate (**6**)) and one flavanone (naringenin (**7**)). The structures of these substances were established by the MS, NMR spectroscopic analysis, and by comparison with data reported in literature. To the best of our knowledge, compounds **1** through **6** have been isolated from this species for the first time. Further studies on the chemical constituents and bioactivities of B. oppositifolia are underway to provide scientific information regarding its use in traditional medicine and to enhance the economic value of this plant.

5. Conflicts of interest

There are no conflicts to declare.

6. Acknowledgments

7. References

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