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PHENOLIC COMPOUNDS FROM *MALLOTUS BARBATUS* (WALL.) MUELL. -ARG. (EUPHORBIACEAE)

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VU MINH TRANG[1,2], LE THU HUONG[1], PHAN MINH GIANG[1], PHAN TONG SON[1]

[1]*Faculty of Chemistry, Hanoi University of Science, Vietnam National University, Hanoi*

[2]*University of Education, Vietnam National University, Hanoi*

ABSTRACT

From the leaves of Mallotus barbatus (Wall.) Muell. -Arg. (Euphorbiaceae) six phenolic compounds, methyl gallate, gallic acid, protocatechuic acid, quercetin, kaempferol, and kaempferol 3-O-β-D-glucopyranoside, and three triterpenoid/steroid compounds, taraxerol, β-sitosterol, and β-sitosterol 3-O-β-D-glucopyranoside were isolated. Their chemical structures were determined by spectroscopic analyses.

Keywords: *Mallotus barbatus*; Euphorbiaceae; flavonol; phenolic acid.

I - INTRODUCTION

Mallotus barbatus (Wall.) Muell. - Arg. (Euphorbiaceae) (Vietnamese name: Bùm bụp gai) is a medium-sized evergreen tree up to 6 m. The root, tree bark, and leaves of *M. barbatus* have been used in the Vietnamese and Chinese traditional medicine [1]. *M. barbatus* species collected in Vietnam was demonstrated to possess antioxidant effect [2]. The antioxidant effect of plants has been known to be correlated with their phenolic constituents and therefore the aim of the present study was to investigate the phenolic constituents of the leaves of *M. barbatus* collected in province Lao Cai, northern Vietnam. We developed a systematic chromatographic fractionation procedure of organic extracts to isolate six phenolic compounds, methyl gallate (**1**), gallic acid (**2**), protocatechuic acid (**3**), quercetin (**4**), kaempferol (**5**), and kaempferol 3-*O*-*β*-D-glucopyranoside (**6**), and three triterpenoid/steroid compounds, taraxerol (**7**), *β*-sitosterol (**8**), and *β*-sitosterol 3-*O*-*β*-D-glucopyranoside (**9**) from a MeOH extract of the leaves of *M. barbatus*. The structures of the isolated compounds (**1**-**9**) were determined by comparing their spectroscopic and chromatographic data with those of literature or authentic samples.

II - EXPERIMENTAL

1. General Procedure

Melting points were determined on a Boetius melting point apparatus and were uncorrected. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra with DEPT program were recorded on a Bruker Avance 500 NMR spectrometer. Chemical shifts are expressed in ppm (δ) relative to tetramethylsilane (TMS) as zero internal standard. Silica gel Merck (Darmstadt, Germany) (63-200 μm and 63-100 μm) was used for CC and silica gel Merck (40 - 63 and 15 - 40 μm) for FC and Mini-C. TLC was performed on precoated Merck DC Alufolien 60 F_{254} sheets and detected by spraying with 1% vanillin in conc. H_2SO_4 or 5% $FeCl_3$ in EtOH.

2. Plant Material

The fresh leaves of *M. barbatus* were collected in Van Ban, province Lao Cai, Vietnam in June 2008 and the plant was identified by Mr. Nguyen Quoc Binh, a botanist of the Institute of Biological Resources and Ecology, Vietnam Academy of Science and Technology, Hanoi, Vietnam.

3. Extraction and Isolation

The fresh leaves were dried in shadow and then oven-dried at 50°C. The dried leaves were powdered and the material (6 kg) was extracted with MeOH (five times, each time for three days) at room temperature. The concentrated MeOH extract was partitioned successively between *n*-hexane/H_2O, CH_2Cl_2/H_2O, EtOAc/H_2O, and *n*-BuOH/H_2O. Removal of the extraction solvents gave *n*-hexane- (146.7 g, extraction yield 2.44% on the basis of the dried material), CH_2Cl_2- (81 g, 1.35%), EtOAc- (48.3 g, 0.8%), and *n*-BuOH- (262.4 g, 4.37%) soluble fractions. A first portion of the EtOAc-soluble fraction (24.2 g) was subjected to silica gel CC using a gradient solvent system of *n*-hexane-EtOAc-HCOOH 20:19:1 and 10:20:1. Twelve pooled fractions were collected on the basis of the TLC analysis from thirty-four column fractions (each 50 ml). Pooled fraction 4 (2.17 g) was subjected to a Sephadex LH-20 column eluted with MeOH; the fractions obtained were purified by silica gel FC using a gradient solvent system of *n*-hexane-EtOAc 1:1, CH_2Cl_2-EtOAc 3:2, and *n*-hexane-EtOAc-HCOOH 10:10:1 and silica gel Mini-C with *n*-hexane-EtOAc 1:1 to afford **1** (52,1 mg), **2** (83,3 mg), and **3** (250,7 mg). Pooled fration 7 (0.66 g) was separated by Sephadex LH-20 CC with MeOH and washed by MeOH to give **9** (6 mg). Another portion of the EtOAc-soluble fraction (24.1 g) was subjected to the same separation procedure (silica gel CC, gradient solvent system of *n*-hexane-EtOAc-HCOOH 20:19:1 and 10:20:1) to give nine pooled fractions from thirty-seven column fractions (each 50 ml). Pooled fraction 3 (1.14 g) was separated by a Sephadex LH-20 column with MeOH to give fifteen fractions, each 20 ml. Fractions 5-8 (0.82 g) was further fractionated by silica gel FC with a gradient of CH_2Cl_2-EtOAc 3:2 and *n*-hexane-EtOAc-HCOOH 10:20:1 to afford a mixture of **1** and **2** (0.48 g) and **2** (0.12 g). Fractions 13-15 (20.8 mg) were purified by silica gel Mini-C with CH_2Cl_2-MeOH 30:1 to give **4** (10.7 mg). Pooled fraction 5 (1.84 g) was separated successively by Sephadex LH-20 CC with MeOH and silica gel FC with a gradient of *n*-hexane-EtOAc 1:1 and *n*-hexane-EtOAc-HCOOH 10:10:1 to give **1** (10 mg), a mixture of **2** and **3** (0.33 g), and **3** (0.13 g). Pooled fraction 8 (0.87 g) was separated successively by Sephadex LH-20 CC with MeOH and purified by silica gel Mini-C with CH_2Cl_2-MeOH 3:2 to give **5** (10 mg). Part of the *n*-butanol-soluble fraction (42.4 g) was separated by CC on highly porous synthetic resin Diaion HP-20 with H_2O, 20%, 40%, 60% MeOH-H_2O, and MeOH. The residue of the 60% MeOH-H_2O eluate (6.64 g) was separated by silica gel CC using a gradient solvent system of *n*-hexane-EtOAc-HCOOH 10:40:1 and EtOAc-H_2O-HCOOH 85:15:10 to give nine fractions. Fraction 1 (10 mg) was recrystallized from MeOH to give **5** (5 mg). Fraction 2 (0.14 g) was separated by silica gel FC with a gradient of *n*-hexane-EtOAc 2:1 and 1:1 to give **4** (5 mg) and **5** (9.7 mg) after recrystallization from MeOH. Fraction 7 (3.2 g) was recrystallized from MeOH to give a crude crystalline powder of **6** (58.2 mg). The mother liquor was concentrated and the residue (2.54 g) was separated on a Sephadex LH-20 column with MeOH and then purified by RP-SPE (Merck Lichrolut® RP-18) using a gradient of 20%, 30%, and 100% MeOH-H_2O to give **6** (0.14 g) after recrystallization from MeOH. The crude crystalline powder (58.2 mg) was separated by silica gel CC with *n*-hexane-EtOAc-HCOOH 10:40:1 and recrystallized from MeOH to give **6** (5 mg). A portion of the CH_2Cl_2-soluble fraction (44.8 g) was subjected to silica gel CC using a gradient solvent system of *n*-hexane-acetone 49:1, 9:1, 6:1 and 3:1 to give thirteen pooled fractions collected from forty-six column fractions (each 100 ml). Pooled fraction 3 (4.5

g) was separated by silica gel CC with a gradient of *n*-hexane-acetone 99:1 and 30:1 to give **7** (30 mg). On recrystallization pooled fraction 7 (0.71 g) gave **8** (30 mg).

Methyl gallate (1): White needles, m.p. 199-201 °C. R_f 0.7 (TLC, silica gel, *n*-hexane-EtOAc-HCOOH 10:10:1). ^{1}H-NMR (CD_3OD): δ 3.83 (3H, s, 7-OCH_3), 7.06 (2H, s, H-2, H-6). ^{13}C-NMR/DEPT (CD_3OD): δ 52.3 (q, C-8), 110.1 (d, C-2, C-6), 121.5 (s, C-1), 139.8 (s, C-3, C-5), 146.5 (s, C-4), 169.1 (s, C-7).

Protocatechuic acid (2): White needles, m.p. 200 - 202°C. R_f 0.35 (TLC, silica gel, *n*-hexane-EtOAc-HCOOH 20:10:1). ^{1}H-NMR (CD_3OD): δ 6.81 (1H, d, *J* = 8.0 Hz, H-5), 7.44 (1H, dd, *J* = 8.0 Hz, 2.0 Hz, H-6), 7.46 (1H, d, *J* = 2.0 Hz, H-2). ^{13}C-NMR/DEPT (CD_3OD): δ 115.8 (d, C-5), 117.8 (d, C-2), 123.2 (s, C-1), 123.9 (d, C-6), 146.1 (s, C-3), 151.6 (s, C-4), 170.3 (s, C-7).

Gallic acid (3): White needles, m.p. 248-250 °C. R_f 0.5 (TLC, silica gel, *n*-hexane-EtOAc-HCOOH 10:10:1). ^{1}H-NMR (CD_3OD): δ 7.08 (2H, H-2, H-6). ^{13}C-NMR/DEPT (CD_3OD): δ 110.3 (d, C-2, C-6), 122.1 (s, C-1), 139.6 (s, C-3, C-5), 146.4 (s, C-4), 170.5 (s, C-7).

Quercetin (4): Yellow amorphous powder. R_f 0.25 (TLC, silica gel, *n*-hexane-EtOAc-HCOOH 20:10:1). ^{1}H-NMR (CD_3OD): δ 6.21 (1H, d, *J* = 2.0 Hz, H-6), 6.41 (1H, d, *J* = 2.0 Hz, H-8), 6.9 (1H, d, *J* = 9.0 Hz, H-5′), 7.65 (1H, dd, *J* = 9.0 Hz, 2.0 Hz, H-6′), 7.75 (1H, d, *J* = 2.0 Hz, H-2′).

Kaempferol (5): Yellow amorphous powder. R_f 0.8 (TLC, silica gel, CH_2Cl_2-EtOAc 3:2). ^{1}H-NMR (CD_3OD): δ 6.19 (1H, d, *J* = 2.0 Hz, H-6), 6.4 (1H, d, *J* = 2.0 Hz, H-8), 6.92 (2H, d, *J* = 9.0 Hz, H-2′, H-6′), 8.1 (2H, d, *J* = 9.0 Hz, H-3′, H-5′). ^{13}C-NMR/DEPT (CD_3OD): δ 94.5 (d, C-8), 99.3 (d, C-6), 104.6 (s, C-10), 116.3 (d, C-3′, C-5′), 123.8 (s, C-1′), 130.7 (d, C-2′, C-6′), 137.2 (s, C-3), 148.1 (s, C-2), 158.3 (s, C-9), 160.6 (s, C-4′), 162.5 (s, C-5), 165.6 (s, C-7), 177.4 (s, C-4).

Kaempferol 3-*O*-β-D-glucopyranoside (6): Yellow amorphous powder. R_f 0.29 (TLC, silica gel, CH_2Cl_2-CH_3OH 6:1). ^{1}H-NMR (DMSO-d_6): δ 3.08-3.32 (5H, H-2″, H-3″, H-4″, H-5″, H-6″a), 3.56 (1H, brd, *J* = 11.0 Hz, H-6″b), 4.22 (1H, s, OH), 4.91 (1H, s, OH), 5.01 (1H, brs, OH), 5.3 (1H, brs, OH), 5.45 (1H, d, *J* = 7.5 Hz, H-1″), 6.2 (1H, d, *J* = 2.0 Hz, H-6), 6.43 (1H, d, *J* = 2.0 Hz, H-8), 6.88 (2H, d, *J* = 8.5 Hz, H-2′, H-6′), 8.03 (2H, d, *J* = 8.5 Hz, H-3′, H-5′). ^{13}C-NMR/DEPT (DMSO-d_6): δ 60.8 (t, C-6″), 69.8 (d, C-4″), 74.2 (d, C-2″), 76.4 (d, C-5″), 77.4 (d, C-3″), 93.6 (d, C-8), 98.7 (d, C-6), 100.9 (d, C-1″), 103.9 (s, C-10), 115.1 (d, C-3′, C-5′), 120.9 (s, C-1′), 130.8 (d, C-2′, C-6′), 133.2 (s, C-3), 156.2 (s, C-9), 156.3 (s, C-2), 159.9 (s, C-4′), 161.2 (s, C-5), 164.1 (s, C-7), 177.4 (s, C-4).

Taraxerol (7): White amorphous powder. R_f 0.4 (TLC, silica gel, *n*-hexane-CH_2Cl_2 1:1). ^{1}H-NMR ($CDCl_3$ + CD_3OD): δ 0.81 (3H, s, 24-CH_3), 0.85 (3H, s, 28-CH_3), 0.93 (3H, s, 27-CH_3), 0.94 (3H, s, 30-CH_3), 0.96 (6H, s, 25-CH_3, 29-CH_3), 0.98 (3H, s, 23-CH_3), 1.12 (3H, s, 26-CH_3), 3.16 (1H, dd, *J* = 11.0 Hz, 5.0 Hz, H-3), 5.5 (1H, dd, *J* = 8.5 Hz, 3.0 Hz, H-15). ^{13}C-NMR/DEPT ($CDCl_3$ + CD_3OD): δ 14.9 (q, C-25), 15.1 (q, C-24), 17.4 (t, C-11), 18.7 (t, C-6), 20.9 (q, C-30), 25.5 (q, C-26), 26.6 (t, C-2), 27.6 (q, C-23), 28.5 (s, C-20), 29.3 (q, C-27), 29.4 (q, C-28), 32.8 (q, C-29), 32.9 (t, C-7), 33.7 (t, C-16), 35.0 (t, C-21), 35.6 (s, C-17), 36.6 (t, C-12), 37.5 (s, C-13), 37.6 (t, C-22), 37.8 (t, C-1), 37.9 (s, C-10), 38.6 (s, C-4), 38.9 (s, C-8), 41.4 (t, C-19), 48.9 (s, C-18), 49.4 (d, C-9), 55.8 (d, C-5), 78.6 (d, C-3), 116.7 (d, C-15), 158.2 (s, C-14).

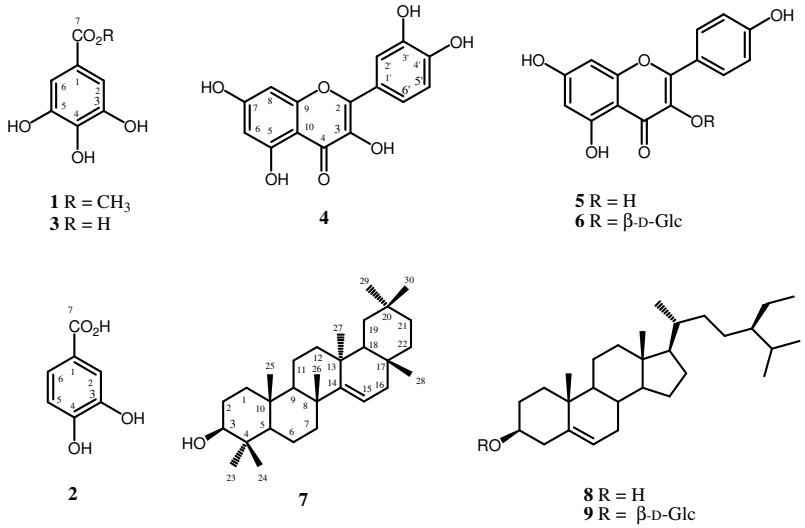
β-Sitosterol (8): White amorphous powder. R_f 0.35 (TLC, silica gel, *n*-hexane-acetone 9:1).

β-Sitosterol 3-*O*-β-D-glucopyranoside (9): White amorphous powder. R_f 0.8 (TLC, silica gel, CH_2Cl_2-CH_3OH 6:1).

III - RESULTS AND DISCUSSION

The MeOH extract of the dried leaves of *M. barbatus* was partitioned between H_2O and *n*-

hexane, CH_2Cl_2, EtOAc, and *n*-BuOH, successively. The CH_2Cl_2-soluble fraction was fractionated by repeated open-column chromatography (CC) to afford **7** and **8**. The EtOAc and *n*-BuOH-soluble fractions were separated by a combination of CC and flash chromatography (FC) on Diaion HP-20, Sephadex LH-20, and silica gel and purified by mini-column chromatography (Mini-C) to afford **1** - **6** and **9**.



Compounds **1** and **3** were isolated as white needles. The ^{1}H-NMR and ^{13}C-NMR spectra of **1** and **3** exhibited similarities in chemical shifts and splitting patterns except for the presence of a methoxyl group in **1** [δ_H 3.83 (3H, s); δ_C 52.3 (q)]. The proton signals at δ_H 7.06 (2H, s) in **1** and 7.08 (2H, s) in **3** were characteristic of methyl gallate (**1**) and gallic acid (**3**) [3].

Compound **2** was isolated as white needles. In the ^{1}H-NMR and ^{13}C-NMR spectra of **2** the presence of a dihydroxybenzoic acid was clearly indicated by seven ^{13}C signals including two oxysubstituted carbons at δ_C 146.1 (s) and 151.6 (s), and a carboxyl group at δ_C 170.3 (s). The 1,3,4-substitution pattern of the benzene ring was seen by the presence of the proton signal at δ_H 7.44 (1H, dd, J = 8.0 Hz, 2.0 Hz) which gave a *meta* coupling with proton signal at δ_H 7.46 (1H, d, J = 2.0 Hz) and an *ortho* coupling with proton signal at δ_H 6.81 (1H, d, J = 8.0 Hz). Thus on the basis of the NMR data **2** was determined to be protocatechuic acid [3].

Compounds **4** and **5** were isolated as yellow amorphous powders. The ^{1}H-NMR spectrum of **4** and **5** suggested flavonol skeleton of the two compounds; protons H-6 and H-8 appeared as two doublets with a *meta* coupling of 2.0 Hz at δ_H 6.21 and 6.41 (**4**) and at δ_H 6.19 and 6.4 (**5**), respectively. However, rings B of **4** and **5** showed different substitution patterns; 1,3,4-trisubstituted benzene ring was seen in **4** and 1,4-disubstituted benzene ring in **5**. Therefore, **4** and **5** were determined to be quercetin [4] and kaempferol [5], respectively. The structure of **5** was further confirmed by comparing its ^{13}C-NMR data with the reported values of kaempferol [6].

Compound **6** was isolated as yellow amorphous powder. The ^{1}H-NMR and ^{13}C-NMR spectra of **6** were similar to those of kaempferol except for the presence of an additional β-glucopyranosyl moiety (δ_C 60.8, 69.8, 74.2, 76.4, 77.4, and 100.9); the β-configuration of the sugar was indicated by the

coupling constant of the anomeric proton at δ_H 5.45 (1H, d, J = 7,5 Hz). The position of the β-glucopyranosyl group remained to be determined. Upfield ^{13}C shift of C-3 and downfield ^{13}C shift of C-2 on going from kaempferol to **6** [6] were indicative for the glycosylation of the 3-hydroxyl group. Therefore, **6** was determined to be kaempferol 3-*O*-β-D-glucopyranoside [6].

Taraxerol (**7**) was determined by comparing its ^1H-NMR and ^{13}C-NMR spectroscopic data with the reported values of taraxerol [7]. β-Sitosterol (**8**) and β-sitosterol 3-*O*-β-D-glucopyranoside (**9**) were determined by direct TLC and co-TLC analysis with those of authentic samples.

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REFERENCES

1. V. C. Vo. Dictionary of Vietnamese medicinal plants, Publishing House Medicine, Ho Chi Minh City (1997).
2. C. Tistaert, B. Dejaegher, N. Nguyen Hoai, G. Chataigne, C. Riviere, V. Nguyen Thi Hong, M. Chau Van, J. Quetin-Leclercq, Y. Vander Heyden. Analytica Chimica Acta, **649**, 24 - 32 (2009).
3. Y. Hu, H. Gao, Z. Tang, X. Sung, L. Wu. Asian Journal of Traditional Medicines, **1**, 3 - 4 (2006).
4. T. Yoshioka, T. Inokuchi, S. Fujioka, Y. Kimura. Z. Naturforsch., **59c**, 509 - 514 (2004).
5. The Flavonoids: Advances in Research, ed. J. B. Harborne, T. J. Mabry. Chapman and Hall, London (1982).
6. Carbon-13 NMR of Flavonoids, ed. P. K. Agrawal, Elservier, Amsterdam (1989).
7. W. Y. Jin, X. F. Cai, M. Na, J. J. Lee, K. Bae, Arch. Pharm. Res., **30**, 412 - 418 (2007).

Corresponding author: **Phan Tong Son**
Faculty of Chemistry, Hanoi University of Science,
Vietnam National University, Hanoi
19 Le Thanh Tong Str., Hoan Kiem Distric, Hanoi, Vietnam.