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Chemical constituents isolated from stems of *Maesa membranacea*

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Abstract:

***Maesa membranacea* A. DC. (Myrsinaceae) has been traditionally used for the treatment of fever and hepatitis. The phytochemical investigation of *Maesa membranacea* stems has led to the isolation of six compounds including kaempferol (1), (-)-epicatechin (2), betulinic acid (3), *p*-hydroxybenzoic acid (4), vanilic acid (5), and protocatechuic acid (6). The chemical structures of these compounds have been determined by nuclear magnetic resonance (NMR), mass spectrometry (MS) data, and by comparison with previously reported works. This is the first report of a chemical study on *Maesa membranacea* as compounds 1-6 listed above were found from the *Maesa* genus for the first time.**

Keywords: **flavonoids, *Maesa membranacea*, phenolic acids, triterpene.**

Classification number: **2.2**

Introduction

Maesa membranacea A. DC. (Myrsinaceae) is an evergreen shrub that grows in China, Vietnam, and Cambodia. In Vietnam, the leaves and roots of this species have been used as traditional medicine for the treatment of fever and hepatitis [1]. Many *Maesa* plants have also been used as a folk remedy in African and Asian countries. Previous chemical studies on the *Maesa* species have revealed the presence of quinone, saponin triterpenoid, and flavonoid compounds [2-5]. The plant's extracts and chemical constituents have displayed various pharmacological properties such as anticancer, antimicrobial, antiviral, and anti-leismania activity [2-5]. However, until now, no chemical study has been reported on *Maesa membranacea*. In this paper, we report the isolation and structure elucidation of six compounds including kaempferol (**1**), (-)-epicatechin (**2**), betulinic acid (**3**), *p*-hydroxybenzoic acid (**4**), vanilic acid (**5**), and protocatechuic acid (**6**) from the stems of *Maesa membranacea*. The structures of these compounds have been determined by NMR, MS analysis, and comparison with previously reported studies.

Experimental design

Plant materials

The stems of *Maesa membranacea* were collected from the Kontum province, Vietnam, in 2012 and were taxonomically identified by Dr. Nguyen Quoc Binh of the Vietnam Museum of Nature - VAST. A voucher specimen (VN-2292) was provided to the Institute of Marine Biochemistry.

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General experimental procedures

The NMR spectra were obtained by a Bruker AM500 FT-NMR spectrometer using TMS as an internal standard. The electrospray ionisation mass spectra (ESI-MS) were obtained on an Agilent 1260 LC/MS system. Column chromatography (CC) was performed on silica gel (Merck, 230-400 mesh), reversed phase silica gel (Merck, RP-C18), or Sephadex LH-20. Precoated silica gel plates (Merck 60 F_{254}) were used for thin layer chromatography. The compounds were detected by a UV lamp or by spraying with 10% sulfuric acid.

Extraction and isolation

The stems of *M. membrancea* (2.0 kg) were extracted with MeOH (10 l × 4 times, 1 day/time) at room temperature. The MeOH solvent was evaporated *in vacuo*. The residue (170 g) was suspended in H_2O and was successively extracted with *n*-hexane and ethyl acetate (3 times x 500 ml/time). The organic solvents were removed *in vacuo* to obtain *n*-hexane (2.8 g) and ethyl acetate residue (18 g), respectively. The water fraction was subjected to a Diaion HP-20 column, eluted with water-MeOH (100:0-0:100) to give a MeOH fraction (6 g).

The ethyl acetate residue (18 g) was subjected to a silica gel column, eluted with a gradient solvent mixture of *n*-hexane-EtOAc (100:0-0:100) to give 16 fractions (E1-E16). Fraction E10 (200 mg) was purified by Sephadex CC and eluted with CH_2Cl_2/MeOH (1/9) to give five sub-fractions denoted as E10.1-E10.5. The sub-fraction E10.3 was fractionated by silica gel CC, eluted with CH_2Cl_2/MeOH (95/5), to yield compound **4** (12 mg) and compound **5** (6 mg). Sub-fraction E10.5 was subjected to Sephadex CC, eluted with CH_2Cl_2/MeOH (2/8) to yield compound **1** (3 mg). Fraction E13 (0.5 g) was separated by Sephadex CC and eluted with CH_2Cl_2/MeOH (2/8) to afford four sub-fractions E13.1- E13.4. Fraction E13.4 (50 mg) was further purified by Sephadex CC using CH_2Cl_2/MeOH (1/9) as mobile phase to afford compound **6** (6 mg). Fraction E15 (0.54 g) was chromatographed on Sephadex CC and eluted with CH_2Cl_2/MeOH (2/8) to afford three sub-fractions E15.1- E15.3. Compound **2** (6 mg) was obtained from sub-fraction E15.3 (60 mg) by crystallisation in acetone/water.

The MeOH fraction (6 g) was separated by reversed phase silica gel CC and eluted with a gradient solvent of water-MeOH (80:20-0:100) to give 15 fractions (E1-E15). Fraction E13 (145 mg) was purified by silica gel CC and eluted with *n*-hexane/acetone (7/3) to yield compound **3** (8 mg).

Kaempferol (**1**): yellow solid; ESI MS *m/z* 287 $[M+H]^+$. ^1H NMR (500 MHz, CD_3OD) δ (ppm): 8.11 (2H, d, *J*=9 Hz, H-2′, 6′), 6.93 (2H, d, *J*=9 Hz, H-3′, 5′); 6.42 (1H, d, *J*=2 Hz, H-8), 6.20 (1H, d, *J*=2 Hz; H-6). ^{13}C NMR (125 MHz, CD_3OD) δ (ppm): 177.3 (C-4), 165.5 (C-7), 162.5 (C-5), 160.5 (C-4′), 158.3 (C-9), 148.1 (C-2), 137.1 (C-3), 130.6 (C-2′,6′), 123.7 (C-1′), 116.3 (C-3′,5′), 104.5 (C-10), 99.2 (C-6), 94.4 (C-8).

(-)-Epicatechin (**2**): white powder, $[\alpha]^{20}_D$-70.50 (*c* 0.20, MeOH), ESI MS (*m/z*): 291 $[M+H]^+$. ^1H NMR (500 MHz, CD_3OD) δ (ppm): 6.99 (1H, d, *J*=1.5 Hz, H-2′), 6.83 (1H, dd, *J*=2.0, 8.0 Hz, H-6′), 6.77 (1H, d, *J*=8.0 Hz, H-5′), 5.96 (1H, d, *J*=2.0 Hz, H-8), 5.94 (1H, d, *J*=2.0 Hz, H-6), 4.83 (1H, br s, 7.5 Hz, H-2), 4.19 (1H, m, H-3), 2.88 (1H, dd, *J*=17.0, 4.5 Hz, H-4a), 2.75 (1H, dd, *J*=16.5, 2.5 Hz, H-4b). ^{13}C NMR (125 MHz, CD_3OD) δ (ppm): 158.0 (C-9), 157.7 (C-7), 157.4 (C-5), 145.9 (C-4′), 145.8 (C-3′), 132.3 (C-1′), 119.4 (C-6′), 115.9 (C-5′), 115.3 (C-2′), 100.1 (C-10), 96.4 (C-6), 95.9 (C-8), 79.8 (C-2), 67.5 (C-3), 29.2 (C-4).

Betulinic acid (**3**): white solid, ESI MS: *m/z* 455 $[M-H]^-$. ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 4.72 (1H, d, *J*=2.0 Hz, Ha-29), 4.59 (1H, br s, Hb-29), 3.19 (1H, m, H-3), 1.67 (3H, s, H-30), 0.97 (3H, s, H-27), 0.96 (3H, s, H-23), 0.94 (3H, s, H-26), 0.82 (3H, s, H-25), 0.75 (3H, s, H-24). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 178.9 (C-28), 150.4 (C-20), 109.5 (C-29), 78.6 (C-3), 55.9 (C-17), 55.1 (C-5), 50.4 (C-9), 48.9 (C-18), 46.7 (C-19), 42.1 (C-14), 40.4 (C-8), 38.5 (C-4), 38.5 (C-1), 37.9 (C-13), 36.9 (C-10), 36.8 (C-22), 34.0 (C-7), 32.0 (C-16), 30.4 (C-21), 29.4 (C-15), 27.8 (C-23), 27.1 (C-2), 25.3 (C-12), 20.7 (C-11), 19.0 (C-30), 18.1 (C-6), 15.9 (C-25), 15.6 (C-26), 15.1 (C-24), 14.4 (C-27).

***p*-Hydroxybenzoic acid** (**4**): white solid. ESI MS *m/z* 139 $[M+H]^+$. ^1H NMR (500 MHz, CD_3OD) δ (ppm): 7.89 (2H, d, *J*=9.0 Hz, H-2,6), 6.84 (2H, d, *J*=8.0 Hz, H-3,5). ^{13}C NMR (125 MHz, CD_3OD) δ (ppm): 170.1 (COOH), 163.3 (C-4), 133.0 (C-2,6), 122.7 (C-1), 116.0 (C-3,5).

Vanilic acid (**5**): white solid. ESI MS *m/z* 169 $[M+H]^+$. ^1H NMR (500 MHz, CD_3OD) δ (ppm): 7.58 (1H, d, *J*=1 Hz, H-2), 7.56 (1H, dd, *J*=2.0, 8.0 Hz, H-6), 6.85 (1H, d, *J*=8.5 Hz, H-5), 3.91 (3H, s, OMe). ^{13}C NMR (125 MHz, CD_3OD) δ (ppm): 168.0 (COOH), 151.5 (C-3), 147.6 (C-4), 124.1 (C-1), 123.0 (C-6), 116.7 (C-2), 114.7 (C-5), 55.4 (OCH_3).

Protocatechuic acid (**6**): white solid. ESI MS *m/z* 155 $[M+H]^+$. ^1H NMR (500 MHz, CD_3OD) δ (ppm): 7.46 (1H, d, *J*=2 Hz, H-2), 7.44 (1H, dd, *J*=2.0, 8.0 Hz, H-6), 6.81 (1H, d, *J*=8.0 Hz, H-5). ^{13}C NMR (125 MHz, CD_3OD) δ (ppm): 169.2 (C-7), 150.4 (C-4), 144.9 (C-3), 122.8 (C-6), 122.0 (C-1), 116.6 (C-5), 114.6 (C-2).

Results and discussion

Compound 1 was isolated from the ethyl acetate extract as a yellow solid. The ESI MS spectrum showed a *quasi*-molecular ion peak with *m/z* 287 $[M+H]^+$, which suggests the molecular formula of **1** is $C_{15}H_{10}O_6$ (M=286). The ^1H NMR spectrum showed signals of a flavonol with two proton signals at δ_H 6.42 (1H, d, *J*=2 Hz, H-8) and 6.20 (1H, d, *J*=2 Hz, H-6), proton signals of an A_2B_2 system at δ_H 8.11 (2H, d, *J*=9 Hz, H-2′, 6′) and 6.93 (2H, d, *J*=9 Hz, H-3′, 5′).

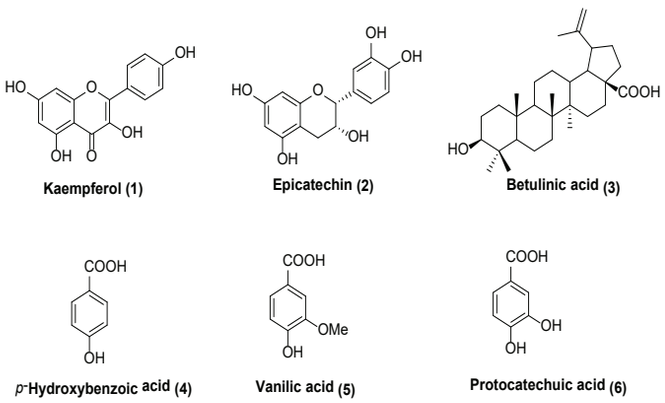

Fig. 1. Chemical structures of compounds 1-6.

The ^{13}C NMR spectrum of **1** revealed 15 carbon signals including a carbonyl signal at δ_C 177.3 (C-4), 5 oxy-bonded carbons at δ_C 165.5 (C-7), 162.5 (C-5), 160.5 (C-4′), 158.3 (C-9), and 148.1 (C-2), and 2 double peaks at δ_C 130.6 (C-2′, 6′) and 116.3 (C-3′, 5′). The structure of compound **1** was assigned as kaempferol. The NMR data of **1** are in agreement with the values published [6].

Compound **2** yielded as a white solid. The ESI MS showed a *quasi*-molecular ion peak of *m/z* 291 $[M+H]^+$,that corresponds to a molecular formula of $C_{15}H_{14}O_6$ (M=290). The ^1H NMR spectrum revealed typical signals of a flavanol including the three aromatic protons in an ABX system at δ_H 6.99 (1H, d, *J*=1.5 Hz, H-2′), 6.83 (1H, dd, *J*=2.0, 8.0 Hz, H-6′), 6.77 (1H, d, *J*=8.0 Hz, H-5′), 2 aromatic proton at δ_H 5.96 (1H, d, *J*=2.0 Hz, H-8), 5.94 (1H, d, *J*=2.0 Hz, H-6). The proton signals of the pyran unit were displayed at δ_H 4.83 (1H, br s, 7.5 Hz, H-2), 4.19 (1H, m, H-3), 2.88 (1H, dd, *J*=17.0, 4.5 Hz, H-4a), and δ_H 2.75 (1H, dd, *J*=16.5, 2.5 Hz, H-4b). The H-2 and H-3 protons were observed as broad peaks, which suggested that these protons were on the same side. The ^{13}C NMR spectrum of **2** showed 15 carbon signals. Based on the spectral analysis and optical rotation data, **2** was identified as (-)-epicatechin. The NMR data of **2** agree with previously reported literature [7].

Compound **3** was obtained from the MeOH fraction as a white solid. The ESI MS showed a *pseudo*-molecular ion peak of *m/z* 455 $[M-H]^-$ and the suggested molecular formula of **3** is $C_{30}H_{48}O_3$ (M=456). The ^1H NMR spectrum revealed the typical signals of a lupan-type structure with 2 olefinic peaks at δ_H 4.72 (1H, d, *J*=2.0 Hz, Ha-29) and 4.59 (1H, br s, Hb-29), and six singlet signals of methyl groups at δ_H 1.67 (3H, H-30), 0.97 (3H, H-27), 0.96 (3H, H-23), 0.94 (3H, H-26), 0.82 (3H, H-25), and 0.75 (3H, H-24). In addition, the signals from the oxymethine group were found at δ_H 3.19 (1H, m, H-3). The ^{13}C NMR and distortionless enhancement by polarization transfer (DEPT) spectra of **3** showed 30 carbon signals including a signal from the carboxylic group at δ_C 178.9 (C-28), peaks of 2 olefinic carbons at δ_C 150.4 (C-20) and 109.5 (C-29), and signals from 6 methyl groups at δ_C 27.8 (C-23), 19.0 (C-30), 15.9 (C-25), 15.6 (C-26), 15.1 (C-24), and 14.4 (C-27). Therefore, compound **3** was determined as betulinic acid. The NMR data of **5** agrees with those from the reported work of [8].

Compounds **4-6** were determined as *p*-hydroxybenzoic acid, vanilic acid, and protocatechuic acid, respectively, by comparison of the NMR data with reported literatures [9, 10] (Fig. 1).

Conclusions

A phytochemical investigation of *M. membranceae* stems led to the isolation of six known compounds identified as kaempferol (**1**), (-)-epicatechin (**2**), betulinic acid (**3**), *p*-hydroxybenzoic acid (**4**), vanilic acid (**5**), and protocatechuic acid (**6**). This is the first chemical study on the *Maesa membranceae* plant. All of the isolated compounds were found from *Measa* genus for the first time.

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The authors declare that there is no conflict of interest regarding the publication of this article.

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