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CHEMICAL CONSTITUENTS FROM THE STEMS OF *CLEISTANTHUS EBERHARDTII*

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Summary

Chemical Constituents from the Stems of *Cleistanthus eberhardtii*

From the stems of *Cleistanthus eberhardtii* (Gagnep.) Croizat, nine known compounds were isolated, including two triterpenoids (lupeol (**1**) and betulinic acid (**2**)), one ellagic acid derivative (3,3′-di-*O*-methylellagic acid-4′-*O*-*α*-L-rhamnopyranoside (**3**)), one steroid (*β*-sitostenone (**4**)), and five benzoic acid derivatives (2,3-dihydroxy-1-(4′-hydroxy-3-methoxyphenyl)propane-1-one (**5**), 3,4-dihydroxybenzaldehyde (**6**), 3,4-dihydrobenzoic acid (**7**), methyl 3,4-dihydroxybenzoate (**8**), and methyl gallate (**9**). The structures of these compounds were determined through MS and NMR spectra analyses and comparison with literature data. Compounds **1**-**3** showed moderate cytotoxic activity against KB and MCF-7 cancer cell lines, with IC_{50} values ranging from 18.3 to 29.2 μg/mL. To date, this is the first report of these compounds from *C. eberhardtii*, and the first phytochemical investigation of *C. eberhardtii* stems.

Keywords: *Cleistanthus eberhardtii*, *Triterpenoid, Ellagic acid derivative, Benzoic acid derivative, Cytotoxic.*

1. Introduction

The genus *Cleistanthus*, belonging to the Phyllanthaceae family (tribe - Bridelieae), was first described in 1848 [1] and is distributed across Africa, Southeast Asia, and Australia [2]. Traditionally, several *Cleistanthus* species have been used in medicine to treat various ailments, including inflammation, fever, skin diseases [3], [4], wounds, and infections [3]. However, comprehensive chemical investigations have been limited, with only ten species, including *C. collinus*, *C. schlechteri*, *C. patulus*, *C. gracilis*, *C. indochinensis*, *C. boivinianus*, *C. concinnus*, *C. sumatranus*, *C. tonkinensis*, and *C. eberhardtii* subjected to detailed analysis [2],[5]. These studies have revealed that arylnaphthalide, furofuranoid, and dibenzylbutane lignans and their glycosides are characteristic constituents of the genus, alongside triterpenoids and alkaloid glucosides [2],[6],[7]. In previous studies, we reported the isolation of new aryltetralin lignans from the fruits of *C. indochinensis* [8], C. *tonkinensis* [9], and *C. eberhardtii* [5] and the evaluation of their cytotoxic activities. Additionally, in the course of a screening program of the Vietnamese flora, the stem extract of *C. eberhardtii* demonstrated significant cytotoxicity against the KB cell line (89.5% inhibition at the concentration of 1 μg/mL). To the best of our knowledge, no study has been performed on the chemical constituents of *C. eberhardtii* stems. Herein, we report the isolation and structural elucidation of nine known compounds from *C. eberhardtii* stems, including lupeol (**1**), betulinic acid (**2**), 3,3′-di-*O*-methylellagic acid-4′-*O*-*α*-L-rhamnopyranoside (**3**), *β*-sitostenone (**4**), 2,3-dihydroxy-1-(4′-hydroxy-3-methoxyphenyl)propan-1-one (**5**), 3,4-dihydroxybenzaldehyde (**6**), 3,4-dihydrobenzoic acid (**7**), methyl 3,4-dihydroxybenzoate (**8**), and methyl gallate (**9**) (Fig. 1).

2. Materials and methods

2.1. Plant materials

The stems of *Cleistanthus eberhardtii* (Gagnep.) Croizat were collected in Phu Loc, Thua Thien Hue, Vietnam in August 2017 and was identified by Dr. Nguyen The Cuong. A voucher specimen (VN-2151B) was deposited at the Institute of Marine Biochemistry, Vietnam Academy of Science and Technology (VAST) in Hanoi.

2.2. General experiment procedures

NMR spectra were recorded on a Bruker 500 or Bruker 600 MHz spectrometer operating at 125 and 150 MHz for ^{13}C-NMR and at 500 and 600 MHz for ^{1}H-NMR. The ^{1}H chemical shifts were

referenced to $CDCl_3$, CD_3OD, and DMSO-d_6 at δ_H 7.27, 3.31, and 2.50 ppm, respectively, while the ^{13}C chemical shifts were referenced to the solvent peaks at δ_C 77.1 ($CDCl_3$), 49.0 (CD_3OD), 39.5 (DMSO-d_6). TLC *silica gel* Merck 60 F_{254} was used for thin-layer chromatography. Column chromatography (CC) was carried out using *silica gel* 40 - 63 μm and Sephadex LH-20.

2.3. Extraction and isolation

Dried and ground stems of *C. eberhardtii* (1.9 kg) were extracted with MeOH (5 times × 6 L, 24 h) at room temperature. The methanol solutions were combined and concentrated under reduced pressure to obtain 100 g of the methanol residue. The methanol residue was suspended in H_2O (500 mL) and then partitioned successively with *n*-hexane (5 times × 500 mL) and EtOAc (5 times × 500 mL). The extracts were concentrated under reduced pressure to give *n*-hexane extract (H, 8.7 g), EtOAc extract (E, 17.4 g), and aqueous extract (W, 76.6 g), respectively. The *n*-hexane extract (H, 8.7 g) was separated over *silica gel* column chromatography (CC) eluting with *n*-hexane/acetone (0% to 100% acetone in *n*-hexane) giving 9 fractions, H1-H9. Fraction H4 (0.67 g) was separated by CC on *silica gel* using a mixture of *n*-hexane/acetone (0% to 100% acetone in *n*-hexane) to give 8 subfractions H4.1-H4.8. Subfractions H4.2 (22 mg) and H4.4 (30 mg) were crystallized with *n*-hexane/acetone (1/1, *v/v*) to give **1** (8.4 mg) and **4** (2.8 mg), respectively. Fraction H9 (0.1 g) was crystallized with *n*-hexane/acetone (1/1, *v/v*) to provide **2** (2.4 mg). The ethyl acetate extract (E, 17.4 g) was separated by CC on *silica gel*, eluted with the solvent mixtures of CH_2Cl_2/MeOH (0% to 100% MeOH in CH_2Cl_2) to give 8 fractions E1-E8. Fraction E4 (0.72 g) was purified by CC on Sephadex LH-20 (MeOH/CH_2Cl_2 4/1, *v/v*) and followed by CC on *silica gel*, eluting with CH_2Cl_2/MeOH (0% to 100% MeOH in CH_2Cl_2) to give **8** (5.6 mg). Fraction E5 (0.87 g) was separated on Sephadex LH-20 column (MeOH) giving 4 subfractions, E5.1-E5.4. Subfraction E5.2 (0.21 g) was subjected to CC on Sephadex LH-20 (MeOH/CH_2Cl_2 9/1, *v/v*) to give 3 subfractions, E5.2.1-E5.2.3. Subfraction E5.2.2 (0.10 g) was separated by CC on *silica gel* using mixtures of CH_2Cl_2/MeOH (0% to 100% MeOH in CH_2Cl_2) to give **3** (3.0 mg), **5** (5.7 mg), and **6** (2.4 mg). Fraction E6 (0.98 g) was subjected to CC on a Sephadex LH-20 (100% MeOH), giving 4 subfractions, E6.1-E6.4. Subfraction E6.3 (0.32 g) was separated by CC on *silica gel* using a mixture of CH_2Cl_2/MeOH (0% to 100% MeOH in CH_2Cl_2) to give 3 subfractions, E6.3.1-E6.3.3. Subfraction E6.3.2 (0.20 g) was purified by CC on Sephadex LH-20 (100% MeOH) and followed by CC on *silica gel*, using a mixture of CH_2Cl_2/MeOH (0% to 100% MeOH in CH_2Cl_2) to provide **7** (3.2 mg). Fraction E7 (2.70 g) was subjected to CC on Sephadex LH-20 (MeOH) and followed by recrystallization in CH_2Cl_2/MeOH (9/1, *v/v*) to provide **9** (3.4 mg).

Lupeol (1): White powder, $[\alpha]_D^{25}$ +28.9 (*c* 0.2, $CHCl_3$) (literature value: $[\alpha]_D^{20}$ +26 (*c* 0.80, $CHCl_3$) [10]), ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H (ppm): 0.76 (3H, s, CH_3-24), 0.79 (3H, s, CH_3-28), 0.83 (3H, s, CH_3-25), 0.95 (3H, s, CH_3-27), 0.97 (3H, s, CH_3-23), 1.03 (3H, s, CH_3-26), 1.69 (3H, s, CH_3-30), 2.38 (1H, td, *J* = 5.5, 11.0 Hz, H-19), 3.19 (1H, dd, *J* = 5.0, 11.5 Hz, H-3α), 4.56 (1H, d, *J* = 2.5 Hz, H-29a), 4.68 (1H, d, *J* = 2.0 Hz, H-29b). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C (ppm): 14.6 (C-27), 15.4 (C-24), 16.0 (C-26), 16.1 (C-25), 18.0 (C-28), 18.3 (C-6), 19.3 (C-30), 20.9 (C-11), 25.2 (C-12), 27.4 (C-2), 27.5 (C-15), 28.0 (C-23), 29.9 (C-21), 34.3 (C-7), 35.6 (C-16), 37.2 (C-10), 38.1 (C-13), 38.7 (C-1), 38.9 (C-4), 40.0 (C-22), 40.9 (C-8), 42.9 (C-14), 43.0 (C-17), 48.0 (C-19), 48.3 (C-18), 50.5 (C- 9), 55.3 (C-5), 79.0 (C-3), 109.3 (C-29), 151.0 (C-20).

Betulinic acid (2): White powder, $[\alpha]_D^{25}$ +17.1 (*c* 0.2, MeOH) (literature value: $[\alpha]_D^{20}$ +18.9 (*c* 0.10, MeOH) [11]), ^{1}H-NMR (500 MHz, CD_3OD) δ_H (ppm): 0.72 (1H, brd, *J* = 9.5 Hz, H-5), 0.77 (3H, s, CH_3-24), 0.88 (3H, s, CH_3-25), 0.97 (3H, s, CH_3-23), 0.99 (3H, s, CH_3-26), 1.03 (3H, s, CH_3-27), 1.71 (3H, s, CH_3-30), 3.03 (1H, dt, *J* = 5.0, 11.0 Hz, H-19), 3.15 (1H, dd, *J* = 5.0, 11.0 Hz, H-3α), 4.61 (1H, d, *J* = 2.0 Hz, H-29a), 4.73 (1H, d, *J* = 2.0 Hz, H-29b). ^{13}C-NMR (125 MHz, CD_3OD) δ_C (ppm): 15.1 (C-27), 16.1 (C-24), 16.6 (C-25), 16.7 (C-26), 19.4 (C-6), 19.5 (C-30), 22.1 (C-11), 26.9 (C-12), 28.0 (C-2), 28.6 (C-23), 30.8 (C-15), 31.7 (C-21), 33.3 (C-16), 35.6 (C-7), 38.1 (C-22), 38.3 (C-10), 39.7 (C-13), 40.0 (C-4), 40.1 (C-1), 41.9 (C-8), 43.6 (C-14), 48.5 (C-19), 50.5 (C-18), 52.0 (C- 9), 56.9 (C-5), 57.5 (C-17), 79.7 (C-3), 110.2 (C-29), 152.0 (C-20), 180.0 (C-28).

3,3′-Di-*O*-methylellagic acid-4′-*O*-α-L-rhamnopyranoside (3): White powder, ESI-MS: *m/z* 475 $[M-H]^-$, ^{1}H-NMR (500 MHz, DMSO-d_6) δ_H (ppm): 1.14 (3H, d, J = 6.0 Hz, CH_3-6″), 3.35 (1H, t, J = 9.0 Hz, H-4″), 3.52 (1H, m, H-5″), 3.72 (1H, dd, J = 3.0, 9.0 Hz, H-3″), 3.95 (1H, m, H-2″), 4.05 (3H, s, 3-OCH_3), 4.07 (3H, s, 3′-OCH_3), 5.56 (1H, brs, H-1″), 7.45 (1H, s, H-5′), 7.76 (1H, s, H-5). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ_C (ppm): 17.9 (CH_3-6″), 60.9 (3′-OCH_3), 61.6 (3-OCH_3), 70.0 (C-2″), 70.3 (C-4″), 70.4 (C-5″), 71.5 (C-3″), 99.8 (C-1″), 110.9 (C-1′), 111.6 (C-5′), 111.7 (C-5), 111.9 (C-1), 112.6 (C-6′), 114.1 (C-6), 140.2 (C-3′), 141.0 (C-2), 141.5 (C-2′), 141.8 (C-3), 150.2 (C-4), 152.8 (C-4′), 158.2 (C-7′), 158.4 (C-7).

***β*-Sitostenone (4):** White powder, ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H (ppm): 0.64 (3H, s, CH_3-18), 0.73 (3H, d, J = 7.0 Hz, CH_3-27), 0.76 (3H, d, J = 7.0 Hz, CH_3-26), 0.79 (3H, t, J = 7.5 Hz, CH_3-29), 0.85 (3H, d, J = 7.5 Hz, CH_3-21), 1.18 (3H, s, CH_3-19), 5.65 (1H, s, H-4). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C (ppm): 11.9 (C-29), 12.0 (C-18), 17.4 (C-19), 18.7 (C-21), 19.0 (C-27), 19.8 (C-26), 21.0 (C-11), 23.1 (C-28), 24.2 (C-15), 26.1 (C-23), 28.2 (C-16), 29.2 (C-25), 32.1 (C-7), 32.9 (C-6), 33.9 (C-2), 34.0 (C-22), 35.6 (C-1), 35.7 (C-8), 36.1 (C-20), 38.6 (C-10), 39.6 (C-12), 42.4 (C-13), 45.8 (C-24), 53.8 (C-9), 55.9 (C-14), 56.0 (C-17), 123.7 (C-4), 171.6 (C-5), 199.6 (C-3).

2,3-Dihydroxy-1-(4′-hydroxy-3-methoxyphenyl) propane-1-one (C-Veratroylglycol) (5): White powder, $[\alpha]_D^{25}$ -48.7 (c 0.15, MeOH) (literature value: $[\alpha]_D^{25}$ -40.0 (c 0.10, MeOH) [12]), ^{1}H-NMR (600 MHz, CD_3OD) δ_H (ppm): 3.75 (1H, dd, J = 5.4, 11.4 Hz, H-3a), 3.90 (1H, dd, J = 3.6, 12.0 Hz, H-3b), 3.93 (3H, s, 3′-OCH_3), 5.12 (1H, t, J = 5.4 Hz, H-2), 6.90 (1H, d, J = 8.4 Hz, H-5′), 7.59 (1H, d, J = 1.8 Hz, H-2′), 7.61 (1H, dd, J = 1.8, 8.4 Hz, H-6′). ^{13}C-NMR (150 MHz, CD_3OD) δ_C (ppm): 56.5 (3′-OCH_3), 66.3 (C-3), 75.5 (C-2), 112.5 (C-2′), 115.9 (C-5′), 125.1 (C-6′), 128.1 (C-1′), 149.3 (C-3′), 153.8 (C-4′), 199.6 (C-1).

3,4-Dihydroxybenzaldehyde (6): White powder, ^{1}H-NMR (500 MHz, CD_3OD) δ_H (ppm): 6.92 (1H, d, J = 7.5 Hz, H-5), 7.32 (1H, d, J = 2.0 Hz, H-2), 7.33 (1H, dd, J = 2.0, 7.5 Hz, H-6), 9.70 (1H, s, CHO).

3,4-Dihydroxybenzoic acid (7): White powder, ^{1}H-NMR (500 MHz, CD_3OD) δ_H (ppm): 6.82 (1H,d, J = 8.0 Hz , H-5), 7.43 (1H, d, J = 2.0 Hz, H-2), 7.45 (1H, dd, J = 2.0, 8.0 Hz, H-6)

Methyl 3,4-dihydroxybenzoate (8): White powder, ^{1}H-NMR (500 MHz, CD_3OD) δ_H (ppm): 3.91 (3H, s, OCH_3), 6.85 (1H, d, J = 9.0 Hz, H-5), 7.56 (1H, d, J = 2.0 Hz, H-2), 7.58 (1H, dd, J = 2.0, 9.0 Hz, H-6).

Methyl gallate (9): White powder, ^{1}H-NMR (500 MHz, CD_3OD) δ_H (ppm): 3.88 (3H, s, OCH_3), 7.06 (2H, s, H-2, H-6).

2.4. Cytotoxic assays

The cytotoxic activity of three compounds **1–3** was evaluated *in vitro* against MCF-7 (human breast cancer cells) and KB (human epithelial carcinoma cells) cell lines. The cells were cultured at 37°C in DMEM medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin in a 5% CO_2 incubator. Cells between 5 and 20 passages were used for the assays. The cytotoxic activity was measured by using a modified MTT assay [13]. Viable cells were seeded in 96-well plates at a density of 3×10^4 cells/mL. Cells were treated with various concentrations of test compounds (2, 8, 32, and 128 µg/mL) and then incubated at 37°C for 72 h in fresh DMEM medium. Cells were subsequently incubated at 37°C with MTT (0.5 mg/mL) for 4 h. After removal of the supernatant, formazan crystals were dissolved in DMSO and the optical density was measured at 540 nm. Ellipticine was used as a positive control.

3. Results and discussion

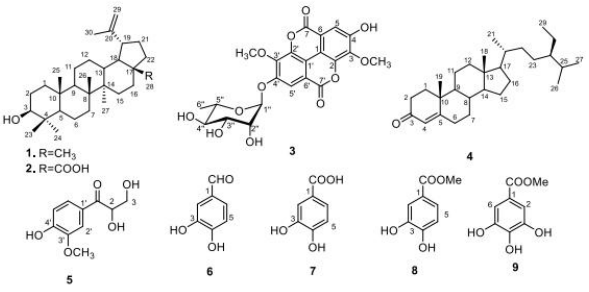

Fig. 1. Structures of the isolated compounds **1**-**9**

Compound **1** was obtained as a white powder and optically active, $[\alpha]_D^{25}$ +28.9 (c 0.2, $CHCl_3$). The ^{1}H-NMR spectrum of **1** revealed the proton signals of seven singlet methyl groups at δ_H 0.76 (3H, s, CH_3-24), 0.79 (3H, s, CH_3-28), 0.83 (3H, s, CH_3-25), 0.94 (3H, s, CH_3 -27), 0.97 (3H, s, CH_3-23), 1.03 (3H, s, CH_3 -26), 1.69 (3H, s, CH_3-30), an oxymethine group at δ_H 3.19 (1H, dd, J = 5.0, 11.5 Hz, H-3), an exo-methylene group at δ_H

4.56 (1H, d, J = 2.5 Hz, H-29a), 4.68 (1H, d, J = 2.0 Hz, H-29b), and the remaining signals in the aliphatic region. Proton H-3 afforded a large (J = 11.5 Hz) and a small (J = 5.0 Hz) coupling constants indicating its *axial* position. The ^{13}C-NMR and DEPT spectra of compound **1** showed the presence of thirty carbon signals, including seven methyl carbons at δ_C 14.6 (C-27), 15.4 (C-24), 16.0 (C-26), 16.1 (C-25), 18.0 (C-28), 19.3 (C-30), 28.0 (C-23), one oxymethine at δ_C 79.0 (C-3), five sp^3 methines at δ_C 38.1 (C-13), 48.0 (C-19), 48.3 (C-18), 50.5 (C-9), 55.3 (C-5), one sp^2 methylene at δ_C 109.3 (C-29), ten sp^3 methylenes at δ_C 18.3 (C-6), 20.9 (C-11), 25.2 (C-12), 27.4 (C-2), 27.5 (C-15), 29.9 (C-21), 34.3 (C-7), 35.6 (C-16), 38.7 (C-1), 40.0 (C-22), one sp^2 carbon at δ_C 151.0 (C-20) and five quaternary carbons at δ_C 38.9 (C-4), 37.2 (C-10), 40.9 (C-8), 42.9 (C-14), and 43.0 (C-17). The chemical shifts of the methyl group CH_3-30 (δ_H 1.69, δ_C 19.3) suggested its linkage to a double bond. Based on these NMR data, compound **1** was identified as a lupane-type triterpenoid. In the ^{1}H-^{1}H COSY spectrum of compound **1**, four spin-spin coupling systems were observed, including H-1/H-2/H-3; H-5/H-6/H-7; H-9/H-11/H-12/H-13/H-18/H-19/H-21/H-22, and H-15/H-16. In the HMBC spectrum, compound **1** exhibited the long-range H-C ^2J and ^3J connectivities associated with the methyl and exo-methylene resonances of a lupane skeleton. The HMBC correlations of Me-23 and Me-24 with C-3/C-4/C-5 deduced that these two methyls were attached to C-4. The remaining five methyl groups could be positioned at C-10, C-8, C-14, C-17, and C-20 on the basis of the HMBC correlations (Fig. 2). The HMBC correlations of CH_2-29 with C-30/C-20/C-19 demonstrated the placement of the exo-methylene group at C-20. The cross peaks of H-3 with C-2/C-4/ Me-23/C-5 indicated the connections of OH to C-3. Detailed analysis of the 2D spectra of **1** and comparison with the literature data confirmed its structure as lupeol [14].

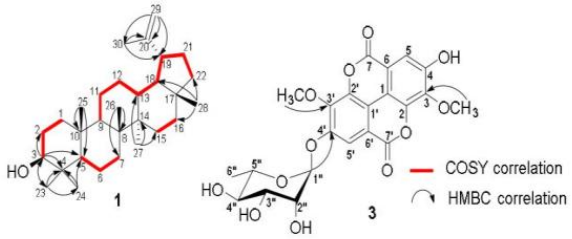

Fig. 2. Selected COSY and HMBC correlations for **1** and **3**

Compound **2** was isolated as a white powder and optically active, $[\alpha]_D^{25}$ +17.1 (c 0.2, MeOH). The ^{1}H-NMR and ^{13}C-NMR spectrum of **2** showed the characteristic signals of a lupane skeleton which were similar to those of compound **1**. The ^{1}H-NMR spectrum revealed the proton signals of six singlet methyls at δ_H 0.77 (3H, s, CH_3-24), 0.88 (3H, s, CH_3-25), 0.97 (3H, s, CH_3-23), 0.99 (3H, s, CH_3-26), 1.03 (3H, s, CH_3-27), 1.71 (3H, s, CH_3-30), one oxymethine at δ_H 3.15 (1H, dd, J = 5.0, 11.0 Hz, H-3), two geminal olefinic protons at δ_H 4.61 (1H, d, J = 2.0 Hz, H-29a), 4.73 (1H, d, J = 2.0 Hz, H-29b). The ^{13}C-NMR and DEPT spectra showed the resonances of thirty carbons, including a carboxyl group at δ_C 180.0 (C-28), six methyl carbons at δ_C 15.1 (C-27), 16.1 (C-24), 16.6 (C-25), 16.7 (C-26), 19.5 (C-30), 28.6 (C-23), one oxymethine at δ_C 79.7 (C-3), five sp^3 methines at δ_C 39.7 (C-13), 48.5 (C-19), 50.5 (C-18), 52.0 (C- 9), 56.9 (C-5), one sp^2 methylene at δ_C 110.2 (C-29), ten sp^3 methylenes at δ_C 19.4 (C-6), 22.1 (C-11), 26.9 (C-12), 28.0 (C-2), 30.8 (C-15), 38.1 (C-22), 31.7 (C-21), 35.6 (C-7), 33.3 (C-16), 40.1 (C-1), one sp^2 quaternary carbon at δ_C 152.0 (C-20), and five sp^3 quaternary carbons at δ_C 38.3 (C-10), 40.0 (C-4), 41.9 (C-8), 43.6 (C-14), 57.5 (C-17). The large coupling constant between H-2 and H-3 (J = 11.4 Hz) indicated an *axial* position for H-3. The disappearance of one methyl group and the presence of one carbonyl group in the structure of compound **2** when compared to those of compound **1** suggested the oxidation of the methyl group to a carboxylic acid. Based on these analyses and comparison with literature data [15], the structure of compound **2** was determined as betulinic acid.

Compound **3** was isolated as a white powder. The ^{1}H-NMR spectrum of **3** exhibited the signals of two methoxy groups at δ_H 4.05 (3H, s, 3-OCH_3), 4.07 (3H, s, 3′-OCH_3), two singlet aromatic protons at δ_H 7.45 (1H, s, H-5′), 7.76 (1H, s, H-5). Additionally, the ^{1}H-NMR spectrum of **3** also showed the characteristic proton signals of a rhamnose moiety at δ_H 1.14 (3H, d, J = 6.0 Hz, CH_3-6″), 3.35 (1H, t, J = 9.0 Hz, H-4″), 3.52 (1H, m, H-5″), 3.72 (1H, dd, J = 3.0, 9.0 Hz, H-3″), 3.95 (1H, m, H-2″), and 5.56 (1H, brs, H-1″). The broadened singlet signal at δ_H 5.56 for the anomeric proton indicated an α-L-rhamnopyranosyl moiety in the structure of compound **3**. The ^{13}C-NMR spectrum of compound **3** revealed twenty-two carbons, including two methyl groups, two carbonyl

groups, two sp^2 methine groups, one methyl group, five oxymethine groups, and ten sp^2 quaternary carbons. These NMR data suggested that compound **3** was an ellagic acid derivative. The HMBC correlation from anomeric proton H-1″ to C-4′ indicated that the rhamnose moiety was attached to C-4′ of aglycone moiety. The two methoxy groups were determined to be attached to C-3 and C-3′ by HMBC correlations of C-3 (δ_C 141.8) with methoxy protons at δ_H 4.05 (3-OCH_3) and C-3′ (δ_C 140.2) with methoxy protons at δ_H 4.07 (3′-OCH_3). A detailed analysis of the ESI-MS, NMR of **3**, and comparison with the reported values indicated that compound **3** was 3,3′-di-*O*-methylellagic acid-4′-*O*-*α*-L-rhamnopyranoside [16].

Compound **4** was obtained as a white powder. The ^{1}H-NMR spectrum of **4** exhibited the presence of six methyls, including two singlet methyls at δ_H 0.64 (3H, s, H-18), 1.18 (3H, s, CH_3-19), three doublet methyls at δ_H 0.73 (3H, d, J = 7.0 Hz, CH_3-27), 0.76 (3H, d, J = 7.0 Hz, CH_3-26), 0.85 (3H, d, J = 7.5 Hz, CH_3-21), one triplet methyl at δ_H 0.79 (3H, t, J = 7.5 Hz, CH_3-29), one singlet olefinic proton at δ_H 5.65 (1H, s, H-4), and the remaining signals in the aliphatic region. The ^{13}C-NMR and DEPT spectrum of compound **4** revealed twenty nine carbons, including six methyls at δ_C 11.9 (C-29), 12.0 (C-18), 17.4 (C-19), 18.7 (C-21), 19.0 (C-27), 19.8 (C-26), eleven methylenes at δ_C 21.0 (C-11), 23.1 (C-28), 24.2 (C-15), 26.1 (C-23), 28.2 (C-16), 32.1 (C-7), 32.9 (C-6), 33.9 (C-2), 34.0 (C-22), 35.6 (C-1), 39.6 (C-12), eight methines [one sp^2 methine at δ_C 123.7 (C-4), seven sp^3 methines at δ_C 29.2 (C-25), 35.7 (C-8), 53.8 (C-9), 55.9 (C-14), 56.0 (C-17), 36.1 (C-20), 45.8 (C-24)], one sp^2 quaternary carbon at δ_C 171.6 (C-5), two sp^3 quaternary carbons at δ_C 38.6 (C-10), 42.4 (C-13), and one carbonyl at δ_C199.6 (C-3). These signals suggested a steroid compound. Detailed analyses of the 1D-NMR spectra data of **4** and comparison with the literature data indicated that compound **4** was *β*-sitostenone [17].

Compound **5** was obtained as a white powder and optically active, $[\alpha]_D^{25}$ -48.7 (*c* 0.15, MeOH). The ^{1}H NMR spectrum of **5** revealed the proton signals of an ABX aromatic ring system at δ_H 6.90 (1H, d, J = 8.4 Hz, H-5′), 7.59 (1H, d, J = 1.8 Hz, H-2′), 7.61 (1H, dd, J = 1.8, 8.4 Hz, H-6′), a methoxy at δ_H 3.93 (3H, s, 3′-OCH_3), an oxymethine at δ_H 5.12 (1H, t, J = 5.4 Hz, H-2) and an oxymethylene at δ_H 3.75 (1H, dd, J= 5.4, 11.4 Hz, H-3a), 3.90 (1H, dd, J = 3.6, 12.0 Hz, H-3b). The ^{13}C-NMR spectrum of **5** indicated the presence of ten carbons, including one methoxy at δ_C 56.5 (3′-OCH_3), three sp^2 methines at δ_C 112.5 (C-2′), 115.9 (C-5′), 125.1 (C-6′), one oxymethine at δ_C 75.5 (C-2), one oxymethylene at δ_C 66.3 (C-3), one carbonyl at δ_C 199.6 (C-1) and three sp^2 quaternary carbons at δ_C 149.3 (C-3′), 153.8 (C-4′), 128.1 (C-1′). Complete analyses of the 1D-NMR spectra of **5** and a comparison with the reported values indicated that compound **5** was 2,3-dihydroxy-1-(4′-hydroxy-3-methoxyphenyl) propane-1-one or C-veratroylglycol [12],[18].

Compounds **6-9** were determined as 3,4-dihydroxybenzaldehyde (**6**) [19], 3,4-dihydrobenzoic acid (**7**) [20],[21], methyl 3,4-dihydroxybenzoate (**8**) [21], methyl gallate (**9**) [22] by analyzing their NMR data and comparing with those reported in the literature. While these compound classes are absent from the fruits of *Cleistanthus eberhardtii*, which predominantly features lignans [5], they are fully consistent with the chemical constituents of the *Cleistanthus* genus [6],[7].

The cytotoxic activities of compounds **1–3** were evaluated against two cancer cell lines: MCF-7 and KB (Table 1). The results showed that compound **1** exhibited potential cytotoxicity against the MCF-7 cell line with an IC_{50} value of 18.3 μg/mL. Compound **2** revealed moderate cytotoxic activity against KB and MCF-7 cell lines with IC_{50} values of 25.8 and 29.2 μg/mL, respectively. Compound **3** significantly exhibited cytotoxicity against the MCF-7 cell line with an IC_{50} value of 28.3 μg/mL.

Table 1. Cytotoxic effects of compounds **1**–**3**

Compounds	IC_{50} (μg/mL)	
	KB	**MCF-7**
1	>128	18.3
2	25.8	29.2
3	28.3	>128
Ellipticine	1.26	1.62

4. Conclusion

In summary, from the stems of *C. eberhardtii*, nine compounds were isolated and structurally determined using NMR spectroscopy, and comparison of their NMR data with the literature data. These compounds included lupeol (**1**), betulinic acid (**2**), 3,3′-di-*O*-methylellagic acid-4′-*O*-*α*-L-rhamnopyranoside (**3**), *β*-sitostenone (**4**), 2,3-dihydroxy-1-(4′-hydroxy-3-methoxyphenyl)propane-1-one (**5**), 3,4 dihydroxybenzaldehyde (**6**), 3,4-dihydrobenzoic acid (**7**), methyl 3,4-dihydroxybenzoate (**8**), and

methyl gallate (**9**). Compounds **1-3** showed moderate cytotoxic activity against KB and MCF-7 cancer cell lines with IC_{50} values ranging from 18.3 to 29.2 µg/mL. Significantly, this is the first report of these compounds from *C. eberhardtii*, and this report represents the first phytochemical investigation of *C. eberhardtii* stems. To further expand our understanding of the chemical and pharmacological properties of this species, future research should focus on analyzing the remaining stem extract for additional phytochemical constituents and evaluating their bioactivities.

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