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ISOLATION, STRUCTURAL DETERMINATION, AND CYTOTOXICITY OF COMPOUNDS FROM THE NON-ALKALOID EXTRACT OF *CINNAMOMUM BEJOLGHOTA*

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Summary

Isolation, Structural Determination, and Cytotoxicity of Compounds from the Non-Alkaloid Extract of *Cinnamomum bejolghota*

Eight compounds were isolated from the non-alkaloid extract of *Cinnamomum bejolghota* stem bark. Their chemical structures were elucidated as ergosta-4,6,8(14),22-tetraen-3-one (**1**), spathulenol (**2**), 5,7-dimethoxy-3',4'-methylenedioxyflavan-3-ol (**3**), afzelin (**4**), 3,4-dimethoxycinnamyl alcohol (**5**), 3,4-dimethoxycinnamaldehyde (**6**), veratraldehyde (**7**), and veratric acid (**8**) on the basis of detailed analysis of the 1D and 2D NMR spectroscopic data in comparison with the literature data. Compound **1** showed activity against the HepG-2 cell line with an IC_{50} value of 21.8 µM. Compound **3** showed weak cytotoxic activity against KB, MCF-7, HepG-2, and SK-LU-1 human cancer cell lines with IC_{50} values of 118.3, 110.7, 90.2, and 95.8 µM, respectively. Compounds **1** and **4** were reported from this plant for the first time.

Keywords: *Cinnamomum bejolghota,* Lauraceae, *Steroid, Sesquiterpenoid, Flavonoid.*

1. Introduction

Cinnamomum bejolghota (Lauraceae) is an evergreen tree distributed widely in Vietnam, China, India, and Sri Lanka. Leaves, seeds, and stem barks of the plant are used not only as a food additive but also as a traditional medicine for treating common colds, toothaches, coughs, and abdominal pain [1],[2]. Previous studies revealed several secondary metabolites, such as terpenoids, phenylpropanoids, alkaloids, lignans, and flavonoids, from several *Cinnamomum* species with antioxidant [3],[4], anticancer [5], anti-bacterial [6],[7], and anti-inflammatory activities [8]. However, little research on the chemical constituents of *C. bejolghota* has been conducted [1],[9]. In a previous study, we reported the isolation of three new alkaloids from the alkaloid-containing extract of *C. bejolghota* [9]. In this study, we report the isolation of one steroid, ergosta-4,6,8(14),22-tetraen-3-one (**1**), one sesquiterpenoid, spathulenol (**2**), two flavonoids, 5,7-dimethoxy-3',4'-methylenedioxyflavan-3-ol (**3**), afzelin (**4**), together with four phenolic compounds, 3,4-dimethoxycinnamyl alcohol (**5**), 3,4-dimethoxycinnamaldehyde (**6**), veratraldehyde (**7**), and veratric acid (**8**) from a non-alkaloid extract of the stem bark of *C. bejolghota* and evaluation of their cytotoxic effects.

2. Material and methods

2.1. Plant materials

The stem bark of *C. bejolghota* (Buch.-Ham. ex Nees) Sweet was collected in Dakrong, Quang Tri, Vietnam in June 2019 and this plant was identified by Dr. Nguyen The Cuong of the Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST). A voucher specimen (VN-1450) was deposited at the Herbarium of the Institute of Ecology and Biological Resources, VAST.

2.2. General experimental procedures

All NMR spectra were recorded on a Bruker 500 MHz or a Bruker 600 MHz spectrometer operating at 125 and 150 MHz for ^{13}C NMR, and at 500 MHz for ^{1}H-NMR. ESI-MS spectra were recorded on an Agilent 1100 LC-MSD Trap spectrometer. Column chromatography (CC) was performed on silica-gel (Kieselgel 60, 230-400 mesh, Merck) or Sephadex LH-20. For thin layer chromatography (TLC), pre-coated silica gel 60 F_{254} (0.25 mm, Merck) plates were used. Medium pressure liquid chromatography (MPLC) was performed on a Biotage-Isolera One system (Sweden).

2.3. Extraction and isolation

950 g of stem bark from *C. bejolghota* were extracted successively with MeOH/NH_3 (99:1) (5 x 3 L x 21h) at room temperature. The solution was concentrated under reduced pressure to obtain 30 g of residue. The residue was suspended in HCl 1N aqueous solution (0.6 L) to acidify the aqueous solution to pH 2-3, and

extracted with ethyl acetate (5 x 0.5 L). The EtOAc solution was concentrated under reduced pressure to obtain 25.3 g of the non-alkaloid extract. The remaining aqueous solution was alkalized with NH_4OH 25% solution until pH 8, and extracted successively with CH_2Cl_2 (5 times x 0.5 L) and EtOAc (5 times x 0.5 L). The organic layers were combined and concentrated under reduced pressure to afford the alkaloid residue (0.89 g).

The non-alkaloid residue (25.3 g) was subjected to MPLC over *silica gel* using mixtures of *n*-hexane/EtOAc (0% to 100% EtOAc in *n*-hexane) and mixtures of EtOAc/MeOH (10% to 100% MeOH in EtOAc) to afford fourteen fractions (F1 - F14). Fraction F4 (0.974 g) was chromatographed on *silica gel* CC with gradient mixtures of *n*-hexane/CH_2Cl_2 (5-100% CH_2Cl_2 in *n*-hexane) to give sixteen subfractions (F4.1 - F4.16). Subfraction F4.14 (14 mg) was purified on *silica gel* CC eluted with *n*-hexane/acetone (85/15, v/v) to obtain compound **1** (2.7 mg). Subfraction F4.9 (19 mg) was separated on *silica gel* CC eluted with *n*-hexane/acetone (96/4, v/v) to yield compound **2** (4.0 mg). Fraction F6 (1.36 g) was subjected to Sephadex LH-20 CC eluted with MeOH to give four subfractions (F6.1 - F6.4). Subfraction F6.2 (54.5 mg) was further separated on *silica gel* CC with *n*-hexane/acetone (75/25, v/v) elution to obtain compound **5** (3.5 mg) and compound **7** (2.0 mg). Subfraction F6.3 (0.228 g) was separated on *silica gel* CC, eluting with gradient *n*-hexane/acetone (5%-100% acetone) to obtain compound **6** (8.0 mg). Subfraction F6.4 (11.5 mg) was separated by preparative TLC with *n*-hexane/acetone (75:25, v/v) to obtain compound **3** (4.0 mg). Fraction F9 (2.09 g) was purified on a Sephadex LH-20 column with MeOH elution, followed by *silica gel* CC, eluting with CH_2Cl_2/EtOAc (99/1, v/v) to yield compound **8** (8.0 mg). Fraction F13 (350 mg) was separated by preparative TLC with CH_2Cl_2/MeOH (75:25, v/v) to obtain compound **4** (23 mg).

Ergosta-4,6,8(14),22-tetraen-3-one (1): Yellow solid; ESI-MS: *m/z* 393 $[M+H]^+$; ^1H NMR (500 MHz, CD_3OD) δ (ppm) 0.88 (3H, d, J = 6.5 Hz, CH_3-28), 0.89 (3H, d, J = 6.5 Hz, CH_3-26), 0.98 (3H, d, J = 6.5 Hz, CH_3-27), 1.03 (3H, s, CH_3-18), 1.04 (3H, s, CH_3-19), 1.10 (3H, d, J = 6.0 Hz, CH_3-21), 1.29 (1H, m, H-17), 1.34 (1H, m, H-12a), 1.52 (1H, m, H-25), 1.57 (1H, m, H-16a), 1.87 (1H, m, H-1a), 1.87 (1H, m, H-16b), 1.91 (1H, m, H-24), 2.10 (1H, m, H-1b), 2.17 (1H, m, H-12b), 2.18 (1H, m, H-9), 2.21 (1H, m, H-20), 2.40 (2H, m, H-15), 2.42 (1H, m, H-2a), 2.43 (1H, m, H-11a), 2.57 (1H, m, H-11b), 2.61 (1H, m, H-2b), 5.29 (1H, m, H-23), 5.74 (1H, s, H-4), 6.12 (1H, d, J = 9.5 Hz, H-6), 6.75 (1H, d, J = 9.5 Hz, H-7). ^{13}C NMR (125 MHz, CD_3OD) δ (ppm) 16.9 (C-18), 18.1 (C-19), 19.3 (C-21), 20.0 (C-11), 20.1 (C-28), 20.4 (C-26), 21.7 (C-27), 26.1 (C-15), 28.8 (C-16), 34.3 (C-25), 34.8 (C-2), 35.1 (C-1), 36.8 (C-12), 38.1 (C-10), 40.6 (C-20), 44.3 (C-24), 45.2 (C-13), 45.7 (C-9), 57.2 (C-17), 123.1 (C-4), 125.3 (C-6), 125.9 (C-8), 133.7 (C-23), 135.9 (C-22), 136.5 (C-7), 157.8 (C-14), 167.8 (C-5), 202.2 (C-3).

Spathulenol (2): Colorless oil; ESI-MS: *m/z* 221 $[M+H]^+$; ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 0.47 (1H, dd, J = 9.5, 11.5 Hz, H-1), 0.71 (1H, m, H-2), 1.01 (1H, m, H-3a), 1.04 (3H, s, CH_3-12), 1.06 (3H, s, CH_3-13), 1.28 (3H, s, CH_3-15), 1.33 (1H, m, H-10), 1.60 (1H, m, H-8a), 1.65 (1H, m, H-7a), 1.77 (1H, m, H-8b), 1.91 (1H, m, H-7b), 1.96 (1H, m, H-3b), 2.02 (1H, m, H-4a), 2.21 (1H, m, H-6), 2.43 (1H, m, H-4b), 4.66 (1H, br s, H-14a), 4.69 (1H, br. s, H-14b). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 16.3 (C-13), 20.2 (C-11), 24.8 (C-3), 26.0 (C-15), 26.7 (C-7), 27.5 (C-2), 28.6 (C-12), 29.9 (C-1), 38.8 (C-4), 41.7 (C-8), 53.4 (C-6), 54.3 (C-10), 80.9 (C-9), 106.2 (C-14), 153.4 (C-5).

5,7-Dimethoxy-3′,4′-methylenedioxyflavan-3-ol (3): Pale yellow solid; ESI-MS: *m/z* 331 $[M+H]^+$; ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 2.91 (2H, m, H-4), 3.77 (3H, s, 5-OCH_3), 3.79 (3H, s, 7-OCH_3), 4.25 (1H, br s, H-3), 4.93 (1H, s, H-2), 5.98 (2H, s, OCH_2O), 6.11 (1H, d, J = 2.5 Hz, H-6), 6.17 (1H, d, J = 2.5 Hz, H-8), 6.85 (1H, d, J = 8.0 Hz, H-5'), 6.96 (1H, dd, J = 1.5, 8.0 Hz, H-6'), 7.05 (1H, d, J = 1.5 Hz, H-2'). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 28.2 (C-4), 55.4 (7-OCH_3), 55.4 (5-OCH_3), 66.5 (C-3), 78.5 (C-2), 92.2 (C-8), 93.3 (C-6), 100.2 (C-10), 101.1 (OCH_2O), 107.2 (C-5'), 108.3 (C-2'), 119.7 (C-6'), 132.2 (C-1'), 147.4 (C-3'), 147.9 (C-4'), 155.2 (C-9), 159.3 (C-5), 159.7 (C-7).

Afzelin (4): Yellow solid; ESI-MS: *m/z* 433 $[M+H]^+$; ^1H NMR (500 MHz, CD_3OD) δ (ppm) 0.95 (3H, d, J = 5.5 Hz, H-6"), 3.23 (1H, m, H-5"), 3.36 (1H, m, H-4"), 3.74 (1H, m, H-3"), 4.25 (1H, m, H-2"), 5.39 (1H, d, J = 2.0 Hz, H-1"), 6.21 (1H, d, J = 1.5 Hz, H-6), 6.37 (1H, d, J = 1.5 Hz, H-8), 6.95 (2H, d, J = 8.5 Hz, H-3', H-5'), 7.77 (2H, d, J = 8.5 Hz, H-2', H-6'). ^{13}C NMR (125 MHz, CD_3OD) δ (ppm) 17.6 (C-6"), 71.9 (C-5"), 72.0 (C-2"), 72.1 (C-3"), 73.2 (C-4"), 94.7 (C-8), 99.8 (C-6), 103.5 (C-1"), 105.9 (C-

10), 116.5 (C-3'/5'), 122.6 (C-1'), 131.9 (C-2'/6'), 136.2 (C-3), 158.5 (C-2), 159.2 (C-9), 161.5 (C-5), 163.2 (C-4'), 165.9 (C-7), 179.6 (C-4).

3,4-Dimethoxycinnamyl alcohol (5): White powder; ESI-MS: *m/z* 195 $[M+H]^+$; ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 3.88 (3H, s, 3-OCH_3), 3.90 (3H, s, 4-OCH_3), 4.31 (2H, m, H-9), 6.25 (1H, dt, J = 6.0, 16.0 Hz, H-8), 6.55 (1H, d, J = 16.0 Hz, H-7), 6.82 (1H, d, J = 8.0 Hz, H-5), 6.92 (1H, dd, J = 1.5, 8.0 Hz, H-6), 6.95 (1H, d, J = 1.5 Hz, H-2). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 55.8 (3-OCH_3), 55.9 (4-OCH_3), 63.8 (C-9), 108.9 (C-5), 111.2 (C-2), 119.7 (C-6), 126.5 (C-8), 129.8 (C-1), 131.2 (C-7), 149.0 (C-3), 149.1 (C-4).

3,4-Dimethoxycinnamaldehyde (6): Yellow powder, ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 3.93 (3H, s, 3-OCH_3), 3.94 (3H, s, 4-OCH_3), 6.62 (1H, dd, J = 8.0, 16.0 Hz, H-8), 6.91 (1H, d, J = 8.0 Hz, H-5), 7.08 (1H, d, J = 2.0 Hz, H-2), 7.16 (1H, dd, J = 2.0, 8.0 Hz, H-6), 7.42 (1H, d, J = 16.0 Hz, H-7), 9.66 (1H, d, J = 8.0 Hz, H-9). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 55.9 (4-OCH_3), 56.0 (3-OCH_3), 110.0 (C-2), 111.2 (C-5), 123.4 (C-6), 126.7 (C-8), 127.1 (C-1), 149.4 (C-4), 152.0 (C-3), 152.8 (C-7), 193.5 (C-9).

Veratraldehyde (7): White powder, ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 3.92 (3H, s, 3-OCH_3), 3.95 (3H, s, 4-OCH_3), 7.15 (1H, d, J = 8.0 Hz, H-5), 7.47 (1H, d, J = 2.0 Hz, H-2), 7.57 (1H, dd, J = 2.0, 8.0 Hz, H-6), 9.83 (1H, s, 1-CHO).

Veratric acid (8): White solid, ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 3.95 (3H, s, 3-OCH_3), 3.96 (3H, s, 4-OCH_3), 6.92 (1H, d, J = 8.5 Hz, H-5), 7.60 (1H, d, J = 2.0 Hz, H-2), 7.78 (1H, dd, J = 2.0, 8.5 Hz, H-6). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 56.0 (3-OCH_3), 56.0 (4-OCH_3), 110.3 (C-5), 112.4 (C-2), 121.7 (C-1), 124.6 (C-6), 148.7 (C-3), 153.7 (C-4), 171.7 (C-7).

2.4. Cytotoxic assay

The cytotoxic activities of compounds **1-4** were evaluated for their cytotoxicity against KB, HepG-2, SK-LU1, and MCF-7 human cancer 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 [10]. Viable cells were seeded in 96-well plates at a density of 3×10^4 cells/mL. Cells were treated with four concentrations of test compounds (2, 8, 32, and 128 µg/mL) and then incubated at 37°C for 72h 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. All cytotoxicity assays were performed in triplicate in three independent experiments.

3. Results and discussion

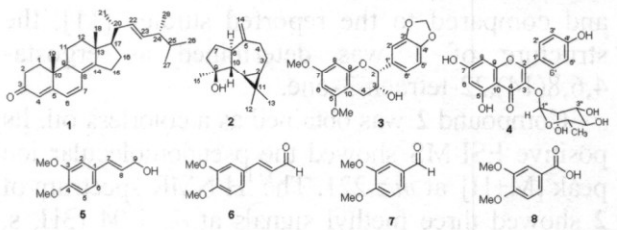

Fig. 1. Chemical structures of compounds **1-8**.

Compound **1** was isolated as a yellow solid. Its positive ESI-MS showed the pseudomolecular ion peak $[M+H]^+$ at *m/z* 393. The ^1H NMR spectrum of **1** showed two singlet methyls at δ_H 1.03 (3H, s, CH_3-18), 1.04 (3H, s, CH_3-19) and four doublet methyl signals at δ_H 0.88 (3H, d, J = 6.5 Hz, CH_3-26), 0.89 (3H, d, J = 6.5 Hz, CH_3-27), 0.98 (3H, d, J = 6.5 Hz, CH_3-28), 1.10 (3H, d, J = 6.5 Hz, CH_3-21), two *trans*-oriented olefinic protons at δ_H 5.29 (1H, dd, J = 15.0, 7.5 Hz, H-22), 5.30 (1H, dd, J = 15.0, 7.5 Hz, H-23), three olefinic protons at δ_H 5.74 (1H, br s, H-4), 6.12 (1H, d, J = 9.5 Hz, H-7), 6.75 (1H, d, J = 9.5 Hz, H-6) and the remaining protons in the aliphatic region at δ_H 1.29-2.61. The ^{13}C NMR and DEPT spectrum of the compound exhibited twenty-eight carbon signals, including one carbonyl group at δ_C 202.3 (C-3), six methyl groups, six methylene groups, five sp^2 methines groups, five sp^3 methines groups, and five non-protonated carbons. The NMR spectra of compound **1** were similar to those of the ergostane skeleton [11]. In the ^1H-^1H COSY spectrum, four spin-spin coupling systems established by the correlations of H-1/H-2; H-6/H-7; H-9/H-11/H-12; H-15/H-16/H-17/H-20/H-22/H-23/H-24/H-25 were also indicated (Fig. 2). In the HMBC spectrum of **1**, correlations from H-4 to C-2/C-3/C-5/C-6; H-7 to C-5/C-9; H-6 to C-4/C-5/C-8/C-10 indicated the location of two double bonds at C-4=C-5 and C-6=C-7. The HMBC correlations of Me-19 to C-1/C-5/C-9/C-10 determined the location of Me-19 at C-10. The Me-18 group was determined to be attached to C-13 by HMBC correlations of

Me-18 to C-12/C-13/C-14/C-17. The remaining four methyl groups could be positioned at C-20, C-24, and C-25 on the basis of the HMBC correlations between Me-21 and C-20/C-17/C-22; between Me-28 and C-23/C-24/C-25; between Me-26 and C-25/C-27/C-24; and between Me-27 and C-25/C-26/C-24, and the correlation of the respective methyl carbons (Me-26 and Me-27) to each other. Based on these data and compared to the reported studies [11], the structure of **1** was determined as ergosta-4,6,8(14),22-tetraen-3-one.

Compound **2** was obtained as a colorless oil. Its positive ESI-MS showed the pseudomolecular ion peak $[M+H]^+$ at *m/z* 221. The ^{1}H NMR spectrum of **2** showed three methyl signals at δ_H 1.04 (3H, s, Me-12), 1.06 (3H, s, Me-13), 1.28 (3H, s, Me-15), one sp^2 methylene group at δ_H 4.66 (1H, br s, H-14a), 4,69 (1H, br s, H-14b). The ^{13}C NMR spectrum of **2** exhibited 15 carbon signals, including three carbon methyls at δ_C 16.3 (C-13), 26.0 (C-15), and 28.6 (C-12), one sp^2 methylene signals at δ_C 106.2 (C-14), four sp^3 methylene signals at δ_C 24.8 (C-3), 26.7 (C-7), 38.9 (C-4), 41.8 (C-8), two sp^3 methine carbons at δ_C 53.4 (C-6), 54.3 (C-10) together with three non-protonated carbons at δ_C 20.3 (C-11), 81.0 (C-9), and 153.4 (C-5). The COSY spectrum established one spin-spin coupling system by the correlations of H-8/H-7/H-6/H-10/H-1/H-2/H-3/H-4 and was also indicated in the structure of **2**. The NMR data of **2** was characteristic of a tricyclic sesquiterpene which is similar to spathulenol. The HMBC cross-peaks of H-14 with C-6/C-5/C-4 suggested the position of the sp^2 methylene group at C-5. The HMBC correlations of Me-15 with C-8/C-9/C-10 demonstrated the placement of this methyl group at C-9. In addition, the HMBC correlations of CH_3-12 and CH_3-13 to C-11/C-1/C-2 determined the locations of CH_3-12 and CH_3-13 at C-11 (Fig. 2). Thus, compound **2** was identified as spathulenol based on comparing the reported data [12] and NMR spectral data of **2**.

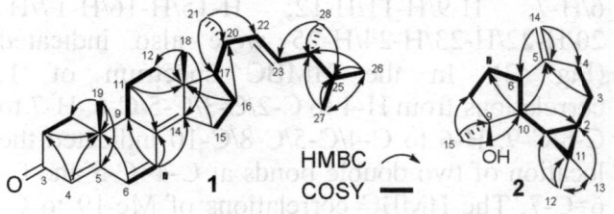

Fig. 2. Key COSY and HMBC correlations of **1** and **2**

Compound **3** was isolated as a yellow solid. The molecular ion peak $[M+H]^+$ was observed at *m/z* 331 in the ESI-MS. The ^{1}H NMR spectrum of **3** showed an ABX aromatic ring system at δ_H 6.85 (1H, d, J = 8.0 Hz, H-5'), 6.96 (1H, dd, J = 1.5, 8.0 Hz, H-6'), 7.05 (1H, d, J = 1.5 Hz, H-2'), two *meta* aromatic protons at δ_H 6.11 (1H, d, J = 2.5 Hz, H-6), 6.17 (1H, d, J = 2.5 Hz, H-8) and two methoxy signals at δ_H 3.77 (3H, s, 5-OCH_3) and 3.79 (3H, s, 7-OCH_3). The presence of one methylenedioxy group at δ_H 5.98 (s, OCH_2O), two oxygenated methine groups, and one methylene group in the aliphatic region was also noted. The ^{13}C NMR and DEPT of **3** showed 18 carbon signals including two methoxy groups at δ_C 55.4 (7-OCH_3), 55.4 (5-OCH_3), one dioxymethylene group at δ_C 101.1 (OCH_2O), one methylene group at δ_C 28.2 (C-4), two oxygenated methine group at δ_C 78.5 (C-2), 66.5 (C-3), five sp^2 methine group and seven non-protonated carbon signals. The *cis* configuration of H-2 and H-3 positions was determined on the basis of singlet signals of H-2 (δ_H 4.93, s) and H-3 (δ_H 4.25, br s) (in the case of *trans* configuration, the proton coupling constant between H-2 and H-3 was larger (J ~ 7.5-8.5 Hz) [12]). The structure of **3** was determined as 5,7-dimethoxy-3',4'-methylenedioxyflavan-3-ol, which was confirmed by comparison with spectroscopic data in the literature [13].

Compound **4** was isolated as a yellow solid. Its positive ESI-MS showed the pseudomolecular ion peak $[M+H]^+$ at *m/z* 433. The ^{1}H NMR spectrum of **4** revealed the proton signals of an A_2B_2 system at δ_H 7.77 (2H, d, J = 8.5 Hz, H-2', H-6'), 6.95 (2H, d, J = 8.5 Hz, H-3', H-5'), 2 aromatic protons in *meta* position at δ_H 6.21 (1H, d, J = 1.5 Hz, H-6), 6.37 (1H, d, J = 1.5 Hz, H-8), an anomeric proton at δ_H 5.39 (1H, d, J = 2.0 Hz, H-1"), four oxygenated signals at δ_H 3.34-4.25, and one methyl signal at δ_H 0.95 (3H, d, J = 5.5 Hz, H-6"). It suggested to present an α-L-rhamnopyranosyl moiety. In the ^{13}C NMR spectrum, six carbon signals at δ_C 17.6 (C-6"), 71.9 (C-5"), 72.0 (C-2"), 72.1 (C-3"), 73.2 (C-4"), and 103.5 (C-1") were attributed to a rhamnopyranoside fragment, 14 aromatic carbon signals and one carbonyl carbon were assigned for a flavonoid skeleton. By comparison of these spectral data with those in the literature [14], compound **4** was identified as kaempferol 3-*O*-α-L-rhamnoside or afzelin.

Compound **5** was obtained as a white powder. Its positive ESI-MS showed the pseudomolecular ion peak $[M+H]^+$ at *m/z* 195. The ^{1}H NMR

spectrum of **5** revealed the signals of an ABX system at δ_H 6.82 (1H, d, J = 8.0 Hz, H-5), 6.92 (1H, dd, J = 1.5, 8.5 Hz, H-6), 6.95 (1H, d, J = 1.5 Hz, H-2), two methoxy groups at δ_H 3.88 (3H, s, 3-OCH_3), 3.90 (3H, s, 4-OCH_3), one oxygenated methylene group at δ_H 4.31 (2H, m, H-9) and two olefinic proton signals at δ_H 6.25 (1H, dt, J = 6.0, 16.0 Hz, H-8), 6.55 (1H, d, J = 16.0 Hz, H-7). The coupling constants (J = 16.0 Hz) indicated the *trans*-orientation of the double bond. The ^{13}C NMR spectrum of **5** exhibited eleven signals, including one oxygenated methylene at δ_C 63.8 (C-9), two methoxy groups at δ_C 55.8 (3-OCH_3), 55.9 (4-OCH_3), five sp^2 methine groups at δ_C 108.9 (C-5), 111.2 (C-2), 119.7 (C-6), 126.5 (C-8), 131.2 (C-7), and three non-protonated carbons. The structure of **5** was determined as 3,4-dimethoxycinnamyl alcohol by comparing these spectral data to the literature [15].

Signals of the ^1D NMR spectra of **6** were close to those of **5**, except for the absence of one oxygenated methylene group and additional signals of one CHO group [δ_H 9.66 (1H, d, J = 8.0 Hz, H-9), δ_C 193.5 (C-9)]. By comparison of these spectral data with those in the literature [16, 17], compound **6** was identified as 3,4-dimethoxycinnamaldehyde.

In addition, the other two phenolic compounds were isolated and elucidated as veratraldehyde (**7**) [18] and veratric acid (**8**) [19] by comparing their NMR data with references.

Compounds **1-4** were evaluated for their cytotoxic activity against KB, MCF-7, HepG-2, and SK-LU-1 human cancer cell lines. Among the tested compounds, compounds **2** and **4** did not show activity against all the tested cell lines. Compound **1** showed activity against HepG-2 cell line with an IC_{50} value of 21.8 µM. Compound **3** showed a weak cytotoxic activity against KB, MCF-7, HepG-2, and SK-LU-1 human cancer cell lines with IC_{50} values of 118.3, 110.7, 90.2, and 95.8 µM, respectively. These activity test results are also consistent with the activity test results on cancer cell lines given according to published documents. Compound **2** was not active against the MCF-7 cell line [21]. Compound **4** did not show activity against KB, MCF-7, HepG-2, and SK-LU-1 human cancer cell lines [22].

4. Conclusion

The investigation of the chemical composition of the non-alkaloid extract of *C. bejolghota* led to the isolation of one ergostane-type steroid, an aromadendrane-type sesquiterpenoid, two flavonoids, together with four other phenolic compounds. Compounds **2** and **4** did not show activity against all the tested cell lines. Compound **1** showed activity against HepG-2 with an IC_{50} value of 21.8 µM. Compound **3** showed weak cytotoxic activity against KB, MCF-7, HepG-2, and SK-LU-1 human cancer cell lines. To the best of our knowledge, compounds **1** and **4** were reported from this plant for the first time.

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ACETYLCHOLINESTERASE INHIBITORY ACTIVITY OF *EMBELIA RIBES* BURM. F. FRUITS COLLECTED IN SOUTHERN VIETNAM AND ITS CHEMICAL COMPONENTS

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Summary

Acetylcholinesterase Inhibitory Activity of *Embelia ribes* Burm. f. Fruits Collected in Southern Vietnam and its Chemical Components

Extracts from *Embelia ribes* Burm. f. fruits in southern Vietnam showed good effects on acetylcholinesterase (AChE) inhibition, with IC_{50} values of 0.23-0.45 mg/ml. Among them, the dichloromethane fraction showed the potent inhibitory activity. The chemical investigation of this fraction resulted in the isolation of five compounds (**1-5**). Their chemical structures were identified as kaempferol (**1**), quercetin (**2**), hydroxymethylfurfural (**3**), 5-(8′-*Z*-heptadecenyl)resorcinol (**4**), and embelin (**5**) based on spectral data analysis and literature references. The *in vitro* AChE inhibitory activity shows embelin may be a principal compound in the most active dichloromethane fraction.

Keywords: *Embelia ribes*, *Acetylcholinesterase, Embelin.*

1. Introduction

Embelia ribes Burm. f. (*E. ribes*) is a woody shrub that belongs to the family Myrsinaceae. The distribution area of this species is relatively wide, and the plant is present in many Southeast Asian and South Asian countries, such as Cambodia, Laos, Thailand, Vietnam, India, and China [1]. In Vietnam, *E. ribes* appears broadly from north to south. It has been used as a Vietnamese traditional medicine for the treatment of inflammatory, bacterial, dental, oral, and throat troubles, pain relief, skin diseases, and parasitic worms [2],[3]. Extracts and embelin isolated from *E. ribes* showed reversible contraceptive effects [3]. *E. ribes* and its main constituent, embelin, have attracted much attention for their effects, such as in the treatment of cancer [4], diabetes [5], and central nervous system diseases [6]. Some studies showed multiple effects of embelin, so it can be considered a potential drug candidate in modern medicine [7],[8]. Recent studies showed that *E. ribes* ethanolic extract and embelin had a significant *in vivo* neuroprotective effect. In particular, the effects of improving the learning ability of *E. ribes*, such as anti-depression and anti-memory decline, e.g., in Alzheimer's disease must be mentioned [9],[10]. More recent studies identified embelin from *E. ribes* as a multi-targeted anti-Alzheimer agent that plays a role in stopping the formation of amyloid-β oligomers and improving cholinergic transmission [11],[12].

Studies on chemical components showed that *E. ribes* fruits contain benzoquinones, benzofurans, resorcinol derivatives, and some