Trop J Nat Prod Res, June 2023; 7(6):3147-3152 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Chemical Constituents and Biological Activities of *Commersonia bartramia* Stems

Kieu C. Nhung[1,2], Tran T. Bach[1,3], Vu T. Hue[4,5], Bui T. Ha[5], Le N. Thanh[1,4]*

[1]*Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Caugiay, Hanoi 10000, Vietnam*
[2]*Ministry of Science and Technology, Hanoi 10000, Vietnam*
[3]*Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Caugiay, Hanoi 10000, Vietnam*
[4]*Institute of Marine Biochemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Caugiay, Hanoi 10000, Vietnam*
[5]*Faculty of Biology, Hanoi National University of Education, Hanoi 10000, Vietnam*

ARTICLE INFO

Article history:
Received 08 April 2023
Revised 31 May 2023
Accepted 05 June 2023
Published online 01 July 2023

ABSTRACT

Commersonia bartramia (Sterculiaceae family) is a wooden tree, widely occurring in Australia, China and South East Asia. The MeOH extract of *C. bartramia* has been reported to show anticancer and antioxidant activities. Phytochemical investigation of *C. bartramia* stems led to the isolation of eight compounds. Their chemical structures were identified as helichrysoside-3′-methyl ether (1), tiliroside (**2**), pinoresinol **(3)**, ursolic acid (4), quercetin (5), kaempferol (6), vanillic acid (7) and 4-hydroxybenzoic acid (8) using NMR and MS spectral analysis. This is the first study about the chemical composition of *C. bartramia*. Among the isolated compounds, quercetin (5) showed good DPPH's radical scavenging activity with IC_{50} values of 11.43 ± 0.95 µg/mL, whereas the MeOH extract, (+)-pinoresinol (3) and kaempferol (6) displayed moderate activity. Quercetin (5) also displayed moderate cytotoxicity with IC_{50} values of 43.64 ± 3.63 to 61.58 ± 5.54 µg/mL. The MeOH extract exhibited weak cytotoxicity against HepG2, MCF7 and A549 cell lines.

Keywords: *Commersonia bartramia*, flavonoids, lignan, triterpene, phenolic

Introduction

Commersonia (Sterculiaceae family) is a genus consisting over 30 species distributed in the South East Asia, South West Pacific with the majority of its species found in Australia. *C. bartramia* is a wooden tree, widely occurring in Australia, China and South East Asian countries like Indonesia, Malaysia and Vietnam.[1] Despite wide distribution, studies about chemical composition and biological activity of plants in *Commersonia* genus have been very limited. A biological study of plants in Philippine showed that the alcoholic extract of *C. bartramia* displayed toxicity in the brine shrimp bioassay as well as inhibition of crown gall tumor growth.[2] Recently, Kim et al. have reported the study of anticancer activity of *C. bartramia* MeOH extract against several cancer cell lines. The *C. bartramia* extract showed 10% cytotoxicity at concentrations of 100 µg/mL, and 4-6% at concentrations ranging from 15-50 µg/mL. It's noted that, the plant extract showed a greater cytotoxic effect on four types of cancer cell lines compared to normal cell lines.[3] Besides that, in 2021 Kadir has documented that the extract of *C. bartramia* exhibited 49.3% radical scavenging activity in DPPH assay.[4]

In the screening program of anticancer activity of Vietnamese plants, the MeOH extract of *C. bartramia* stems also showed cytotoxic activity against HepG2, MCF7 and A549 cancer cell lines with IC_{50} values ranging from 106.09 ± 4.74 µg/mL to 116.42 ± 5.96 µg/mL. Therefore, chemical investigation of *C. bartramia* stems was carried out. Eight compounds 1-8 were isolated and elucidated by NMR and MS spectral analysis and comparison with reported data. The isolated compounds were evaluated for cytotoxic and antioxidant activities.

*Corresponding author. E mail: lethanh@imbc.vast.vn
Tel: +84983882573

Citation: Nhung KC, Bach TT, Hue VT, Ha BT, Thanh LN. Chemical Constituents and Biological activities of *Commersonia bartramia* Stems. Trop J Nat Prod Res. 2023; 7(6): 3147-3152 http://www.doi.org/10.26538/tjnpr/v7i6.13

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Material and Methods

Plant materials

The plant stems were collected at Me Linh district, Vinh Phuc province, Vietnam (GPS: 21°21'38.8"N 105°44'55.9"E) in January 2022 and identified as *Commersonia bartramia* (L.) Merr. (Sterculiaceae) by Prof. Tran The Bach, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology and Dr. Bui Thu Ha, Faculty of Biology, Hanoi National University of Education. A voucher specimen (KCN-01) was preserved at the Institute of Ecology and Biological Resources.

General experimental procedures

Optical rotations were recorded using a JASCO P-2000 polarimeter (JASCO, Tokyo, Japan). Melting point were recorded on a Melting points were measured with a Mel-Tem 3.0 apparatus (Thermo Fisher Scientific, USA). NMR spectra were recorded by a Bruker AVANCE III HD 500 MHz or Bruker AVANCE NEO 600 MHz spectrometers (Bruker, Billerica, MA, USA) using TMS as an internal standard. The electrospray ionization mass spectra (ESI-MS) were obtained on an Agilent 1260 series single quadrupole LC/MS system (Agilent, CA, USA). Column chromatography (CC) was performed on silica gel (Merck, 230-400 mesh), reversed phase C18 (YMC, RP-18, 150 µm), Sephadex® LH20, or Diaion HP-20 resins. Thin layer chromatography was performed using precoated silica gel plates (Merck 60 F_{254}). Preparative HPLC was obtained on an Agilent 1260 infinity II system using DAD detector and YMJH08S04 column (20 × 250 mm) (Agilent, CA, USA). Compounds were visualized by spraying with 10% sulfuric acid and heating. All solvents used were laboratory grade reagents and were distilled prior to use.

Extraction and isolation

The dried, powdered *C. batramia* stems (5 kg) was macerated three times with methanol (MeOH) (each time 20 L for 24 hours) at room temperature. The combined extracts were filtered and concentrated under reduced pressure. The residue was suspended in water (1 L) and the suspension was successively partitioned with hexane and ethyl acetate (EtOAc). After evaporation of solvents *in vaccuo*, the crude extract of hexane fraction (20.1 g), EtOAc fraction (E, 29.3 g) and water layer were obtained. The water layer was subjected to diaion HP-20 column, eluted with a solvent system of MeOH: H_2O (0:1-1:0, v/v) to give MeOH fraction (W3, 4.1 g).

The EtOAc extract (29.3 g) was subjected to silica gel CC and eluted with a gradient solvent system of hexane/EtOAc (100:1 – 0:1) to afford 16 fractions E1–E16, respectively. Fraction E8 (0.62 g) was fractionated by silica gel CC eluting with hexane/EtOAc (8:2, v/v) to give six fractions E8.1-E8.6. Compound 4 (5.2 mg) was obtained from fraction E8.3 by recrystallization in acetone. Fraction E9 (1.10 g) was separated by silica gel CC, and eluted with hexane/acetone (9:1, v/v) to give nine fractions E9.1-E9.9. Compound 8 (3.6 mg) was obtained from fraction E9.9 by recrystallization in CH_2Cl_2. Fraction E9.7 (0.48 g) was chromatographed by silica gel CC, eluted with CH_2Cl_2/acetone (19:1, v/v) to afford five smaller fractions E9.7.1-E9.7.5. Fraction E9.7.5 (64 mg) was purified by silica gel CC, eluted with CH_2Cl_2/acetone (19:1, v/v) to yield compound 7 (3.4 mg). Fraction E11 (0.62 g) was fractionated by silica gel CC eluting with hexane/EtOAc (8:2, v/v) to give six fractions E11.1-E11.6. Fraction E11.3 (64 mg) was purified by Sephadex® LH-20 CC, eluted with MeOH/CH_2Cl_2 (9:1, v/v) to yield compound 3 (3.2 mg). Fraction E13 (1.90 g) was separated with Sephadex® LH-20 CC and eluted with MeOH/CH_2Cl_2 (9:1, v/v) to afford seven fractions E.13.1-E13.7. Fraction E13.2 (133 mg) was purified by Sephadex® LH-20 CC and eluted with MeOH to afford compound 6 (3.3 mg). Fraction E13.7 (15 mg) was further purified by Sephadex® LH-20 CC and eluted with MeOH to afford compound 5 (4.5 mg).

Fraction W3 (4.1 g) was subjected silica gel CC and eluted with a gradient solvent system of EtOAc/acetone (100:1 – 0:1) to afford 4 fractions W3.1–W3.4. Fraction W3.2 (1.3 g) was purified by Sephadex® LH-20 CC and eluted with MeOH to give five sub-fractions W3.2.1-W3.2.5. Fraction W3.2.4 (0.12 g) was chromatographed on reversed phase silica gel CC eluting with acetone/water (1:2, v/v) to afford three sub-fractions W3.2.4.1- W3.2.4.3. Fraction W3.2.4.3 (15 mg) was purified by preparative HPLC eluting with 30 % acetonitrile in water to obtain compound 1 (2,7 mg, t_R = 49.37 min) and 2 (2.1 mg, t_R = 52.24 min) (Figure S27).

Cytotoxic assay

Cytotoxic activities of MeOH extract and isolated compounds against HepG2, MCF7 and A549 cancer cell lines (ATCC) were evaluated using the MTT assay, that was documented in the previous publication.[5] Briefly, cells were maintained in Dulbecco's D-MEM medium (Sigma), supplemented with 10% fetal calf serum, L-glutamine (2 mM), penicillin G (100 UI/mL) and streptomycin (100 μg/mL) (Sigma). The isolated compounds were diluted in dimethyl sulfoxide (Merck) at the following concentrations: 128 µg/mL, 32 µg/mL, 8 µg/mL, 2 µg/mL, and 0.5 µg/mL, and used for the cytotoxicity assays. After 48 h incubation at 37 °C in air/CO_2 (95:5) with or without test compounds, MTT reagent (Sigma) was added to the each well (0.5 mg/mL). Cell growth was estimated by colorimetric measurement of formazan. Optical density was determined at 570 nm using a microplate reader. The IC_{50} value was defined as the concentration of a sample necessary to inhibit the cell growth to 50% of the control. Ellipticine was used as a positive control.

Antioxidant activity

The ability of compounds to scavenge the DPPH radicals was carried out according to the previously described method.[6] Compounds and MeOH extract were dissolved in DMSO (Merck) and diluted into concentrations at concentrations of 128 µg/mL, 32 µg/mL, 8 µg/mL, 2 µg/mL, and 0.5 µg/mL. In each well of the 96-well microplate, 10 µl sample was incubated with 200 µl DPPH (Merck) (0.1 mM in MeOH) at 25°C for 30 minutes. The absorbance was measured by Biotek spectrophotometer at 517 nm. The percentage of DPPH quenching activity was calculated by the following formula:

Inhibitory percentage SC (%) = [(Ao -A_1)/Ao] ×100.

where A_O was defined as the absorbance of control reaction, and A_1 represented for the absorbance in the presence of test or standard sample.

Each experiment was repeated three times with resveratrol serving as the positive control. The EC_{50} value, also known as the concentration of tested samples that induced half maximal response has been calculated from linear regression of the serial SC values versus the concentrations.

Statistical analysis

In the cytotoxicity and antioxidant assays, the IC_{50} and EC_{50} are presented as the mean ± standard deviation (S.D) by using Microsoft Excel (Microsoft Corporation, 2020). The statistical significance of all treatment effects was evaluated by Student's t-test with a probability limit for the significance of $p < 0.05$, $p < 0.001$.

Results and Discussion

Chemical structure identification

The MeOH extract was purified by combined chromatographic methods (silica gel CC, C-18 RP silica gel CC, Sephadex® LH-20 CC, preparative HPLC) to obtain eight compounds (1–8; Figure 1).

Characterisation of compounds 1−8

Helichrysoside-3'-methyl ether (1): White solid. R_f: 0.28 (acetone/water: 1/1). ESI-MS *m/z* 623 $[M-H]^-$. ^{1}H-NMR (600 MHz, CD_3OD) δ (ppm): 7.87 (1H, d, *J* = 1.8 Hz, H-2′), 7.58 (1H, dd, *J* = 1.8, 8.4 Hz, H-6′), 7.40 (1H, d, *J* = 15.6 Hz, H-7‴), 7.32 (2H, d, *J* = 9.0 Hz, H-2‴, H-6‴), 6.86 (1H, d, *J* = 8.4 Hz, H-5′), 6.82 (2H, d, *J* = 9.0 Hz, H-3‴, H-5‴), 6.30 (1H, br s, H-8), 6.15 (1H, d, *J* = 1.2 Hz, H-6), 6.08 (1H, d, *J* = 15.6 Hz, H-8‴), 5.32 (1H, d, *J* = 7.9 Hz, H-1″), 4.32-4.25 (2H, m, H-6″), 3.54-3.48 (3H, m, H-2″, H-3″, H-5″), 3.93 (3H, s, OMe), 3.36 (1H, m, H-4″). ^{13}C-NMR (150 MHz, CD_3OD) δ (ppm): 179.4 (C-4), 168.8 (C-9‴), 167.0 (C-7), 163.9 (C-5), 161.2 (C-4‴), 158.8 (C-2), 157.6 (C-9), 150.9 (C-4′), 148.3 (C-3′), 146.6 (C-7‴), 135.2 (C-3), 131.2 (C-2‴, C-6‴), 127.1 (C-1‴), 123.9 (C-6′), 123.0 (C-1′), 116.8 (C-3‴, C-5‴), 116.0 (C-5′), 114.6 (C-8‴), 114.3 (C-2′), 105.2 (C-10), 104.0 (C-1″), 100.4 (C-6), 95.1 (C-8), 78.0 (C-3″), 75.8 (C-5″, C-2″), 71.9 (C-4″), 64.3 (C-6″), 56.7 (OMe).

Tiliroside (2): Pale yellow solid, R_f: 0.28 (acetone/water: 1/1). ESI-MS *m/z* 593 $[M-H]^-$. ^{1}H-NMR (600 MHz, CD_3OD) : δ (ppm): 8.01 (2H, d, *J* = 8.4 Hz, H-2′, H-6′), 7.42 (1H, d, *J* = 15.6 Hz, H-7‴), 7.33 (2H, d, *J* = 9.0 Hz, H-2‴, H-6‴), 6.84 (2H, d, *J* = 8.4 Hz, H-3′, H-5′), 6.82 (2H, d, *J* = 8.4 Hz, H-3‴, H-5‴), 6.32 (1H, br s, H-8), 6.15 (1H, br, H-6), 6.09 (1H, d, *J* = 15.6 Hz, H-8‴), 5.24 (1H, d, *J* = 7.9 Hz, H-1″), 4.32-4.19 (2H, m, H-6″), 3.49-3.47 (3H, m, H-2″, H-3″, H-5″), 3.33 (1H, overlapped, H-4″). ^{13}C-NMR (150 MHz, CD_3OD) δ (ppm): 179.3 (C-4), 168.8 (C-9‴), 166.9 (C-7), 163.5 (C-5), 161.4 (C-4′), 161.2 (C-4‴), 159.2 (C-2), 158.5 (C-9), 146.6 (C-7‴), 135.2 (C-3), 132.2 (C-2′,C-6′), 131.2 (C-2‴,C-6‴), 127.1 (C-1‴), 122.8 (C-1′), 116.8 (C-3‴, C-5‴), 116.0 (C-3′, C-5′), 114.7 (C-8‴), 105.1 (C-10), 104.0 (C-1″), 100.3 (C-6), 94.9 (C-8), 78.0 (C-3″), 75.8 (C-5″), 75.7 (C-2″), 71.7 (C-4″), 64.3 (C-6″).

(+)-Pinoresinol (3): White solid, $[\alpha]_D^{25}$: 70° (*c* 0.2, $CHCl_3$). R_f: 0.57 (hexane/acetone: 8/2). mp: 120-122 °C. ESI-MS *m/z* 359 $[M+H]^+$. ^{1}H-NMR (500 MHz, $CDCl_3$), δ (ppm): 6.90 (2H, d, *J* = 2.0 Hz, H-2, H-2′), 6.88 (2H, d, *J* = 8.0 Hz, H-5, H-5′), 6.82 (2H, dd, *J* = 1.5 Hz, *J* = 8.0 Hz, H-6, H-6′), 5.68 (2H, s, OH), 4.73 (2H, d, *J* = 4.5 Hz, H-7, H-7′), 4.25 (2H, q, *J* = 7.0 Hz, 9.0 Hz, H-9*β*), 3.90 (6H, s, OMe), 3.89 (2H, q, *J*= 4.0 Hz, *J*= 9.5 Hz, H-9*α*), 3.11 (2H, m, H-8, H-8′). ^{13}C-NMR (125MHz, $CDCl_3$), δ (ppm): 146.7 (C-4, C-4′), 145.3 (C-3, C-3′), 132.9 (C-1, C-1′), 119.0 (C-6, C-6′), 114.3 (C-5, C-5′), 108.6 (C-2, C-2′), 85.9 (C-7, C-7′), 71.7 (C-9, C-9′), 55.9 (OMe), 54.1 (C-8, C-8′).

Ursolic acid (4): Pale yellow solid. R_f: 0.57 (hexane/acetone: 8/2). mp: 286-288 °C. ESI-MS *m/z* 455 $[M-H]^-$. ^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 5.25 (1H, t, *J* = 3.5 Hz, H-12), 3.17 (1H, dd, *J* = 4.5 Hz, *J* = 11.5 Hz, H-3), 1.14 (3H, s, H-27), 1.00 (3H, s, H-23), 0.99 (3H, d, *J* = 6.5 Hz, H-30), 0.98 (3H, s, H-24), 0.91 (3H, d, *J* = 6,5 Hz, H-29) 0.87 (3H,

s, H-26), 0.70 (3H, s, H-24). ^{13}C-NMR (125 MHz, CD_3OD): 180.4 (C-28), 139.7 (C-13), 126.9 (C-12), 79.7 (C-3), 56.7 (C-5), 54.4 (C-18), 49.4 (C-9), 49.0 (C-17), 43.2 (C-14), 40.8 (C-8), 40.4 (C-19), 40.4 (C-20), 40.0 (C-22), 39.8 (C-4), 39.8 (C-10), 38.1 (C-1), 34.3 (C-7), 31.8 (C-21), 29.2 (C-15), 28.8 (C-23), 27.9 (C-2), 25.3 (C-16), 24.4 (C-11), 24.1 (C-27), 21.5 (C-30), 19.5 (C-6), 17.8 (C-26), 17.6 (C-29), 16.4 (C-25), 16.0 (C-24).

Quercetin (5): Yellow solid. mp: > 300 ºC. ESI-MS *m/z* 303 $[M + H]^+$. ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 12.46 (1H, s, 5-OH), 7.67 (1H, d, *J* = 2.0 Hz, H-2'), 7.54 (1H, dd, *J* = 2.0 Hz, 8.4 Hz, H-6'), 6.88 (1H, d, *J* = 8.4 Hz, H-5'), 6.40 (1H, d, *J* = 2.0 Hz, H-8), 6.18 (1H, d, *J* = 2.0 Hz, H-6).^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 176.4 (C-4), 164.4 (C-7), 161.3 (C-5), 156.7 (C-9), 148.2 (C-2), 147.3 (C-4'), 145.6 (C-3'), 136.2 (C-3), 122.4 (C-1'), 120.5 (C-6'), 116.1 (C-5'), 115.6 (C-2'), 103.5 (C-10), 98.6 (C-6), 93.8 (C-8).

Kaempferol (6)*:* Yellow solid. mp: 272-275 ºC. ESI-MS *m/z* 287 $[M + H]^+$. ^{1}H-NMR (600 MHz, acetone-d_6) δ (ppm): 8.06 (2H, dd, *J* = 9.0 Hz; 2.5 Hz, H-2', 6'), 7.01 (2H, dd, *J* = 9.0 Hz, 2.0 Hz, H-3', 5'), 6.62 (1H, s, H-8), 6.23 (1H, s, H-6).^{13}C-NMR (150 MHz, acetone-d_6) δ (ppm): 183.2 (C-4), 165.2 (C-7), 162.0 (C-5), 158.7 (C-4′), 158.5 (C-5), 150.9 (C-2), 136.5 (C-3), 129.6 (C-2′, 6′), 123.3 (C-1′), 116.8 (C-3′, C-5′), 105.3 (C-10), 103.6 (C-6), 100.6 (C-8).

Vanillic acid (7)*:* Brown solid. R_f: 0.41 (hexane/acetone: 6/4). mp: 207-210 ºC. ESI-MS (*m/z*): 167 $[M-H]^-$. ^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 7.58-7.56 (2H, m, H-2, H-6), 6.86 (1H, d, *J* = 8.5 Hz, H-5), 3.91 (s, 3H). ^{13}C-NMR (125 MHz, CD_3OD), δ (ppm): 170.1 (COOH), 152.6 (C-3), 148.6 (C-4), 125.2 (C-6), 123.1 (C-1), 115.8 (C-5), 113.8 (C-2), 56.4 (OMe).

4-Hydroxybenzoic acid (8)*:* Brown solid. R_f: 0.3 (CH_2Cl_2/acetone: 5/1). mp: 211-214 ºC. ESI-MS *m/z* 137 $[M-H]^-$. ^{1}H-NMR (500MHz, CD_3OD) δ (ppm): 7.90 (2H, d, *J* = 8.0 Hz, H-2, H-6), 6.83 (2H, d, *J* = 8.0 Hz, H-3, H-5). ^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 169.9 (COOH), 163.1 (C-4), 133.0 (C-2, C-6), 122.6 (C-1), 116.0 (C-3, C-5).

Compound 1 was isolated as a white solid. The ESI-MS spectrum revealed a *pseudo*-molecular ion at *m/z* 623 $[M-H]^-$ (Figure S1), suggested the molecular formula of 1 is $C_{31}H_{28}O_{14}$ (M= 624). The ^{1}H NMR spectrum showed characteristic signals of a flavononol glycoside with 2 *meta*-aromatic protons at δ_H 6.30 (1H, br s, H-8) and 6.15 (1H, d, *J* = 1.8 Hz, H-6), three protons of an ABX system at δ_H 7.87 (1H, d, *J* = 1.8 Hz, H-2′), 7.58 (1H, dd, *J* = 1.8, 8.4 Hz, H-6′), 6.86 (1H, d, *J* = 8.4 Hz, H-5′). Sugar part was observed with anomer proton at δ_H 5.32 (1H, d, *J* = 7.9 Hz, H-1″) and other protons at δ_H 4.32-4.25 (2H, m, H-6″), 3.54-3.48 (3H, m, H-2″, H-3″, H-5″). In addition, a *trans-p*-coumaroyl moiety was revealed with 2 olefinic protons at δ_H 7.40 (1H, d, *J* = 15.6 Hz, H-7‴) and 6.08 (1H, d, *J* = 15.6 Hz, H-8‴), four proton of an A_2B_2 system at δ_H 7.32 (2H, d, *J* = 9.0 Hz, H-2‴, H-6‴) and 6.82 (2H, d, *J* = 9.0 Hz, H-3‴, H-5‴). A methoxy group was found at δ_H 3.93 (3H, s, OMe) (Figure S2). The ^{13}C NMR showed thirty carbon signals including fifteen signals of quercetin structure with a carbonyl carbon at δ_C 179.4 (C-4) and 14 aromatic carbon; nine carbon signals of a *trans-p*-coumaroyl moiety at δ_C 168.8 (C-9‴), 146.6 (C-7‴), 131.2 (C-2‴, C-6‴), 127.1 (C-1‴), 116.8 (C-3‴, C-5‴) and 114.6 (C-8‴), six carbons of a sugar at δ_C 104.0 (C-1″), 78.0 (C-3″), 75.8 (C-5″, C-2″), 71.9 (C-4″) and 64.3 (C-6″) and a methoxy group at δ_C 56.7 (Figure S3). The methoxy group was confirmed at C-3′ by the HMBC correlation of methoxy signal (δ_H 3.78) to C-3′ (δ_C 147.7). The anomer proton H-1″ correlated with C-3 of 3′-methyl quercetin aglycone. HMBC cross peaks of H-6″ (δ_H 4.32-4.25) to C-9‴ (δ_C 168.8) indicated that ester group at hydroxyl group of C-6″ (Figure 2 and S5). Therefore, compound 1 was assigned as helichrysoside-3′-methyl ether, which was only isolated from *Croton* species (Euphorbiaceae).[7,8] Helichrysoside-3′-methyl ether (1) showed potent antioxidant activity against *β*-carotene bleaching. This compound also displayed weak acetylcholinesterase (AChE) inhibitory activity.[8]

Compound 2 was obtained as a pale yellow solid. The ESI-MS (Figure S6) showed a *pseudo*-molecular ion at *m/z* 593 $[M-H]^-$, corresponding to $C_{30}H_{26}O_{13}$ molecular formula. The NMR spectra (Figure S7, S8) of 2 showed signals of a flavononol glucoside, that were similar to those of compound 1, except quercetin aglycon part was replaced by kaempferol structure. Four protons of an A_2B_2 system was revealed at δ_H 8.01 (2H, d, *J* = 8.4 Hz, H-2′, H-6′) and 6.84 (2H, d, *J* = 8.4 Hz, H-3′, H-5′). Compound 2 was determined as tiliroside by comparison of NMR data with those reported in literature.[9] Tiliroside has been found from *Croton* species[7,8] and several *Helicteres spp*. of the Sterculiaceae family.[10] Tiliroside showed better antioxidant and AChE inhibitory activity than those of helichrysoside-3′-methyl ether (1).[8] In addition, tiliroside exerted antiobesity, antidiabetic and anti-inflammatory effects.[11-13]

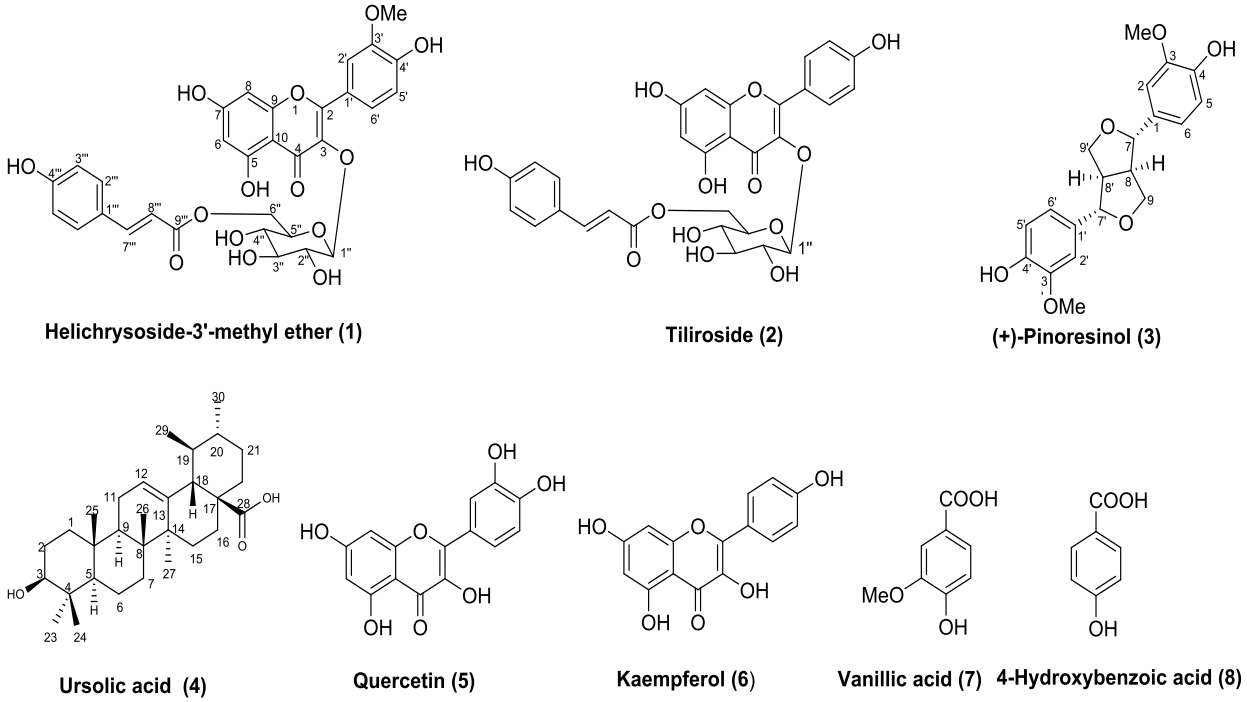

Figure 1: Chemical structures of isolated compounds 1-8

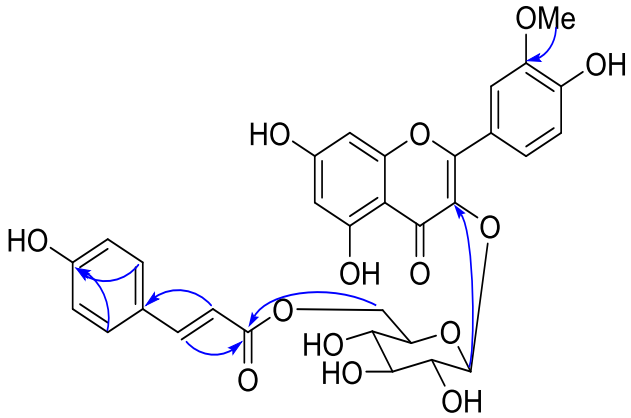

Figure 2: Key HMBC correlations of compound 1

Compound 3 was isolated as a white solid. The molecular formula of 3 was deduced as $C_{20}H_{22}O_6$ based on a protonated molecular ion peak at *m/z* 359 $[M+H]^+$ in the ESI-MS spectrum (Figure S9) and NMR data. The ^1H and ^{13}C NMR spectra (Figure S10-S12) revealed signals of eleven protons and ten carbons, respectively, therefore these signals are double peaks. In the NMR spectra, signals of two identical ABX systems (δ_H 6.90 (d, *J* = 2.0 Hz, H-2, H-2′)/δ_C 108.6, δ_H 6.88 (d, *J* = 8.0 Hz, H-5, H-5′)/δ_C 114.3, 6.82 (dd, *J* = 1.5 Hz, *J* = 8.0 Hz, , H-6, H-6′)/δ_C 119.0), two oxymethine protons (δ_H 4.73 (d, *J* = 4.5 Hz, H-7, H-7′)/δ_C 85.9); two oxymethylene protons (δ_H 4.25 and 3.89 (H-9, H-9′)/ δ_C 71.7), two methoxy groups (δ_H 3.90/δ_C 55.9) and two methine protons (δ_H 3.11 (H-8, H-8′)/δ_C 54.1) were observed. Comparing spectral and optical rotation data[14,15], compound 3 was identified as (+)-pinoresinol. (+)-Pinoresinol is a common lignan found in plants and posseses anti-inflammatory, anticancer, antioxidant properties.[16-18]

Compound 4 was obtained as a pale yellow solid. The ^1H NMR spectrum (Figure S14) showed typical signals of a ursane-type triterpene with seven methyl groups including five singlets at δ_H 1.14 (3H, s, H-27), 1.00 (3H, s, H-23), 0.98 (3H, s, H-24), 0.87 (3H, s, H-26), and 0.70 (3H, s, H-24) and two doublets at δ_H 0.99 (3H, d, *J* = 6.5 Hz, H-30) and 0.91 (3H, d, *J* = 6,5 Hz, H-29). In addition, an olefinic proton at δ_H 5.25 (1H, t, *J* = 3.5 Hz, H-12) and an oxymethine proton at δ_H 3.17 (1H, dd, *J* = 4.5 Hz, *J* = 11.5 Hz, H-3) were found. The ^{13}C NMR, DEPT spectra (Figure S15-16) exhibited thirty carbon signals including a carboxylic carbon at δ_C 180.4 (C-28), two olefinic carbons at δ_C 139.7 (C-13) and 126.9 (C-12), an oxymethine group at δ_C 79.7 (C-3), and seven methyl groups at δ_C 28.8 (C-23), 24.1 (C-27), 21.5 (C-30), 17.8 (C-26), 17.6 (C-29), 16.4 (C-25) and 16.0 (C-24). The ESI-MS spectrum revealed a *pseudo*-molecular ion at *m/z* 455 $[M-H]^-$, suggested the molecular formula of 4 is $C_{30}H_{48}O_3$ (Figure S13). Compound 4 was assigned as ursolic acid by the comparison of NMR data with those published.[19,20] Ursolic acid exhibited anticancer activity, and anti-inflammatory effects.[21]

Compounds 5-8 were elucidated as quercetin (5)[22], kaempferol (6)[22], vanillic acid (7)[23] and 4-hydroxy benzoic acid (8)[23] by comparison NMR and MS spectral data (Figure S17-S26) with those reported. Flavonoids quercetin and kaempferol are bioactive compounds, that showed antioxidant, antibacterial, cardioprotective and antihypertensive properties.[24,25] Phenolic acids 7 and 8 also possess antioxidant, cardioprotective and anti-inflammatory activities. [26,27] To the best of our knowledge, this is the first chemical report of *C. bartramia* plant.

Biological activities

The isolated compounds from the MeOH extract of *C. bartramia* stems were evaluated for cytotoxicity and antioxidant activity. As shown in the Table 1, the MeOH extract of *C. bartramia* exhibited moderate antixidant activity with IC_{50} of 54.27 ± 2.45 µg/mL. Among the isolated compounds, quercetin (5) showed good DPPH's radical scavenging activity with IC_{50} values of 11.43 ± 0.95 µg/mL, stronger than reference compound, reveratrol, whereas (+)-pinoresinol (3) and kaempferol (6) displayed moderate activity with IC_{50} values of 63.14 ± 3.59 µg/mL and 50.29 ± 3.54 µg/mL, respectively. Our antioxidant results were quite similar with previous reports.[3] Quercetin has been documented to have radical scavenging effect with EC_{50} of 8.1 µg/mL[28] or 14.52 ± 2.12 µg/mL,[29] that are close to our results. Aderogba *et al.* reported that helichrysoside-3′-methyl ether (1) and tiliroside (2) scavenged only 26.78% and 25.96% of the DPPH's free radicals at the concentration of 200 mM, respectively.[7] In our assay, helichrysoside-3′-methyl ether (1) and tiliroside (2) showed scavenging activity of 35.3% and 28.1% at the concentration of 128 µg/mL, respectively (Table S1).

In the cytotoxic test, the MeOH extract exhibited weak cytotoxicity against tested cell lines with IC_{50} values of 106.09 ± 4.74 to 116.42 ± 5.96 µg/mL (Table 1 and S2). Quercetin showed moderate cytotoxicity with IC_{50} values of 43.64 ± 3.63 to 61.58 ± 5.54 µg/mL, that are similar to results of Son and Anh.[29] In Aderogba's study, helichrysoside-3′-methyl ether (1) and tiliroside (2) displayed weak cytotoxicity against African green monkey (Vero) cells with IC_{50} values of 189.54 µg/mL and 169.45 µg/mL respectively.[7] Similarly, these two compounds (1-2) showed only 20.5-42.8% and 21.4-48.3% inhibition at the 128 µg/mL concentration, respectively on HepG-2, MCF-7 and A-549 cancer cell lines (Table S2). (+)-Pinoresinol (3) and ursolic acid (4) and have been reported to have different cytotoxic against various cancer cells[30,31] but in our cytotoxic assay, these isolated compounds were not active at the 128 µg/mL concentration (Table 1). Overall, quercetin was proven as an antioxidant and anticancer compound in our assays.

Table 1: Cytotoxicity and antioxidant activities of MeOH extract and isolated compounds 1-8

Compounds	IC_{50} (µg/mL)			EC_{50} (µg/mL)
	HepG2	MCF7	A549	DPPH' RSA
1	>128	>128	>128	>128
2	>128	>128	>128	>128
3	>128	>128	>128	63.14 ± 3.59
4	>128	>128	>128	>128
5	55.25 ± 3.88	61.58 ± 5.54	43.64 ± 3.63	11.43 ± 0.95
6	>128	>128	>128	50.29 ± 3.54
7	>128	>128	>128	>128
8	>128	>128	>128	>128
MeOH extract	116.42 ± 5.96	107.96 ± 8.23	106.09 ± 4.74	54.27 ± 2.45
Ellipticine	0.40 ± 0.03	0.32 ± 0.02	0.42 ± 0.04	-
Resveratrol	-	-	-	13.39 ± 0.86

Conclusion

A chemical investigation of *C. bartramia* stems led to the isolation of eight compounds including helichrysoside-3′-methyl ether (1), tiliroside (2), pinoresinol (3), ursolic acid (4), quercetin (5), kaempferol (6), vanillic acid (7) and 4-hydroxy benzoic acid (8). The structures were determined by MS and NMR spectral data and comparison with published literature. The chemical composition of a plant in the *Commersonia* genus was studied for the first time. Biological assays revealed that quercetin (5) showed good antioxidant activity with IC_{50} values of 11.43 ± 0.95 µg/mL and moderate cytotoxicity with IC_{50} values of 43.64 ± 3.63 to 61.58 ± 5.54 µg/mL. The MeOH extract of *C. bartramia* stems, (+)-pinoresinol (3) and kaempferol (6) displayed moderate antioxidant activity. The MeOH extract also exhibited weak cytotoxicity against HepG2, MCF7 and A549 cell lines with IC_{50} values of 106.09 ± 4.74 to 116.42 ± 5.96 µg/mL. Our findings suggest that the active compounds from *C. bartramia* can prove useful in the research and development of antioxidant and anticancer agents. Further chemical and biological investigations on other parts of *C. bartramia* will be reported in due course.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

This research is funded by Ministry of Science and Technology, code ĐTĐL.CN-72/22.

References

1. Guymer GP. New species of *Commersonia* JR Forst. & G. Forst. (Sterculiaceae) from eastern Australia and Vanuatu. Austrobaileya 2005; 231–250.
2. Gonzalez MAS, Buenafe MEG, Ysrael MC. Plants from Palauig, Zambales. Acta Manilana, 1993; 41: 17-22.
3. Kim S, Park SG, Song YJ, Park JK, Choi CH, Lee S, Hoffman RM. Analysis of anticancer activity and chemical sensitization effects of *Dendropanax morbifera* and *Commersonia bartramia* extracts. Anticancer Res. 2018; 38: 3853–3861.
4. Kadir DH. Statistical evaluation of main extraction parameters in twenty plant extracts for obtaining their optimum total phenolic content and its relation to antioxidant and antibacterial activities. Food Sci Nutr. 2021; 9: 3491–3499.
5. Oanh VTK, Thoa HT, Hang NTM, Phuong DTL, Lien NTP, Popova M, Trusheva B, Bankova V, Le TN. New dihydrochromene and xanthone derivatives from *Lisotrigona furva* propolis. Fitoterapia 2021, 149: 104821.
6. Ha NTT, Cuong PV, Tra NT, Cham BT. Chemical constituents from methanolic extract of *Garcinia mackeaniana* leaves and their antioxidant activity. Vietnam J Sci Technol. 2020; 58: 411–418.
7. Aderogba MA, McGaw LJ, Bezabih M, Abegaz BM. Isolation and characterisation of novel antioxidant constituents of *Croton zambesicus* leaf extract. Nat Prod Res. 2011; 25: 1224–1233.
8. Ndhlala AR, Aderogba MA, Ncube B, Van Staden J. Anti-oxidative and cholinesterase inhibitory effects of leaf extracts and their isolated compounds from two closely related *Croton* species. Molecules 2013; 18: 1916–1932.
9. Thao TTP, Luu NT, Chi NL, Hau VTB, Thuy NTT. The first phytochemical study of *Elaeagnus latifolia* in Vietnam. Vietnam J Chem. 2021; 59: 376-382.
10. Fernandes DA, Souza MRS, Teles YCF, Oliveira LHG, Lima JB, Conceição AS, Nunes FC, Silva TMS, de Souza MFV. New sulphated flavonoids and larvicidal activity of *Helicteres velutina* K. Schum (Sterculiaceae). Molecules 2018; 23: 2784.
11. Yuca H, Özbek H, Demirezer LÖ, Kasil HG, Güvenalp Z. Trans-tiliroside: A potent α-glucosidase inhibitor from the leaves of *Elaeagnus angustifolia* L. Phytochemistry 2021; 188: 112795.
12. Goto T, Teraminami A, Lee JY, Ohyama K, Funakoshi K, Kim YI, Hirai S, Uemura T, Yu R, Takahashi N, Kawada T. Tiliroside, a glycosidic flavonoid, ameliorates obesity-induced metabolic disorders via activation of adiponectin signaling followed by enhancement of fatty acid oxidation in liver and skeletal muscle in obese–diabetic mice. J Nutr Biochem. 2012; 23(7): 768-776.
13. Velagapudi R, Aderogba M, Olajide OA. Tiliroside, a dietary glycosidic flavonoid, inhibits TRAF-6/NF-κB/p38-mediated neuroinflammation in activated BV2 microglia. Biochim Biophys Acta - Gen Subj. 2014; 1840(12): 3311-3319.
14. Tung TH, Hue CT, Giap TH, Thoa HT, Dung NA, Hang NTM, Hung NV, Thanh LN. Lignans isolated from the ethyl acetate extract of *Knema pachycarpa* fruit. Vietnam J Chem. 2017; 55: 400–406.
15. Harsono A, Rahman A, Putri RJ, Tjahjandarie TS, Saputri RD, Ahmat N, Tanjung M. Isolation of Lignans from The Stem Bark of Willughbeia coriacea and Their Cytotoxic Activity. Trop J Nat Prod Res. 2023; 7(2): 2394-2396.
16. During A, Debouche C, Raas T, Larondelle Y. Among plant lignans, pinoresinol has the strongest antiinflammatory properties in human intestinal Caco-2 cells. J Nutr. 2012; 142(10): 1798-1805.
17. Jang WY, Kim MY, Cho JY (2022). Antioxidant, anti-Inflammatory, anti-menopausal, and anti-cancer effects of lignans and their metabolites. Int J Mol Sci. 2022; 23(24): 15482.
18. López-Biedma A, Sánchez-Quesada C, Beltrán G, Delgado-Rodríguez M, Gaforio JJ. (2016). Phytoestrogen (+)-pinoresinol exerts antitumor activity in breast cancer cells with different oestrogen receptor statuses. BMC Complement Altern Med. 2016; 16: 1-14.
19. Seebacher W, N. Simic N, Weis R, Saf R, Kunert O. Complete assignments of 1H and 13C NMR resonances of oleanolic acid, 18*α*-oleanolic acid, ursolic acid and their 11-oxo derivatives. Magn. Reson. Chem. 2003; 41: 636–638.
20. Iftikhar A, Falodun A, Siyo B, Jules BKJ, Hussain H, Langer P. (2019). Isolation of phytochemical constituents from Hunteria umbellata K. Schum. Trop J Nat Prod Res. 2019; 3(3): 95-98.
21. Yin R, Li T, Tian JX, Xi P, Liu RH. Ursolic acid, a potential anticancer compound for breast cancer therapy. Crit Rev Food Sci Nutr. 2018; 58(4): 568-574.
22. Xiao ZP, Wu HK, Wu T, Shi H, Hang B, H. Aisa A. Kaempferol and quercetin flavonoids from *Rosa rugosa*, Chem Nat Comp. 2006; 42: 736–737.
23. Nguyen DH, Zhao BT, Le DD, Kim KY, Kim YH, Yoon YH, Ko JY, Woo KS, Woo MH. Phenolic constituents and their anti-inflammatory activity from *Echinochloa utilis* grains. Nat Prod Sci. 2016; 22: 140–145.
24. Jan R, Khan M, Asaf S, Lubna, Asif S, Kim KM (2022). Bioactivity and therapeutic potential of kaempferol and quercetin: new insights for plant and human health. Plants 2022; 11(19): 2623.
25. Espley RV, Butts CA, Laing WA, Martell S, Smith H, McGhie TK, Zhang J, Paturi G, Hedderley D, Bovy A. Dietary flavonoids from modified apple reduce inflammation markers and modulate gut microbiota in mice. J. Nutr. 2014; 144: 146–154.

26. Al-Haidari RA, Al-Oqail MM. New benzoic acid derivatives from Cassia italica growing in Saudi Arabia and their antioxidant activity. Saudi Pharm J. 2020; 28(9): 1112-1117.
27. Yalameha B, Nejabati HR, Nouri M. Cardioprotective potential of vanillic acid. Clin Exp Pharmacol Physiol. 2023; 50(3): 193-204.
28. Choi CW, Kim SC, Hwang SS, Choi BK, Ahn HJ, Lee MY, Park SH, Kim SK. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. Plant Sci. 2002; 163: 1161–1168.
29. Son LH, Anh NP. Phytochemical composition, in vitro antioxidant and anticancer activities of quercetin from methanol extract of *Asparagus cochinchinensis* (Lour.) Merr. tuber. J Med Plant Res. 2012; 7(46): 3360-3366.
30. Deveci E, Tel-Çayan G, Duru ME, Öztürk M. Chemical constituents of *Porodaedalea pini* mushroom with cytotoxic, antioxidant and anticholinesterase activities. J. Food Meas Charact. 2019; 13: 2686-2695.
31. Lee SM, Min BS, Kho YH. Brine shrimp lethality of the compounds from *Phryma leptostachya* L. Arch Pharm Res. 2002; 25: 652-654.