

**In Vitro Cytotoxic Activity of Constituents of the Aerial Parts of *Glycosmis parviflora***Phu Q. D. Nguyen¹, Hoai T. Nguyen¹, Linh T. K. Nguyen¹, Hung Q. Vo¹, Anh T. Le², Thao T. Do³, Duc V. Ho^{1*}¹Faculty of Pharmacy, Hue University of Medicine and Pharmacy, Hue University, 06 Ngo Quyen, Hue City, Vietnam²Quang Tri Center of Science and Technology, Mien Trung Institute for Scientific Research, VAST, Dien Bien Phu, Dong Ha, Quang Tri, Vietnam³Institute of Biotechnology, Vietnam Academy of Science and Technology, Hanoi, Vietnam

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ABSTRACT

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Glycosmis parviflora (Sims) Little is used in Vietnamese ethnomedicine to treat several ailments. We investigated the aerial parts of *G. parviflora* and obtained three triterpenes, a sesquiterpene, and a flavonoid C-glycoside. The terpenes were friedelin, arborinol, isoarborinol, and spathulenol, and the glycoside was vitexin. The compounds were identified by one- and two-dimensional nuclear magnetic resonance spectroscopy and by comparison with previous reports. Spathulenol exhibited moderate cytotoxic activity against LU-1, MDA-MB-231, MKN7, HepG2, and HeLa human cancer cells with 50% inhibitory concentration (IC₅₀) values ranging from 31.88 to 42.33 µg/mL. Therefore, *G. parviflora* and spathulenol might be useful for developing novel anticancer agents.

Keywords: Triterpene, Sesquiterpene, Flavonoid, Spathulenol, Cytotoxicity, Vietnam.

Introduction

According to estimates from the World Health Organization in 2018, cancer incidence and mortality are rapidly growing worldwide, with 18.1 million new cases and 9.6 million deaths.¹ Recently, since the first report on 31 December 2019 in Wuhan, China, the outbreak of COVID-19 has become an international concern. As of 12th August 2020, there were approximately 20 million confirmed cases and over 730,000 deaths from COVID-19.² The pandemic has also made the situation worse on patients with cancer in terms of their inability to receive necessary medical services resulting in the delay of diagnosis and treatment, and suspended clinical trials.³ In the fight against cancer, medicinal plants have been an important source possessing an enormous chemical diversity of secondary metabolites which could be invaluable for novel therapeutic candidates in cancer therapy.⁴ Among the 148 genera of the Rutaceae family, the genus *Glycosmis* comprises approximately 50 species which are shrubs or trees and mainly distributed in warm and temperate regions.⁵ Previous studies of *Glycosmis* species have reported the occurrence of alkaloids, amides, terpenoids, coumarins, and flavonoids.^{6,7} Their crude extracts and purified compounds also showed anticancer, antibacterial, antifungal, antiviral, antioxidant, hepatoprotective, and anti-inflammatory activities, suggesting that *Glycosmis* genus might be a noteworthy resource for drug discovery.⁸ One of the *Glycosmis* species, namely *Glycosmis parviflora*, has been found in Tropical America, Jamaica, Cuba, China, Japan, Myanmar, Java, and Vietnam.^{8,9} It has been used for a long time in Vietnamese ethnomedicine to treat cough, flu, and indigestion.¹⁰ There were several studies reporting the identification of chemical constituents from different parts of *G. parviflora* such as carbalexine A–C,⁸ glycothiomin-A, glycothiomin-B, and ritigalin.¹¹

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In the search for anticancer components from plants in Vietnam, we report the isolation of four terpenoids and a flavonoid C-glycoside from the aerial parts of *G. parviflora* as well as their cytotoxic activity against various human cancer cell lines including LU-1 (human lung adenocarcinoma), MDA-MB-231 (human breast carcinoma), MKN7 (human gastric cancer), HepG2 (human hepatocellular carcinoma) and HeLa (human cervix carcinoma).

Materials and Methods

General experimental procedures

1D and 2D NMR spectra were acquired on a Bruker Avance 500 spectrophotometer (Bruker, Massachusetts, USA) with tetramethylsilane as an internal reference. HRESIMS spectra were recorded on Agilent 6545 Accurate-Mass spectrometers (Agilent, CA, USA). Column chromatography was performed using silica gel (240-430 mesh, Merck - Germany and ODS-60-14/63, Fujisilisa Japan), Sephadex LH-20 (Dowex 50 WX2-100, Sigma-Aldrich, USA). Analytical thin-layer chromatography was performed on pre-coated silica gel 60 F₂₅₄ and RP-18 F₂₅₄ plates (thickness 0.25 or 0.50 mm, Merck KGaA, Darmstadt, Germany). The identification of spots on TLC plates was under UV radiation at 254 and 365 nm wavelengths, and by spraying with 10% H₂SO₄ in ethanol and subsequently heating the plate at about 80°C with a heat gun. The human cancer cell lines, LU-1, MDA-MB-231, MKN7, HepG2, and HeLa were kindly provided by Prof. Delfino D.V. (Perugia University, Italia) and Prof. Pezzuto J.M., Long Island University, US state of New York, USA). Fetal bovine serum, Dulbecco's Modified Eagle's Medium and phosphate buffer saline were purchased from Life Technologies, Inc. (Gaithersburg, MD, USA). The ELISA Plate Reader (Bio-Rad, California, USA) was used to measure the optical density and viability of the cells in the cytotoxicity assay.

Plant material

The aerial parts of *Glycosmis parviflora* were collected from Quang Tri province, Vietnam (N16°45'35.6"N E106°33'54.7"W) in February 2019 and were immediately subjected to drying. The plant material was identified by Dr. Chinh Tien Vu (Institute of Ecology and Biological Resources, VAST, Viet Nam) and a voucher specimen

(GP-01) was deposited at the Faculty of Pharmacy, Hue University of Medicine and Pharmacy, Hue University, Vietnam.

Extraction and isolation

Dried powder from aerial parts of *G. parviflora* (6.0 kg) was macerated with methanol (MeOH; 3 × 12.0 L, 72 h each) at room temperature. The MeOH extract was filtered and concentrated in a rotary evaporator at 50°C under reduced pressure to obtain 300 g dark crude extract. This extract was suspended in water and distributed with *n*-hexane (5.0 L three times) and ethyl acetate (EtOAc; 5.0 L three times). The *n*-hexane, EtOAc, and water layers were concentrated under vacuum, generating *n*-hexane (H; 75 g), EtOAc (E; 17 g), and water (W; 179 g) residues. We subjected the *n*-hexane extract to flash silica gel column chromatography by increasing the amount of acetone in the *n*-hexane (100:0, 40:1, 20:1, 10:1, 5:1, 1:1 v/v, each, 1.5 L) to yield six fractions, H1–H6. Fraction H3 (26.3 g) was purified by silica gel open-column chromatography with a gradient of *n*-hexane-acetone (40:1 → 5:1 v/v, 0.5 L) to obtain eight fractions. In the H3A and H3D fractions, white precipitates appeared and were recrystallized from acetone to yield **1** (30.4 mg) and **3** (50.7 mg). Fraction H3C (2.9 g) was separated into five subfractions, H3C1–H3C5, on a silica gel column eluting with *n*-hexane-acetone (40:1 v/v, 2.0 L). Fraction H3C2 (500 mg) was a white solid and was recrystallized in acetone to obtain **2** (15.1 mg). Fraction H3C3 (1.0 g) was added to a reversed-phase RP-18 silica gel column and eluted with acetone-MeOH-water (1:1:0.1 v/v, 1.5 L) to obtain **4** (86.0 mg). The E extract was placed on a silica gel column, eluted with a CH₂Cl₂-MeOH gradient (100:0, 40:1, 10:1, 0:100 v/v, each 0.5 L) to obtain four fractions, E1–E4. Fraction E3 (3.2 g) was chromatographed on a Sephadex LH-20 column and eluted with CH₂Cl₂-MeOH (1:1 v/v, 1.0 L) to give three smaller fractions, E3A–E3C. Subfraction E3B (0.8 g) was further purified on a silica gel column with EtOAc saturated with water-MeOH (20:1 v/v, 1.0 L) as the eluent to yield four subfractions, E3B1–E3B4. Fraction E3B3 precipitated crude crystals, which were recrystallized from MeOH to give compound **5** (10.2 mg).

SRB assay for evaluating cytotoxic activity

The cytotoxic activity of isolated compounds **1–5** from *G. parviflora* was determined by sulforhodamine B (SRB) assay as described by Monks *et al.* based on the estimation of the binding of cellular protein with SRB dye.¹² Cancer cell lines (LU-1, MDA-MB-231, MKN7, HepG2, and HeLa) were maintained in Dulbecco's modified Eagle's medium containing 2 mM L-glutamine, 1.5 g/L Na₂CO₃, and 10% fetal bovine serum. The medium was changed at 48-h intervals. The cells were dissociated with 0.05% trypsin-ethylenediaminetetraacetic acid, subcultured every 3 to 5 days at a ratio of 1:3, and incubated at 37°C in a humidified atmosphere of 5% CO₂ for 48 h. Cells were seeded in growth medium (180 µL) in 96-well microplates (4 × 10⁴ cells/well). Test samples were added to each well, and the plates were reincubated under the same conditions. After incubation with isolates for 72 h, the medium was removed, and the cell monolayers were fixed with cold 20% (w/v) trichloroacetic acid for 1 h at 4°C. Next 1X SRB solution was added to each well, and the plates were incubated at room temperature for 30 min. Subsequently, the wells were washed with aqueous acetic acid (1% v/v) to remove excess dye. The protein-bound dye produced was dissolved in 10 mM Tris base solution. The absorbance of the control and treated wells at λ = 515 nm was measured. Dimethyl sulfoxide (10%) was used as the blank and ellipticine as the positive control, replacing the test compounds. Cytotoxicity was evaluated at doses of 100, 20, 4, and 0.8 µg/mL, and IC₅₀ values were calculated with Table Curve version 4.0. The inhibition rate of cancer cells was calculated by the following formula:

$$\text{IR}\% = (100\% - [(\text{Abs}_t - \text{Abs}_0)/(\text{Abs}_c - \text{Abs}_0)] \times 100),$$

Where; IR: Inhibition rate of cell growth, Abs_t: average optical density value at day 3; Abs₀: average optical density value at time-zero; Abs_c: average optical density value of the blank DMSO control sample.

Statistical analysis

All experiments were set up in triplicate. The IC₅₀ values were presented as mean ± standard deviation.

Results and Discussion

Compound **1** was isolated as a white powder from the *n*-hexane-soluble portion. The ¹H-NMR in CDCl₃ revealed the typical signals of seven tertiary methyl groups [δ_{H} 0.73, 0.87, 0.95, 1.01, 1.03, 1.05, and 1.18 (each, 3H, s)] and a secondary methyl group [δ_{H} 0.88 (3H, d, $J = 6.5$ Hz)]. The ¹³C-NMR and HSQC experiments indicated 30 signals for eight methyl, eleven methylene, four methine, and seven quaternary carbons. The presence of one carbonyl carbon was clearly seen via the signal at δ_{C} 213.3. Based on the 1D-NMR spectroscopic data, compound **1** was identified as friedelin.¹³

Compound **2** was obtained as a white powder. The ¹H-NMR spectrum displayed the characteristic signals of one olefinic proton [δ_{H} 5.27 (1H, d, $J = 6.5$ Hz, H-11), one oxygenated methine [δ_{H} 3.42 (1H, dd, $J = 2.5, 3.0$ Hz, H-3)], eight methyl groups [δ_{H} 0.76, 0.77, 0.82, 0.88, 0.96, 1.05 (each, 3H, s), and 0.83, 0.89 (each, 3H, d, $J = 6.5$ Hz)]. Notably, the small J value (2.5, 3.0 Hz) between H-3 and H-2 suggested the β -equatorial orientation of H-3 in chair conformer of A ring. The ¹³C-NMR and HSQC spectra indicated 30 signals [8 CH₃, 9 CH₂, 7 CH, and 6 C]. The existence of a tri-substituted double bond was deduced by the signals at δ_{C} 149.0 (s, C-9), and 114.3 (d, C-11), whereas the signal at δ_{C} 76.4 (d, C-3) was assigned for carbinol group. Therefore, compound **2** was concluded as arborinol.¹⁴

Compound **3** was obtained as a white powder. The ¹³C-NMR data of **3** was similar to those of **2**, except for C-1, C-3, C-5, and C-24. This observation implied that compounds **2** and **3** are C-3 epimers. Furthermore, the coupling constant values of H-3 ($J_{3a/2a} = 11.5$ Hz, $J_{3a/2c} = 4.0$ Hz) confirmed the α -axial orientation of this proton. Compound **3** was thus determined to be isoborinol.¹⁵

Compound **4** was isolated as odourless oil. Some important signals were found in the ¹H-NMR spectrum, including an *exo*-methylene group at δ_{H} 4.66 (1H, br.s, H-14a) and 4.69 (1H, br.s, H-14b) as well as three angular methyl groups at δ_{H} 1.04 (s, H₃-13), 1.05 (s, H₃-12), and 1.28 (s, H₃-15). The ¹³C-NMR spectrum showed 15 carbon signals from which two sp² carbons (δ_{C} 153.6, 106.4) and one oxygenated sp³ carbon (δ_{C} 81.1) were deduced. Consequently, compound **4** was established as spathulenol.¹⁶

Compound **5** was isolated as a yellow amorphous powder. The ¹H-NMR spectrum in DMSO-*d*₆ demonstrated the signals of a *para*-substituted benzene ring [δ_{H} 8.02 (2H, d, $J = 8.5$ Hz) and 6.89 (2H, d, $J = 8.5$ Hz)], two aromatic protons [δ_{H} 6.77 and 6.27 (each, 1H, s)] and an anomeric proton [δ_{H} 6.27 (1H, d, $J = 10.0$ Hz)]. The analysis of the ¹³C-NMR and the HSQC spectra indicated 21 signals corresponding to one oxymethylene carbon (δ_{C} 61.3), six sp² methine carbons [δ_{C} 128.9 (2C), 115.8 (2C), 102.4, and 98.1], five oxymethine carbons (δ_{C} 81.8, 78.7, 73.4, 70.8, 70.5), and nine non-hydrogenated carbons (δ_{C} 182.1, 163.9, 162.5, 161.1, 160.4, 156.0, 121.6, 104.0, and 102.4). The above spectroscopic data as well as the lack of the acetal signal in the ¹³C-NMR spectrum suggested that compound **5** was a flavone C-glycoside. Further analysis led to the assignment of this compound as 5,7,4'-trihydroxy-8-C- β -D-glucopyranosyl flavone (trivial name as vitexin), which is consistent with previous investigation.¹⁷

Spectral data for compounds 1-5

Friedelin (**1**): White powder; ¹H-NMR (CDCl₃, 500 MHz): δ_{H} 1.68 (1H, m, H-1b), 1.97 (1H, m, H-1a), 2.39 (1H, ddd, $J = 2.0, 5.0, 13.5$ Hz, H-2a), 2.30 (1H, dd, $J = 7.0, 13.5$ Hz, H-2b), 2.24 (1H, q, $J = 6.5$ Hz, H-4), 0.73 (3H, s, H-24), 0.87 (3H, s, H-25), 1.01 (3H, s, H-26), 1.05 (3H, s, H-27), 1.18 (3H, s, H-28), 0.95 (3H, s, H-29), 1.03 (3H, s, H-30); ¹³C-NMR (CDCl₃, 125 MHz): δ_{C} 22.4 (CH₂-1), 41.7 (CH₂-2), 213.3 (C-3), 58.4 (CH-4), 42.3 (C-5), 41.5 (CH₂-6), 18.4 (CH₂-7), 53.3 (CH-8), 37.6 (C-9), 59.7 (CH-10), 35.8 (CH₂-11), 30.7 (CH₂-12), 39.9 (C-13), 38.5 (C-14), 32.6 (CH₂-15), 36.2 (CH₂-16), 30.2 (C-17), 43.0 (CH-18), 35.5 (CH₂-19), 28.3 (C-20), 33.0 (CH₂-21), 39.4 (CH₂-22), 7.0 (CH₃-23), 14.8 (CH₃-24), 18.1 (CH₃-25), 20.4 (CH₃-26), 18.8

(CH₃-27), 32.3 (CH₃-28), 35.2 (CH₃-29), 31.9 (CH₃-30); HRESIMS *m/z* 449.3757 [M+Na]⁺ (calcd. for C₃₀H₅₀O₂Na, 449.3759).

Arborinol (**2**): White powder; ¹H-NMR (CDCl₃, 500 MHz): δ_H 3.42 (1H, dd, *J* = 2.5, 3.0 Hz, H-3), 5.27 (H, d, *J* = 6.5 Hz, H-11), 0.96 (3H, s, H-23), 0.88 (3H, s, H-24), 1.05 (3H, s, H-25), 0.82 (3H, s, H-26), 0.77 (3H, s, H-27), 0.76 (3H, s, H-28), 0.83 (3H, d, *J* = 6.5 Hz, H-29), 0.89 (3H, d, *J* = 6.5 Hz, H-30); ¹³C-NMR (CDCl₃, 125 MHz): δ_C 30.6 (CH₂-1), 25.9 (CH₂-2), 76.4 (CH-3), 38.0 (C-4), 46.7 (CH-5), 21.5 (CH₂-6), 26.8 (CH₂-7), 41.2 (CH-8), 149.0 (C-9), 39.8 (C-10), 114.3 (CH-11), 36.2 (CH₂-12), 36.9 (C-13), 38.5 (C-14), 29.8 (CH₂-15), 36.1 (CH₂-16), 43.0 (C-17), 52.3 (CH-18), 20.4 (CH₂-19), 28.4 (CH₂-20), 59.8 (CH-21), 30.9 (CH-22), 28.4 (CH₃-23), 22.7 (CH₃-24), 22.1 (CH₃-25), 17.2 (CH₃-26), 15.5 (CH₃-27), 14.2 (CH₃-28), 23.1 (CH₃-29), 22.3 (CH₃-30); HRESIMS *m/z* 449.3755 [M+Na]⁺ (calcd. for C₃₀H₅₀O₂Na, 449.3759).

Isobarborinol (**3**): White powder; ¹H-NMR (CDCl₃, 500 MHz): δ_H 3.20 (1H, dd, *J* = 4.0, 11.5 Hz, H-3), 5.23 (1H, d, *J* = 6.5 Hz, H-11), 0.99 (3H, s, H-23), 0.80 (3H, s, H-24), 1.03 (3H, s, H-25), 0.81 (3H, s, H-26), 0.77 (3H, s, H-27), 0.76 (3H, s, H-28), 0.83 (3H, d, *J* = 6.5 Hz, H-29), 0.89 (3H, d, *J* = 6.5 Hz, H-30); ¹³C-NMR (CDCl₃, 125 MHz): δ_C 36.2 (CH₂-1), 26.9 (CH₂-2), 79.1 (CH-3), 39.2 (C-4), 52.5 (CH-5), 21.6 (CH₂-6), 28.0 (CH₂-7), 41.2 (CH-8), 149.0 (C-9), 39.8 (C-10), 114.5 (CH-11), 36.2 (CH₂-12), 37.0 (C-13), 38.4 (C-14), 29.8 (CH₂-15), 36.1 (CH₂-16), 43.0 (C-17), 52.3 (CH-18), 20.3 (CH₂-19), 28.4 (CH₂-20), 59.8 (CH-21), 30.9 (CH-22), 28.4 (CH₃-23), 15.8 (CH₃-24), 22.3 (CH₃-25), 17.2 (CH₃-26), 15.5 (CH₃-27), 14.2 (CH₃-28), 23.1 (CH₃-29), 22.3 (CH₃-30); HRESIMS *m/z* 449.3757 [M+Na]⁺ (calcd. for C₃₀H₅₀O₂Na, 449.3759).

Spathulenol (**4**): Odourless oil; ¹H-NMR (CDCl₃, 500 MHz): δ_H 2.21 (1H, ddd, *J* = 10.5, 10.5, 6.0 Hz, H-1), 1.63 (1H, m, H-2a), 1.90 (1H, m, H-2b), 1.57 (1H, m, H-3a), 1.78 (1H, m, H-3b), 1.31 (1H, t, *J* = 11.0 Hz, H-5), 0.47 (1H, dd, *J* = 11.0, 9.5 Hz, H-6), 0.71 (1H, m, H-7), 1.02 (1H, m, H-8a), 1.98 (1H, m, H-8b), 2.04 (1H, t, *J* = 13.0 Hz, H-9a), 2.42 (1H, ddd, *J* = 13.0, 6.0, 0.5 Hz, H-9b) 1.05 (3H, s, H-12), 1.04 (3H, s, H-13), 4.66 (1H, br s, H-14a), 4.69 (1H, br s, H-14b); ¹³C-NMR (CDCl₃, 125 MHz): δ_C 53.6 (CH-1), 26.9 (CH₂-2), 41.9 (CH₂-3), 81.1 (C-4), 54.5 (CH-5), 30.1 (CH-6), 27.7 (CH-7), 25.0 (CH₂-8), 39.0 (CH₂-9), 153.6 (C-10), 20.4 (C-11), 28.8 (CH₃-12), 16.5 (CH₃-13), 106.4 (CH₂-14), 26.2 (CH₃-15); HRESIMS *m/z* 221.1902 [M+H]⁺ (calcd. for C₁₅H₂₅O, 221.1905).

Vitexin (**5**): Yellow amorphous powder; ¹H-NMR (DMSO-*d*₆, 500 MHz): δ_H 13.16 (1H, s, 5-OH), 6.77 (1H, s, H-3), 6.27 (1H, s, H-6), 8.02 (2H, d, *J* = 8.5 Hz, H-2'/H-6'), 6.89 (2H, d, *J* = 8.5 Hz, H-3'/H-5'), 4.69 (1H, d, *J* = 10.0 Hz, H-1'), 3.84 (1H, dd, *J* = 10.0, 9.0 Hz, H-2''), 3.29 (1H, overlapped, H-3''), 3.34 (1H, overlapped, H-4''), 3.25

(1H, m, H-5''), 3.53 (1H, dd, *J* = 11.0, 5.5 Hz, H-6'a), 3.77 (1H, dd, *J* = 11.0, 6.0 Hz, H-6'b); ¹³C-NMR (DMSO-*d*₆, 125 MHz): δ_C 163.9 (C-2), 102.4 (CH-3), 182.1 (C-4), 160.4 (C-5), 98.1 (CH-6), 162.5 (C-7), 104.6 (C-8), 156.0 (C-9), 104.0 (C-10), 121.6 (C-1'), 128.9 (CH-2', CH-6'), 115.8 (CH-3', CH-5'), 161.1 (C-4'), 73.4 (CH-1''), 70.8 (CH-2''), 78.7 (CH-3''), 70.5 (CH-4''), 81.8 (CH-5''), 61.3 (CH₂-6''); HRESIMS *m/z* 431.0990 [M-H]⁻ (calcd. for C₂₁H₁₉O₁₀, 431.0978).

A friedelane-type triterpene (**1**) and two arborinane-type triterpenes (**2**, **3**) have also been reported from *G. arborea*¹⁸ and *G. montana*.¹⁹ Compound **4** is an aromadendrane-type sesquiterpene, which has been detected in the essential oils of *G. pentaphylla*²⁰ and *G. lucida*.²¹ Compound **5** was found in several medicinal plants such as *Ficus deltoidea*,²² *Spirodela polyrhiza*²³ and *Acer palmatum*.²⁴ However, it is noteworthy to mention that this is an initial report on the isolation of these constituents from *G. parviflora*.

All the isolated compounds (**1–5**) were evaluated for their cytotoxicity against LU-1, MDA-MB-231, MKN7, HepG2, and HeLa cell lines using the sulforhodamine B assay. The cytotoxicity and IC₅₀ values of the isolates are listed in Table 1. Compound **4** had significant inhibitory effects on all cell lines, with IC₅₀ values of 31.88 ± 3.31 to 42.33 ± 2.05 µg/mL. At 100 µg/mL, compound **4** inhibited almost all cancer cell lines by 80.73% to 94.39%. Compounds **3** and **5** showed weak cytotoxicity against four cell lines (MDA-MB-231, MKN7, HepG2, and HeLa), with IC₅₀ values of 69.78 ± 7.02 to 99.59 ± 1.17 µg/mL. Compounds **1** and **2** did not have cytotoxic effects on any of the cell lines (IC₅₀ > 100 µg/mL). Comparison of the cytotoxicity of **2** with that of **3** indicated that the β orientation of the hydroxyl group of **3** was slightly more active than the α orientation of **2**.

Friedelin (**1**) showed significant cytotoxicity against brine shrimp nauplii with LC₅₀ and LC₉₀ values of 13.80 and 213.79 µg/mL, respectively. Furthermore, friedelin showed moderate free radical scavenging ability in a 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.²⁵ Friedelin also demonstrated moderate antibacterial activity against methicillin-resistant *Staphylococcus aureus*, *Helicobacter pylori*, and *Escherichia coli* (minimum IC₅₀ 10 µg/mL).²⁶ Isoarborinol (**3**) reportedly has anti-amoebic effects on *Entamoeba histolytica* at 0.05 mg/mL without affecting mammalian cells.²⁷ Spathulenol (**4**) exhibits significant scavenging activity according to DPPH (IC₅₀ 85.60 µg/mL) and malondialdehyde (IC₅₀ 26.13 µg/mL) assays. In addition, spathulenol at 100 mg/kg had marked anti-inflammatory effects in a model of Cg-induced mouse pleurisy. Spathulenol has moderate activity against the OVCAR-3 cell line (IC₅₀ 49.30 µg/mL).²⁸ It also inhibits proliferation of lymphocytes and induces their apoptosis, possibly by a caspase-3 independent pathway.²⁹ Vitexin (**5**) decreases the viability and migration of human oral cancer OC2 cells via the p53-PAI1-MMP2 cascade³⁰ and has antitumor effects by targeting apoptosis of U937 cells.³¹

Table 1: *In vitro* cytotoxicity of compounds (**1–5**) isolated from *Glycosmis parviflora*

Compound	IC ₅₀ ^a (µg/mL) ± SD				
	LU-1	MDA-MB-231	MKN7	HepG2	HeLa
1	> 100	> 100	> 100	> 100	> 100
2	> 100	> 100	> 100	> 100	> 100
3	> 100	93.81 ± 6.11	92.28 ± 8.00	96.41 ± 4.62	99.59 ± 1.17
4	41.86 ± 2.58	42.33 ± 2.05	39.95 ± 2.90	33.18 ± 1.82	31.88 ± 3.31
5	> 100	69.78 ± 7.02	79.65 ± 6.76	92.08 ± 5.25	95.19 ± 4.72
Ellipticine ^b	0.38 ± 0.04	0.39 ± 0.04	0.39 ± 0.03	0.45 ± 0.03	0.43 ± 0.02

^a IC₅₀ (concentration that inhibits 50% of cell growth).

^b Positive control.

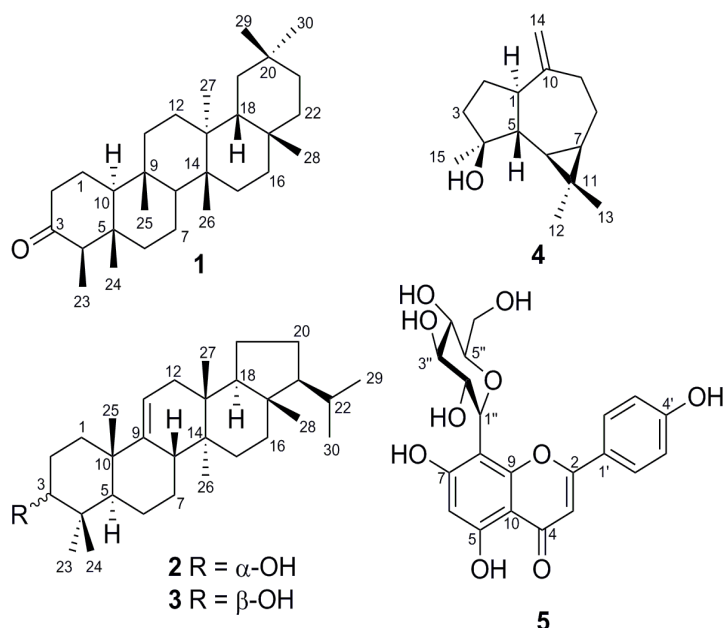


Figure 1: Structures of isolated compounds (1–5) from *Glycosmis parviflora*

Conclusion

In this study, five secondary metabolites were isolated from the aerial parts of *G. parviflora*. This is the first report of the isolation and characterization of these compounds from this plant material. The *in vitro* cytotoxicity of all isolates was evaluated, and spathulenol exhibited moderate activity against the five cancer cell lines tested.

Conflict of interest

The authors declare no conflicts 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.

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References

- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: Globocan of estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018; 68(6):394-424.
- WHO. Coronavirus Disease (COVID-19) Dashboard. Available from: <https://covid19.who.int/> (accessed 2020/08/12).
- Richards M, Anderson M, Carter P, Ebert BL, Mossialos E. The impact of the Covid-19 pandemic on cancer care. *Nat Cancer.* 2020; 1(6):565-567.

- Ito C, Itoigawa M, Sato A, Hasan CM, Rashid MA, Tokuda H, Mukainaka T, Nishino H, Furukawa H. Chemical constituents of *Glycosmis arborea*: Three new carbazole alkaloids and their biological activity. *J Nat Prod.* 2004; 67(9):1488-1491.
- Pacher T, Bacher M, Hofer O, Greger H. Stress induced carbazole phytoalexins in *Glycosmis species*. *Phytochem.* 2001; 58(1):129-135.
- Yasir M, Tripathi MK, Singh P, Shrivastava R. The genus *Glycosmis* (Rutaceae): A comprehensive review on its phytochemical and pharmacological perspectives. *The Nat Prod J.* 2019; 9(2):98-124.
- Zhang D and Hartley TG. *Glycosmis Corréa*. *Ann. Mus. Natl. Hist. Nat.* 6: 384. 1805, nom. cons. In: Wu ZY, Peter HR, Deyuan H. *Flora of China*. Eds. Missouri Botanical Garden Press; 2008. 11:80-83 p.
- Ho PH. An Illustrated Flora of Vietnam [in Vietnamese] (2nd ed.). Tre Publishing House: Ho Chi Minh City, Viet Nam; 2003. 952 p.
- Hofer O and Greger H. Sulfur-Containing Amides from *Glycosmis Species* (Rutaceae). In: Chang CWJ, Greger H, Hofer O, Herz W, Falk H, Kirby GW, Moore RE. *Fortschritte der Chemie organischer Naturstoffe / Progress in the Chemistry of Organic Natural Products*, Eds. Springer Vienna: Vienna; 2000. 187-223 p.
- Monks A, Scudiero D, Skehan P, Shoemaker R, Paull K, Vistica D, Hose C, Langley J, Cronise P, Vaigro-Wolff A, Gray-Goodrich M, Campbell H, Mayo J, Boyd M. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *J Natl Cancer I.* 1991; 83(11):757-766.
- Akihisa T, Yamamoto K, Tamura T, Kimura Y, Iida T, Nambara T, Chang FC. Triterpenoid Ketones from *Lingnania chungii* MoClure: Arborinone, Friedelin and Glutinone. *Chem Pharm Bull (Tokyo).* 1992; 40(3):789-791.
- Farruque R, Chowdhury R, Sohrab MH, Hasan CM, Rashid MA. Triterpene constituents from the leaves of *Melicope indica*. *Pharmazie.* 2003; 58(7):518-520.
- Gomes RA, Teles YCF, Pereira FdO, Rodrigues LAdS, Lima EdO, Agra MdF, Souza MdFVd. Phytoconstituents from *Sidastrum micranthum* (A. St. – Hil.) Fryxell (Malvaceae) and antimicrobial activity of pheophytin A. *Braz J Pharm Sci.* 2015; 51(4):861-867.
- Goud TV, Reddy NS, Krishnaiah P, Venkateswarlu Y. Spathulenol: a rare sesquiterpene from soft coral *Sinularia kavarattiensis*. *Biochem Syst Ecol.* 2002; 30(5):493-495.
- Cuong LCV, Trang DT, Cuc NT, Nhiem NX, Yen PH, Anh HLT, Huong LM, Minh CV, Kiem PV. Flavonoid glycosides from *Antidesma ghaesembilla*. *Vietnam J Chem.* 2015; 53(2e):94-97.
- Pakrashi SC and Samanta TB. Acid induced empimerization and rearrangements of arborinol, the novel triterpene from *Glycosmis arborea* (ROXB.) DC. *Tetrahedron Lett.* 1967; 8(38):3679-3684.
- Fan Q-F, Song Q-S, Zuo G-Y, Zheng J-Y, Na Z, Hu H-B. Chemical constituents of the twigs and leaves of *Glycosmis montana*. *Chem Nat Compd.* 2015; 51(3):550-551.
- Ahmed R, Choudhury S, Vajczikova I, Leclercq PA. Essential oils of *Glycosmis pentaphylla* (Cor.). A new report from Assam, India. *J Essent Oil Res.* 2000; 12(4):471-474.
- Guo S-S, Zhang W-J, Yang K, Liang J-Y, You C-X, Wang C-F, Li Y-P, Geng Z-F, Deng Z-W, Du S-S. Repellence of the main components from the essential oil of *Glycosmis lucida* Wall. ex Huang against two

- stored product insects. Nat Prod Res. 2017; 31(10):1201-1204.
20. Choo CY, Sulong NY, Man F, Wong TW. Vitexin and isovitexin from the leaves of *Ficus deltoidea* with in-vivo α -glucosidase inhibition. J Ethnopharmacol. 2012; 142(3):776-781.
21. Kim J, Lee I, Seo J, Jung M, Kim Y, Yim N, Bae K. Vitexin, orientin and other flavonoids from *Spirodela polyrhiza* inhibit adipogenesis in 3T3-L1 cells. Phytother Res. 2010; 24(10):1543-1548.
22. Kim JH, Lee BC, Kim JH, Sim GS, Lee DH, Lee KE, Yun YP, Pyo HB. The isolation and antioxidative effects of vitexin from *Acer palmatum*. Arch Pharm Res. 2005; 28(2):195-202.
23. Anjum A, Sultan MZ, Hasan CM, Rashid MA. Antibacterial and cytotoxic constituents from *Bridelia verrucosa* Haines growing in Bangladesh. Dhaka Univ J Pharm Sci. 2017; 16(1):61-68.
24. Odeh IC, Tor-Anyiin TA, Igoli JO, Anyam JV. In vitro antimicrobial properties of friedelan-3-one from *Pterocarpus santalinoides* L'Herit, ex Dc. Afr J Biotechnol. 2016; 15(14):531-538.
25. Zavala-Ocampo LM, Aguirre-Hernández E, Pérez-Hernández N, Rivera G, Marchat LA, Ramírez-Moreno E. Antiamoebic activity of *Petiveria alliacea* leaves and their main component, Isoarborinol. J Microbiol Biotechnol. 2017; 27(8):1401-1408.
26. do Nascimento KF, Moreira FMF, Alencar Santos J, Kassuya CAL, Croda JHR, Cardoso CAL, Vieira MdC, Góis Ruiz ALT, Ann Foglio M, de Carvalho JE, Formagio ASN. Antioxidant, anti-inflammatory, antiproliferative and antimycobacterial activities of the essential oil of *Psidium guineense* Sw. and spathulenol. J Ethnopharmacol. 2018; 210:351-358.
27. Ziaei A, Ramezani M, Wright L, Paetz C, Schneider B, Amirghofran Z. Identification of spathulenol in *Salvia mirzayanii* and the immunomodulatory effects. Phytother Res. 2011; 25(4):557-562.
28. Yang S-H, Liao P-H, Pan Y-F, Chen S-L, Chou S-S, Chou M-Y. The novel p53-dependent metastatic and apoptotic pathway induced by vitexin in human oral cancer OC2 cells. Phytother Res. 2013; 27(8):1154-1161.
29. Lee C-Y, Chien Y-S, Chiu T-H, Huang W-W, Lu C-C, Chiang J-H, Yang J-S. Apoptosis triggered by vitexin in U937 human leukemia cells via a mitochondrial signaling pathway. Oncol Rep. 2012; 28(5):1883-1888.