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Original Research Article

In Vitro Cytotoxic Activity of Constituents of the Aerial Parts of *Glycosmis parviflora*

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ABSTRACT

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 (IC_{50}) 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 F_{254} and RP-18 F_{254} 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% H_2SO_4 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″) 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

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(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 CH_2Cl_2-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 CH_2Cl_2-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 Na_2CO_3, 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% CO_2 for 48 h. Cells were seeded in growth medium (180 µL) in 96-well microplates (4×10^4 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 IC_{50} values were calculated with Table Curve version 4.0. The inhibition rate of cancer cells was calculated by the following formula:

$$IR\% = (100\% - [(Abs_t - Abs_0)/(Abs_c - Abs_0)] \times 100),$$

Where; IR: Inhibition rate of cell growth, Abs_t: average optical density value at day 3; Abs_0: 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 IC_{50} 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 ^{1}H-NMR in $CDCl_3$ 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 ^{13}C-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 ^{1}H-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 ^{13}C-NMR and HSQC spectra indicated 30 signals [8 CH_3, 9 CH_2, 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 ^{13}C-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, $J_{3a/2e}$ = 4.0 Hz) confirmed the α-axial orientation of this proton. Compound **3** was thus determined to be isoarborinol.[15]
Compound **4** was isolated as odourless oil. Some important signals were found in the ^{1}H-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, H_3-13), 1.05 (s, H_3-12), and 1.28 (s, H_3-15). The ^{13}C-NMR spectrum showed 15 carbon signals from which two sp^2 carbons (δ_C 153.6, 106.4) and one oxygenated sp^3 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 ^{1}H-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 ^{13}C-NMR and the HSQC spectra indicated 21 signals corresponding to one oxymethylene carbon (δ_C 61.3), six sp^2 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 ^{13}C-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; ^{1}H-NMR ($CDCl_3$, 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); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ_C 22.4 (CH_2-1), 41.7 (CH_2-2), 213.3 (C-3), 58.4 (CH-4), 42.3 (C-5), 41.5 (CH_2-6), 18.4 (CH_2-7), 53.3 (CH-8), 37.6 (C-9), 59.7 (CH-10), 35.8 (CH_2-11), 30.7 (CH_2-12), 39.9 (C-13), 38.5 (C-14), 32.6 (CH_2-15), 36.2 (CH_2-16), 30.2 (C-17), 43.0 (CH-18), 35.5 (CH_2-19), 28.3 (C-20), 33.0 (CH_2-21), 39.4 (CH_2-22), 7.0 (CH_3-23), 14.8 (CH_3-24), 18.1 (CH_3-25), 20.4 (CH_3-26), 18.8

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(CH_3-27), 32.3 (CH_3-28), 35.2 (CH_3-29), 31.9 (CH_3-30); HRESIMS *m/z* 449.3757 $[M+Na]^+$ (calcd. for $C_{30}H_{50}ONa$, 449.3759).

Arborinol (**2**): White powder; ^{1}H-NMR ($CDCl_3$, 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); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ_C 30.6 (CH_2-1), 25.9 (CH_2-2), 76.4 (CH-3), 38.0 (C-4), 46.7 (CH-5), 21.5 (CH_2-6), 26.8 (CH_2-7), 41.2 (CH-8), 149.0 (C-9), 39.8 (C-10), 114.3 (CH-11), 36.2 (CH_2-12), 36.9 (C-13), 38.5 (C-14), 29.8 (CH_2-15), 36.1 (CH_2-16), 43.0 (C-17), 52.3 (CH-18), 20.4 (CH_2-19), 28.4 (CH_2-20), 59.8 (CH-21), 30.9 (CH-22), 28.4 (CH_3-23), 22.7 (CH_3-24), 22.1 (CH_3-25), 17.2 (CH_3-26), 15.5 (CH_3-27), 14.2 (CH_3-28), 23.1 (CH_3-29), 22.3 (CH_3-30); HRESIMS *m/z* 449.3755 $[M+Na]^+$ (calcd. for $C_{30}H_{50}ONa$, 449.3759).

Isoarborinol (**3**): White powder; ^{1}H-NMR ($CDCl_3$, 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); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ_C 36.2 (CH_2-1), 26.9 (CH_2-2), 79.1 (CH-3), 39.2 (C-4), 52.5 (CH-5), 21.6 (CH_2-6), 28.0 (CH_2-7), 41.2 (CH-8), 149.0 (C-9), 39.8 (C-10), 114.5 (CH-11), 36.2 (CH_2-12), 37.0 (C-13), 38.4 (C-14), 29.8 (CH_2-15), 36.1 (CH_2-16), 43.0 (C-17), 52.3 (CH-18), 20.3 (CH_2-19), 28.4 (CH_2-20), 59.8 (CH-21), 30.9 (CH-22), 28.4 (CH_3-23), 15.8 (CH_3-24), 22.3 (CH_3-25), 17.2 (CH_3-26), 15.5 (CH_3-27), 14.2 (CH_3-28), 23.1 (CH_3-29), 22.3 (CH_3-30); HRESIMS *m/z* 449.3757 $[M+Na]^+$ (calcd. for $C_{30}H_{50}ONa$, 449.3759).

Spathulenol (**4**): Odourless oil; ^{1}H-NMR ($CDCl_3$, 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); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ_C 53.6 (CH-1), 26.9 (CH_2-2), 41.9 (CH_2-3), 81.1 (C-4), 54.5 (CH-5), 30.1 (CH-6), 27.7 (CH-7), 25.0 (CH_2-8), 39.0 (CH_2-9), 153.6 (C-10), 20.4 (C-11), 28.8 (CH_3-12), 16.5 (CH_3-13), 106.4 (CH_2-14), 26.2 (CH_3-15); HRESIMS *m/z* 221.1902 $[M+H]^+$ (calcd. for $C_{15}H_{25}O$, 221.1905).

Vitexin (**5**): Yellow amorphous powder; ^{1}H-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); ^{13}C-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 (CH_2-6″); HRESIMS *m/z* 431.0990 $[M-H]^-$ (calcd. for $C_{21}H_{19}O_{10}$, 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 IC_{50} values of the isolates are listed in Table 1. Compound **4** had significant inhibitory effects on all cell lines, with IC_{50} 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 IC_{50} 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 (IC_{50} > 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 LC_{50} and LC_{90} 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 IC_{50} 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 (IC_{50} 85.60 μg/mL) and malondialdehyde (IC_{50} 26.13 μg/mL) assays. In addition, spathulenol at 100 mg/kg had marked antiinflammatory effects in a model of Cg-induced mouse pleurisy. Spathulenol has moderate activity against the OVCAR-3 cell line (IC_{50} 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 cascade[30] 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] IC_{50} (concentration that inhibits 50% of cell growth).
[b] Positive control.

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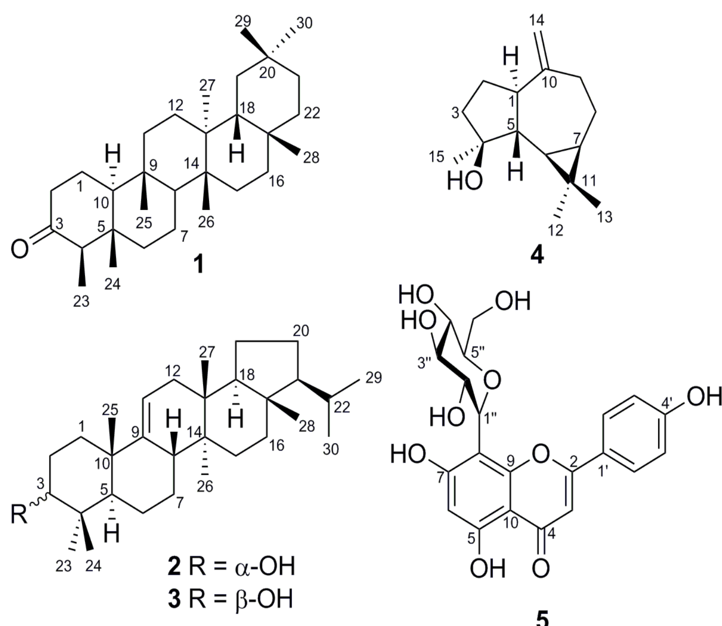

Figure 1: Structures of isolated compounds (**1–5**) from *Glycosmis parviflora*

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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