EXPLORATION OF COMPONENTS CONTRIBUTING TO POTENT CYTOTOXICITY OF GARDENIA THUNBERGIA L. F. AGAINST HUMAN LEUKEMIA AND HEPATOMA

Shaymaa M. Mohamed¹, Samir A. Ross²,³ and Nesma M. Mohamed¹,*

¹Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut 71515, Egypt.
²National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi, University, Mississippi 38677, USA
³Department of BioMolecular Sciences, Division of Pharmacognosy, School of Pharmacy, University of Mississippi, University, Mississippi 38677, USA

Promising cytotoxic effects of several Gardenia species (Rubiaceae) have been established by many studies. The current study evaluated MTT-based cytotoxic activities of the crude extract from Gardenia thunbergia L. f. aerial parts and four fractions thereof, including n-hexane, dichloromethane (DCM), ethyl acetate, and aqueous, against human leukemia (HL-60) and hepatoma (HepG2) cell lines, as well as the normal (WI38) cell line. Both non-polar fractions, n-hexane and dichloromethane, showed tumor-selective toxicities against both tested cancerous cell lines. These results sparked our interest in chemically characterising these bioactive fractions to reveal their cytotoxic components. The composition of n-hexane-soluble fraction was investigated via GC-MS analysis, while column chromatographic separation was used to isolate the components of DCM-soluble fraction. These isolated phytochemicals were identified via spectroscopic analyses. Besides, the chemotaxonomic value of the detected phytochemicals and their reported cytotoxic profiles were discussed.

Keywords: Gardenia thunbergia; Rubiaceae; Cytotoxicity; Human leukemia; HepG2 cells; Chemotaxonomy

INTRODUCTION

Researcher's efforts have been oriented to explore the cytotoxicity of natural products to mitigate the habitual toxicity of chemotherapeutics and circumvent their acquired resistance. Gardenia genus is a member of the tribe Gardenieae (subfamily: Ixoroideae, family: Rubiaceae) which comprises over 140 species involving several limitedly explored plants such as G. thunbergia L. f.¹ Several Gardenia species have proved to have potent cytotoxic phytochemicals²–⁷. Previous studies on Gardenia species revealed the presence of diverse phytochemicals including monoterpenes⁸, triterpenes⁹, saponins², flavonoids¹⁰, iridoids, and lignans¹¹. This study revealed promising in vitro cytotoxic activities of the non-polar fractions of G. thunbergia L. f. aerial parts against HL-60 and HepG2 cell lines. Therefore, we chemically investigated the n-hexane- and dichloromethane-soluble fractions. As a result, the n-hexane profile was characterized through GC-MS analysis and five compounds were isolated from the dichloromethane fraction; three of them were reported for the first time from the plant.
MATERIALS AND METHODS

General procedures
The NMR spectral analysis was achieved on 400 and 500 MHz Bruker Avance DRX spectrometer (MA, USA) using the deuterated NMR solvents: CDCl₃, CD₂OD, and DMSO-d₆ (Cambridge Isotope Lab., Inc., MA) and TMS as an internal standard. Chromatographic adsorbents: silica gel G₆₀ (60–120 mesh, Merck, Darmstadt, Germany). TLC was performed using 0.25 mm aluminum pre-coated silica 60 F₂₅₄ sheets (E-Merck, Darmstadt, Germany). Solvents for extraction and isolation procedures were analytical grade, Fisher Scientific (Advic, El Nasr Pharm. Co., Cairo-Egypt). The cell lines: HL-60, HepG2, and WI38 were obtained from American Type Culture Collection. The cells were cultured using DMEM medium (Invitrogen/Life Technologies). Cytotoxic activities were assessed utilizing In Vitro MTT based TOX-1 Kit, (Sigma-Aldrich Corp.).

Plant material collection and extraction
The aerial parts were collected in May 2018 from Aswan Botanical Garden, Aswan, Egypt and identified by Dr. Hafeez Rofaeel. An existing specimen (voucher No. 29186) is available at the herbarium of Flora and Phytotaxonomy Researches, Horticulural Research Institute, Agricultural Research Center, Dokki (Cairo), Egypt. The air-dried aerial parts (3 kg) were ground and macerated in 500 mL distilled water and the resulting residue was suspended in 500 mL distilled water and fractionated into n-hexane (13 g), DCM (8 g), ethyl acetate (25 g), and aqueous (90 g) fractions using liquid-liquid partitioning.

Isolation of DCM compounds
The cytotoxic DCM fraction (8 g) was fractionated into four groups D₁–D₄ using silica gel column chromatography (CC) (400 g), eluted with n-hexane–DCM gradient systems (1:1, 3:7, and 1:9), then DCM, followed by a gradient of DCM in EtOAc (95:5, 9:1, 85:5, 8:2, and 1:1), to collect fractions of 250 mL and monitor their TLC profiles. D₁ (1.8 g) was chromatographed on silica gel (90 g), eluted with n-hexane then n-hexane–DCM (7:3), to collect 26 subfractions grouped into three groups D₁–(I–III) based on their TLC pictures. D₁-I (612 mg) was purified using silica gel CC (30 g), eluted with n-hexane–DCM (1:1), to afford 1 (9 mg). D₁-II (79 mg) was purified over silica gel (15 g), eluted with n-hexane–DCM (1:1), to give 2 (5 mg). Fractionation of D₂ (1.4 g) via silica gel (70 g), eluted with n-hexane–EtOAc (20:1 till 10:1), resulted in five subfractions D₂-IV-V. D₂-III (459 mg) was purified by silica gel (25 g) to yield 3 (12 mg). D₂-IV (213 mg) was purified over silica gel (12 g), eluted with DCM–EtOAc (40:1), to furnish 4 (16.5 mg). Repeated chromatographic separation of D₃ (1.2 g) using silica gel CC, eluted with n-hexane–EtOAc (15:1), afforded 5 (15 mg).

Spectroscopic characterization of the isolated compounds
Lupeol (1): White amorphous powder; C₃₀H₄₈O; ¹H-NMR (CDCl₃; 400 MHz) δₜₜ: 4.56 (d, J = 2.0 Hz, H-29a), 4.68 (d, J = 2.0 Hz, H-29b), 3.18 (dd, J = 4.9, 10.8 Hz, H-3), 1.67, 1.02, 0.96, 0.94, 0.82, 0.78, 0.75 (each 3H, s); DEPTQ-135 (CDCl₃; 100 MHz) δ₂: 38.8 (CH₂, C-1), 27.6 (CH₂ C-2), 79.1 (CH, C-3), 38.9 (C-4), 55.4 (C-5), 18.4 (C-6), 34.3 (C-7), 41.0 (C-8), 50.6 (C-9), 37.3 (C-10), 21.1 (C-11), 25.3 (C-12), 38.2 (C-13), 43.0 (C-14), 27.6 (C-15), 35.7 (C-16), 43.1 (C-17), 48.4 (C-18), 48.1 (C-19), 151.1 (C-20), 30.0 (C-21), 40.1 (C-22), 28.2 (C-23), 15.6 (C-24), 16.3 (C-25), 16.1 (C-26), 14.8 (C-27), 18.1 (C-28), 109.5 (C-29), 19.6 (C-30).

Stigmasterol (2): White amorphous powder; C₂₅H₄₈O; ¹H-NMR (CDCl₃; 500 MHz) δₜₜ: 5.34 (s, H-6), 5.14 (dd, J = 14.5, 8.5 Hz, H-22), 5.00 (dd, J = 15.2, 8.8 Hz, H-23), 3.51 (br s, H-3); DEPTQ-135 (CDCl₃; 125 MHz) δ₂: 37.4 (C-1), 31.7 (C-2), 71.9 (C-3), 42.4 (C-4), 140.9 (C-5), 121.8 (C-6), 32.1 (C-7), 32.0 (C-8), 50.3 (C-9), 36.6 (C-10), 21.2 (C-11), 39.9 (C-12), 42.4 (C-13), 56.9 (C-14), 25.5 (C-15), 28.4 (C-16), 56.2 (C-17), 12.0 (C-18), 19.5 (C-19), 40.6 (C-20), 20.0 (C-21), 138.4 (C-22), 129.4 (C-23), 51.4 (C-24), 32.5 (C-25), 20.3 (C-26), 21.3 (C-27), 24.4 (C-28), 12.1 (C-29).

Scopoletin (3): White amorphous powder; C₁₀H₁₄O₄; ¹H-NMR (DMSO-d₆; 500 MHz) δₜₜ: 3.80 (s, CH₃O-6), 6.20 (d, J = 9.5 Hz, H-3), 7.88 (d, J = 9.5 Hz, H-4), 7.19 (s, H-5), 6.77 (s, H-6).
Preparation of unsaponifiable and saponifiable matter

Preparation of unsaponifiable matter (USM)

Alkaline hydrolysis was applied to saponify a portion of the n-hexane fraction (3 g). The n-hexane fraction was refluxed with 0.5 N alc. KOH for 3 h on a boiling water bath. Most of the alcohol was distilled away, and the remaining liquid was diluted with twice its volume of water before being extracted repeatedly with ether until it was exhausted. Ether was distilled away, leaving a 1.2 g dark orange residue that represents the unsaponifiable matter (USM)\(^2\).

Preparation of saponifiable matter

After removing the USM, the alkaline aqueous solution (soap) was acidified with 10% H\(_2\)SO\(_4\). The liberated fatty acids were extracted using small amounts of ether in a row. The combined ethereal extracts were washed with distilled water until the wash was litmus paper neutral. The ether was distilled away, and the total fatty acid residue (TFA) was dried, yielding a yellowish brown residue of 0.76 g\(^1\)\(^2\).

Preparation of fatty acid methyl esters (FAMEs)

FAMEs were prepared by refluxing the TFA residue with 100 mL CH\(_3\)OH and 10 mL H\(_2\)SO\(_4\) for 4 h, extracting with ether, and then evaporating the ether\(^1\)\(^2\).

**GC-MS analysis**

The GC-MS analysis was performed with a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TR-5 MS column (30 m L., 0.32 mm I.D., 0.25 µm film thickness). Helium served as the carrier gas, with a flow rate of 1 mL/min and a split ratio of 1:10. The temperature was programmed as: 60 °C for 1 min; rising at 4 °C /min to 240 °C and held for 1 min. The injector and detector were held at 210 °C. Diluted samples (1:10 hexane, v/v) of 1 µL of the mixtures were always injected. Electron ionization (EI) at 70 eV yielded mass spectra with a spectral range of m/z 40–450.

**In vitro MTT-formazan anti-proliferative assay**

The 70% methanol extract of the aerial parts of *G. thunbergia* L. f. together with its four fractions were assessed for their cytotoxic effects on two cancerous cell lines (HL-60 and HepG2) as well as WI38 normal cells via the MTT assay as previously described \(^1\). In brief, cultures were incubated and brought into a sterile laminar flow hood. Each MTT [M5655] vial was reconstituted in 3 mL of medium without phenol red and serum. The reconstituted MTT was added in an amount equal to 10% of the culture medium volume followed by 2–4 hrs incubation period. The cultures were removed from incubator and the formed formazan crystals were dissolved in MTT solubilization solution [M-8910] of equal volume to the original culture medium. Finally, the absorbance of the solubilized formazan was measured spectrophotometrically at 570 nm.

**RESULTS AND DISCUSSION**

**Identification of isolated compounds (1–5)**

The structural elucidation of the isolated compounds (1–5) (Fig. 1) was based on spectroscopic analyses (\(^1\)H and DEPTQ-135) compared with the data published in the literatures. These compounds were identified as lupeol\(^1\)\(^4\) (1), stigmasterol\(^1\)\(^5\) (2), scopoletin\(^1\)\(^6\) (3), syringaldehyde\(^1\)\(^6\) (4), and vanillic acid\(^1\)\(^7\) (5). Compounds (1–3) have recently been reported from the leaves of the plant\(^1\)\(^8\). In terms of their

H-8); DEPTQ-135 (DMSO-\(d_6\); 125 MHz) \(\delta_C\): 56.0 (CH\(_3\)O-6), 160.7 (C-2), 111.7 (C-3), 144.5 (C-4), 109.6 (C-5), 145.3 (C-6), 151.2 (C-7), 102.8 (C-7), 149.5 (C-9), 110.5 (C-10).

Syringaldehyde (4): Yellow amorphous powder; C\(_{12}\)H\(_8\)O\(_2\); \(^1\)H-NMR (CD\(_2\)OD; 400 MHz) \(\delta_H\): 7.15 (s, H-2, H-6), 3.88 (s, CH\(_3\)O-3, 5), 9.69 (s, CHO); DEPTQ-135 (CD\(_3\)OD; 100 MHz) \(\delta_C\): 129.1 (C-1), 108.1 (C-2, 6), 149.4 (C-3, 5), 143.5 (C-4), 192.9 (–CHO), 56.7 (CH\(_3\)O-3, 5).

Vanillic acid (5): White amorphous powder; C\(_{12}\)H\(_8\)O\(_2\); \(^1\)H-NMR (CD\(_2\)OD; 400 MHz) \(\delta_H\): 7.45 (d, \(J\) = 2.0 Hz, H-2), 6.72 (d, \(J\) = 8.8 Hz, H-5), 7.44 (dd, \(J\) = 8.8, 2.0 Hz, H-6), 3.77 (s, OMe); DEPTQ-135 (CD\(_3\)OD; 100 MHz) \(\delta_C\): 123.0 (C-1), 113.7 (C-2), 148.6 (C-3), 152.6 (C-4), 115.8 (C-5), 125.2 (C-6), 170.1 (COOH), 56.3 (CH\(_3\)O-3).

**RESULTS AND DISCUSSION**

**Identification of isolated compounds (1–5)**

The structural elucidation of the isolated compounds (1–5) (Fig. 1) was based on spectroscopic analyses (\(^1\)H and DEPTQ-135) compared with the data published in the literatures. These compounds were identified as lupeol\(^1\)\(^4\) (1), stigmasterol\(^1\)\(^5\) (2), scopoletin\(^1\)\(^6\) (3), syringaldehyde\(^1\)\(^6\) (4), and vanillic acid\(^1\)\(^7\) (5). Compounds (1–3) have recently been reported from the leaves of the plant\(^1\)\(^8\). In terms of their
reported distribution in other species, all detected compounds could be regarded as chemotaxonomic markers for the genus. Lupeol has been reported from *G. saxatilis*\(^{19}\), *G. aqualla*\(^{20}\), *G. ternifolia*\(^{21}\), and *G. volkensii*\(^{22}\) have all been found to have stigmasterol. *G. jasminoides* and *G. carinata* have both been found to contain syringaldehyde\(^{24,23}\). Scopoletin has been detected from *G. volkensii* and *G. carinata*\(^{4,22}\). Vanillic acid has been reported from *G. jasminoides* and *G. carinata*\(^{4,23}\). Co-occurrence of the three phenolic compounds 3–5 in *G. carinata* and the plant under study could have a chemotaxonomic implication to guide subsequent sub-classification of *Gardenia*.

**Fig. 1:** Structures of isolated compounds

**Fig. 2:** GC/MS chromatogram of the USM
Investigations of USM and FAMEs

GC-MS analysis of the USM (Fig. 1) revealed a diverse chemical profile that comprising different classes of volatile compounds. The principal detectable phytochemicals, each have 1% peak relative area or higher, that account for 69.62% of the total are given in Table 1. Most of these compounds are aromatic hydrocarbons (31.5%), which include several alkylbenzenes and a few polycyclic aromatic hydrocarbons. The most abundant alkyl benzene, with a relative concentration of 6.37%, was 1,2,3,4-tetramethylbenzene. The oxygenated volatile compounds were the second most detected components (23.86%) including terpenoid alcohols, aldehydes, and esters. The most prevalent terpenoid alcohols were β-eudesmol (3.18%), trans-farnesol (2.76%), and cubenol (2.66%). In addition, various hydrocarbons with a relative content of 14.11% were identified in the USM, the most abundant of them was 3-ethyl-5-(2-ethylbutyl)-octadecane (3.77%).

Table 1: GC/MS analysis results of the major representative compounds of the unsaponifiable matter (USM) of the n-hexane extract of *G. thunbergia* aerial parts

| RT (min) | Compound Name                                      | Molecular formula | Area% |
|---------|----------------------------------------------------|-------------------|-------|
| 9.31    | Decane                                             | C_{10}H_{12}      | 1.01  |
| 10.15   | 2-Ethyl-1-hexanol                                  | C_{6}H_{14}O      | 1.55  |
| 10.43   | 1-Iodo-2-methylundecane                            | C_{12}H_{24}I     | 1.33  |
| 10.83   | p-Tolyl acetaldehyde                               | C_{9}H_{10}O      | 1.77  |
| 11.10   | 1-Ethyl-2,3-dimethyl-benzene                       | C_{10}H_{14}      | 1.44  |
| 11.69   | 1-Ethyl-2,4-dimethyl-benzene                       | C_{10}H_{14}      | 1.51  |
| 11.94   | α-Cymene                                           | C_{10}H_{14}      | 2.14  |
| 12.53   | 2-Ethyl-1,4-dimethyl- Benzene                      | C_{10}H_{14}      | 1.18  |
| 12.99   | 1,2,3,4-Tetramethylbenzene                         | C_{10}H_{14}      | 6.37  |
| 13.09   | 1-Ethyl-3,5-dimethyl- Benzene                      | C_{10}H_{14}      | 2.79  |
| 13.56   | 1-Methyl-4-(2-propenyl)- Benzene                   | C_{10}H_{14}      | 2.47  |
| 14.00   | 1,2,4,5-tetramethyl-Benzene                        | C_{10}H_{14}      | 3.99  |
| 14.52   | 2-(4′-Methylphenyl)-Propanal                       | C_{10}H_{18}O     | 1.04  |
| 14.92   | 1H-Indene, 1-Methylene                             | C_{10}H_{8}       | 4.35  |
| 15.25   | 3,4-Dimethylcumene                                 | C_{11}H_{16}      | 1.43  |
| 15.57   | Pentamethyl-Benzene                                | C_{11}H_{16}      | 1.49  |
| 16.71   | 4,6-Dimethylindane                                 | C_{12}H_{14}      | 1.16  |
| 18.02   | 1-Phenyl-4-penten-1-ine                            | C_{11}H_{10}      | 1.33  |
| 23.16   | α-Selinene                                         | C_{15}H_{20}      | 1.28  |
| 24.67   | Elemol                                             | C_{15}H_{18}O     | 1.18  |
| 24.83   | Nerolidol                                         | C_{15}H_{20}O     | 1.00  |
| 25.13   | (3E,7E)-4,8,12-Trimethyltrideca-1,3,7,11-tetraene  | C_{16}H_{26}      | 1.02  |
| 25.58   | Geranyl isovalerate                                | C_{15}H_{20}O     | 1.21  |
| 26.74   | Cubenol                                           | C_{15}H_{22}O     | 2.66  |
| 27.33   | β-Eudesmol                                        | C_{15}H_{22}O     | 3.18  |
| 28.63   | trans-Farnesol                                     | C_{15}H_{22}O     | 2.76  |
| 31.04   | Neophytadiene                                      | C_{20}H_{18}      | 1.19  |
| 31.17   | E,E,Z-1,3,12-Nonadecatriene-5,14-diol              | C_{19}H_{32}O     | 1.22  |
| 31.93   | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol             | C_{20}H_{32}O     | 1.16  |
| 33.37   | 12-methyloctadeca-2,13-dien-1-ol                   | C_{19}H_{32}O     | 1.42  |
| 36.14   | Linoleoyl chloride                                 | C_{18}H_{32}ClO   | 2.42  |
| 36.98   | Phytol                                            | C_{20}H_{40}O     | 1.29  |
| 40.27   | Dotriacontane                                      | C_{32}H_{66}      | 3.27  |
| 40.62   | 5-Ethyl-5-(2-ethylbutyl)- Octadecane               | C_{26}H_{34}      | 3.77  |
| 43.86   | 17-Pentatriacontene                                | C_{35}H_{60}      | 1.24  |
On the other hand, both saturated and unsaturated fatty acid methyl esters together with other compounds were observed through the GC/MS analysis of FAMEs (Fig. 3). The compounds having ≥ 1% peak area that constituted 60.6% of the total are listed in Table 2. Among these compounds, saturated fatty acids dominated with a relative content of 30.56%, whereas the unsaturated fatty acids constituted 12.29% of the total. Methyl 5-(2-undecyclopropyl) pentanoate (6.94%), nonanedioic acid dimethyl ester (4.38%), and docosanoic acid methyl ester (3.22%) were the most abundant saturated fatty acids. 3-(octadecyloxy)-Oleic acid-propyl ester (5.56%) was the major unsaturated fatty acid followed by (9E,12E)-9,12-octadecadienoyl chloride (2.56%) and methyl (6E,9E,12E)-6,9,12-octadecatrienoate (2.02%).

![Fig. 3: GC/MS chromatogram of the FAMEs](image)

**Table 2:** GC/MS analysis results of the major representative compounds of the saponifiable matter of the n-hexane extract of *G. thunbergia* aerial parts

| RT (min) | Compound | Molecular formula | Area % |
|---------|----------|-------------------|--------|
| 12.08   | Methyl benzoate | C₈H₈O₂ | 3.16 |
| 12.83   | 1-(3-methyl phenyl)-ethanone | C₉H₁₀O | 4.41 |
| 15.01   | Methyl salicylate | C₈H₇O₃ | 1.56 |
| 15.69   | Nonanoic acid methyl ester | C₁₀H₂₀O₂ | 2.89 |
| 18.45   | Methyl 8-methyl-nonanoate | C₁₃H₂₄O₂ | 1.09 |
| 24.14   | Nonanedioic acid dimethyl ester | C₂₂H₄₀O₄ | 4.38 |
| 28.09   | Methyl tetradecanoate | C₁₅H₃₀O₂ | 3.05 |
| 29.37   | Methyl octadecanoate | C₁₇H₃₄O₂ | 1.20 |
| 32.12   | Methyl-pentadec-14-ynoate | C₁₈H₃₈O₂ | 2.58 |
| 34.71   | Methyl 5-(2-undecyclopropyl) pentanoate | C₂₀H₃₈O₂ | 6.94 |
| 35.63   | Methyl 14-methylhexadecanoate | C₂₀H₃₈O₂ | 1.93 |
| 36.13   | (Z,Z)-9,12-Octadecadienoyl chloride | C₁₉H₃₈ClO | 1.09 |
| 38.59   | (9E,12E)-9,12-Octadecadienoyl chloride | C₁₉H₃₈ClO | 2.56 |
| 38.71   | Methyl (7E,10E)-7,10-octadecadienoate | C₁₉H₃₈O₂ | 1.06 |
| 38.95   | Methyl dihydrosterculate | C₂₀H₃₈O₂ | 1.45 |
| 39.07   | Docosanoic acid methyl ester | C₂₂H₄₀O₂ | 3.22 |
| 39.33   | (9E)-8-Methyl-9-tetradecenyl acetate | C₁₇H₃₈O₂ | 1.29 |
| 39.47   | Methyl (6E,9E,12E)-6,9,12-octadecatrienoate | C₁₉H₃₈O₂ | 2.02 |
| 41.31   | 3-(octadecyloxy)-Oleic acid-propyl ester | C₂₀H₃₈O₂ | 5.56 |
| 41.67   | Docosanoic acid-1,2,3-propanetriyl ester | C₃₀H₄₄O₆ | 1.83 |
| 42.28   | Isochiapin B | C₁₀H₁₆O₂ | 1.24 |
| 43.57   | 1,54-Dibromotetrapentacontane | C₃₆H₄₀Br₂ | 1.29 |
| 44.75   | 3-acetoxy-7,8-epoxylanostan-11-ol | C₃₃H₄₄O₄ | 5.74 |
Identification of the USM and FAMEs components was de-convoluted using AMDIS software (www.amdis.net) and identified by its retention indices (relative to n-alkanes C8-C22) and mass spectra matching (Wiley spectral library collection and NSIT library database). It is worth noting that identified compounds in both unsaponifiable and saponifiable matters are first to be reported from *G. thunbergia*.

**In vitro MTT-formazan anti-proliferative assay**

As a result of cytotoxic evaluation, *n*-hexane and DCM fractions displayed selective cytotoxic effects on both cancerous cell lines. All results were compared to staurosporin which was used as a positive control in this assay. The cytotoxic potencies of tested fractions are expressed in terms of IC$_{50}$ values, as shown in Table 3.

To date, phytochemical investigation on this valuable plant is limited. Therefore, we sought to characterize these bioactive non-polar fractions to explore the responsible cytotoxic components. Chromatographic separation of the non-polar DCM fraction yielded five compounds comprising lupeol (1), stigmasterol (2), scopoletin (3), syringaldehyde (4), and vanillic acid (5).

Based on previous studies, lupeol and syringaldehyde could be contributing components for the DCM fraction’s potent selective cytotoxic activity. Lupeol has been shown growth-inhibitory activity and apoptosis on HepG2 cells$^{24}$. Syringaldehyde has displayed remarkable cytotoxic activity against HL-60 with an IC$_{50}$ value of 6.01 µg/mL and also has shown some cytotoxicity against HepG2 with an IC$_{50}$ value of 47.29 µg/mL$^{25,26}$. Vanillic acid was inactive against HL-60$^{25,27}$. A previous study has reported no activity of stigmasterol on HepG2 cells and weak activity on HL-60 cells$^{28}$. Previous studies have shown that scopoletin was inactive against both HL-60 and HepG2$^{26,27}$.

| Sample          | HL60 [µg/mL] | SI* | HepG2 [µg/mL] | SI* | WI38 [µg/mL] |
|-----------------|--------------|-----|---------------|-----|--------------|
| Crude extract   | 43.32 ± 3.07 | 1.4 | 24.80 ± 1.77  | 2.4 | 60.71 ± 2.42 |
| *n*-Hexane fraction | 4.38 ± 0.21 | 7.8 | 3.19 ± 0.16  | 10.7 | 3.99 ± 2.25  |
| DCM fraction    | 9.05 ± 0.63  | 5.8 | 5.53 ± 0.28  | 9.6 | 52.84 ± 3.17 |
| EtOAc fraction  | 50.76 ± 3.17 | 3.4 | 36.11 ± 1.92 | 4.7 | 171.51 ± 9.26 |
| Aqueous fraction| 24.16 ± 1.95 | 2.4 | 22.58 ± 1.63 | 2.6 | 57.84 ± 2.69 |
| Staurosporin    | 7.49 ± 0.45  | 2.9 | 3.17 ± 0.22  | 6.8 | 21.62 ± 1.54 |

*SI (selectivity index): IC$_{50}$ WI38/IC$_{50}$ tumor cell.

Results are expressed as mean ± SD which were derived from the dose-response curve of triplicate analyses.

**Conclusion**

The current study enriched the knowledge about the chemical composition and cytotoxic activity of *G. thunbergia* aerial parts. The non-polar fractions (*n*-hexane and DCM) showed selective in vitro cytotoxic activities against HL-60 and HepG2 cells. The detection of lupeol and syringaldehyde in the DCM-soluble fraction justified the observed cytotoxicity.

**Declaration of competing interest**

No potential conflict of interest was reported by the authors.

**Acknowledgments**

This research was supported financially by Egyptian Government and the National Centre of Natural Products Research (NCNPR), School of Pharmacy, University of Mississippi, USA.

**REFERENCES**

1. S. Mongrand, A. Badoc, B. Patouille, C. Lacomblez, M. Chavent and J. J. Bessoule, "Chemotaxonomy of the Rubiaceae family based on leaf fatty acid composition", *Phytochemistry*, 66(5), 549-559 (2005).
2. S. Kaennakam, T. Aree, J. Yahuafai, P.
Siripong and S. Tip-pong, "Erythrosaponins A–J, triterpene saponins from the roots and stem bark of Gardenia erythroclada", Phytochemistry, 152, 36–44 (2018).

3- T. Nuayrai, R. Sappapan, T. Vilaivan and K. Pudhom, "Gardenoins E-H, cycloartane triterpenes from the apical buds of Gardenia obtusifolia", Chem Pharm Bull, 59(3), 385–387 (2011).

4- N. Kongkum, P. Tuchinda, M. Pohmakotr, V. Reutrakul, P. Piyachaturawat, S. Jariyawat, K. Suksen, C. Yoosook, J. Kasisit, C. Napaswad, "DNA topoisomerase IIα inhibitory and anti-HIV-1 flavones from leaves and twigs of Gardenia carinata", Fitoterapia, 83(2), 368–372 (2012).

5- T. Nuayrai, R. Sappapan, T. Vilaivan and K. Pudhom, "Cycloartane triterpenes from the exudate of Gardenia thailandica", Phytochem Lett, 4(1), 26–29 (2011).

6- L. S. Zhang, Y. L. Wang, Q. Liu, C. X. Zhou, J. X. Mo, L. G. Lin and L. S. Gan, "Three new 3,4-seco-cycloartane triterpenoids from the flower of Gardenia jasminoides", Phytochem Lett, 23, 172–175 (2018).

7- K. Pudhom, T. Nuayrai and K. Matsubara, "Cytotoxic and anti-angiogenic properties of minor 3,4-seco-cycloartanes from Gardenia sootepensis exudate", Chem Pharm Bull, 60(12), 1538–1543 (2012).

8- L. Yang, K. Peng, S. Zhao, L. Chen and F. Qiu, "Monoterpeneid from the fruit of Gardenia jasminoides Ellis (Rubiaceae)", Biochem Syst Ecol, 50, 435–437 (2013).

9- S. Thanansurapong, P. Tuchinda, V. Reutrakul, M. Pohmakotr, P. Piyachaturawat, A. Chairoungdua, K. Suksen, R. Akkarawongsapat, J. Limthongkul, C. Napaswad and N. Nuntasaen, "Cytotoxic and anti-HIV-1 activities of triterpenoids and flavonoids isolated from leaves and twigs of Gardenia sessiliflora", Phytochem Lett, 35, 46–52 (2020).

10- R. Gonçalves de Oliveira-Júnior, N. Marcoult-Fréville, G. Prunier, L. Beaugeard, E. Beserra de Alencar Filho, E. D. Simões Mourão, S. Michel, L. J. Quintans-Júnior, J. R. Guedes da Silva Almeida, R. Grougnet and L. Picot, "Polymethoxyflavones from Gardenia oudiepe (Rubiaceae) induce cytoskeleton disruption-mediated apoptosis and sensitize BRAF-mutated melanoma cells to chemotherapy", Chem Biol Interact, 325, 109109 (2020).

11- Y.-G. Cao, Y.-J. Ren, Y.-L. Liu, M.-N. Wang, C. He, X. Chen, X.-L. Fan, Y.-L. Zhang, Z.-Y. Hao, H.-W. Li, X.-K. Zheng and W.-S. Feng, "Iridoid glycosides and lignans from the fruits of Gardenia jasminoides Eills", Phytochemistry, 190, 112893 (2021).

12- A. R. Johnson and J. B. Davenport, "Biochemistry and methodology of lipids", 578 (1971).

13- L. Tolosa, M. T. Donato and M. J. Gómez-Lechón, In Methods in Molecular Biology (Humana Press Inc., 2015), vol. 1250, pp. 333–348.

14- S. M. Abdullahi, A. M. Musa, M. I. Abdullahi, Sule M I and Y. M. Sani, "Isolation of Lupeol from the Stem-bark of Lonchocarpus sericeus (Papilionaceae)", Sch Acad J Biosci, 1, 18–19 (2013).

15- X. J. Li, Z. Z. Liu, K. W. Kim, X. Wang, Z. Li, Y. C. Kim, C. S. Yook and X. Q. Liu, "Chemical Constituents from Leaves of Pileostegia viburnoides hook.F.et thoms", Nat. Prod. Sci, 22(3), 154–161 (2016).

16- J. Panyo, K. Matsunami and P. Panichayupakaranant, "Bioassay-guided isolation and evaluation of antimicrobial compounds from Ixora megalophylla against some oral pathogens", Pharm Biol, 54(9), 1522–1527 (2016).

17- S. W. Chang, K. H. Kim, I. K. Lee, S. U. Choi, S. Y. Ryu and K. R. Lee, "Phytochemical constituents of Bistorta manshuriensis", Nat Prod Sci, 15(4), 234–240 (2009).

18- N. Tajuddeen, T. Swart, H. C. Hoppe and F. R. van Heerden, "Phytochemical and antiplasmodial investigation of Gardenia thunbergia L. f. leaves", Nat Prod Res, 1–9 (2021).

19- A. Suksamrarn, T. Tanachatchairatana and S. Kanokmedhakul, "Antiplasmodial triterpenes from twigs of Gardenia saxatilis", JEthnopharmacol, 88(2-3), 275–277 (2003).
20- J. N. Nyemb, L. M. Magnibou, E. Talla, A. T. Tchinda, R. T. Tchuenguem, C. Henoumont, S. Laurent and J. T. Mbafor, "Lipids constituents from Gardenia aqualla Stapf & Hutch", Open Chem, 16(1), 371–376 (2018).
21- C. Ochieng, J. Midiwo and P. Owuor, "Anti-plasmodial and larvicidal effects of surface exudates of Gardenia ternifolia aerial parts", Res J Pharmacol, 4(2), 45-50 (2010).
22- B. F. Juma and R. R. T. Majinda, " Constituents of Gardenia volkensii: Their brine shrimp lethality and DPPH radical scavenging properties", Nat Prod Res, 21(2), 121–125 (2007).
23- Z. YM, Z. ZL, Y. YQ, L. GM, C. CJ and W. YY, "[Study on the chemical components of Gardenia jasminoides].", Zhong Yao Cai, 36(2), 225–227 (2013).
24- Y. He, F. Liu, L. Zhang, Y. Wu, B. Hu, Y. Zhang, Y. Li and H. Liu, "Growth inhibition and apoptosis induced by Lupeol, a dietary triterpene, in human hepatocellular carcinoma cells", Biol Pharm Bull, 34(4), 517–522 (2011).
25- Y. Chen, J. Xu, H. Yu, C. Qing, Y. Zhang, L. Wang, Y. Liu and J. Wang, "Cytotoxic phenolics from Bulbophyllum odoratissimum", Food Chem, 107, 169–173 (2008).
26- L. Ge, L. Xiao, H. Wan, J. Li, K. Lv, S. Peng, B. Zhou, T. Li and X. Zeng, "Chemical constituents from Lonicera japonica flower buds and their anti-hepatoma and anti-HBV activities", Bioorg Chem, 92, 103198 (2019).
27- T. Akihisa, K. Kawashima, M. Orido, H. Akazawa, M. Matsumoto, A. Yamamoto, E. Ogihara, M. Fukatsu, H. Tokuda and J. Fuji, "Antioxidative and melanogenesis-inhibitory activities of caffeoylquinic acids and other compounds from Moxa", Chem Biodivers, 10(3), 313–327 (2013).
28- L. Lin, Q. Gao, C. Cui, H. Zhao, L. Fu, L. Chen, B. Yang, W. Luo and M. Zhao, "Isolation and identification of ent-kaurane-type diterpenoids from Rabdosia serra (MAXIM.) HARA leaf and their inhibitory activities against HepG-2, MCF-7, and HL-60 cell lines", Food Chem, 131(3), 1009–1014 (2012).
استكشاف المكونات التي تساهم في نشاط سمية الخلايا لنبات غاردنيا تونبرغ ضد اللوكيميا البشرية والورم الكبي.

شيماء محمد، سمير أنيس روس، نسمة محمد

قسم العقاقير، كلية الصيدلة، جامعة أسيوط، مصر.

المركز الوطني لبحوث المنتجات الطبية، معهد أبحاث العلوم الصيدلانية، كلية الصيدلة، جامعة الميسسيبي، الميسسيبي 38677، الولايات المتحدة الأمريكية.

قسم العلوم الجزيئية الحيوية، كلية الصيدلة، جامعة الميسسيبي، الميسسيبي 38677، الولايات المتحدة الأمريكية.

أثبتت العديد من الدراسات التأثيرات السامة على الخلايا السرطانية للعديد من أنواع جنس الغاردنيا (بتيغ الفصيلة الفوية). فشلت الدراسة الحالية فاعلية المستخلص الأخام للغاردنيا تونبرغ (Gardenia thunbergia L. f) وكذلك أربع مستخلصات مشتقة منه، تشمل مستخلصات الهكسان، كلوريد الميثيلين، خلات الأيت والمستخلص المائي، في قتل سلالات خلايا سرطان الدم البشري (HeLa)، وخلايا سرطان الكبد (HepG2)، بالإضافة إلى سلالة حيوية حميدة (HL60) وخلايا سرطان الكبد (W138)، بالإضافة إلى سلالة خلية حيوية مختبرية (W138). أظهر المستخلصان (الهكسان وكلوريد الميثيلين) سمية إنتقائية للخلايا الخبيثة ضد كلا السلالتين المختبرتين.

أثارت هذه النتائج اهتماماً بتوصيف المستخلصات الفعالة كيميائياً للكشف عن مكوناتها السامة للخلايا السرطانية. تم تحديد مكونات مستخلص الهكسان عن طريق تقنية الكرومتوغرافيا الغازية المتصلة بمطيافية الكتلة وتم قراءة المركبات النانوية مستخلص كليود الميثيلين باستخدام كرومتوغرافيا جراميكي. تم التعرف على المواد الكيميائية النباتية المعزولة من خلال التحليلات الطيفية. إلى جانب ذلك، تم إشراف على القيمة التصنيفية الكيميائية للمكونات المكتشفة وفاعليتها السابقة دراستها كمواد سامة للخلايا السرطانية.