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

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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 phytochemicals[2–7]. Previous studies on *Gardenia* species revealed the presence of diverse phytochemicals including monoterpenes[8], triterpenes[9], saponins[2], flavonoids[10], iridoids, and lignans[11]. 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.

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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: $CDCl_3$, CD_3OD, and DMSO-d_6 (Cambridge Isotope Lab., Inc., MA) and TMS as an internal standard. Chromatographic adsorbents: silica gel G_{60} (60–120 mesh, Merck, Darmstadt, Germany). TLC was performed using 0.25 mm aluminum pre-coated silica 60 F_{254} sheets (E-Merck, Darmstadt, Germany). Solvents for extraction and isolation procedures were analytical grade, Fisher Scientific (Adwic, 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, Horticultural Research Institute, Agricultural Research Center, Dokki (Cairo), Egypt. The air-dried aerial parts (3 kg) were ground and macerated repeatedly in 70% (v/v) aqueous methanol at room temperature. The extracts were combined and concentrated under vacuum at 40 °C, to get a dry residue of 140 g. 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 D_1–D_4 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. D_1 (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 D_1-(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 D_2 (1.4 g) via silica gel (70 g), eluted with *n*-hexane–EtOAc (20:1 till 10:1), resulted in five subfractions D_2-(I–V). D_2-III (459 mg) was purified by silica gel (25 g) to yield **3** (12 mg). D_2-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 D_3 (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; $C_{30}H_{50}O$; ^{1}H-NMR ($CDCl_3$; 400 MHz) δ_H: 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 ($CDCl_3$; 100 MHz) δ_C: 38.8 (CH_2, C-1), 27.6 (CH_2, 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; $C_{29}H_{48}O$; ^{1}H-NMR ($CDCl_3$; 500 MHz) δ_H: 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 ($CDCl_3$; 125 MHz) δ_C: 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; $C_{10}H_8O_4$; ^{1}H-NMR (DMSO-d_6; 500 MHz) δ_H: 3.80 (*s*, CH_3O-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-8); DEPTQ-135 (DMSO-d_6; 125 MHz) δ_C: 56.0 (CH_3O-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-8), 149.5 (C-9), 110.5 (C-10).

Syringaldehyde (**4**): Yellow amorphous powder; $C_9H_{10}O_4$; ^{1}H-NMR (CD_3OD; 400 MHz) δ_H: 7.15 (*s*, H-2, H-6), 3.88 (*s*, CH_3O-3, 5), 9.69 (*s*, CHO); DEPTQ-135 (CD_3OD; 100 MHz) δ_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_3O-3, 5).

Vanillic acid (**5**): White amorphous powder; $C_8H_8O_4$; ^{1}H-NMR (CD_3OD; 400 MHz) δ_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_3OD; 100 MHz) δ_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_3O-3).

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)[12].

Preparation of saponifiable matter

After removing the USM, the alkaline aqueous solution (soap) was acidified with 10% H_2SO_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[12].

Preparation of fatty acid methyl esters (FAMEs)

FAMEs were prepared by refluxing the TFA residue with 100 mL CH_3OH and 10 mL H_2SO_4 for 4 h, extracting with ether, and then evaporating the ether[12].

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 programed 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 [13]. In brief, cultures were incubated and brought into a sterile laminar flow hood. Each MTT [M-5655] 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[14] (**1**), stigmasterol[15] (**2**), scopoletin[16] (**3**), syringaldehyde[16] (**4**), and vanillic acid[17] (**5**). Compounds (**1**–**3**) have recently been reported from the leaves of the plant[18]. 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[4&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*.

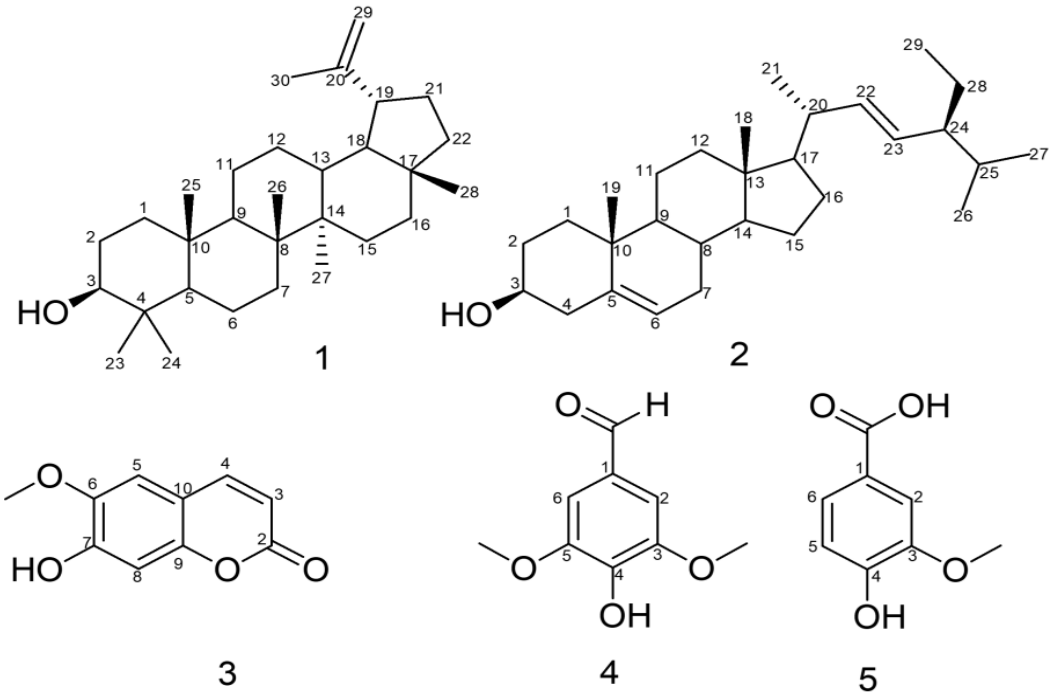

Fig. 1: Structures of isolated compounds

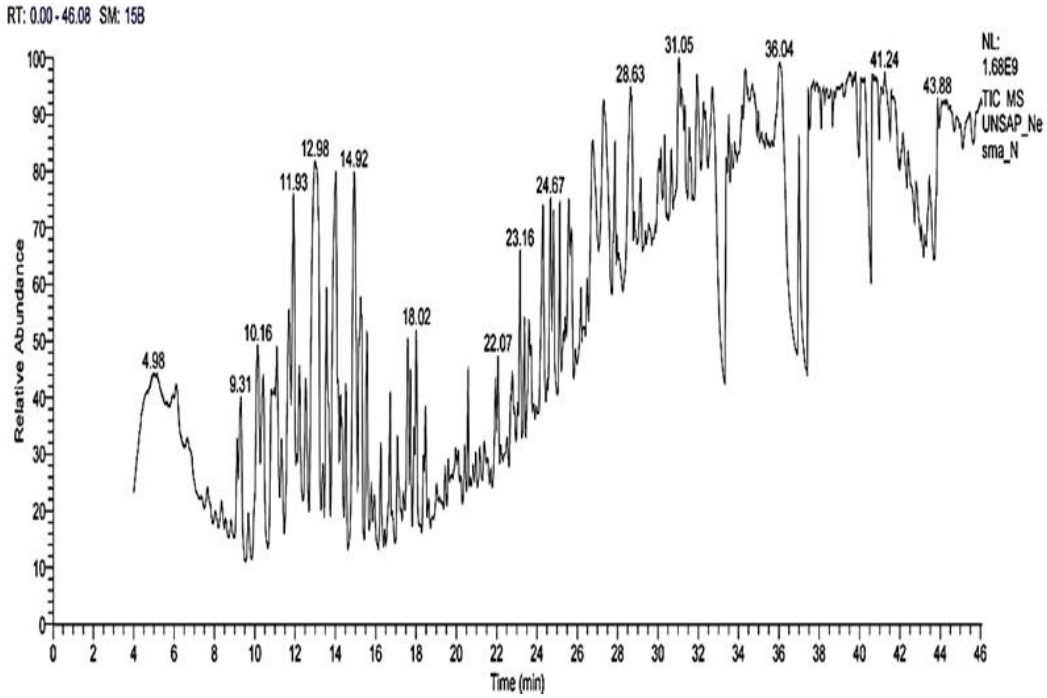

Fig. 2: GC/MS chromatogram of the USM

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	$C_{10}H_{22}$	1.01
10.15	2-Ethyl-1-hexanol	$C_8H_{18}O$	1.55
10.43	1-Iodo-2-methylundecane	$C_{12}H_{25}I$	1.33
10.83	*p*-Tolyl acetaldehyde	$C_9H_{10}O$	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	*o*-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	$C_{10}H_{12}$	2.47
14.00	1,2,4,5-tetramethyl-Benzene	$C_{10}H_{14}$	3.99
14.52	2-(4'-Methylphenyl)-Propanal	$C_{10}H_{12}O$	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	$C_{11}H_{14}$	1.16
18.02	1-Phenyl-4-penten-1-yne	$C_{11}H_{10}$	1.33
23.16	α-Selinene	$C_{15}H_{24}$	1.28
24.67	Elemol	$C_{15}H_{26}O$	1.18
24.83	Nerolidol	$C_{15}H_{26}O$	1.00
25.13	(3*E*,7*E*)-4,8,12-Trimethyltrideca-1,3,7,11-tetraene	$C_{16}H_{26}$	1.02
25.58	Geranyl isovalerate	$C_{15}H_{26}O_2$	1.21
26.74	Cubenol	$C_{15}H_{26}O$	2.66
27.33	*β*-Eudesmol	$C_{15}H_{26}O$	3.18
28.63	*trans*-Farnesol	$C_{15}H_{26}O$	2.76
31.04	Neophytadiene	$C_{20}H_{38}$	1.19
31.17	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	$C_{19}H_{34}O_2$	1.22
31.93	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	1.16
33.37	12-methyloctadeca-2,13-dien-1-ol	$C_{19}H_{36}O$	1.42
36.14	Linoleoyl chloride	$C_{18}H_{31}ClO$	2.42
36.98	Phytol	$C_{20}H_{40}O$	1.29
40.27	Dotriacontane	$C_{32}H_{66}$	3.27
40.62	3-Ethyl-5-(2-ethylbutyl)- Octadecane	$C_{26}H_{54}$	3.77
43.86	17-Pentatriacontene	$C_{35}H_{70}$	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-undecylcyclopropyl) 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 (9*E*,12*E*)-9,12-octadecadienoyl chloride (2.56%) and methyl (6*E*,9*E*,12*E*)-6,9,12-octadecatrienoate (2.02%).

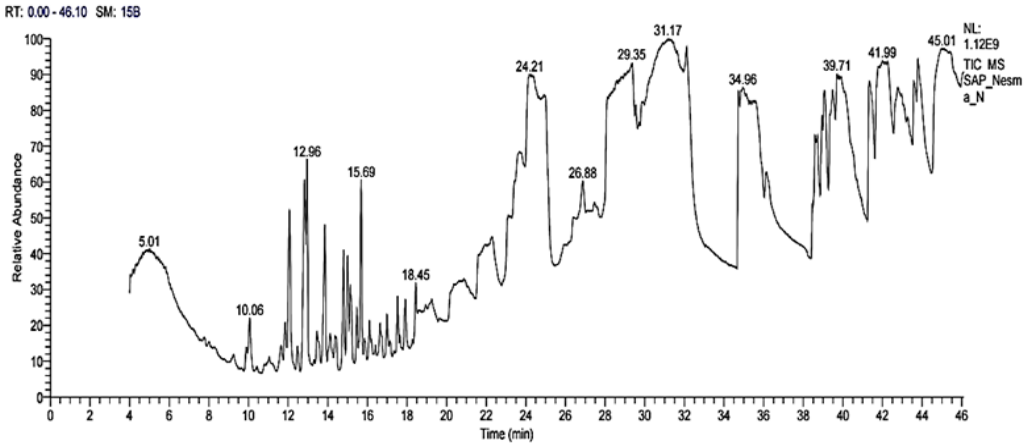

Fig. 3: GC/MS chromatogram of the FAMEs

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	$C_8H_8O_2$	3.16
12.83	1-(3-methyl phenyl)-ethanone	$C_9H_{10}O$	4.41
15.01	Methyl salicylate	$C_8H_8O_3$	1.56
15.69	Nonanoic acid methyl ester	$C_{10}H_{20}O_2$	2.89
18.45	Methyl 8-methyl-nonanoate	$C_{11}H_{22}O_2$	1.09
24.14	Nonanedioic acid dimethyl ester	$C_{11}H_{20}O_4$	4.38
28.09	Methyl tetradecanoate	$C_{15}H_{30}O_2$	3.05
29.37	Methyl octadecanoate	$C_{19}H_{38}O_2$	1.20
32.12	Methyl-pentadec-14-ynoate	$C_{16}H_{28}O_2$	2.58
34.71	Methyl 5-(2-undecylcyclopropyl) pentanoate	$C_{20}H_{38}O_2$	6.94
35.63	Methyl 14-methylhexadecanoate	$C_{18}H_{36}O_2$	1.93
36.13	(*Z*,*Z*)- 9,12- Octadecadienoyl chloride	$C_{18}H_{31}ClO$	1.09
38.59	(9*E*,12*E*)-9,12-Octadecadienoyl chloride	$C_{18}H_{31}ClO$	2.56
38.71	Methyl (7*E*,10*E*)-7,10-octadecadienoate	$C_{19}H_{34}O_2$	1.06
38.95	Methyl dihydrosterculate	$C_{20}H_{38}O_2$	1.45
39.07	Docosanoic acid methyl ester	$C_{23}H_{46}O_2$	3.22
39.33	(9*E*)-8-Methyl-9-tetradecenyl acetate	$C_{17}H_{32}O_2$	1.29
39.47	Methyl (6*E*,9*E*,12*E*)-6,9,12-octadecatrienoate	$C_{19}H_{32}O_2$	2.02
41.31	3-(octadecyloxy)-Oleic acid-propyl ester	$C_{39}H_{76}O_3$	5.56
41.67	Docosanoic acid-1,2,3-propanetriyl ester	$C_{69}H_{134}O_6$	1.83
42.28	Isochiapin B	$C_{19}H_{22}O_6$	1.24
43.57	1,54-Dibromotetrapentacontane	$C_{54}H_{108}Br_2$	1.29
44.75	3-acetoxy-7,8-epoxylanostan-11-ol	$C_{32}H_{54}O_4$	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].

Table 3: Cytotoxic activities of the crude extract and fractions of *G. thunergia* towards HL60 and HepG2.

Sample	HL60		HepG2		WI38
	[µg/mL]	SI*	[µg/mL]	SI*	[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	33.99 ± 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.

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استكشاف المكونات التي تساهم في نشاط سمية الخلايا لنبات جاردينيا تونبرغ ضد اللوكيميا البشرية والورم الكبدي

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أثبتت العديد من الدراسات التأثيرات السامة على الخلايا السرطانية للعديد من أنواع جنس الغاردينيا (يتبع الفصيلة الفَويّة). قيمت الدراسة الحالية فاعلية المستخلص الخام لنبات غاردينيا تونبرغ (الاسم العلمي *Gardenia thunbergia* L. f) وكذلك أربع مستخلصات مشتقة منه، تشمل مستخلصات الهكسان، كلوريد الميثيلين، خلات الايثل والمستخلص المائي، في قتل سلالات خلايا سرطان الدم البشري (HL-60) وخلايا سرطان الكبد (HePG-2)، بالإضافة إلى سلالة خلوية حميدة (WI38). أظهر المستخلصان (الهكسان وكلوريد الميثيلين) سمية إنتقائية للخلايا الخبيثة ضد كلا السلالتين المختبرتين. أثارت هذه النتائج اهتمامنا بتوصيف المستخلصين الفاعلين كيميائيًا للكشف عن مكوناتهما السامة للخلايا السرطانية. تم تحديد مكونات مستخلص الهكسان عن طريق تقنية الكروماتوغرافيا الغازية المتصلة بمطياف الكتلة وتم فصل المركبات الثانوية لمستخلص كلوريد الميثيلين باستخدام كروماتوغرافيا العمود. تم التعرف على المواد الكيميائية النباتية المعزولة من خلال التحليلات الطيفية. إلى جانب ذلك، تمت الإشارة الى القيمة التصنيفية الكيمائية للمكونات المكتشفة وفاعليتها السابق دراستها كمواد سامة للخلايا السرطانية.