

Anticancer activity and phytochemical composition of wild *Gundelia tournefortii*

SALEH ABU-LAFI¹, BARAA RAYAN², SLEMAN KADAN², MALEK ABU-LAFI³ and ANWAR RAYAN^{2,4}

¹Faculty of Pharmacy, Al-Quds University, Abu Dies, Palestine; ²Drug Discovery Informatics Lab, Qasemi Research Center, Al-Qasemi Academic College, Baqa-El-Gharbia 30100; ³Faculty of Medicine, Al-Quds University, Abu Dies, Palestine; ⁴Drug Discovery Informatics Laboratory, Institute of Applied Research - Galilee Society, Shefa-'Amr 20200, Israel

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Abstract. Artichoke-like wild thistles are often used in Palestinian cuisine. One of the most commercially recognized species of these wild edible thistles is *Gundelia tournefortii*, a common plant in the Mediterranean region. *G. tournefortii*, or 'Akoob' in Arabic, remains uncultivated, harvested wild by local populations and considered highly valuable due to its reputed health benefits. The present study aimed to investigate the anticancer effects of *G. tournefortii* on the human colon carcinoma HCT-116 cell line. Methanol and hexane extracts were identified to exert considerable antitumor activity against the HCT-116 cancer cell line, while the aqueous extract was inactive. The phytochemical profiles of the methanol and hexane extracts were investigated using gas chromatography-mass spectrometry. A total of 6 of the 27 natural compounds identified, including sitosterol, stigmasterol, lupeol, gitoxigenin, α -amyrin and artemisinin, have been previously validated as being active against cancerous cells. Therefore, the presence of these phytochemicals in *G. tournefortii* is of importance in its role in preventing and treating cancer.

Introduction

Gundelia tournefortii (Asteraceae) thistle is a vegetable that is similar to artichoke and grows in the semi-desert climate of numerous countries in the Mediterranean region (1). Particularly in Palestine, *G. tournefortii*, or 'Akoob' in Arabic, remains uncultivated, harvested by local populations, and is considered highly valuable due to its reputed health benefits (2). Previously, it was listed as 1 of the 10 species with the highest mean cultural importance in northern Palestine (2), and it is a component of a number of traditional recipes, which differ

according to different localities. In Palestinian traditional medicine and ethno-botany, this plant is believed to possess nutritive and curing benefits for diabetes, epilepsy, stomach and intestinal diseases (3,4). According to the literature, it has been validated to exert antioxidant, hepatoprotective and antibacterial effects (1,5). A previous study (2) conducted among Palestinians eating Akoob on a regular basis revealed a consensus belief of its capability to prevent and cure cancer. However, an intensive search of PubMed (using the terms *gundelia tournefortii* and cancer; on 12th September 2016) indicates that there is no single study on the effects of *G. tournefortii* extracts against cancer. The present study aimed to investigate the anticancer effects of *G. tournefortii* on the human colon cancer HCT-116 cell line. Gas chromatography-mass spectrometry (GC-MS) was utilized to explore the potential phytochemicals responsible for the anticancer activity. A total of 27 constituents were identified in *G. tournefortii*, of which 6 phytochemicals, including sitosterol, stigmasterol, lupeol, gitoxigenin, α -amyrin and artemisinin have been demonstrated to exhibit anticancer activities. To the best of our knowledge, the present study was the first to investigate the potential benefits of consuming wild edible *G. tournefortii* for cancer, and to analyze the phytochemical contents known for their anticancer effects.

Materials and methods

Plant material. The *G. tournefortii* plant was collected from the mountains of Nablus in northern Palestine between February and March 2014. The prickles were removed with scissors, and the stems and head sections (the parts of the plant that are usually consumed) were reserved. The processed plant was dried in the shade at room temperature.

Extract preparation. The air-dried aerial parts of *G. tournefortii* were ground into a fine powder using mixer grinder machine. Three equal fractions, weighing 25 ± 0.01 g, of the ground plant were then transferred into Erlenmeyer flasks, and treated with 250 ml methanol (MeOH of purity >99.9%), H₂O or hexane (C₆H₁₄, 99% purity) solvents separately. The flasks were then sonicated for 2 h at 50°C, and left in glass bottles (covered with aluminum foil) in the dark for 24 h to ensure complete extraction of the compounds. The extracts were filtered by passing the solvents through a 0.4- μ m filter. A total of 10 ml was removed

Correspondence to: Professor Anwar Rayan, Drug Discovery Informatics Laboratory, Institute of Applied Research-Galilee Society, 1 Galilee Street, Shefa-'Amr 20200, Israel
E-mail: a_rayan@qsm.ac.il

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from each solvent type for analysis of antioxidant activity. The remaining volume from each solvent type was evaporated under reduced pressure (range, 0.05 and 0.2 atm) and dissolved in dimethyl sulfoxide for *in vitro* experiments. The yields of the extracts were calculated by dividing the weight of the extracted material following vacuum evaporation on the crude material and found to be 3.2, 1.8 and 12% (wt/wt) for the methanol, hexane and water extracts, respectively. The stock crude extracts were preserved in sealed glass containers and kept at -20°C until use.

MTT assay. MTT is a water-soluble tetrazolium salt, which is converted to insoluble purple formazan by cleavage of the tetrazolium ring by succinate dehydrogenase within the mitochondria. The formazan product accumulates only in healthy cells. The assay was optimized for the cell lines used in the experiments. MTT was applied to assess cell viability as described in a previous study (6). HCT-116 (colorectal carcinoma cell-line from the American Type Culture Collection termed in brief ATCC® CCL247™) cells (2×10^4 /well) were plated in 200 μ l medium/well in 96-well plates and were allowed to adhere to the plate for 24 h. The medium was purchased from Biological Industries Israel Beit Haemek Ltd, Kibbutz Beit Haemek, Israel. Plant extracts were added at increasing concentrations (0-1,000 μ g/ml) for 22 h. The cell medium was then replaced with 100 μ l fresh medium/well containing 0.5 mg/ml MTT and cultivated with the cells for an additional 4 h in an incubator in the dark at 38°C. The supernatant was removed and 100 μ l isopropanol/HCl [2% HCl (0.1 M) in isopropanol] was added to each well. The absorbance at 620 nm (A_{620}) was measured with microtiter plate reader (Anthos Labtec Instruments, Austria). For each plate, two wells without cells served as blanks. All experiments were repeated three times in triplicate. The effects of the plant extracts on cell viability were expressed using the following formula: Percent viability = (A_{620} of treated sample / A_{620} of non-treated sample) $\times 100$.

GC-MS analysis. Solutions of the *G. tournefortii* methanol and hexane extracts were selected and examined with GC-MS using an Agilent 7890A GC system coupled with the Agilent 5975C inert MSD with Triple-Axis Detector mass spectrometer (Agilent Technologies, Inc., Santa Clara, CA, USA). The GC was performed on an Agilent J&W GC HP-5 column [30 m \times 0.32 mm (inner diameter), with 0.25- μ m film thickness; Agilent Technologies, Inc., Santa Clara, CA, USA]. The carrier gas was helium at a flow rate of 1.2 ml/min, and the injection volume was 1 μ l. The injection port and the MS interface temperatures were 300°C and the ionization voltage was 70 eV. The samples were injected in the split mode with a ratio of 10:1. Mass spectra were recorded every second over a range set at (m/z) of 45-800 Da. The oven heating stage was activated with an initial temperature of 50°C for 5 min, then increased to 320°C at a rate of 5°C/min, and then maintained at 320°C for an additional 20 min. The total duration of the GC-MS protocol was 79 min, and the solvent delay time was 7 min.

The percentages of the phytochemical components were computed from the GC peak areas normalization. The normalization is done automatically by the software while dividing the peak area of each peak by the total area and multiply by 100. Library searches were performed using the Mass Spectral

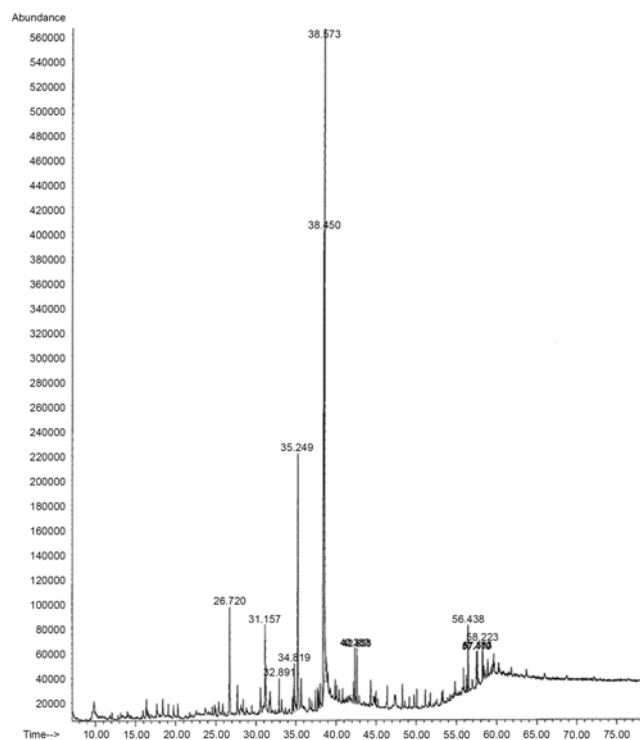


Figure 1. Total ion chromatogram of *Gundelia tournefortii* hexane extract.

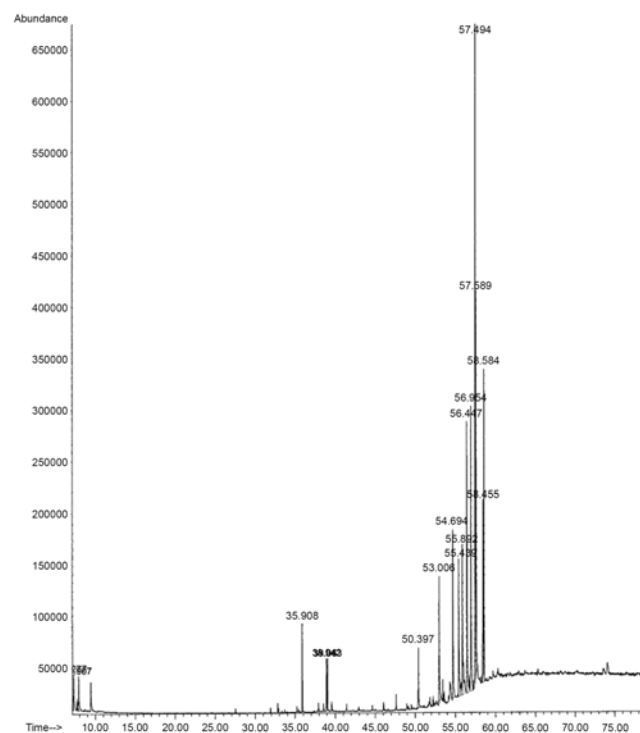


Figure 2. Total ion chromatogram of *Gundelia tournefortii* methanol extract.

Library of the National Institute of Standards and Technology (Gaithersburg, MD, USA).

Results and Discussion

In vitro anticancer activity of the different *G. tournefortii* extracts. The effects of *G. tournefortii* extracts on HCT-116

Table I. Phytochemicals of *Gundelia tournefortii* from the hexane extract by gas chromatography-mass spectrometry.

No.	Retention time (mins)	Component name	Abundance, %
1	35.91	Methyl palmitate	1.89
2	38.94	Methyl linoleate	1.27
3	39.06	Ethyl oleate	1.65
4	50.39	NI	1.96
5	53.01	Heptadecane	4.21
6	54.69	Stigmasterol ^a	5.75
7	55.44	β -sitosterol ^a	5.09
8	55.89	α -amyrin ^a	5.76
9	56.45	Lupeol ^a	9.17
10	56.95	Olean-12-en-3-yl acetate	9.58
11	57.49	Thunbergol	24.41
12	57.59	Hop-22(29)-en-3. β -ol	11.31
13	58.45	NI	6.58
14	58.58	A'-neogammacer-22(29)-en-3-ol, acetate, (3. β .,21. β .)-	8.341

NI, not identified. ^aAnticancer component.

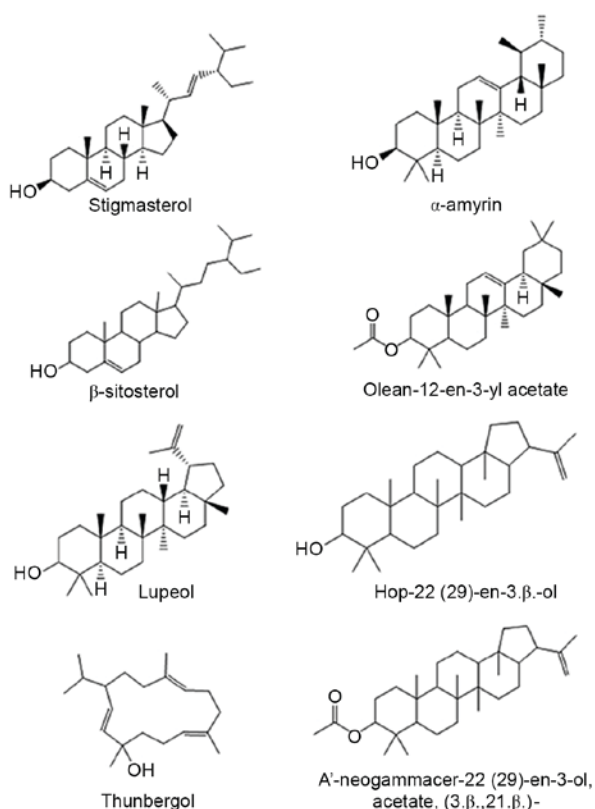


Figure 3. Chemical structure of the major components in *Gundelia tournefortii* hexane extract.

human colon cancer cells were determined using the MTT assay. The HCT-116 cell line was exposed to *G. tournefortii* methanol, hexane and aqueous extracts (0-1,000 $\mu\text{g/ml}$ for 22 h. The half maximal effective concentrations obtained by the MTT assay were 303.3 ± 12 $\mu\text{g/ml}$ for the methanol extract and 313.3 ± 18.6 $\mu\text{g/ml}$ for the hexane extract. However, the aqueous extract only reduced cell viability to 74.8% at

concentration of 1,000 $\mu\text{g/ml}$, thus demonstrating inactivity on cancer cells.

Phytochemical analysis of *G. tournefortii* using GC-MS. The phytochemical screening using GC-MS in the electron impact mode revealed 27 compounds in the *G. tournefortii* methanol and hexane extracts, 19 of which, to the best of our knowledge, were detected for the first time in *G. tournefortii* (Figs. 1-4), with the remaining 6 components [including stigmasterol (PubChem CID: 5280794), β -sitosterol (PubChem CID: 222284), palmitic acid, linoleic acid, α -linolenic acid and stearic acid] having been identified previously (5). Figs. 1 and 2 indicate the total ion chromatograms of the hexane and methanol extracts. The analysis of these two extracts revealed 14 and 13 major components in the hexane and methanol extracts, respectively (Tables I and II; Figs. 3 and 4). Compounds such as sterols, triterpenes, esters and carboxylic acids were observed in the *G. tournefortii* extracts in large quantities. For example, two common sterols known as sitosterol and stigmasterol (Fig. 3), were observed in the hexane extract constituting ~10% of its components (Table I). It has been demonstrated that sitosterol acts as an inhibitor of tumor promotion *in vivo* and that it inhibits carcinogenesis (7,8). Furthermore, stigmasterol significantly inhibits tumor promotion in two-stage carcinogenesis in mice (7,9). A mixture of sitosterol and stigmasterol was indicated to possess anti-inflammatory activity following topical application (10). Therefore, it is expected that the presence of such sterols in *G. tournefortii* may be important for its anticancer activities.

Lupeol (PubChem CID: 222284; Table I; Fig. 3), an additional phytochemical, which to the best of our knowledge has never been identified to be present in *G. tournefortii*, weighed ~10% of the hexane extract. It has been revealed to act as a novel androgen receptor, which inhibits the proliferation of human prostate cancer cells by targeting β -catenin signaling during carcinogenesis (11).

Table II. Phytochemicals of *Gundelia tournefortii* from the methanol extract by gas chromatography-mass spectrometry.

No.	Retention time, min	Component name	Abundance, %
1	26.72	Dodecanoic acid	5.16
2	31.16	Tetradecanoic acid	1.58
3	32.89	6,10,14-trimethylpentadecan-2-one	1.18
4	34.82	(Z)-11-hexadecenoic acid	2.41
5	35.25	Palmitic acid	11.70
6	38.45	Linoleic acid	26.88
7	38.57	(9Z)-9,17-octadecadienal	39.28
8	42.38	Artemisinin ^a	2.39
9	42.62	Matricarin	1.97
10	56.44	Hop-22(29)-en-3.β.-ol	2.81
11	57.48	NI	1.53
12	57.58	NI	1.59
13	58.22	Gitoxigenin ^a	1.51

NI, not identified. ^aAnticancer component.

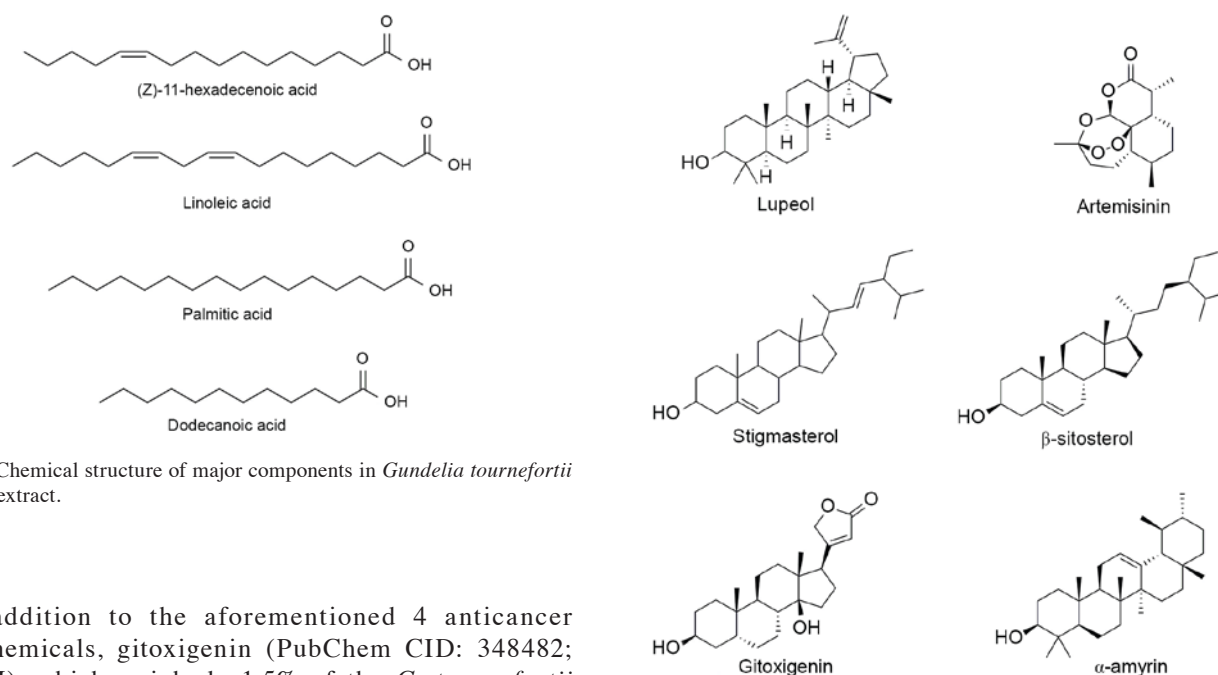


Figure 4. Chemical structure of major components in *Gundelia tournefortii* methanol extract.

In addition to the aforementioned 4 anticancer phytochemicals, gitoxigenin (PubChem CID: 348482; Table II), which weighed ~1.5% of the *G. tournefortii* methanol extract, was suggested to elicit significant anticancer activity (12) against renal adenocarcinoma and other cancer cell lines, with half-maximal inhibitory concentration (IC_{50}) values in the μM range. Furthermore, α -amyrin (PubChem CID: 73170; Table I; Fig. 3), which composed ~5.7% of the hexane extract, was identified previously to have significant anticancer activity (13) on four cancer cell lines (MCF-7, BEL-7402, SPC-A-1 and SGC-7901), with IC_{50} values of 7.2 ± 0.12 , 8.2 ± 0.29 , 7.6 ± 0.06 and $5.0 \pm 0.12 \mu M$, respectively.

The methanol extract contained 6 carboxylic acids, together representing almost half of the whole extract (48.9%). Artemisinin (PubChem CID: 68827; Table II), an active promising photochemical, was also identified for the first time, to the best of our knowledge, in *G. tournefortii* extracts, comprising 2.39%. This compound is currently undergoing investigation for its use in the treatment of cancer (14).

Figure 5. Chemical structures of 6 of the 27 phytochemicals validated as being active against cancerous cells: Sitosterol, stigmasterol, lupeol, gitoxigenin, α -amyrin, and artemisinin.

Additionally, artemisinin was mentioned in >300 studies as a compound with potential anticancer activities (PUBMED search using the terms: Artemisinin AND [anticancer OR cytotoxic], on 21 September 2016).

Summary. To the best of our knowledge, the present study was the first to highlight the anticancer activity of *G. tournefortii*. Methanol and hexane extracts exhibited anticancer capacities against the HCT-116 cancer cell line, while the aqueous extract was inactive. Using GC-MS, miscellaneous phytochemicals in the methanol and hexane extracts were identified. The results

obtained indicate the abundance of important active ingredients in this plant. The existence of potent chemicals, such as sitosterol, stigmasterol, lupeol, gitoxygenin, α -amyrin and artemisinin (Fig. 5) may act additively or synergistically, and therefore would be important factors in the preventative and anticancer properties of *G. tournefortii*. The results of the present study are in concordance with the survey conducted on participants who regularly consumed Akoob, with the belief that it possesses anticancer benefits (2). Additional studies aiming to identify the mechanism of action, and to evaluate the efficacy and toxicity of *G. tournefortii* in *in vivo* models, are required.

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