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CYTOTOXIC METABOLITES FROM THE AERIAL PART OF *ONOPORDUM HETERACANTHUM* GROWING IN SAUDI ARABIA

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ABSTRACT. Six known metabolites: β-amyrin-3-O-stearate (**1**), taraxasterol acetate (**2**), lupeol (**3**), 27-*nor*-3β-hydroxy-25-oxocycloartane (**4**), stigmasterol (**5**), and scutellarein-6-methyl ether (**6**) were isolated from the $CHCl_3$ and EtOAc fractions of the aerial parts of *Onopordum heteracanthum* C.A. Mey (Asteraceae). Compounds **1** and **4** are reported for the first time from the genus *Onopordum*, while compounds **2**, **3**, **5**, and **6** are reported for the first time from *O. heteracanthum*. Their chemical structures were established based on comprehensive spectroscopic analyses, together with comparison to literature data. The cytotoxic activity of these compounds was assessed against breast adenocarcinoma (MCF-7), human hepatocellular carcinoma (HepG2), and colorectal adenocarcinoma (HCT-116) tumor cell lines using the sulphorhodamine B assay (SRB). Compound **4** exhibited notable cytotoxic activity against MCF-7, HepG2, and HCT-116 cells (IC_{50} values of 2.1 ± 0.06, 2.5 ± 0.06, and 2.9 ± 0.05 µM, respectively), compared with doxorubicin (IC_{50}s 0.2 ± 0.008, 0.6 ± 0.05, and 0.18 ± 0.005 µM, respectively). Compounds **1**, **3**, and **6** showed moderate activity, whereas compounds **2** and **5** were weak or inactive. These results highlighted the potential of *O. heteracanthum* as a source of bioactive triterpenoids with promising anticancer activities.

KEY WORDS: *Onopordum heteracanthum*, Triterpenes, Cycloartane, Flavonoid, Cytotoxicity

INTRODUCTION

Medicinal plants have played a significant role in traditional and modern healthcare systems for millennia. Their substantial divergence and affordability make them promising renewable resources for finding novel therapeutic agents [1, 2].

The Compositae family (Asteraceae) is one of the largest flowering plant families, including more than 1600 genera and 23,000 species, and is well known for its significant pharmacological and chemical diversity [3, 4]. The Kingdom of Saudi Arabia has a diverse flora, supporting a wide range of medicinal plants, such as trees, shrubs, and herbs, because of the variation in its climatic and ecological conditions [5]. The genus *Onopordum* comprises 40 species, widely distributed across southern Europe, northern Africa, the Canary Islands, the Caucasus, and southwest and central Asia, of which *O. heteracanthum* and *O. sibthorpianum* are reported in Saudi Arabia [6].

The species of the *Onopordum* genus has attracted considerable attention due to their nutritional, ethnopharmacological, and biological relevance [7, 8]. Its plants are used to treat gynecological, gastric, respiratory, renal, and urinary disorders, as well as diabetes [8-10]. Additionally, they possess diverse bioactivities, including apoptotic, antioxidant,

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antiproliferative, antimicrobial, cytotoxic, larvicidal, antitumor, analgesic, anticholinergic, hemostatic, antipyretic, anti-inflammatory, antiepileptic, anticonvulsant, and hypotensive properties [11-16].

Phytochemical investigations of the genus *Onopordum* have revealed the isolation of diverse secondary metabolites, including flavonoids, sesquiterpenoids, steroids, coumarins, phenylpropanoids, fatty acids, triterpenes, tannins, and nitrogen-containing compounds [5, 9, 17–23].

Onopordum illyricum, *O. acanthium*, and *O. alexandrinum* are among the most studied species of this genus. *Onopordum illyricum* L. (onopordo maggiore, cardo maggiore) is a Mediterranean medicinal plant traditionally used for biliary disorders, respiratory and urinary inflammation, and skin ulcers, as well as digestive and cough sedative [12, 24]. Onopordum *acanthium* L. (Scotch thistle), which grows in Asia and Europe, possesses cardiotonic, anti-inflammatory, and antitumor activities [7, 9, 13, 14]. Additionally, its inflorescences contain onopordosin (a proteolytic enzyme complex) that may be utilized as milk-clotting agents in the dairy industry [7]. *Onopordum heteracanthum* C. A. Mey is an erect, branching, spiny-leaved herb, with white-downy leaves and spiny, winged stems [4], which is one of the underexplored *Onopordum* species. Its extract demonstrated cytotoxic activity against A2780 (ovarian cancer) and MCF7 cell lines and antibacterial activity [4]. However, phytochemical studies on *O. heteracanthum* are still limited, with only a few reports describing the isolation of fatty acids, volatile constituents, and flavonoids [5, 22].

Therefore, the present study aimed to isolate and characterize secondary metabolites from the aerial parts of *O. heteracanthum* growing in Saudi Arabia using chromatographic and spectroscopic techniques and to evaluate their cytotoxic activity against selected human cancer cell lines (MCF-7, HepG2, and HCT-116) using the sulphorhodamine B (SRB) assay.

MATERIALS AND METHODS

General experimental procedures

1D (^{1}H and ^{13}C) and 2D (HSQC and HMBC) NMR spectra (chemical shifts in ppm and coupling constants in Hz) were recorded on Bruker Avance DRX 850 MHz spectrometers (Bruker BioSpin, Billerica, MA, USA). GCMS was performed using Clarus 500 GC/MS (PerkinElmer, Shelton, CT) with TurboMass software (version 4.5.0.007, PerkinElmer). An elite 5 MS GC capillary column (30 m × 0.25 mm i.d., film thickness 0.5 μm; PerkinElmer) was employed. The carrier gas was helium at a flow rate of 2 mL/min with a split ratio of 1:40. Temperature conditions were as follows: inlet temperature, 200 °C; ion source temperature, 150 °C; trap emission, 100 °C; and electron ionization energy, 70 eV. The column temperature program was set at 50 °C (held for 5 min), then increased to 220 °C at a rate of 20 °C/min and maintained for 5 min. The injector temperature was 220 °C. Mass spectra were recorded over a scan range of m/z 50-650. Column chromatographic separations were performed on silica gel 60 (0.04-0.063 mm) and Sephadex LH-20 (Merck, Darmstadt, Germany). TLC was performed on precoated TLC plates with silica gel 60 F254 (0.2 mm, Merck). The compounds were detected by UV absorption at l_{max} 255 and 366 nm, followed by spraying with anisaldehyde/H_2SO_4 reagent and heating at 140 °C for 2-3 min.

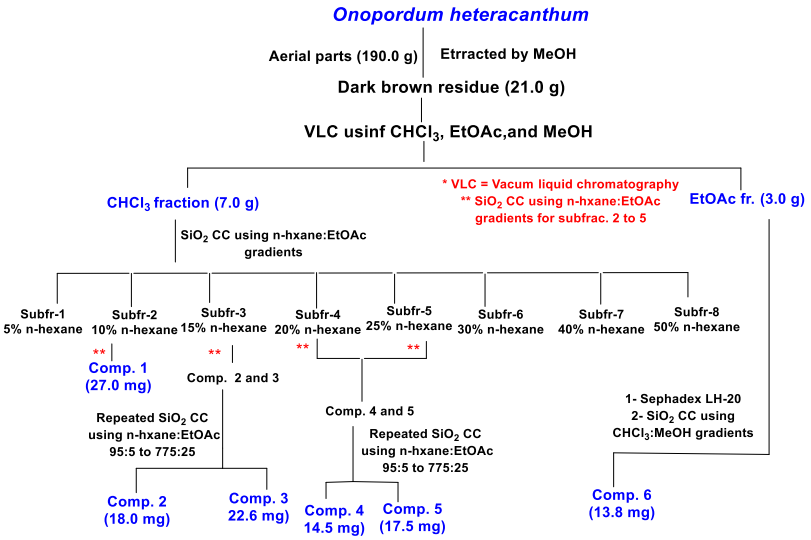
Plant material

Onopordum heteracanthum aerial parts were collected from Al-Taif governorate, Saudi Arabia, in April 2016. The plant was identified by a taxonomist at King Abdulaziz University, Department of Natural Products and Alternative Medicine, Saudi Arabia, based on morphological characteristics and comparison with authenticated reference data [6]. The plant identification was confirmed by staff members of the Biology Department, Faculty of Sciences and Arts, Saudi

Arabia. A voucher sample (OH-0634) was archived in the Department of Natural Products and Alternative Medicine, University of King Abdulaziz.

Extraction and isolation

The air-dried powdered aerial parts of *O. heteracanthum* (190.0 g) were extracted at room temperature by methanol (6 × 2.5 L). The MeOH extract was evaporated and concentrated under reduced pressure to afford a dark brown residue (21.0 g). The residue was suspended in distilled H_2O (150 mL) and successively partitioned between $CHCl_3$ and EtOAc to yield $CHCl_3$ (7.0 g), EtOAc (3.7 g), and aqueous (7.7 g) fractions. The $CHCl_3$ fraction (5.5 g) was chromatographed over a silica gel column chromatography (SiO_2 CC; 100 g, 100 × 3 cm) using *n*-hexane: EtOAc gradient to obtain eleven sub-fractions: OHC-1 to OHC-11. Sub-fraction OHC-3 (402 mg) was subjected to SiO_2 CC (30 g × 50 ×2 cm) using *n*-hexane: EtOAc gradient (100% to 90:10) to get compound **1** (27.0 mg). Sub-fraction OHC-4 (207 mg) was chromatographed on SiO_2 CC (20g, 50 × 2cm), using n-hexane: EtOAc gradient (99:1 to 97:3) to give compound **2** (18.0 mg). SiO_2 CC (50 g, 50 × 3 cm) of sub-fraction OHC-6 (522 mg) using *n*-hexane: EtOAc gradient (95:5 to 85:15) to yield compound **3** (22.6 mg). Sub-fraction OHC-7 (301 mg) was separated on SiO_2 CC (40 g, 50 × 3 cm) using *n*-hexane: EtOAc gradient (95:5 to 85:15) to obtain compound **4** (14.9 mg). Sub-fraction OHC-9 (290 mg) was subjected to SiO_2 CC (20 g, 50 × 2 cm) and eluted with *n*-hexane: EtOAc gradient (95:5 to 80:20) to give compound **5** (17.5 mg). The EtOAc fraction (3.0 g) was subjected to a Sephadex LH-20 column (30 g, 50 × 2 cm) using $CHCl_3$: MeOH (10:90) to obtain five sub-fractions: OHE-1 to OHE-5. Sub-fraction OHE-4 (270 mg) was further separated on SiO_2 CC (20 g, 50 × 2 cm) using $CHCl_3$: MeOH gradient (99:1 to 90:10) to furnish compound **6** (13.8 mg).



Scheme 1. Extraction and isolation of compounds (**1-6**).

*Alkaline hydrolysis of compound **1***

A solution of **1** (10 mg) in 3% KOH/MeOH (4 mL) was allowed to stand for 15 min at room temperature, then neutralized with 1 N HCl/MeOH. The reaction mixture was extracted with $CHCl_3$. The solvent was evaporated, and the obtained residue was chromatographed on SiO_2 CC

using *n*-hexane: EtOAc gradient to furnish β-amyrin and methyl ester of stearic acid. β-amyrin was identified by EIMS analysis and co-TLC with the authentic sample, while the methyl ester of stearic acid was identified by GCMS analysis [25].

In vitro cytotoxic activity

Cell culture

Human hepatocellular carcinoma (HepG2), colorectal adenocarcinoma (HCT-116), and breast adenocarcinoma MCF-7 cell lines were obtained from the American Type Culture Collection (ATCC). Cells were maintained in RPMI-1640 medium supplemented with penicillin (100 units/mL) and heat-inactivated fetal bovine serum (10% v/V) in a humidified, 5% (v/V) CO_2 atmosphere at 37 °C. Doxorubicin was used as a positive control [26, 27].

Cytotoxicity assay

The cytotoxicity of the isolated compounds was tested against the selected human tumor cells using the SRB assay. Healthy, growing cells were cultured in a 96-well tissue culture plate (3000 cells/well) for 24 h before treatment with the test compounds to allow cell attachment to the plate. Cells were exposed to five concentrations of each compound (0.01, 0.1, 1, 10, and 100 μM), and untreated cells served as a control. Triplicate wells were incubated with the different concentrations for 72 h and subsequently fixed with trichloroacetic acid (10% w/V) for 1 h at 4 °C. After several washings, cells were stained by 0.4% (w/V) SRB solution for 10 min in a dark place. Excess stain was washed with 1% (v/V) glacial acetic acid. After drying overnight, the SRB-stained cells were dissolved with Tris-HCl buffer, and the absorbance was measured at 540 nm using a microplate reader. The dose-response curves were constructed, and the IC_{50} values (the concentration required to reduce cell viability by 50%) were calculated by nonlinear regression analysis using SigmaPlot 12.0 software.

RESULTS AND DISCUSSION

Chromatographic separation of $CHCl_3$ and EtOAc fractions on SiO_2 CC using n-hexane: EtOAc or $CHCl_3$: MeOH gradients afforded six compounds (**1**-**6**). Their structures were elucidated based on comprehensive spectroscopic analyses, including ^{1}H and ^{13}C-NMR, HSQC, and HMBC experiments, and confirmed by comparison with previously reported data.

The ^{1}H-NMR spectrum of compound **1** displayed characteristic signals for eight tertiary methyl groups at δ_H 0.86 (H-23), 0.97 (H-24), 0.86 (H-25), 0.98 (H-26), 1.13 (H-27), 0.83 (H-28), 0.87 (H-29), and 0.85 (H-30) (each, s); one oxygenated methine proton at δ_H 4.49 (dd, J = 10.2, 5.1 Hz, H-3); and an olefinic proton at δ_H 5.18 (t, J = 3.4 Hz, H-12). The ^{13}C-NMR and HSQC spectra revealed the presence of 46 carbons resonances, consistent with a triterpenoid ester skeleton, including eight methyl groups at δ_C 28.1 (C-23), 16.8 (C-24), 16.7 (C-25), 15.5 (C-26), 26.0 (C-27), 28.4 (C-28), 33.5 (C-29), and 23.7 (C-30), ten methylene groups at δ_C 38.3 (C-1), 23.6 (C-2), 18.3 (C-6), 32.5 (C-7), 23.5 (C-11), 26.9 (C-15), 26.2 (C16), 46.8 (C-19), 34.7 (C-21), and 37.8 (C-22); three methine carbons which appeared at δ_C 55.3 (C-5), 47.6 (C-9), and 47.2 (C-18), one oxymethine at δ_C 80.6 (C-3) and one olefinic at δ_C 121.6 (C-12), and eight quaternary carbons displayed at δ_C 37.2 (C-4), 39.8 (C-8), 36.9 (C-10), 145.2 (C-13), 41.7 (C-14), 32.6 (C-17), 31.1 (C-20), and 173.7 (C-1'). Moreover, it showed resonances for methylene group at δ_H 2.28 (t, J = 7.7 Hz H-2'), and strong methylene protons signal around δ_H 1.2-1.31 were indicative of the presence of a fatty acid, which was also supported by the appearance of ^{13}C signals due to an ester carbonyl group at δ_C173.7, a long chain of methylene groups at δ_C 29.7-29.2, and a terminal methyl at δ_C 14.2. Alkaline hydrolysis of compound **1** afforded β-amyrin and methyl

stearate, confirming the presence of a C_{18} fatty acid chain. The downfield shift of H-3 at δ_H (4.49) in **1** revealed the attachment of the fatty acid at C-3 of **1**. The HMBC correlation between H-3 (δ_H 4.49) and the ester carbonyl carbon C-1′ (δ_C 173.7) further confirmed the attachment of the fatty acid moiety at C-3.'. Based on the above spectral features and by comparison of the data with literature [28], compound **1** was identified as *β*-amyrin-3-O-stearate (Figure 1) [25].

Compound **2** was obtained as a white amorphous powder. The analysis of the ^{1}H-NMR spectrum showed the presence of six singlet methyl groups δ_H 0.84-1.03 and one doublet methyl group at δ_H 0.85 (d, J = 6.8 Hz, H-29). Two exomethylene protons were observed at δ_H (4.60 and 4.61, each brs) and one oxymethyl group at δ_H 4.49. The ^{13}C-NMR spectrum displayed thirty carbon signals, including seven methyl groups at δ_C 28.0 (C-23), 16.5 (C-24), 15.9 (C-25), 16.4 (C-26), 14.7 (C-27), 25.5 (C-28), and 19.5 (C-29), eleven methylenes at δ_C 39.1 (C-1), 23.7 (C-2), 18.0 (C-6), 34.0 (C-7), 21.5 (C-11), 26.2 (C-12), 26.6 (C-15), 38.5 (C-16), 25.6 (C-21), 38.9 (C-22), and 107.1 (C-30), six methines at δ_C 55.5 (C-5), 50.4 (C-9), 39.4 (C-13), 48.7 (C-18), and 38.3 (C-19), one oxygen bearing carbon at δ_C 81.0 (C-3), six quaternary carbons at δ_C 37.7 (C-4), 40.9 (C-8), 37.0 (C-10), 42.0 (C-14), 34.5 (C-17), and 154.7 (C-20), and one olefinic carbon at δ_C 154.7. The resonances at δ_C 171.0 and 21.4/δ_H 2.03 further confirmed the presence of an acetyl carbonyl group and its associated methyl carbon. The downfield shift of the oxymethine signal at C-3, which appeared at δ_H 4.49 (m, H-3), supports the connectivity of the acetyl group at C-3. From these data, it was evident that compound **2** was the acetylated derivative of taraxasterol. Comparison with reported spectroscopic data confirmed the structure of compound **2** as taraxasterol acetate (3β-acetoxy-20(30)-taraxastene) [29].

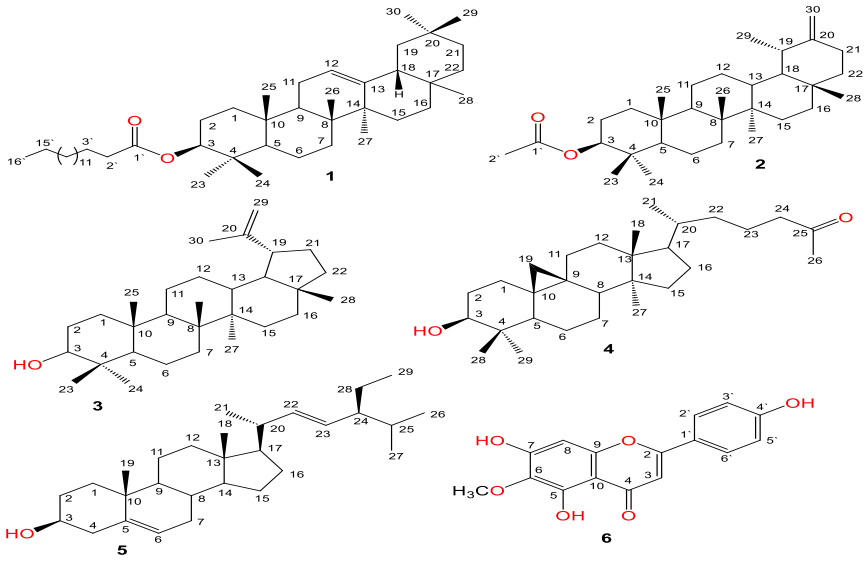


Figure 1. Chemical structures of the isolated compounds **1-6**.

Compound **3** was obtained as a white amorphous powder. The ^{1}H-NMR spectrum displayed seven singlet tertiary methyl group resonances at δ_H 0.79-1.03 (H-25), characteristic of a pentacyclic triterpene skeleton. A multiplet proton at δ_H 2.38 was assigned to H-19β. The H-3 proton appeared as a multiplet at δ_H 3.46, indicating the presence of a hydroxyl group. Additionally, two doublets were observed at δ_H 4.69 and 4.57 (each d, J = 1.7 Hz), representing the exocyclic double bond protons (H-29A and H-29B). The ^{13}C-NMR showed 30 carbon signals, including seven methyl groups at δc 28.3 (C-23), 16.0 (C-24), 15.9 (C-25), 14.7 (C-26), 14.1 (C-

27), 18.0 (C-28), and 22.2 (C-30), ten methylenes at δc 38.0 (C-1), 25.4 (C-2), 18.3 (C-6), 34.1 (C-7), 22.7 (C-11) 25.1 (C-12), 27.4 (C-15), 35.6 (C-16), 33.3 (C-21), and 40.0 (C-22), six methine groups at δc 76.3 (C-3), 55.4 (C-5), 50.2 (C-9), 37.2 (C-13), 49.0 (C-18), and 48.3 (C-19), and six quaternary carbons at δc 37.3 (C-4), 41.0 (C-8), 31.9 (C-10), 42.9 (C-14), 43.0 (C-17), and 151.1 (C-20). The signals at δ_C 151.1 (C-20) and 109.3 (C-29) confirmed the presence of an exocyclic double bond, while the signal at δ_C 76.3 indicated an oxygenated C-3 methine carbon. Based on the above spectral features and by comparison of the data with literature [30], compound **3** was identified as lupeol.

Table 1. NMR spectral data of the isolated compounds **1**-**3** ($CDCl_3$ at 850 and 214 MHz).

Comp.	**1**		**2**		**3**	
No.	d_H (m, *J* in Hz)	$_C\delta$	d_H (m, *J* in Hz)	$_C\delta$	d_H (m, *J* in Hz)	$_C\delta$
1	1.63 m, 1.05 m	38.3 CH_2	1.55 m, 1.05 m	39.1 CH_2	-	38.0 CH_2
2	1.88 m, 1.61 m	23.6 CH_2	1.64 m	23.7 CH_2	-	25.4 CH_2
3	4.49 dd (10.2, 5.1)	80.6 CH	4.49 m	81.0 CH	3.46 m	76.3 CH
4	-	37.2 C	-	37.7 C	-	37.3 C
5	0.85 m	55.3 CH	0.80 m	55.5 CH	0.81 m	55.4 CH
6	1.54 m, 1.40 m	18.3 CH_2	1.50 m, 1.38 m	18.0 CH_2	-	18.3 CH_2
7	1.51 m, 1.33 m	32.5 CH_2	1.38 m	34.0 CH_2	-	34.1 CH_2
8	-	39.8 C	-	40.9 C	-	41.0 C
9	1.59 m	47.6 CH	1.34 m	50.4 CH	-	50.2 CH
10	-	36.9 C	-	37.0 C	-	31.9 C
11	1.85 m	23.5 CH_2	1.62 m, 1.53 m	21.5 CH_2	-	22.7 CH_2
12	5.18 t (3.4)	121.6 CH	1.67 m	26.2 CH_2	-	25.1 CH_2
13	-	145.2 C	2.08 m	39.4 CH	-	37.5 CH
14	-	41.7 C	-	42.0 C	-	42.9 C
15	1.78 m	26.9 CH_2	1.71 m	26.6 CH_2	-	27.4 CH_2
16	1.99 m, 0.94 m	26.2 CH_2	1.58 m, 1.41 m	38.5 CH_2	-	35.6 CH_2
17	-	32.6 C	-	34.5 C	-	43.0 C
18	1.95 dd (13.6, 4.3)	47.2 CH	0.98 d (7.6)	48.7 CH	-	49.0 CH
19	1.67 m, 1.02 m	46.8 CH_2	1.35 m, 1.02 m	38.3 CH	2.38 m	48.3 CH
20	-	31.1 C	-	154.7 C	-	151.1 C
21	1.32 m, 1.08 m	34.7 CH_2	1.11 m	25.6 CH_2	-	33.3 CH_2
22	1.42 m, 1.22 m	37.8 CH_2	1.72 m, 1.22 m	38.9 CH_2	-	40.0 CH_2
23	0.86 s	28.1 CH_3	0.84 s	28.0 CH_3	0.94 s	28.3 CH_3
24	0.97 s	16.8 CH_3	0.87 s	16.5 CH_3	0.84 s	16.0 CH_3
25	0.86 s	16.7 CH_3	1.03 s	15.9 CH_3	1.03 s	15.9 CH_3
26	0.98 s	15.5 CH_3	0.85 s	16.4 CH_3	0.83 s	14.7 CH_3
27	1.13 s	26.0 CH_3	0.92 s	14.7 CH_3	0.79 s	14.1 CH_3
28	0.83 s	28.4 CH_3	1.02 s	25.5 CH_3	0.96 s	18.0 CH_3
29	0.87 s	33.5 CH_3	0.85 d (6.8)	19.5 CH_3	4.69, 4.57 each d (1.7)	109.3 CH_2
30	0.85 s	23.7 CH_3	4.61, 4.60 each brs	107.1 CH_2	1.68 s	22.2 CH_3
1`	-	173.7 C	-	171.0 C	-	-
2`	2.28 t (7.7)	34.9 CH_2	2.03 s	21.4 CH_3	-	-
3`	1.62 m	25.2 CH_2	-	-	-	-
$(CH_2)_{11}$	1.2-1.31 m	29.7-29.2 $-CH_2-$	-	-	-	-
	1.25 m	31.9 CH_2		-	-	-
15`	1.28 m	22.7 CH_2	16`	-	-	-
16`	0.88 t (6.8)	14.2 CH_3	15`	-	-	-

Compound **4** was obtained as a white amorphous powder. The ^{1}H-NMR spectrum exhibited cyclopropyl protons at δ_H 0.33 (d, *J* = 3.4 Hz, H-19A) and 0.55 (d, *J* = 3.4 Hz, H-19B) characteristic of a cycloartane-type triterpene skeleton [31]. Five singlet methyl groups at δ_H 0.95 (H-18), 2.18 (H-26), 0.88 (H-27), 0.96 (H-28), and 0.81 (H-29), along with a doublet methyl group at δ_H 0.89 (d, *J* = 6.8 Hz, H-21). An oxygenated methine proton was observed at δ_H 3.28 (dd, *J* = 4.3,11.1 Hz, H-3), indicating the presence of an OH group. The ^{13}C-NMR experiment showed presence of 29 carbon signals, including five singlet methyls at δc 18.1 (C-18), 31.9 (C-26), 19.3 (C-27), 25.4 (C-28), and 14.0 (C-29) and one doublet methyl at δc 18.3 (C-21); 12 methylenes at δc 31.9 (C-1), 30.4 (C-2), 21.1 (C-6), 26.0 (C-7), 26.5 (C-11), 32.9 (C-12), 35.6 (C-15), 28.1 (C-16), 29.9 (C-19), 35.9 (C-22), 22.7 (C-23), and 40.5 (C-24), five methines at δc 78.9 (C-3), 47.1 (C-5), 48.0 (C-8), 52.2 (C-17), and 36.0 (C-20), and six quaternary carbons at δc 40.5 (C-4), 20.0 (C-9), 26.1 (C-10), 45.3 (C-13), 48.8 (C-14), and 208.7 (C-25). The signal at δ_C 208.7 indicated the presence of a ketone carbonyl group at C-25. The HMBC correlations of H-19 to C-1, C-5, C-8, C-9, and C-10 confirmed the existence of the cyclopropyl moiety in **4**. Based on the above evidence, compound **4** was assigned as 27-*nor*-3β-hydroxy-25-oxocycloartane [32].

Table 2. NMR spectral data of the isolated compounds (**4**-**6**).

Comp.	**4***		**5***		**6****		
No.	d_H (m, *J* in Hz)	δ_C			No	d_H (m, *J* in Hz)	δ_C
1	1.24 m	31.9 CH_2	1.84 m, 1.08 m	37.3 CH_2	2	-	163.8 C
2	1.55 m, 1.40 m	30.4 CH_2	1.80 m, 1.42 m	31.0 CH_2	3	6.59 brs	102.4 CH
3	3.28 dd (4.3, 11.1)	78.9 CH	3.52 m	71.8 CH	4	-	182.1 C
4	-	40.5 C	2.28 m, 2.23 m	42.3 CH_2	5	-	145.2 C
5	1.27 m	47.1 CH	-	140.8 C	6	-	131.4 C
6	1.59 m, 0.78 m	21.1 CH_2	5.36 d (3.4)	121.8 CH	7	-	152.5 C
7	1.08 m	26.0 CH_2	1.53 m	31.9 CH_2	8	6.78 brs	94.3 CH
8	1.49 m	48.0 CH	1.65 m	29.7 CH	9	-	152.8 C
9	-	20.0 C	0.93 m	50.1 CH	10	-	105.7 C
10	-	26.1 C	-	36.5 C	1`	-	121.3 C
11	1.98 m, 1.11 m	26.5 CH_2	1.28 m, 1.22 m	23.1 CH_2	2`, 6`	7.93 d (8.5)	128.5 CH
12	1.61 m	32.9 CH_2	2.00 m, 1.17 m	39.7 CH_2	3`, 5`	6.93 d (8.5)	115.9 CH
13	-	45.3 C	-	39.6 C	4`	-	161.2 C
14	-	48.8 C	0.99 m	56.9 CH	6-OCH_3	3.75 s	60.0 CH_3
15	1.28 m	35.6 CH_2	1.55 m, 1.07 m	24.4 CH_2	5-OH	13.07 s	-
16	1.89 m, 1.28 m	28.1 CH_2	1.71 m, 1.31 m	28.9 CH_2	-	-	-
17	1.57 m	52.2 CH	1.12 m	56.0 CH	-	-	-
18	0.95 s	18.1 CH_3	0.70 s	11.9 CH_3	-	-	-
19	0.55 d (3.4) 0.33 d (3.4)	29.9 CH_2	1.01 s	19.4 CH_3	-	-	-
20	1.48 m	36.0 CH	2.02 m	40.5 CH	-	-	-
21	0.89 d (6.8)	18.3 CH_3	1.02 d (6.8)	19.8 CH_3	-	-	-
22	1.40 m	35.9 CH_2	5.15 dd (14.0, 8.5)	138.3 CH	-	-	-
23	1.47 m, 1.27 m	22.7 CH_2	5.02 dd (14.0, 8.5)	129.3 CH	-	-	-
24	2.35 m, 2.08 m	40.5 CH_2	1.52 m	51.3 CH	-	-	-
25	-	208.7 C	1.98 m	33.7 CH	-	-	-
26	2.18 s	31.9 CH_3	0.85 d (7.0)	19.4 CH_3	-	-	-
27	0.88 s	19.3 CH_3	0.83 d (7.0)	18.8 CH_3	-	-	-
28	0.96 s	25.4 CH_3	1.63 m, 1.18 m	25.4 CH_2	-	-	-

29	0.81	14.0 CH_3	0.84 t (6.9)	12.3 CH_3	-	-	-

*Compounds **4** and **5** were measured in $CDCl_3$ at 850 and 214 MHz; **Compounds **6** in DMSO-d_6 at 850 and 214 MHz

Compound **5** was obtained as white needles (17.5 mg). The ^{1}H-NMR spectral data revealed general characteristics of steroidal compounds. It indicated resonance of two compounds based on integral and disparity in hydrogen peaks. Six methyl groups were observed at δ_H 0.70 (H-18), 1.01 (H-19), 1.02 (d, *J* = 6.8 Hz, H-21), 0.85 (d, *J* = 7.0 Hz, H-26), 0.83 (d, *J* = 7.0 Hz, H-27), and 0.84 (t, *J* = 6.9 Hz, H-29). An oxygenated methine proton was observed at δ_H 3.52 (H-3), indicating the presence of a hydroxyl group. The three downfield olefinic protons at δ_H 5.36 (d, *J* = 3.4 Hz, H-6), 5.15 (dd, *J* = 14.0, 8.5 Hz, H-22), and 5.02 (dd, *J* = 14.0, 8.5 Hz, H-23) suggested the presence of two double bonds. The ^{13}C-NMR spectrum showed resonance for 29 carbons, including four olefinic carbons at δc 140.8 (C-5), 121.8 (C-6), 138.3 (C-22), and 129.3 (C-23), and an oxygenated carbon at δC 71.8 (C-3), six methyl groups, nine methylenes, seven methines, and two quaternary carbons. These are characteristics of a sterol bearing two double bonds and a hydroxyl group. Comparison of the obtained spectroscopic data with reported values confirmed the structure of compound **5** as stigmasterol [33,34].

Compound **6** was obtained as yellowish needles. The ^{1}H-NMR spectrum displayed two singlet aromatic protons, at δ_H 6.59 and 6.78, assigned to H-8 and H-3, respectively. The aromatic protons for ring-B appeared as two doublets at δ_H 7.93 (d, *J*= 8.5 Hz) and δ_H 6.93 (d, *J*= 8.5 Hz); each signal integrating for two protons, corresponding to H-2'/H-6' and H-3'/H-5', respectively. A singlet at δ_H 3.75 indicated the presence of a methoxy group at C-6. The sharp singlet integrated for one proton at δ_H 13.07 was characteristic of a chelated hydroxyl group at C-5. The ^{13}C-NMR spectrum displayed signals for nine quaternary carbons, including a carbonyl group at δ_C 182.1 assigned to C-4, consistent with a flavone skeleton. Several oxygenated aromatic carbons at δ_C 163.8, 161.2, 145.2, 152.5, and 152.8 are assigned to C-2, C-4', C-5, C-7, and C-9, respectively, suggesting a highly substituted aromatic system. The ^{13}C-NMR spectrum showed six methine carbons, including two pairs of equivalent aromatic carbons at δ_C 128.5 (C-2′/C-6′) and 115.9 (C-3′/C-5′), and two additional aromatic methines at δ_C 102.4 (C-3) and 94.3 (C-8). Also, the downfield methyl at δ_C 60.0 was assigned to the methoxy group at C-6. Based on these spectral data and comparison with literature values, compound **6** was identified as scutellarein-6-methyl ether [35].

Cytotoxic activity

The cytotoxic activity of the isolated compounds was examined using the SRB assay against three human cancer cell lines, MCF-7 (breast cancer), HepG2 (hepatocellular carcinoma), and HCT-116 (colon cancer), over a concentration range of 0.01 to 100 μM. The tested compounds showed variable cytotoxicity profiles against the above tumor cell lines (Table 3; Figures 2-4).

Table 3. IC_{50} of the isolated compounds against different cancer cell lines (μM).

Compounds	IC_{50} (μM)		
	HCT-116	HepG2	MCF-7
Doxorubicin	0.18 ± 0.005	0.6 ± 0.05	0.2 ± 0.008
1	25.3 ± 5.10	22.8 ± 1.50	6.3 ± 0.10
2	87.7 ± 2.40	≥100	≥100
3	13.4 ± 0.20	27.3 ± 0.60	29.6 ± 0.50
4	2.9 ± 0.05	2.5 ± 0.06	2.1 ± 0.06
5	≥100	≥100	32.6 ± 4.50
6	18.7 ± 0.30	35.5 ± 0.30	29.2 ± 0.90

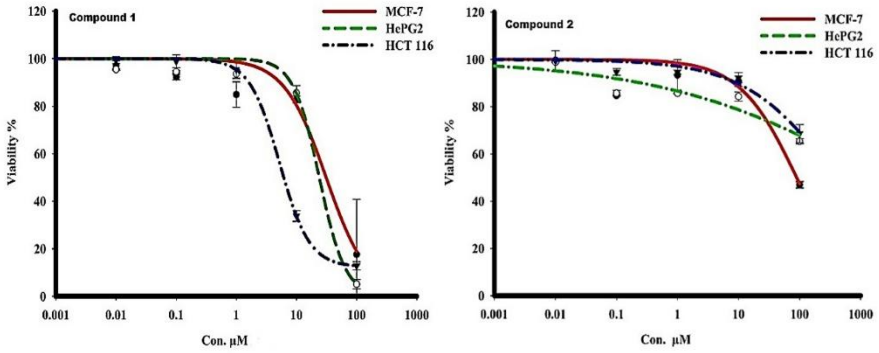


Figure 2. Dose-response curves showing cytotoxic activity of compounds **1** and **2** towards MCF-7, HepG2, and HCT 116 cell lines. Data are presented as mean ± standard error of the mean (SEM) based on three replicates (n = 3).

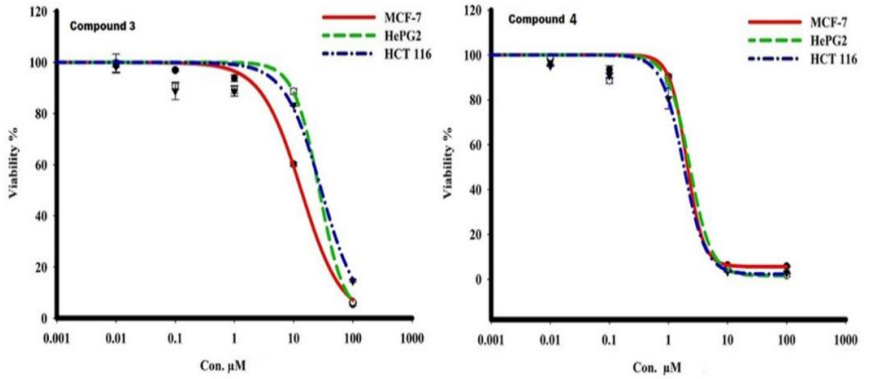


Figure 3. Dose-response curves showing cytotoxic activity of compounds **3** and **4** towards MCF-7, HepG2, and HCT 116 cell lines. Data are presented as mean ± standard error of the mean (SEM) based on three replicates (n = 3).

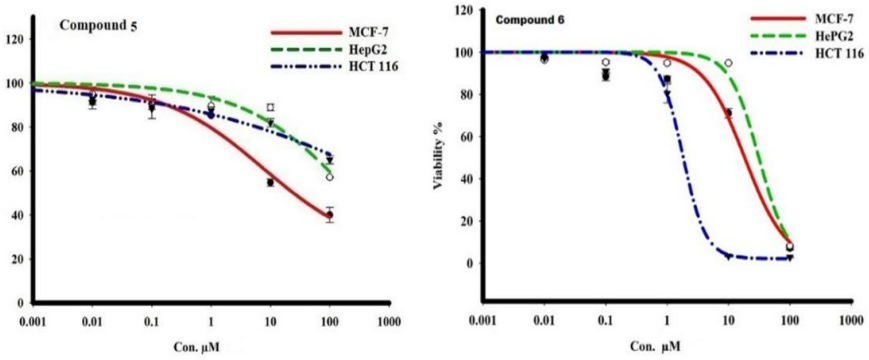


Figure 4. Dose-response curves showing cytotoxic activity of compounds **5** and **6** towards MCF-7, HepG2, and HCT 116 cell lines. Data are presented as mean ± standard error of the mean (SEM) based on three replicates (n = 3).

Among the tested compounds, compound **4** showed the most potent cytotoxic activity against MCF-7, HepG2, and HCT-116 cells (IC_{50}s 2.1 ± 0.06 μM, 2.5 ± 0.06 μM, and 2.9 ± 0.05 μM, respectively) (Figure 3). Previous studies reported cycloartane triterpenoids among the tetracyclic triterpenes, exhibiting cytotoxic activity against various cancer cell lines [36–38]. For example, a study by Sun et al. reported that the cycloartane triterpenoid ADHC-AXpn, isolated from *Cimicifuga foetida*, remarkably inhibited MCF-7 cell growth in a dose- and time-dependent manner. This compound induced mitochondrial-mediated apoptosis and G2/M cell-cycle arrest, along with suppressing the phosphorylation of Akt, ERK1/2, and Raf proteins in MCF-7 cells [37].

In addition, compounds **1**, **3**, and **6** exhibited moderate cytotoxic activity, with IC_{50}'s ranging from 13.4 ± 0.2 μM to 35.5 ± 0.3μM. Compound **1** had promising activity against MCF-7 cells (IC_{50} 6.3 ± 0.1 μM). Compound **2** displayed weak cytotoxicity, with an IC_{50} of more than one hundred μM against HepG2 and MCF-7 cells, and 87.7 ± 2.4 μM against HCT-116 cells (Figure 2). Similarly, compound **5** demonstrated a negligible to weak antiproliferative effect on all cancer cell lines (IC_{50} >100 μM) against HCT-116 and HepG2, and moderate activity against MCF-7 cells (IC_{50} 32.6 ± 4.50 μM) (Figure 4).

CONCLUSION

The current work reported the isolation and characterization of six secondary metabolites from the aerial parts of *O. heteracanthum*, including triterpenoids, a sterol, and a flavonoid derivative. Their structures were assigned using various spectroscopic techniques, revealing the presence of both cycloartane-type and pentacyclic triterpenes within this species. Also, this study represents the first report of compounds **1** and **4** from the genus *Onopordum* and compounds **2**, **3**, **5**, and **6** from *O. heteracanthum*. The cytotoxic assessment revealed antiproliferative activities of the tested compounds. Among them, compound **4** exhibited notable cytotoxic activity against MCF-7, HepG2, and HCT-116 cell lines, with IC_{50} values in the low micromolar range, highlighting its potential as an anticancer lead compound. However, compounds **1**, **3**, and **6** revealed moderate activity, while compounds **2** and **5** showed negligible or weak activity. The notable activity of compound **4** may be assigned to its cycloartane skeleton and the existence of a ketone functionality. Overall, these results highlighted *O. heteracanthum* as a significant source of bioactive metabolites. Future studies are required to further explore its secondary metabolites, with particular emphasis on mechanistic studies and in vivo validation to assess their therapeutic potential.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

All data generated or analyzed during this study are included in this article.

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