

Original Research

# Chemical Constituents of the Volatile and Nonvolatile, Cytotoxic and Free Radical Scavenging Activities of Medicinal Plant: *Ranunculus millefoliatus* and *Acanthus dioscoridis*

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## Abstract

In this first reported study, the hydrodistilled volatile oils from the fragrant aerial parts (*Ranunculus millefoliatus*) and leaves (*Acanthus dioscoridis*) were investigated by GC-MS and GC-FID. In total 78 constituents, 46 and 44 compounds representing 92.5 and 92.57% of the volatile oil were identified from *A. dioscoridis* and *R. millefoliatus* respectively. The main volatile constituents of ADL-Oils were caryophyllene oxide (3.1%), E -phytol acetate (3.6%), (E)- $\beta$ -ionone (3.7%), spathulenol (8.8%), phytol (15.7%), palmitic acid (23.0%). The major compounds of RMA-Oils were palmitic acid (4.89%), (E)-nerolidol (6.89%),  $\alpha$ -copaen-11-ol (11.96%),  $\gamma$ -eudesmol (12.84%) and  $\alpha$ -eudesmol (35.98%). The RMA-oils and ADL-oils displayed the cytotoxicity with  $IC_{50}$  12.28 and 15.44  $\mu$ g/ml respectively. The antioxidant showed that the methanolic extract from RMA was stronger than ADL, with  $IC_{50}$  values (54 and 118  $\mu$ g/ml) respectively. Likewise, the antioxidant activity of RMA-oils was found to be stronger than ADL-oils (43 and 105  $\mu$ g/ml) respectively, compared with BHT ( $IC_{50}$ , 24  $\mu$ g/ml), as a positive control.

**Keywords:** *Ranunculus millefoliatus*, *Acanthus dioscoridis*, Phytoconstituents, cytotoxicity, free radical activity

## Introduction

Several medicinal plants grow naturally in the environment from Kurdistan Region-Iraq. There are a variety of medicinal plants employed among the Kurdish culture [1]. The traditional herbalists use these medicinal plants to treat several human diseases and they have a rich medical history in Kurdistan traditional medicine [2-3]. Therefore, it is imperative to avoid wasting the Kurdish cultural heritage by keeping a written memory of folklore associated with medicinal plants and confirming their therapeutic uses with scientific evidence [4-5]. Phytochemical profiling of plant species potentially containing bioactive principles ought to be encouraged. Besides, phytochemistry has developed as a definite discipline, and it is involved with the volatile and nonvolatile compounds present in plants, as well as research on the isolation of bioactive compounds in the plant has become more important in Kurdish culture [6-8].

The healthful values of the plants depend on the presence of phytochemical substances, including nonvolatile compounds like ellagitannins, steroids, saponins, flavonoids, alkaloids, and phenolic resins; and volatile organic compounds like terpenes and other small compounds that can produce a wide range of biochemical effects on the human body. As well as, phenolic compounds have been found to possess antioxidant activity [9]. Stilbenoid and polyphenolic compounds having a variety of reactive groups in their structures are commonly recognized for their antioxidant properties. Reactive oxygen species (ROS) as an example, hydroxyl radical ( $\text{HO}^\cdot$ ) is frequently generated spontaneously in the living cell during metabolism and plays a very important role in cell signaling and potential stress to the cell's machinery. Antioxidant compounds act to shield cells from the harm caused by free radical-induced oxidative stress, by forming stable radicals that serve as dead-ends to chain reactions that propagate the production of radical species in the cell [10].

*Acanthus dioscoridis* L. is the most common species of the genus *Acanthus* in the family Acanthaceae, with around 30 species of flowering plants [11] and in Iraq, the genus is represented by a single species [12]. Plants in this family are perennials and produce groups of nectar-rich white or purple flowers in a raceme atop a central stem, surrounded by spiny leaves. *Acanthus* genus is distributed mostly in the tropical and subtropical regions of the world [13]. A recent review [14] on *Acanthus* genus has been reported, which described some bioactive compounds such as acanthicifoline (alkaloid) quercetin (flavonoid) and  $\beta$ -sitosterol (steroid). *Acanthus* species are widely used as traditional medicines for the treatment of rheumatism, lymph node inflammations, snakebite, palsy, hepatic disorders, asthma attack, and bellyache in different countries [15]. Also, in the Kurdistan region,

a decoction of the leaves of *Acanthus dioscoridis* L is typically used for skin disease [16].

*Ranunculus millefolius* Banks et Sol., or Jerusalem buttercup, belongs to the Ranunculaceae family. The largest genus *Ranunculus* consists of 600 species [17]. It is represented by 29 species growing in Iraq [18]. Members of the genus are variously known as spearworts, buttercups, and crowfoots. The plants are primarily perennials or annuals, producing composite flowers atop rosettes of leaves. The genus is distributed throughout the southern temperate regions and northern hemisphere in the tropics, where they are usually limited to higher elevations in the mountains. According to the reported review, many pharmacologically active compounds were isolated and characterized from *Ranunculus* plants [19]. Plants of the genus *Ranunculus* (Ranunculaceae) are widely used in traditional medicines, all around the world, due to different pharmacological activities, for example, anti-rheumatism, intermittent fever, antipyretic, and rubefacient in Asian. Furthermore, *Ranunculus millefoliatus* is employed among Kurdish people as an herbal medicine for the treatment of rheumatism [20].

To the best of our knowledge, there are no scientific documented reports about the volatile and nonvolatile compounds, cytotoxic and radical scavenging activity of both plants, except *Acanthus dioscoridis*, the total phenolic and flavonoid contents have been reported, which was collected in Turkey [15]. Therefore, we focused our study on assessing the volatile and nonvolatile extract of these two plants and their radical scavenging and cytotoxicity of volatile oils against the human cancer line MCF-7, utilizing the DPPH and MTT (3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)-assay respectively. Hence, the study will also provide scientific validation for traditional Kurdish medicine.

## Materials and Methods

### Chemicals

Chemical compounds were purchased from Sigma-Aldrich (Steinheim, Germany).

### Plant Material Collection

*Ranunculus millefoliatus* and *Acanthus dioscoridis* were collected in April and May from Safeen and Rayan Mountain, Erbil/Kurdistan region of northern Iraq, respectively. The plants were identified by botanists Prof. Dr. Abdul Hussain Al Khayat and Dr. Abdullah Shukr. The voucher specimens, accession number (7202) for *A. dioscoridis* and (7224) for *R. millefoliatus* [16]. were deposited at the Herbarium of Department of Biology, College of Education, Salahaddin University, Kurdistan-Iraq. The plants

were air-dried under the shade at room temperature (20-25°C). After drying, the plants were ground into a fine powder using a laboratory grinding mill and sieved to provide homogeneous powder for analyzing. Powdered materials of the plants were stored in bottles in a dark room, and at room temperature and then analyzed.

#### Preparation of Extracts

The extract was prepared from leaves of *A. dioscoridis* and aerial parts of *R. millefoliatum* by maceration. For each 20 g of the powdered plant bodies were separately extracted using 250 mL of methanol. The extraction was carried out during 72 h at room temperature. The extract was filtered using a Whatman filter paper and then concentrated in a vacuum at 40°C using a rotary evaporator. The residue obtained was stored in a freezer until further tests.

#### Non-Volatile Phytochemical Screening

For nonvolatile phytochemical analysis of the methanolic extract was carried out using standard procedures to identify of the existence or nonexistence of various constituents such as saponins, alkaloid, flavonoid, terpenoids, tannins, phlobatannin, steroid, phenols proteins, carbohydrate, proteins and glycosides compounds [21-22].

#### Isolation of Volatile Oils

Samples of aerial parts (100 g) were cut into segments of approximately 1 cm and subjected to hydrodistillation in a Clevenger-type apparatus for 3 h as described in the British Pharmacopoeia (1993). The distilled essential oil was dried ( $\text{Na}_2\text{SO}_4$ ) and keep in closed dark vials at 4°C until the analysis.

#### GC and GC/MS Analyses

Analysis of the volatile oils by using gas chromatography was performed employing a thermoquest with a flameionization detector (FID). The detector and injector temps. were 300 and 250°C, respectively. The analysis was administered using a fused silica capillary DB-5 column (60 m 9 0.25 mm; film thickness 0.25  $\mu\text{m}$ ). Oven temp. was programmed from 60 to 250°C at the speed of 5°C/min, and eventually control isothermally for 2 min.  $\text{N}_2$  was used as the carrier gas at a flow rate of 1 ml/min. The GC-MS analysis was performed employing a Thermoquest-Finnigan gas chromatograph equipped with the above-named column, used under identical conditions. Such as, the carrier gas. Ionization voltage was kept at 70 eV. Ion source and interface temps. were kept 200 and 250°C, resp. Mass range was scanned from 43 to 456 m/z [23-24].

#### Identification of Volatile Compounds

The essential oil constituents were identified by comparison of the relative retention indices calculated with respect to homologous of n-alkanes ( $\text{C}_6\text{--C}_{25}$ ). According to MS library search (Wiley and Adams) and comparison of mass spectrum reported in the literature. The relative amounts of individual components (%) were determined using area percentage method relative to the total peak area from the GC-FID analysis [25-26], without using a correction factor

#### Cytotoxic Activity of the Volatile Oils

The MTT test is a colorimetric method for monitoring cell survival. The basis of this test is to change the color of the yellow Tetrazolium salt to the violet crystals of culture medium. After several days, MCF-7 cells (from the Pasteur Institute of Iran) were plated in 96-well plates. For feeding, DMED plus FBS were added and incubated for 24 hours (5%  $\text{CO}_2$ , 37°C). Four concentrations of the essential oils that ranging from 5 to 122  $\mu\text{g/mL}$  were obtained and after filtering 20  $\mu\text{l}$  of them were added to the wells. After the treatment with the essential oil and putting the plates in the incubator for 24 h, 20  $\mu\text{l}$  of the MTT salt dissolved in PBS was added to the wells and incubated for 5 hours for the formation of Formazan. Then, the medium was removed, and 100  $\mu\text{l}$  of DMSO was added to dissolve the metabolite. In the last step, their absorption was read at 570 nm. Untreated cells (<1 % DMSO) were used as control. Five replicate wells were used for each concentration tested, and 50% inhibition of cell growth ( $IC_{50}$ ) was used as the analysis parameter [27-28].

#### Radical Scavenging Activity Assay

Free radical scavenging, DPPH method was submitted [29-30]. In brief, different concentrations of each single extract was added to 1 mL of 90  $\mu\text{M}$  DPPH solution and made up with methanol (95% v/v) to a final volume of 3 ml. The mixture was shaken immediately after adding DPPH solution and was allowed to stand for 1 h at 25°C in the dark place, then the absorbance was read at 517 nm against the blank. The tests were repeated three times and recorded for each sample, commercially antioxidant BHT (butylated hydroxytoluene) used as a positive control. The radical scavenging capacity (RSC) was calculated using the following equation:

Radical scavenging activity% =  $(A_{blank} - A_{sample})/A_{blank} \times 100$ ,  $IC_{50}$  which expresses the concentration of the extract that causes 50% inhibition, was obtained from the plot of RSC versus extract concentrations.

#### Statistical Analysis

All data were expressed as the mean $\pm$ SD (standard deviation), and one-way ANOVA (analysis of variance)

Table 1. Non-volatile phytochemical screening of from leaves (ADL) and aerial parts (RMA), Presence (+); Absence (-).

Nonvolatile phytochemical constituents	Test method	ADL	RMA
Carbohydrates	Fehling's solutions	-	+
Glycosides	Keller-kilani	-	-
Phenolics	Ferric chloride	+	+
Tannins	Ferric chloride	-	+
Alkaloids	Dragendorff's	-	+
Proteins & amino acids	Ninhydrin test	+	-
Saponins	Foam test	+	-
Flavonoids	Alkaline reagent	+	+
Steroids	Salkowski's test	+	+
Phlobatannins	Precipitate test	-	-
Terpenoids	Salkowski's test	-	-

followed by the Dun-nett's test was used for statistical analysis using Excel software. Values of  $p < 0.05$  were considered statistically significant.

### Results and Discussion

In this study, qualitative nonvolatile and quantitative volatile compounds from both *A. dioscoridis* (ADL) and *R. millefoliatum* (RMA) are reported for the first time, the screening of preliminary phytochemical tests for methanol extract was studied by a standard method. Results showed from eleven phytochemicals, only five of them have been existence, such as phenolics, flavonoids, saponins, steroids, proteins and amino acids

in *A. dioscoridis* (ADL). While the six phytochemicals were observed in the methanol extract of *R. millefoliatum* (RMA), they are steroids, carbohydrates, flavonoids, phenols, tannins and alkaloids (Table 1).

Of this plants; since the alkaloids show various pharmacological activities [31]. Furthermore, the presences of phenolics and flavonoids in both herbal plants confirm several of the medicinal uses of these two plants, such as anti-inflammatory, antibacterial, antiviral, antiallergic, cytotoxic antitumor, treatment of neurodegenerative diseases and antioxidant activity [4, 32-34].

An important portion of this investigation was the measuring of cytotoxicity for both volatile compounds. The cytotoxicity of the volatile oils was carried out

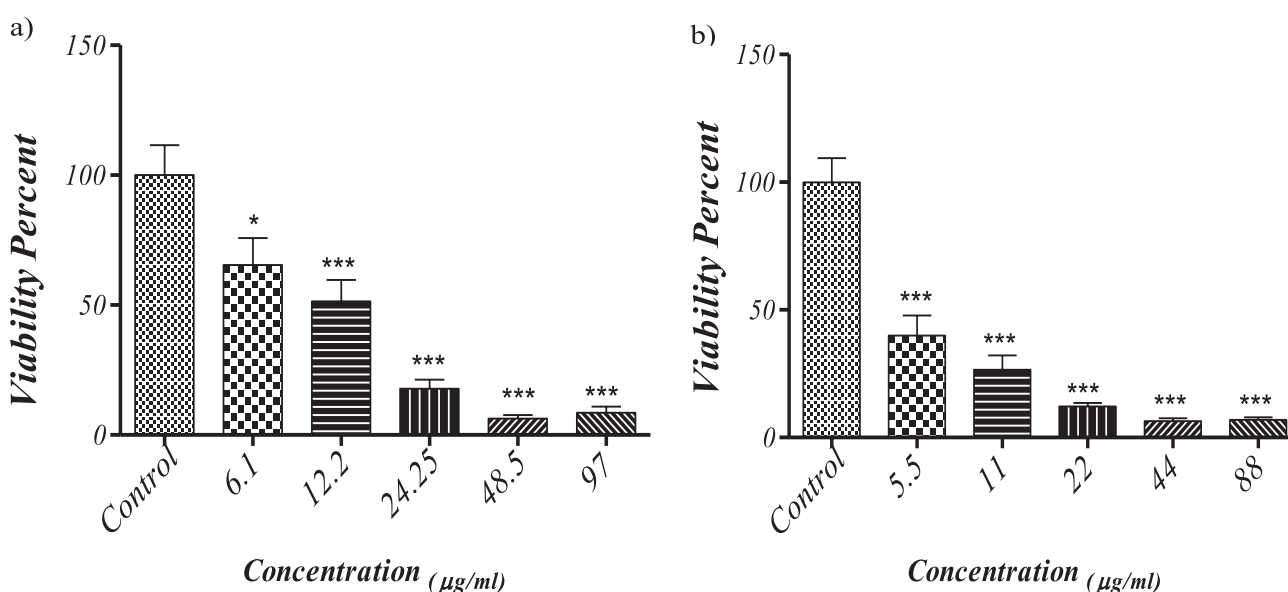


Fig. 1. Inhibitory effect of (a) ADL-Oils and (b) RAM-Oils, on MCF7 cell growth number of viable cells is expressed as a percentage of vehicle control. Mean  $\pm$  standard deviation (SD) of 5 independent experiments.

Table 2. Radical scavenging and cytotoxic activities of methanolic extract from leaves (ADL) and aerial parts (RMA), Volatile oils, (ADL-Oils and RMA-Oils), BHT (tert-butylated hydroxytoluene): positive control.

Compound	RSA ( $IC_{50}$ $\mu\text{g/ml}$ )	Cytotoxicity ( $IC_{50}$ $\mu\text{g/ml}$ )
ADL-Oils	105	15.44
RMA-Oils	43	12.28
ADL	118	-
RMA	54	-
BHT	24	-

against an MCF-7 cell line at different concentrations to determine the growth inhibition rate. Dose-response histogram created between the range of 5 and 122  $\mu\text{g mL}^{-1}$  for the volatile oils expresses the decreasing number of viable cells with increasing concentration of volatile oils (Fig. 1). The volatile oils significantly exhibited high cytotoxicity in

comparison with the control (untreated cells). The results  $IC_{50}$  values (efficient-concentration that causes a 50% decrease in cell growth) showed of the RMA-oils (12.28  $\mu\text{g/ml}$ ) was better active than ADL-oils (15.44  $\mu\text{g/ml}$ ) under the experimental conditions. Moreover, these results from ADL-oils strongly related to phytol and palmitic acid [35-36] are major portion and  $\alpha$ -eudesmol [37] was main compound from RMA-oils. The resulting  $IC_{50}$  values, which were calculated by probit analysis ( $P < 0.05$ ), clearly indicated that both essential-oils exhibited a remarkably high and having correlation between radical scavenging and anticancer activity (Table 3). The  $IC_{50}$  value was projected from a concentration-response bar chart using Graph Pad Prism software.

In the present study, the antioxidant activity of *A. dioscoridis* (ADL) and *R. millefoliatum* (RMA) from methanol extracts and volatile oils was evaluated in comparison to BHT as positive control by DPPH assay. The resulting  $IC_{50}$  values (Table 2), clearly indicated that both plant extracts exhibited a strong and selective *in vitro* antioxidant activity. Notably, the minimal

Table 3. Volatile oils from leaves (ADL-Oils) and aerial parts (RMA-Oils).

NO.	Components name	RT	RI	Peak area percentage % ( $\pm\text{SD}^a$ )	
				ADL-Oils	RMA-Oils
1	1-Octen-3-ol	4.85	974	0.7 $\pm$ 0.1	-
2	$\alpha$ -Terpineol	7.46	1086	0.2 $\pm$ 0.0	0.08 $\pm$ 0.1
3	Linalool	9.78	1095	0.9 $\pm$ 0.1	0.04 $\pm$ 0.0
4	Nonanal	7.53	1100	-	0.03 $\pm$ 0.0
5	Ethyl octanoate	10.05	1196	-	0.24 $\pm$ 0.0
6	Linalool acetate	11.42	1254	-	0.04 $\pm$ 0.0
7	<i>E</i> - $\beta$ -Damascenone	14.67	1386	0.3 $\pm$ 0.0	0.04 $\pm$ 0.0
8	Dodecanal	15.22	1408	0.6 $\pm$ 0.0	1.91 $\pm$ 0.1
9	Geranyl acetone	16.33	1453	1.0 $\pm$ 0.1	0.05 $\pm$ 0.0
10	<i>ar</i> -Curcumene	17.07	1479	-	0.1 $\pm$ 0.0
11	<i>E</i> - $\beta$ -Ionone	17.19	1487	3.7 $\pm$ 0.2	0.31 $\pm$ 0.0
12	10,11-epoxy-Calamenene	17.33	1491	-	0.52 $\pm$ 0.0
13	$\alpha$ -Muurolene	17.52	1500	-	0.11 $\pm$ 0.0
14	Butylated hydroxytoluene	17.8	1514	0.3 $\pm$ 0.0	-
15	Sesquicineole	17.81	1515	-	0.73 $\pm$ 0.1
16	6-Methyl- $\alpha$ -ionone	17.95	1520	0.3 $\pm$ 0.0	-
17	$\delta$ -Cadinene	18.06	1522	-	0.39 $\pm$ 0.0
18	2 <i>E</i> ,4 <i>E</i> -Dodecadienol	18.03	1523	0.2 $\pm$ 0.0	0.28 $\pm$ 0.0
19	$\alpha$ -Copaen-11-ol	18.43	1539	0.4 $\pm$ 0.0	11.96 $\pm$ 1.1
20	Elemol	18.75	1548	-	0.16 $\pm$ 0.0
21	trans-Cadinene ether	18.86	1557	1.1 $\pm$ 0.1	-
22	Germacrene B	19.02	1559	0.8 $\pm$ 0.0	-

Table 3. Continued.

23	(E)-Nerolidol	19.10	1561	-	6.89±0.5
24	Caryolan-8-ol	19.25	1571	-	0.1±0.0
25	Spathulenol	19.43	1577	8.8±0.7	0.37±0.0
26	Caryophyllene oxide	19.55	1582	3.1±0.2	-
27	Fokienol	20.26	1596	-	1.87±0.1
28	n-Hexadecane	19.75	1600	0.2±0.0	-
29	5-epi-7-epi- $\alpha$ -Eudesmol	19.86	1607	-	0.49±0.0
30	Humulene epoxide II	20.10	1608	1.0±0.1	-
31	$\beta$ -Biotol	20.22	1612	2.0±0.1	-
32	1,10-di-epi-Cubenol	20.14	1618	-	0.46±0.0
33	$\beta$ -Cedrene epoxide	20.43	1621	0.3±0.0	-
34	$\gamma$ -Eudesmol	20.63	1630	0.6±0.1	12.84±0.9
35	Hinesol	20.91	1640	-	2.83±0.0
36	Cubenol	20.82	1645	0.3±0.1	-
37	Cedr-8(15)-en-10-ol	21.00	1650	0.5±0.1	-
38	Himachalol	21.08	1652	1.9±0.2	-
39	$\alpha$ -Eudesmol	21.41	1652	-	35.98±1.32
40	Allohimachalol	21.14	1661	1.3±0.1	-
41	14-hydroxy-(Z)-Caryophyllene	21.22	1666	0.2±0.0	-
42	Tetradecanol	21.44	1671	0.5±0.0	-
43	Helifolenol A	21.58	1674	-	0.61±0.0
44	(2Z,6Z)-Farnesal	21.64	1684	0.6±0.0	-
45	$\alpha$ -Bisabolol	21.84	1685	-	1.26±0.1
46	Germacre-4(15),5,10(14)-trien-1 $\alpha$ -ol	21.90	1685	2.4±0.1	-
47	n-Heptadecane	22.00	1700	-	0.33±0.0
48	Eudesma-7(11)-en-4-ol	22.34	1700	1.9±0.1	-
49	(2Z,6E)-Farnesol	22.62	1722	-	0.19±0.0
50	Isobicyclgermarenal	23.08	1733	1.2±0.1	-
51	Aristolone	23.51	1762	-	0.03±0.0
52	$\beta$ -Acoradienol	23.58	1762	1.3±0.1	0.03±0.0
53	$\beta$ -Bisabolenal	23.69	1768	0.1±0.0	-
54	Pentadecanol	23.71	1773	2.6±0.1	-
55	cis-Thujopsenic acid	23.75	1863	2.9±0.1	-
56	8S,13-Cedrane-diol	23.81	1897	1.1±0.1	-
57	(5E,9E)-Farnesyl acetone	23.85	1913	1.9±0.1	-
58	Butyl dodecanoate	23.90	1786	-	1.98±0.1
59	Octadecane	24.11	1800	-	0.08±0.0
60	Isopropyl tetradecanoate	24.49	1828	-	0.03±0.0
61	Cyclopentadecanolide	24.67	1832	-	0.04±0.0
62	Nonadecane	26.16	1900	-	1.08±0.1

Table 3. Continued.

63	(5E,9E)- Farnesyl acetone	26.59	1913	-	0.13±0.0
64	Methyl hexadecanote	26.69	1921	-	0.37±0.0
65	Phytol	25.12	1942	15.7±0.9	-
66	Palmitic acid	27.95	1959	23.0±1.2	4.89±0.9
67	Isopropyl hexadecanoate	28.49	2024	0.3±0.0	-
68	Linoleic acid	30.04	2132	0.9±0.1	0.27±0.0
69	1-Docosene	30.14	2189	0.7±0.0	-
70	Octadecanol acetate	30.79	2209	0.3±0.0	-
71	E-Phytol acetate	30.30	2218	3.6±0.1	0.29±0.0
72	Tricoisane	33.47	2300	0.1±0.0	0.75±0.0
73	Pentacosane	36.74	2500	0.6±0.0	1.39±0.1
74	Heptacosane	38.30	2700	0.1±0.0	-
	Total			92.50	92.57

RT: Retention time on a DB-5 column; RI: retention indices from literature (DB-5 column).

<sup>a</sup>Standard deviation for three replications.

$IC_{50}$  activity assay displayed a stronger DPPH radical scavenging capacity under the same testing conditions. The results of the antioxidant assay showed that the methanolic extract of the RMA was stronger than the extract of ADL, with  $IC_{50}$  values (54 and 118  $\mu\text{g/ml}$ ) respectively. Meanwhile, the volatile oils from RMA-Oils showed higher free radical scavenging activity compared to ADL-Oils (43 and 105  $\mu\text{g/ml}$ ) respectively, compared with BHT ( $IC_{50}$ , 24  $\mu\text{g/ml}$ ), as a positive control. The strong scavenging capacity of the extracts on DPPH• was possible due to the hydrogen donating ability of the polyphenolic (phenolic and flavonoid) compounds present in the plant extracts and essential oils.

**Chemical Composition of the volatile fractions.** The leaves *A. dioscoridis* (ADL) and aerial parts *R. millefoliatius* (RMA) were separately hydrodistilled, as described in the experimental part, to yield, two light yellow oils (ADL-Oils and RMA-Oils), in yields of 0.5% (w/w) and 0.7% (w/w) respectively, related to the dry weight of the plants.

GC-MS Analysis led to the identification of 46 components of the ADL-Oils and 44 components of the RMA-Oils (Table 3), representing 92.20% and 92.57%, respectively, of the two oil compositions. At all the different volatile compounds, at quite different concentrations, were identified by comparing their calculated Kovats Retention Indices (KIs) [38-40], with literature values, and their mass spectra with the databases in GC/MS, Mass Spectral Library and Wiley Registry of Mass Spectral Data. The quantified by GC-FID, from the percentage composition of the individual compounds. The volatile constituents are listed in Table 3, according to their elution order of the DB-5 column. The main volatile constituents of ADL-Oils were

caryophyllene oxide (3.1%), E-phytol acetate (3.6%), (E)- $\beta$ -ionone (3.7%), spathulenol (8.8%), phytol (15.7%), hexadecanoic acid (23.0%). The major components of RMA-Oils were hexadecanoic acid (4.89%), (E)-nerolidol (6.89%),  $\alpha$ -copaen-11-ol (11.96%),  $\gamma$ -eudesmol (12.84%) and  $\alpha$ -eudesmol (35.98%). These variations within the composition of the 2 extracted oils explain the readily-perceptible distinct odors of the two plants. The chemical structures of the representative main compounds are shown in Fig. 2.

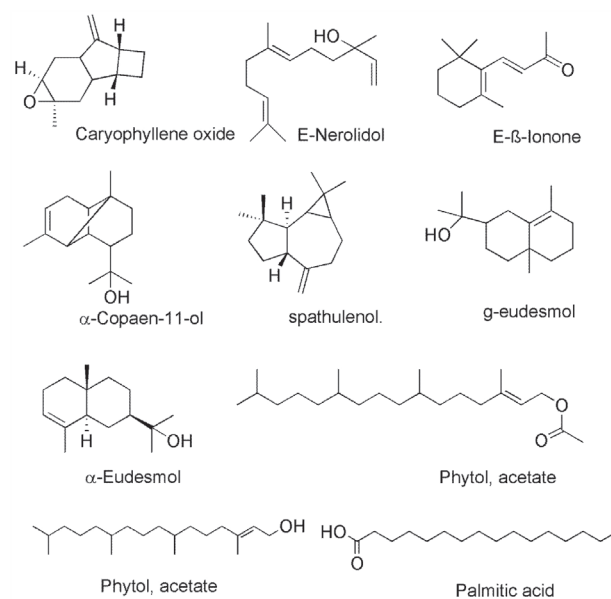


Fig. 2. Chemical structures of representative main components of oils extracted from leaves of *Acanthus dioscoridis* and aerial parts of *Ranunculus millefoliatius*.

## Conclusions

The results suggest that, *Acanthus dioscoridis* and *Ranunculus millefoliatus*, contain a wide range of biologically active compounds. The main volatile compounds of ADL-Oils were caryophyllene oxide (3.1%), *E*-phytol acetate (3.6%), (*E*)- $\beta$ -ionone (3.7%), spathulenol (8.8%), phytol (15.7%), hexadecanoic acid (23.0%). The major components of RMA-Oils were hexadecanoic acid (4.89%), (*E*)-nerolidol (6.89%),  $\alpha$ -copaen-11-ol (11.96%),  $\gamma$ -eudesmol (12.84%) and  $\alpha$ -eudesmol (35.98%). Nonvolatile compounds such as steroids, saponins, flavonoids, phenols, tannins and alkaloids, qualitatively have been found in both plants. Additionally, this work demonstrates that the essential oil of both plants are a good correlation of volatile oils to removing radical and cytotoxic to MCF-7 cells. Further studies are needed to detect the effective plant chemicals of the oils and to elucidate their vital roles in achieving cytotoxicity. As well as, the extracts from ADL and RMA showed have noteworthy antioxidant activity, particularly the methanolic extract and volatile oils from aerial parts of *Ranunculus millefoliatus*. This investigation on the phytochemicals of two different species is one of the first research on medicinal plants growing on mountains in Iraqi Kurdistan. Moreover, it represents an important stage for more intensive plant chemical and bio-assay activity of the rather unexpected Kurdish folk medicine.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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