Original Article

Chemical Composition, Antioxidant, Antibacterial, and Anticancer Activities of *Scorzonera calyculata* Boiss. and *Centaurea irritans* Wagenitz. Extracts, Endemic to Iran

Anahita Ayromlou, Shiva Masoudi, Amir Mirzaie[1]

Department of Chemistry, Islamic Azad University, Central Tehran Branch, Tehran, [1]Department of Biology,Islamic Azad University, Roudehen Branch, Roudehen, Iran

Abstract

Background & Purpose: This research focused on the composition for the essential oils, which was obtained by solvent-free microwave extraction (SFME) from the aerial parts of *Scorzonera calyculata*, and hydrodistilled oils from the aerial parts and roots of *Centaurea irritans*, from Astraceae family, were investigated by gas chromatography (GC) and GC/mass spectrometry (MS). In addition, the biological activities of the methanolic extracts from the aerial parts of *S. calyculata* and *C. irritans* were determined. **Methods:** Total phenolic content was determined by the Folin–Ciocalteu procedure. Antibacterial activity of the methanolic extracts was carried out by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Cytotoxicity of the methanolic extract of S. calyculata against human lung cancer cells (A549) and the methanolic extract of *C. irritans* against breast lung cancer cells (MCF-7) were measured using 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. **Results:** The obtained results of GC/MS technique showed that the SFME oil of *S. calyculata*, was rich in regard to nonterpenoid and sesquiterpene components. Both oils from the aerial parts and roots of *C. irritans* were rich in regard to oxygenated monoterpenes. The *S. calyculata* and *C. irritans* extracts showed moderate antioxidant activities with an inhibitory concentration (IC50) value of 1.48 and 1.99 mg/mL, using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and 73.51 and 44.48 µmol Fe (II)/g dry mass using ferric-reducing antioxidant power (FRAP) assay, respectively. The extracts showed high toxicity against gram-positive bacteria and the IC50 value of extracts cytotoxicity was found to be 9.8 and 10.3 mg/mL, respectively. **Conclusion:** It appeared that the investigated samples could be as a promising drug for pharmaceutical industry.

Keywords: *Antibacterial activity, anticancer activity, antioxidant capacity, Centaurea irritans Wagenitz, essential oil, Scorzonera calyculata Boiss*

Introduction

The *Scorzonera* is a genus belonging to the family Asteraceae, and it grows mainly in dry areas of Europe and Asia. It also contains approximately 175 species distributed throughout Europe, Asia, and Africa.[1,2] The genus *Scorzonera* is represented in the flora of Iran by 50 species, in which 22 of them are endemic.[3,4] Some species of *Scorzonera* are used for cooking vegetables and in traditional medicine in Europe and Asia. Furthermore, these plants are used in the Mongolian and Chinese folk medicine to prolong the period of lactation. Moreover, this species is also used as antifebrile, against bacterial and viral infections, as well as in the treatment of the poisonous ulcer, gastric, and intestinal disorders.[5-7] *Scorzonera hispanica* L. is the most recognized species that grows naturally and widely in Europe where it has been cultivated since the seventeenth century as a food source. *Scorzonera undulata* is a perennial species, diploid, and a highly polymorphic plant. *S. undulata* is mostly purposed as food source; however, in Tunisia, the roots are appreciated for their sweetness; they are either eaten raw or cooked in water. They are also used for preparing a decoction for its benefits as depurative. The ashes of burned roots are very effective in the treatment of burns.[8] *Scorzonera austriaca* is widely distributed in the northeast and northwest in China. It has been widely used for curing fever, carbuncle, and mastitis as a traditional herbal medicine. Also *S. austriaca* is used to treat hepatitis B as a folk medicine.[9,10] In Turkish folk medicine, members of the genus *Scorzonera* are used for treating a variety of illnesses, including arteriosclerosis, kidney

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Address for correspondence:
Dr. Shiva Masoudi, Department of Chemistry, Islamic Azad University, Central Tehran Branch, P.O. Box 86831-14676, Tehran, Iran.
E-mail: Shi.masoudi@iauctb.ac.ir

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diseases, hypertension, diabetes mellitus, and rheumatism, as well as for pain relief and healing different injuries.[11,12] Antioxidant, analgesic, anti-inflammatory, and wound healing activities of some *Scorzonera* species have been reported previously.[13-15] Antioxidant activity and antimicrobial effects in *Scorzonera suberosa*, *Scorzonera latifolia*, and *Scorzonera laciniata* have been reported from Turkish region. According to the result, using plant extraction has high antioxidant and antiradical effects. All plant samples have more pronounced antimicrobial effect on *Escherichia coli* and *Bacillus megaterium*. In addition, these samples show antitoxic property and protective effect of cell viability in the probiotic yeast culture.[16] The genus *Scorzonera* is known for the presence of variety of compounds, and previous investigations have led to the isolation of triterpenoids,[17-19] coumarins,[20] lignans,[21] sesquiterpene lactones, and sesquiterpene glucosides,[5,22] flavonoid glucosides,[23-25] dimeric guaianolides,[26] and phenolic compounds.[27-29] *Centaurea* L. is a large genus, which comprised several species, many of which are used in folk medicine. It is one of the biggest genera of family Asteraceae as this genus has over 400–700 species and 199 taxons.[30] Seventy-four species of the genus *Centaurea* are found in Iran, among which 38 are endemic. Various *Centaurea* species have certain biological activities and are used as anti-inflammatory,[31] antimicrobial,[32] as diuretic and mild astringent,[33] antihepatotoxic,[34] antioxidant,[35] and cytotoxic agent.[36] Extensive chemical investigations of *Centaurea* species led to the isolation and identification of various types of compounds including alkaloids,[37] lignans,[38] aglycone flavonoids,[39] and sesquiterpene lactones.[40-42] *Centaurea cyanus* flower, also known as blue cornflower or bachelor's button, grows as a wild and common garden plant throughout Europe.[43] Owing to its intense blue flowers, it is used as an ornamental plant, for coloring sugar and confectionaries, in teas and salads, and to garnish dishes.[43,44] Several therapeutic activities have also been attributed to the flowers, including the treatment of indigestion, gallbladder dysfunction, kidney regulation, menstrual disorder regulation, increasing immunity, and for the efficient cleaning of wounds.[44,45]

Throughout this study, the analysis of the essential oils obtained by solvent-free microwave extraction (SFME) from leaves and flowers of *Scorzonera calyculata* and hydrodistilled oils from the aerial parts and roots of *Centaurea irritans* are reported. Also the antioxidant, antibacterial, and anticancer potentials of the methanolic extract from the aerial parts of the plants were investigated. To the best of our knowledge, this is the first report on the oil composition and biological activities of *S. calyculata* and *C. irritans* growing wild in Iran.

Materials and Methods

Plant preparation

The aerial parts of *S. calyculata* and the aerial parts and roots of *C. irritans*, which are endemic to Iran, were collected during the flowering stage from Mehran, Province of Ilam, west of Iran, in June and July 2017, respectively. Voucher specimens have been deposited at the Herbarium of the Research Institute of Forests and Rangelands (TARI), Tehran, Iran. Plant specimens were authenticated by Dr. Vali-Allah Mozaffarian from the same institute.

Isolations of the essential oils

Distillation

Air-dried aerial parts (100 g) and roots (70 g) of *C. irritans* were separately subjected to hydrodistillation using a Clevenger-type apparatus for 4 h. After decanting and drying off the oils over anhydrous sodium sulfate, the corresponding yellowish-colored oils were recovered in a yield of 0.16% and 0.10% (wt/wt), respectively.

Solvent-free microwave extraction

SFME extraction was performed in a Milestone ETHOS 1600 batch reactor (Milestone Srl, Sorisole (BG), Italy), which is a multimode microwave reactor operating at 2455 MHz with a maximum delivered power of 1000 W, variable in 10 W increments. The dimensions of the polytetrafluoroethylene-coated cavity are 35 × 35 × 35 cm. During the experiment, time, temperature, pressure, and power were controlled by using the "easy-WAVE" software package (GPS Limited, London, UK). Temperature was monitored with the aid of a shielded thermocouple (ATC-300, Java Multi Mandiri Cv, Indonesia) inserted directly into the sample container. In a typical SFME procedure, 250 g of dry leaves and flowers of *S. calyculata* were moistened before extraction by soaking in water for 1 h, then draining off the excess water. This step is essential for giving the leaves and flowers the initial moisture. Moistened leaves and flowers also were next placed in a reactor without any added solvent or water. The essential oil is collected, dried with anhydrous sodium sulfate, and stored at 0°C until used.

Gas chromatography

Gas chromatography (GC) analysis was performed on Schimadzu 15A (Shimadzu, Kyoto, Japan) gas chromatograph equipped with a split/splitless injector (25°C) and a flame ionization detector (250°C). Nitrogen was used as carrier gas (1 mL/min), and the capillary column used was a DB-5 (50 m × 0.2 mm, film thickness, 0.32 µm). The column temperature was kept at 60°C for 3 min and then heated to 220°C with a 5°C/min rate and kept constant at 220°C for 5 min. Relative percentage amounts were calculated from the peak area using a Shimadzu C-R4A Chromatopac, without the use of correction factors.

Gas chromatography-mass spectrometry

GC/mass spectrometry (MS) analysis was carried out using a Hewlett-Packard 5973 MSD detector (Agilent Technologies, Santa Clara, CA, USA) with a HP-5 MS column (30 m × 0.25 mm, film thickness, 0.25 µm). The column temperature was kept at 60°C for 3 min and programmed to 220°C at a rate of 5°C/min and kept constant at 220°C/min for 5 min. The injector and GC/MS interphase were also maintained at

Table 1: Chemical composition (%) of the oils obtained by solvent-free microwave extraction from leaves and flowers of *Scorzonera calyculata*

No.	Compounds	RI	SFME (%)
1	α-Thujene	931	0.10
2	α-Pinene	939	0.17
3	Sabinene	976	t
4	1-Octen-3-ol	978	t
5	β-Pinene	980	0.14
6	Decane	999	0.32
7	α-Phellandrene	1005	0.12
8	α-Terpinene	1018	0.25
9	ρ-Cymene	1026	1.73
10	Limonene	1031	0.38
11	1,8-Cineole	1033	0.13
12	(Z)-β-Ocimene	1040	t
13	γ-Terpinene	1062	1.86
14	Acetophenone	1065	1.88
15	Nonanal	1098	0.57
16	Menthone	1154	0.12
17	(E)-2-Nonenal	1158	t
18	1-Nonanol	1171	0.14
19	Terpin-4-ol	1177	t
20	Dodecane	1199	0.17
21	Safranal	1203	0.11
22	Decanal	1204	0.30
23	Pulegone	1237	0.19
24	(E)-ocimenone	1239	t
25	Neral	1240	0.13
26	Carvone	1242	0.18
27	2,6,6-trimethyl-1-cyclohexene-1-acetaldehyde	1258	0.12
28	(E)-2-decenal	1261	0.18
29	(E)-citral	1275	0.18
30	Tridecane	1299	t
31	(E,E)-2,4-dodecadienal	1314	0.13
32	(E)-2-undecenal	1356	0.47
33	2-butyl-2-octenal	1370	0.17
34	α-copaene	1376	0.16
35	(E)-β-damascenone	1380	0.11
36	Tetradecane	1399	0.26
37	6,10-dimethyl-2-undecanone	1401	0.56
38	Dodecanal	1407	0.22
39	(E)-β-damascone	1409	0.18
40	β-Caryophyllene	1418	1.24
41	β-Gurjunene	1432	0.10
42	Geranyl acetone	1453	0.96
43	α-Humulene	1454	0.38
44	Dodecanol	1473	0.33
45	(E)-β-ionone	1485	6.77
46	Pentadecane	1500	0.39
47	1,5-epoxy salvial-4(14)ene	1560	0.47
48	(Z)-3-hexen-1-ol benzoate	1568	t
49	Spathulenol	1576	0.70
50	Caryophyllene oxide	1581	16.84
51	Hexadecane	1600	0.93
52	Humulene epoxide II	1606	1.29
53	Epi-cedrol	1611	0.13
54	Tetradecanal	1613	0.51
55	1-Butyl heptyl-benzene	1630	0.12

Table 1: Continued

No.	Compounds	RI	SFME (%)
56	*cis*-Methyl dihydro jasmonate	1654	t
57	Salicylic acid, hexyl ester	1675	0.78
58	1-Methyl decyl-benzene	1698	0.17
59	Methyl tetradecanoate	1726	0.17
60	1-Butyl octyl-benzene	1731	T
61	(E)-2-hexyl cinnamaldehyde	1740	0.61
62	Ambroxide	1756	0.10
63	Octadecane	1800	0.26
64	Iso-propyl tetradecanoate	1835	0.39
65	6,10,14-trimethyl-2-pentadecanone	1878	27.73
66	Nonadecane	1900	1.17
67	Methyl hexadecanoate	1927	1.12
68	Phytol	1949	0.55
69	Pentadecanoic acid	1970	0.13
70	Hexadecanoic acid	1980	0.92
71	Iso-amyl octanoate	2030	T
72	Methyl linoleate	2092	0.42
73	(Z,Z,Z)-9,12,15-octadecatrienoic acid, methyl ester	2098	1.34
74	Neophytadiene	2111	7.68
75	(E)-Phytol acetate	2221	0.11
76	Tetracosane	2400	0.26
77	Pentacosane	2500	0.24
	Total		87.04
	Monoterpene hydrocarbons		4.75
	Oxygenated monoterpenes		1.04
	Sesquiterpene hydrocarbons		1.88
	Oxygenated sesquiterpenes		20.68
	Diterpenes		8.34
	Nonterpenoid compounds		50.35

t = trace (<0.1%), RI = retention indices as determined on a DB-5 column using a homologous series of *n*-alkanes

270°C. The flow rate of helium, as carrier gas, was 1 mL/min, with a split ratio of 1/50. The ionization voltage was 70 eV. The ion source temperature was 250°C, and the transfer line temperature was 280°C. In addition, the mass range (m/z) was 45–465 amu (atomic mass unit) at a speed of 2.8 scan/s. The retention indicates that all the components determined according to the van den Dool method, using *n*-alkanes as standards. As a result, the compounds were identified (RRI, DB5) by comparison with the data reported in the literature and by comparison of their MS with either the Wiley library or with the published mass spectra.[46,47]

Preparation of methanolic extracts

Extracts were prepared by cold percolation method. The aerial parts of *S. calyculata* and the aerial parts of *C. irritans* were dried at room temperature and ground with a blender. Approximately 30 g of the powders were macerated in 200 mL of methanol 80% in water for 48 h in room temperature by shaking. The extracts were filtered, and methanol was evaporated at 40°C in rotary evaporator. The extracts were lyophilized and kept in dark at +4°C until tested.

Solvents and chemicals

Ferric chloride ($FeCl_3 \cdot 6H_2O$), ascorbic acid, gallic acid, methanol, Folin–Ciocalteu reagent, sodium acetate trihydrate ($C_2H_3NaO_2 \cdot 3H_2O$), acetic acid, hydrochloric acid, ferrous sulfate ($FeSO_4 \cdot 7H_2O$), and sodium carbonate were purchased from Merck (Darmstadt, Germany); 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), 2, 4, 6-tri [2-pyridyl]-s-triazine (TPTZ), and 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical (Sigma–Aldrich, Sternheim, Germany). All the other chemicals were of analytical grade or more pure.

Determination of total phenolic contents

The amount of total phenolic content (TPC) of both plant extracts was determined according to the Folin–Ciocalteu method[48] and expressed as gram of gallic acid equivalent (GAE) per gram of dry weight of extract. A volume of 50 µL of the extracts (different concentrations), 450 µL of distilled water, and 500 µL of Folin–Ciocalteu reagent 0.5 N were mixed and incubated at room temperature for 15 min. A total of 500 µL of standard sodium carbonate 1% was added, incubated in the dark for 30 min at room temperature, and absorbance was measured at 765 nm using UV spectrophotometer (UV-1800 Shimadzu). TPC of the samples was calculated from calibration curve of gallic acid. All samples were analyzed in triplicates. The gallic acid standard curve was established by plotting concentration (mg/mL) versus absorbance (nm) as follows:

Table 2: Comparative percentage composition of the aerial part and root oils of *Centaurea irritans*

No	Compounds	RI	Aerial part oil (%)	Root oil (%)
1	1-Decene	991	2.39	0.34
2	Decane	999	0.62	4.58
3	(Z)-β-Ocimene	1040	0.35	_
4	Nonanal	1098	_	1.61
5	Isoneral	1190	0.75	_
6	Dodecane	1199	_	0.33
7	Neral	1240	28.08	17.52
8	Geranial	1270	38.62	22.26
9	Neryl acetate	1365	1.14	_
10	α-Copaene	1376	0.41	0.31
11	Geranyl acetate	1383	_	0.35
12	Butanoic acid, octyl ester	1387	_	0.46
13	*cis*-α-Bergamotene	1415	_	0.12
14	β-Caryophyllene	1418	0.49	_
15	*trans*-α-Bergamotene	1436	0.31	_
16	1-Pentadecene	1465	_	1.47
17	Dodecanol	1473	0.61	_
18	1,5-epoxy-salvial-4(14)ene	1525	3.46	0.70
19	*trans*-Calamenene	1532	_	1.03
20	(Z)-Nerolidol	1534	_	0.67
21	Spathulenol	1576	2.25	_
22	Caryophyllene oxide	1581	2. 84	0.43
23	Salvial-4(14)en-1-one	1587	2.25	_
24	*cis*-Isolongifolanone	1604	0.70	_
25	β-Oplopinone	1606	2.03	0.58
26	10-epi-γ-eudesmol	1619	1.08	_
27	γ-Eudesmol	1630	_	0.43
28	epi-γ-cadinol	1640	0.58	_
29	Cedr-8(15)-en-9-α-ol	1644	0.42	_
30	α-Muurolol	1645	1.15	_
31	β-Eudesmol	1649	_	1.86
32	Selin-11-en-4-α-ol	1652	_	1.42
33	8-hydroxy isobornyl isobutyrate	1673	3.88	33.12
34	1-Heptadecene	1693	_	0.25
35	Heptadecane	1700	0.43	0.33
36	Octadecane	1800	0.49	0.29
37	epi-α-bisabolol acetate	1801	0.50	_
38	6,10,14-trimethyl-2-pentadecanone	1840	3.11	1.22
39	Isobutyl phthalate	1894	_	1.15
40	Nonadecane	1900	0.39	_
41	Butyl phthalate	1996	_	0.79
42	Eicosane	2000	0.30	_
43	Henicosane	2100	0.37	0.61
44	Tricosane	2300	_	0.46
45	Pentacosane	2500	_	0.36
46	Heptacosane	2700	_	0.96
	Total		100.0	96.01
	Monoterpene hydrocarbons		0.35	_
	Oxygenated monoterpenes		72.47	73.25
	Sesquiterpene hydrocarbons		1.21	1.46
	Oxygenated sesquiterpenes		17.26	6.09
	Nonterpenoid compounds		8.71	15.21

RI = Retention indices as determined on a DB-5 column using a homologous series of *n*-alkanes

($Y = 35.96X - 0.0096$, $R^2 = 0.998$), where Y is absorbance and X is concentration in GAE.

2, 2-Diphenyl-1-picrylhydrazyl radical scavenging assay

The antioxidant activity of the methanolic extracts of both plants was measured in terms of hydrogen donation or free radical scavenging ability by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) stable radical.[49] The reaction mixture comprised 20 µL of various concentrations of methanolic extracts and 250 µL of methanol solution of DPPH 1 mM. The reaction mixture was incubated for 30 min in the dark at room temperature. The absorbance was measured at 517 nm using UV spectrophotometer (UV-1800 Shimadzu) against control. Ascorbic acid was used as standard control. Inhibition of DPPH-free radical in percent was calculated by the following formula:

$$\text{DPPH scavenging effect (\%)} = [(Ac - As) / Ac]100$$

where Ac is the absorbance of the control and As is the absorbance of the tested sample. All tests were carried out in triplicate. The inhibition concentration at 50% inhibition (IC_{50}) was the parameter used to compare the radical scavenging activity.

Ferric-reducing antioxidant power assay

The ability of both plant extracts to reduce Fe (III)/tripyridyl triazine complex was assessed by ferric-reducing antioxidant power (FRAP) assay.[50] The stock solution included 300 mM acetate buffer (pH = 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$, solution. The fresh working solution was prepared by mixing 200 mL of acetate buffer, 20 mL of TPTZ, and 20 mL of $FeCl_3 \cdot 6H_2O$. The temperature of the solution was raised to 37°C before use. A volume of 5 µL of the extracts (different concentrations) were mixed with 295 µL of distilled water and were allowed to react with 1000 µL of the FRAP solution for 30 min in the dark. The absorbance of reaction mixtures was measured at 593 nm by UV spectrophotometer (UV-1800 Shimadzu), using FRAP working solution as blank. FRAP of the extracts was determined thrice in comparison with $FeSO_4 \cdot 7H_2O$ standard curve. The standard curve was linear between 0.005 and 0.06 µmol $FeSO_4 \cdot 7H_2O$. Results were expressed as µmol Fe (II)/g dry mass and compared with ascorbic acid as positive control.

Antibacterial activity assay

The microdilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the methanolic extracts from the aerial parts of *S. calyculata* and the aerial parts of *C. irritans*, using 96-well microtitration plates.[51,52] The antibacterial activities of the extracts were tested against three gram-positive and three gram-negative bacteria. The gram-positive bacteria included *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 23857, and *Streptococcus pyogenes* ATCC 19615, and the gram-negative bacteria included *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 15442, and *Listeria monocytogenes* ATCC 19115. The bacteria were obtained from the Iranian Research Organization of Science and Technology. At first, the selected bacteria were cultured in tryptic soy broth at 37°C, and the concentration of these cultures was adjusted to 10^8 CFU/mL using phosphate-buffered saline (PBS). The bacterial suspension was inoculated into a 96-well microplate, which contained serial dilution of *S. calyculata* and *C. irritans* extracts (3.125–200 mg/mL). The microplate was incubated at 37°C for 24 h. The MIC value was defined as the lowest concentration of the extract that showed no visible turbidity after incubation. To determine MBC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes, which did not show any growth, and incubated on Mueller–Hinton agar by streaking. The plates were incubated at 37°C for 24 h again. After incubation, the concentration at which no visible growth was seen was noted as MBC. All the experiments were carried out in triplicates, and the mean was calculated. In addition, ampicillin (5–160 µg/mL) was used as standard positive control.

Anticancer activity test

Colorimetric MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay was used for the evaluation of anticancer activity of the methanolic extracts from the aerial parts of *S. calyculata* and the aerial parts of *C. irritans* against human lung cancer cell line (A549) and breast cancer cell line (MCF-7), respectively. A549 cells and MCF-7 cells were plated in 96-well tissue culture plate separately at a density of 1×10^4 cells/well and incubated at 37°C for 24 h. Subsequently, the cells were treated with various concentration of extracts ranging from 3.125 to 200 mg/mL. After incubation for 24 h, the cells were incubated with MTT for 4 h at 37°C. After removing the medium from the plate, 100 µL of DMSO was

Table 3 : Antibacterial activity of the methanolic extract of *Scorzonera calyculata*

Microorganisms	MIC (mg/mL)	MBC (mg/mL)	Ampicillin (µg/mL)
Staphylococcus aureus	25	50	5
Listeria monocytogenes	100	200	10
Bacillus subtilis	100	200	10
Klebsiella pneumoniae	200	200	20
Pseudomonas aeruginosa	100	100	20
Streptococcus pyogenes	50	100	10

MIC = minimum inhibitory concentration, MBC = minimum bactericidal concentration

added to each well. Finally, the absorbance of each well was measured at 570 nm by UV spectrophotometer plate reader (Stat Fax 4200, Awareness Technologies, Westport, CT, USA), and the cell cytotoxicity of the extracts was calculated using the following formula:

$$\% \text{ Cell viability} = \text{mean OD} / \text{control OD} \times 100$$

where OD is the optical density. Moreover, the IC_{50} value of the extracts was evaluated.

Results

Composition of the essential oils

The identified volatile components and their peak area percentages of the leaves and flowers of *S. calyculata* obtained by SFME, and those of aerial parts and roots of *C. irritans* obtained by hydrodistillation are given in Tables 1 and 2, respectively. The components are listed in order of their elution on the DB-5 column. As shown in Table 1, 87.04% (77 components) of the solvent free microwave extraction oil of *S. calyculata* was identified. The main components in the oil were 6,10,14-trimethyl-2-pentadecanone (27.73%) and caryophyllene oxide (16.84%). Other notable constituents were neophytadiene (7.68%) and (E)-β-ionone (6.77%). According to these results, the oil was rich with respective to nonterpenoid compounds (50.35%), whereas the sesquiterpene fractions were 22.56%. The monoterpene and diterpene fractions of the oil were relatively small, representing 5.79% and 8.34% of the total oil. It can be seen from Table 2, that the composition of the aerial part and the root oils of *C. irritans* are more different in quantity than quality. Twenty-nine compounds were identified in the aerial part oil of the plant, which is representing 100% of the whole oil composition. The main compounds were geranial (38.62%) and neral (28.08%). In the root oil, 30 compounds were identified, representing 96.01% of the whole oil composition. The main compounds were 8-hydroxy isobornyl isobutyrate (33.12%), geranial (22.26%) and neral (17.52%). As can be seen from the aforementioned information, both oils were characterized by large amounts of oxygenated monoterpenes (72.47% and 73.25%, respectively), whereas the sesquiterpene and nonterpenoid fractions of the oils, representing 18.47% and 7.55% and 8.71% and 15.21% of the total oils, respectively.

Total phenolic content

The TPC of the methanolic extracts of the aerial parts of *S. calyculata* and the aerial parts of *C. irritans* was

Table 4: Antibacterial activity of the methanolic extract of the aerial parts of *Centaurea irritans*

Microorganisms	MIC (mg/mL)	MBC (mg/mL)	Ampicillin (µg/mL)
Staphylococcus aureus	100	200	5
Listeria monocytogenes	100	200	10
Bacillus subtilis	50	100	10
Klebsiella pneumoniae	200	200	20
Pseudomonas aeruginosa	100	100	20
Streptococcus pyogenes	100	200	10

MIC = minimum inhibitory concentration, MBC = minimum bactericidal concentration

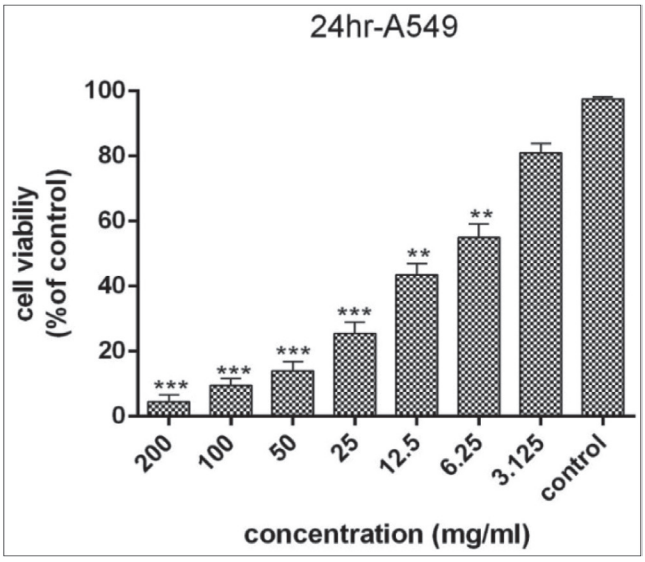

Figure 1: Survival percentage of A549 cells against various concentrations of the extract of *Scorzonera calyculata* within 24 h. *n* = 3; ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$. Results have been reported as survival rate compared with control samples

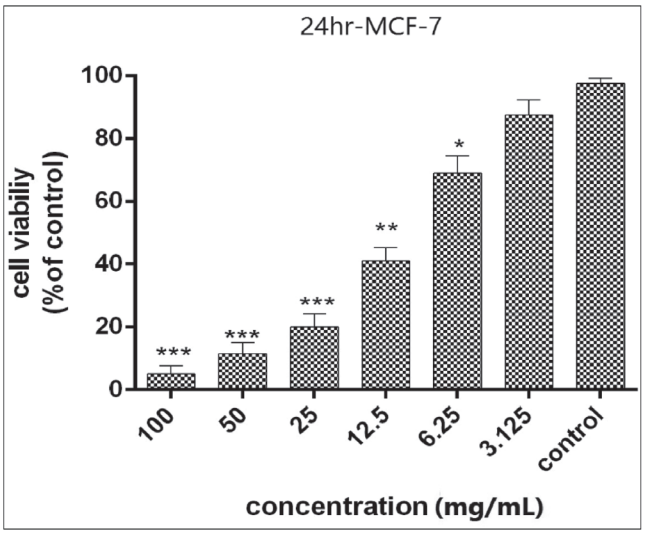

Figure 2: Survival percentage of MCF-7 cells against various concentrations of the extract of *Centaurea irritans* within 24 h. *n* = 3; ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$. Results have been reported as survival rate compared with control samples

determined to be 4.68 and 3.78 mg GAE/g sample, respectively. The phenolic assay involving an electron-transfer reaction was evaluated using the Folin–Ciocalteu reagent. The TPC measures both types of antioxidants, hydrophobic and hydrophilic, in complex with Fe^{2+}. Phenols and flavonoids are known to inhibit lipid peroxidation by quenching lipid peroxy radicals and to reduce or chelate iron in lipoxygenase enzyme and thus prevent initiation of lipid peroxidation reaction.[53]

2, 2-Diphenyl-1-picrylhydrazyl results

The results of DPPH test of the methanolic extracts of the aerial parts of *S. calyculata* and the aerial parts of *C. irritans* were dose dependent. The IC50 value of the extracts was 1.48 and 1.99 mg/mL, respectively. These values were found to be more than ascorbic acid as standard (0.09 mg/mL).

Ferric ion reducing power

FRAP values of the samples were 73.51 and 44.48 μmol Fe (II)/g dry mass, respectively. The FRAP value of the methanolic extracts was significantly lower than that of ascorbic acid (7370.84 μmol/g). FRAP assay is widely used in the evaluation of the antioxidant component in dietary polyphenols.[54]

Antibacterial activity

The MIC test was conducted to determine the lowest concentration of the extracts that inhibits the bacterial growth. In addition, the MBC is the lowest concentration of an antibacterial agent required to kill a particular bacterium. The results showed that antibacterial activity of extracts were dose dependent. The antibacterial activity showed that the extract of aerial parts of *S. calyculata* had the most effect on *S. aureus* ATCC 25923 with lower MIC value. The highest and lowest MIC values were belonged to *K. pneumoniae* and *S. aureus*, respectively [Table 3]. Also, the antibacterial activity revealed that the extract of aerial parts of *C. irritans* had the most effect on *B. subtilis* with lower MIC value. The highest and lowest MIC values belonged to *K. pneumoniae* and *B. subtilis*, respectively [Table 4]. Ugur *et al.* showed that ethanolic and chloroform extract of *Scorzonera sandrasica* had significant antibacterial activity against multiresistant strains of *Stenotrophomonas maltophila*.[55]

Anticancer activity

The anticancer activity of the extracts was performed using MTT method in various concentrations. As indicated in Figure 1, after incubation of cells in different concentrations for 24 h, the significant cytotoxicity was observed in 200 mg/mL compared with control (untreated cells) ($P < 0.05$). Moreover, the IC50 value cytotoxicity of extract of the aerial parts of S. calyculata on A549 cell line was 9.8 mg/mL. In Figure 2, the significant cytotoxicity was observed in 100 mg/mL compared with control (untreated cells) ($P < 0.05$). Moreover, the IC50 value cytotoxicity of extract of the aerial parts of *C. irritans* on MCF-7 cell line was 10.3 mg/mL.

Discussion

Only a few studies on the chemical composition of the oils of *Scorzonera* species have previously been reported. The chemical composition and antibacterial activities of volatile components from capitula and aerial parts of *S. undulata* have been described. In fact, 36 constituents were identified in the oil and the main components of them were methyl hexadecanoate (30.4%), methyl linoleate (23.9%) and heneicosane (12.2%). The *Scorzonera undulata* oil exhibited an interesting antibacterial activity against gram-positive and gram-negative bacteria but no antifungal activity was detected.[56] Previous investigation on oils of the *Centuarea* genus showed varying compositions. The oil of *C. imperialis*, growing in Iran, was found to contain caryophyllene oxide (23.2%), germacrene D (19.7%) and β-caryophyllene (14.1%) as the major constituents.[57] Water-distilled oils obtained from the aerial parts of *C. depressa* and *C. solstitialis* and flower of *C. ispahanica* have been the subject of our previous studies. Piperitone (35.2%) and elemol (14.1%) were detected in *C. depressa*, as the major components.[58] The oil of *C. solstitialis* was found to contain hexadecanoic acid (30.8%) and caryophyllene oxid (25.2%) as the major constituents.[59] The dominant compounds in the oil of *C. ispahanica* were benzyl benzoate (26.5%), hexadecanoic acid (17.1%), benzyl salicylate (16.6%) and caryophyllene oxide (12.8%).[60] The oil from the aerial parts of C. grinensis from Croatia, contained 4-vinyl guaiacol (21.5%), hexadecanoic acid (16.2%) and acetophenone (12.5%) as the major components. The dominant compounds in the oil of *C. apiculate* from Bulgaria, were caryophyllene oxide (15.8%), spathulenol (14.5%) and humulene epoxide II (9.4%).[61]

Several studies have shown the anticancer activity of *Scorzonera* species. Yang *et al*.[62] indicated that the cytotoxicity of *Scorzonera divaricata* Turcz. against HL-60 and Hep-G2 cell lines was 14.6μg/mL.

Recently we analyzed the chemical composition of the ethanolic extract of the aerial parts of *S. calyculata* by GC/MS.

A total of 27 compounds were identified in the *S. calyculata* extract, among which, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (30.79%) was the major one.[63]

This flavonoid compound contains therapeutic application such as, antioxidant, antimicrobial, anti-inflammatory and antiproliferative.[64] The other notable compounds in the extract of the plant were propylamin-N[9-borabicyclo{3,3,1}non-9yl (7.15%) which has antimicrobial activity,[64]hexadecanoic acid (6.12%), which has anti-inflammatory, antioxidant, antipsychotic and anti-allergic activities,[65] and neophytadiene (3.86%) which has strong bactericidal, anti-inflammatory and antifungal activities.[66]

More ever, *S. calyculata* extract is full of polyphenolic compounds, thus all the aforementioned compounds could have potentially been the cause of antioxidant, antibacterial and anticancer properties of the *S. calyculata*.

According to our recently reported on the chemical composition of the ethanolic extract of the aerial parts of *S. calyculata*, many of the identified components belonged to the polyphenolic compounds, such as 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, which was the major one. Polyphenolic compounds can play a key role in determining the biological properties of the *S. calyculata* extract.

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Conflicts of interest

There are no conflicts of interest.

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