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Determination of essential oil compositions as well as phenolic and flavonoid contents of *Inula viscosa* L. and *Inula graveolens* L. from the coastal region of Latakia - Syria

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ABSTRACT

The essential oils (EOs) of dry leaves of *Inula viscosa* L. and *Inula graveolens* L. obtained by hydro-distillation were investigated using GC-MS within two different harvest seasons, namely summer and autumn (August and October, respectively). The results showed that the essential oils were mainly composed of monoterpenes and sesquiterpenes. Twenty-four and fifty active components, representing 63.22% and 85.41% of the essential oils, were identified in *I. viscosa* L., whereas, fifty-two and thirty-five active components, representing 96.46% and 58.26% of the essential oils, were identified in *I. graveolens* L throughout the two harvest seasons respectively. The quantitative determination of methanolic extracts of *I. viscosa* L. and *I. graveolens* L. leaves was performed, where the total phenolic contents (TPC) and total flavonoid contents (TFC) were measured. Methanolic extracts of *I. viscosa* L. were found to be richer in phenolic and flavonoid contents than those of *I. graveolens* L. in the two considered seasons.

Keywords: medical plants, essential oil, phenolic content, flavonoid content, harvest season

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INTRODUCTION

In the persistent attempts to improve the efficacy of drug discovery and medical practices, healthy organic foods, plants and dietary supplements enriched with medicinal ingredients have attracted attention all over the world, especially plants that are used in traditional medicine to prevent and treat many human diseases[1,2]. Medicinal plant are natural sources that are rich of active phytochemicals (secondary metabolites) that are known to have important medical properties[3].

The Asteraceae (Compositae) family is one of the largest flowering plant families, with over 1600 genera and 2500 species worldwide. Members of the Asteraceae family have been used in diets and medicine for centuries. Most Asteraceae family members demonstrate strong antioxidant, anti-inflammatory, antimicrobial and wound healing properties. Their pharmacological effects can be attributed to their range of phytochemical compounds, including polyphenols, phenolic acids, flavonoids, triterpenes and sesquiterpene lactones[4].

Inula L. is a large genus from Asteraceae family with more than one hundred species[5]. *Inula* L. species grow in Africa, Asia, Europe and the Mediterranean region. They were first used in Roman, Greek and Chinese traditional medicine to treat various diseases[6]. Extracts of the species are particularly rich in terpenoids, in particular eudesmane acids, ilicic acid and α-costic acid[7], Several *Inula* L. species are known for their therapeutic properties, for example *Inula viscosa* L. and *Inula graveolens* L.

Inula viscosa L. Aiton (Asteraceae) (Synonym: *Dittrichia viscosa* L. Greuter), is a strong-smelling perennial plant. Its leaves are sticky, oval and pointed. At the top of the stem there are numerous yellow flowered heads[8]. By contrast, *Inula graveolens* L. Desf. (Synonym: *Dittrichia graveolens* L. Greuter), is an annual aromatic plant with a foul camphor odor. Its leaves are sticky, oval and pointed, while its flowers appear in September / October and have yellow petals. Its yellow flowers do not produce seeds until September, while other plants from this genus complete their life cycles by the end of September[9,10]. Both plant species grow on cultivated land, abandoned roadsides and rural areas[11].

In traditional medicine, *I. viscosa* L. has many uses. These include antispasmodic, sedative, antiseptic, treating bronchial disorders, expectorant, antipyretic, antispasmodic, antidiabetic, diuretic, anthelmintic and anti-aging. The fresh leaves are used for wound healing by being applied to the wound area[12,13]. Meanwhile, *I. graveolens* L. is widely used for its anti-inflammatory effects and in aromatherapy for the treatment of asthma. It is also used as Broncho

spasmolytic, mucolytic, rheumatic fever, reducing blood sugar, dissolving internal blood clots, treating of urinary tract infections and wound infections[14].

Essential oil, aqueous and organic extracts of *I. viscosa* L. different parts showed antifungal and antibacterial activities in vitro[12,15,16]. In addition, antioxidant and anticancer activities were observed in ethanolic and methanolic extracts in relation to the presence of flavonoids and Sesquiterpenoids[6]. The ethanolic extract induces programmed cell death in kinetoplastids[17], and causes strong anticancer effects on Burkitt lymphoma cell line through inhibition of cell proliferation and induction of cell apoptosis[18]. Methanolic extract of the plant's leaves has an antihypertensive effect[19], essential oil and hexane extract from leaves have insecticidal properties, and play an important role in control of the bacteria that cause olive knot disease[20,21].

Essential oil, methanolic and ethanolic extracts of *I. graveolens* L. have antioxidant properties[22]. Methanolic, ethanolic and acetone extracts show antifungal properties against *Fusarium poae* that causes Fusarium head blight (FHB) disease, which is an important and insidious disease affecting mainly wheat and other cereals worldwide. Essential oils have anti candida, anti-bacterial, anti-cancer effects as well as acetylcholinesterase and tyrosinase inhibitor effects[23-26].

Both species are found in several areas in Syria and contain some pharmacologically active compounds, including phenols, flavonoids, terpenoids and tannins[11]. The present study is undertaken to report the essential oil compositions, phenolic and flavonoid contents of methanolic extracts from the leaves of the Syrian species *I. viscosa* L. and *I. graveolens* L. growing in Wadi Al-Janayin (AL-Qardaha region, Latakia - Syria). This study has not been reported previously.

METHODOLOGY

Plant material

The leaves of both plant species were collected in August (summer, before flowering) and October (Autumn, during flowering) of 2021 from populations growing wild in Wadi Al-Janayin (AL-Qardaha region, Latakia - Syria). Both plant species were identified by Prof. Aziza Ibrahim Youssef, a member of the academic staff at the Faculty of Pharmacy, Tishreen University (Syria). The leaves were dried in ambient air in the pharmacognosy laboratory and sheltered from light and moisture.

Chemicals

All of the chemicals were purchased from Sigma Aldrich Co. (St. Louis, MO, USA), and the solvents were from E. Merck (Darmstadt, Germany). All of the reagents were prepared in deionized distilled water to eliminate the contamination of metal ions.

Extraction of essential oils

According to the British Pharmacopoeia, essential oils were extracted from 100 g of dry leaves of each plant species (separately) by hydro-distillation in Clevenger-type apparatus for a period of 4 hours with 1 L of distilled water .The essential oils were dried over anhydrous sodium sulfate, filtered and stored at 4 °C until the time of analysis by gas chromatography-mass spectrometry (GC/MS).

GC/MS analysis

The GC/MS analysis of the oil samples were carried out at the Institute of Marine Research, Faculty of Agriculture, Tishreen University, Latakia – Syria. From Agilent technologies, A Hewlett-Packard 6890N gas chromatograph coupled to a 5975-mass spectrometry detector, and a capillary HP-5 column (5%-phenyl)-methylpolysiloxane) (30m×250μm×0.25μm) were used. The carrier gas was helium, with a flow velocity of 1.2 mL/min. The temperature of the injector and the detector was set to 250°C and 280°C respectively. The heating program started at 70°C for 2 min, then the temperature was increased to 280°C at a rate of 4°C/min. The chemical components were, then, identified based on a comparison of their retention indices relative to (C6–C24) n-alkanes with those in literatures, and by matching their mass spectra with those recorded in the mass spectral libraries.

Extraction of phenolic and flavonoid compounds

The method proposed by Oniszczuk and Podgórski was adopted[27]. A 1 g of powdered leaves of each plant species was subject to extraction using 20 mL of 80% methanol following the reflux extraction method at 70°C for 20 min. The methanolic extracts were filtered and evaporated to dryness under reduced pressure in a rotary evaporator at 40°C. The extracts obtained by evaporation have a gelatinous liquid appearance of dark brown color.

Determination of total phenolic contents

The total phenolic contents were determined using the Folin-Ciocalteu (F-C) method, which was previously described by Singleton et al.[28] with slight modi-

fications. The calibration curve was plotted using gallic acid standard (50-700 µg/mL). An amount of 0.1 mL of the plant dry extracts that are dissolved in methanol 80% was mixed with 2 mL of 2% sodium carbonate solution. 2 mL of 10% F-C reagent was added to the mixture 10 mins later and shaken well. After 15 minutes of incubation in a dark place at room temperature, the absorbance of the blue mixtures was read against the blank at 750 nm using UV/visible spectrophotometer. The total phenolic concentrations were calculated from the calibration curve (Abs = 0.0007x + 0.0365, R^2 = 0.998). The concentrations were expressed as milligram gallic acid equivalent per gram dry extract (mg GAE. g DE^{-1})[29]. The process was repeated three times for each sample.

Determination of total flavonoid contents

The quantification of total flavonoids was obtained by a colorimetric assay after reaction with aluminum chloride using a method described by Hongbin Zhu et al.[30] with slight modifications. To draw the standard curve, a standard solution (0.16 mg/mL) of rutin was prepared. Seven portions (of volumes: 1, 2, 3, 5, 7, 8, 10 mL) of the rutin standard solution were removed in seven 25-mL volumetric flasks. The following steps were, then, performed.

- 1.6 mL of 5% $NaNO_2$ solution were added to each flask, shaken up and then left to settle for 6 min.
- 1.25 mL of 10% $AlCl_3.6H_2O$ solution were added to each flask, shaken and left to settle for 6 min.
- 7.5 mL of 4.3% NaOH solution was added to each flask, followed by the addition of water to the scale.

The mixture was shaken, and left to settle for 15 min. The absorbance was read against the blank at 498 nm using UV/visible spectrophotometer. The concentration of flavonoids in each plant sample was calculated from the standard curve (y = 0.0109x + 0.0112, R^2 = 0.998) after adding 1 mL of the plant dry extract (dissolved in methanol 80%) to a volumetric flask (25 mL) with the same previous additions described in drawing rutin standard curve section. The concentrations were expressed as milligram rutin equivalent per gram dry extract (mg RE. g DE^1). The process was repeated three times for each sample.

RESULTS and DISCUSSION

Essential oil analysis

The hydro-distillation of the leaves of two plant species yielded light yellow-colored essential oils. The GC/MS analysis of those essential oils regarding the two different harvest seasons gave the following results. The analysis of essential oils of *I. viscosa* L. harvested in the summer showed the presence of twenty-four active components, representing 63.22% of *I. viscosa* L. essential oils, whereas for the autumn *I. viscosa* L. there were fifty active components representing 85.41% of *I. viscosa* L. essential oils. With regard to *I. graveolens* L., the corresponding figures were fifty-two and thirty-five active components, representing 96.46% and 58.26% of essential oils. The analysis revealed also the predominant presence of 3,5-di-tert-butyl-4-hydroxybenzaldehyde (10.48%), 2,6-di-tert-butyl-4-ethylphenol (7.76%), methyl-cyclopentane (7.01%) and α-selinene (5.61%) regarding *I. viscosa* L. that was harvested in the summer. As for the autumn harvest, the identified compounds were caryophyllene oxide (33.2%), heneicosane (6.29%), aromadendrene (5.6%) and dodecanoic acid (3.21%). The corresponding results for *I. graveolens* L. were borneol (35.21%), bornyl acetate (22.86%), anethol (5.57%) and adamantane (3.88%) for the summer harvest; and bornyl acetate (16.13%), β-cubebene (5.25%), adamantane (4.4%) and borneol (3.77%) for the autumn harvest.

Table 1. Compounds identified in the essential oils of *I. viscosa* L. and *I. graveolens* L. leaves using GC/MS in the order of their elution from the column

Peak	Compound	Chemical Group	RI* Range[31]	RI	Rt	Area %			
						Iv-S	Iv-A	Ig-S	Ig-A
1.	**Methyl-Cyclopentane**	Cyclic alkane	520-636	523.07	1.503	**7.01**	-	-	**3.52**
2.	Toluene	Aromatic hydrocarbon	672-779	621.32	1.925	0.30	-	-	0.22
3.	6-Methyl-3,5-heptadiene-2-one	Enone	720-790	761.3	4.643	-	-	0.20	-
4.	1,3-Cycloheptadiene	Cyclic alkane	832-921	887.74	7.939	-	-	-	0.15
5.	Nerol oxide	Monoterpenoid Alcohol	1146-1154	775.9	8.557	-	-	0.17	-
6.	Camphene hydrate	Monoterpene	1144-1148	1022.87	8.763	-	-	0.27	-
7.	**Borneol**	Monoterpenoid Alcohol (Bicyclic monoterpenoid)	1152-1164	1077.1	9.495	-	0.11	**35.21**	**3.77**
8.	α-Terpineol	Monoterpenoid Alcohol	1178-1188	1026.68	9.724	-	0.10	0.11	-
9.	p-Mentha-1,5-dien-8-ol	Monoterpenoid Alcohol	1155-1164	1036.1	10.382	-	-	0.35	-
10.	Jasmolone	Monoterpenoid Alcohol	1095-1120	1075.05	11.658	-	0.09	-	-
11.	**Bornyl acetate**	Bicyclic monoterpenoid ester	1264-1283	1174.6	12.116	-	0.64	**22.86**	**16.13**
12.	Theaspirane	Norisoprenoid (Oxaspiro compound)	1190-1285	1213.27	12.276	-	0.07	-	-
13.	**Anethol**	Phenyl propene (monomethoxybenzene)	1273–1303	1383.41	13.375	0.52	1.92	**5.57**	0.61
14.	2,4-Di-tertbutylphenol	Alkylbenzene	1300-1400	1038.48	13.605	-	-	-	0.78
15.	Isobornyl propionate	Monoterpenoid ester	1405-1490	1472	13.981	-	0.16	1.01	-
16.	Caryophyllene	Bicyclic Sesquiterpene	1392–1426	1316.88	14.130	-	0.27	1.27	0.48
17.	**3,5-Di-tert-butyl-4-hydroxy-benzaldehyde**	Hydroxybenzaldehyde	1520-1600	1586.86	14.887	**10.48**	-	-	1.3
18.	**2,6-Di-tert-butyl-4-ethylphenol**	Alkylbenzene	1530-1596	1511	15.005	**7.76**	-	-	0.29
19.	**α-Selinene**	Sesquiterpene	1477–1510	1698.4	16.001	**5.61**	-	0.16	0.25

Peak	Compound	Chemical Group	RI* Range[31]	RI	Rt	Area %			
20.	**Guaia-10(14),11-diene**	Sesquiterpene	1560-1609	1511	16.018	-	**2.45**	-	-
21.	Spathulenol	Tricyclic Sesquiterpenoid alcohol	1562–1590	1556.3	16.156	1.60	-	0.30	-
22.	α-Curcumene	Sesquiterpene	1468–1494	1533.97	16.264	0.66	1.03	0.26	-
23.	α-Longipinene	Sesquiterpene	1337–1362	1552.3	16.476	-	0.19	-	-
24.	Apiole	Phenylpropene	1608–1634	1583.5	16.672	-	-	-	0.25
25.	β –Farnesene	Sesquiterpene	1438–1460	1227.7	16.711	-	0.36	-	-
26.	α-Copaen-11-ol	Tricyclic Sesquiterpenoid alcohol	1556–1594	1571.4	16.813	1.36	-	-	-
27.	γ-Muurolene	Sesquiterpene	1461–1487	1510	16.854	0.76	-	0.11	-
28.	Prenyl benzoate	Benzoic acid ester	1530-1589	1556.8	17.231	-	-	0.61	-
29.	Trans-β-Ionone	Sesquiterpene	1256-1308	1296	17.523	-	-	0.31	-
30.	6-Prop-2-enyl-1,3-benzodioxol-5-ol	Benzodioxole	1290-1356	1303	17.535	-	0.31	-	-
31.	Dodecanedioic acid	Dicarboxylic fatty acid	1030-1110	1081	17.923	-	-	-	0.38
32.	(+)-Nerolidol	Sesquiterpenoid alcohol	1225-1298	1241.4	18.015	1.45	1.30	-	-
33.	**2-Pentylpyridine**	Pyridine	1498-1556	1529	18.146	**5.42**	-	-	-
34.	Neryl propionate	Carboxylic ester	1024-1098	1069	18.473	-	-	0.71	-
35.	**α-Curjunene**	Sesquiterpene	1300-1398	1338	18.620	4.45	-	-	-
36.	Alloaromadendrene oxide	Sesquiterpene oxide	1543-1587	1571	18.862	-	-	0.17	-
37.	Caryophylladienol	Sesquiterpenoid alcohol	1456-1540	1528.75	18.960	-	-	-	0.50
38.	**β-Cubebene**	Sesquiterpene	1370–1393	1559.87	19.205	-	-	-	**5.25**
39.	2-Methoxy-3-methylpyrazine	pyrazines (Aromatic ether)	1572-1603	1590.26	19.371	-	0.48	-	-
40.	Thymol	Monoterpenoid phenol	1272–1304	603.5	19.389	-	-	0.63	1.34
41.	3-Carene	Monoterpene	1002–1025	1092.9	19.892	-	0.24	-	-

Peak	Compound	Chemical Group	RI* Range[31]	RI	Rt	Area %			
42.	γ-Elemene	Sesquiterpene	1327–1344	1027.34	19.931	0.73	-	-	-
43.	γ-Caryophyllene	Sesquiterpene	1405–1440	1525.68	20.035	-	-	0.68	-
44.	(+)-γ-Cadinene	Sesquiterpene	1498–1531	1561.3	20.339	-	-	0.28	0.21
45.	**Aromadendrene**	Sesquiterpene	1419–1465	1549.5	20.533	1.27	**5.6**	0.21	0.67
46.	**(+)-Epi-bicyclosesquiphel-landrene**	Sesquiterpene	1564-1599	1571.56	20.733	-	-	**2.66**	-
47.	Geranyl acetate	Monoterpenoid ester	1358–1388	1556	20.813	-	-	0.80	0.23
48.	Terpinylisobutyrate	Monoterpenoid ester	1256-1298	1278	20.876	-	0.65	-	-
49.	**Adamantane**	Polycyclic alkane	1376-1443	1406	20.894	**3.07**	**2.7**	**3.88**	**4.4**
50.	α-Cadinol	Sesquiterpenoid alcohol	1635–1664	1070	21.197	-	-	0.25	-
51.	(E)-Stilbene	Diarylethene	1530-1579	1543.22	21.330	-	-	-	0.46
52.	**Menthol**	Monoterpenoid Alcohol	1169–1194	1500	21.506	-	**2.78**	-	-
53.	**(+)-Beta-selinene**	Sesquiterpene	1473–1496	1000	21.506	-	-	**2.65**	-
54.	2-Isopropyl-tricy-clo[4.3.1.1(2,5)]undec-3-en-10-ol	Fatty acid Alcohol	1540-1590	1588.15	21.632	-	0.42	-	-
55.	Isoaromadendrene epoxide	Sesquiterpenoid	1398-1434	1409.3	21.649	1.56	0.48	0.94	-
56.	α-Farnesene	Sesquiterpene	1486–1497	1557.6	21.815	1.75	-	0.54	-
57.	Batyl-alcohol (Batilol)	Alkylglycerol	1550-1587	1573.7	21.938	-	-	-	0.94
58.	3-Isopropyltricyclo[4.3.1.1(2,5)] undec-3-en-10-ol	Alcohol	2120-2189	2139.7	21.982	0.59	-	-	-
59.	10s,11s-Himachala-3(12),4-diene	Sesquiterpenoid	1390-1450	1416.92	22.049	-	-	0.35	-
60.	Geranyl butyrate	Monoterpenoid ester	1550-1598	1578.8	22.381	-	-	0.32	-
61.	Dodecane	Alkane	1420-1487	1447.7	22.471	-	-	-	1.94
62.	(+)-Ar-tumerone	Sesquiterpenoid ketone	1203-1280	1236.67	22.570	-	-	0.57	-
63.	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-	Monoterpenoid ketone	1530-1599	1575.2	22.742	-	-	0.24	-

Peak	Compound	Chemical Group	RI* Range[31]	RI	Rt	Area %			
64.	Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-	Alkane	1498-1546	1527.76	22.799	-	0.55	-	-
65.	2-Pentadecanone, 6,10,14-trimethyl	Ketone	1548-1606	1586.67	22.948	-	-	0.33	-
66.	2-Undecanone, 6,10-dimethyl-	Dialkyl Ketone	1779-1850	1815.58	22.971	-	0.74	-	-
67.	β -Patchoulene	Tricyclic sesquiterpene	1450–1464	1362.6	23.096	-	0.24	-	-
68.	Longifolenaldehyde	Sesquiterpenoid Aldehyde	1500-1590	1548.47	23.171	-	-	0.18	-
69.	α-Humulene	Monocyclic sesquiterpene	1435–1470	1514.46	23.251	-	0.59	-	-
70.	Benzoic acid	Aromatic carboxylic acid	1510-1614	1574.87	23.730	1.81	-	-	-
71.	Hexahydrofarnesylacetone	Sesquiterpenoid lactone	1831–1855	1776.6	23.893	0.59	-	-	-
72.	3-Phenylpropyl isovalerate	Fatty acid ester	1820-1870	1835.28	23.943	-	-	0.57	-
73.	Tetradecane	Alkane	1404-1467	1438.3	24.035	-	0.90	-	0.90
74.	1-Heptatriacotanol	Fatty alcohol	1400-1470	1438.5	24.184		0.48	-	-
75.	Aromadendrene oxide-(1)	Sesquiterpenoid	3750-3790	3785.78	24.424	-	-	0.41	-
76.	**α –Guaiene**	Sesquiterpene	1424–1454	1511.18	24.464	-	**2.71**	-	-
77.	Methyl decanoate	Fatty acid methyl ester	1323–1329	1566.74	24.784	-	0.52	-	-
78.	Linalool	Monoterpenoid alcohol	1088–1109	1150.32	24.945	0.80	-	-	-
79.	Z-7-Pentadecenol	Fatty acid alcohol	1000-1096	1029.37	25.105	-	0.65	-	-
80.	Farnesol	Sesquiterpenoid alcohol	1678–1700	1591.6	25.494	-	-	0.29	-
81.	Methyl 14-methyl pentadecanoate	Fatty acid methyl ester	1478-1548	1509.17	25.530	-	-	-	1.74
82.	Propyl cinnamate	Alkyl cinnamate (Phenylpropanoid)	1750-1809	1798.63	25.889	-	-	0.26	-
83.	Hexadecane	Alkane	1198-1230	1202.83	25.894	-	0.82	-	0.90
84.	Palmitic Acid	Saturated long-chain fatty acid	1660-1690	1675.19	26.066	-	-	1.21	-

Peak	Compound	Chemical Group	RI* Range[31]	RI	Rt	Area %			
85.	Octathiocane	Homomono-cyclooc-tasulfur	1623-1689	1640	26.123	0.57	-	-	0.35
86.	**Geranylgeraniol**	Diterpenoid Alcohol	1809-1850	1818.24	26.209	-	**2.68**	0.13	-
87.	**Dodecanoic acid**	Fatty acid	1557–1587	2085.04	26.598	-	**3.21**	-	-
88.	Retene	polycyclic aromatic hydrocarbon	1200-1250	1213.8	26.667	-	-	0.35	-
89.	**Heptadecane**	Alkane	1840-1906	1890.31	27.101	-	0.15	-	**3.36**
90.	Diisobutyl phthalate	Phthalate ester	1700-1790	1719.57	27.148	-	-	0.85	-
91.	**Caryophyllene oxide**	Sesquiterpenoid oxide	1563–1595	1691.54	27.342	**3.10**	**33.2**	**3.51**	1.53
92.	Heptanoic-acid	Carboxylic acid	1499-1549	1504.17	27.360	-	-	-	1.24
93.	3-Phenylpropyl isobutyrate	Cinnamyl phenylpropyl	700-789	761.1	27.777	-	-	1.86	-
94.	2-Methylbenzyl benzoate	Benzoate ester	1350-1396	1375.9	28.046	-	0.30	-	-
95.	Benzyl salicylate	Benzoate ester / phenol	1857–1881	1532.1	28.132	-	-	0.12	-
96.	Phytol	Acyclic diterpene alcohol	2104–2136	1449.36	28.315	-	0.97	-	-
97.	1-Hexadecene	Alkene	2000-2080	2036.14	28.504	-	0.42	-	-
98.	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	Alcohol	1612-1678	1646	28.841	-	0.39	-	-
99.	Phthalic acid, ethyl isopropyl ester	Phthalic acid esters	1761-1790	1784	29.242	-	0.27	-	-
100.	**Octadecane**	Alkane	1343-1379	1362.65	29.319	-	**2.76**	-	**2.49**
101.	1,7-Octadien-3-one, 2-methyl-6-methylene-	Ketone	1800-1870	1813.56	29.365	-	-	0.34	-
102.	9-Nonadecene	Alkene	1045-1079	1066.76	29.660	-	1.26	0.45	-
103.	Phenylethyl salicylate	Benzoate ester / phenol	1932-1967	1951.9	29.808	-	-	0.27	-
104.	Pyrene, hexadecahydro-	Pyrene	1512-1567	1540	29.946	-	0.40	-	-
105.	Longipinene epoxide	Sesquiterpenoid	1602-1688	1619.1	30.157	-	0.50	-	-
106.	Nonadecane	Alkane	1510-1550	1516	31.067	-	1.05	-	0.56

Peak	Compound	Chemical Group	RI* Range[31]	RI	Rt	Area %			
107.	Eicosane	Alkane	1912-1990	1988.7	36.299	-	-	0.09	0.35
108.	**Heneicosane**	Alkane	2020-2074	2050.25	37.024	-	**6.29**	0.42	0.43
109.	Tetracosane	Alkane	2156-2190	2186.76	37.756	-	0.31	0.17	0.16
110.	Hexacosane	Alkane	2402-2440	2412.5	37.869	-	-	-	0.18
111.	1-Heptacosanol	Fatty alcohol	2610-2649	2617.8	38.671	-	0.10	-	-
112.	Octacosane	Alkane	2750-2799	2791.37	42.608	-	0.6	-	-
Number of active compounds						24	50	52	35
Main chemical groups in each essential oil		**Total Monoterpenes %**				**0.80**	**4.77**	**61.97**	**21.47**
		Hydrocarbon Monoterpenes %				-	0.24	0.27	-
		Oxygenated Monoterpenes %				0.80	4.53	61.7	21.47
		Total Sesquiterpenes %				**24.89**	**48.92**	**16.1**	**8.89**
		Hydrocarbon Sesquiterpenes %				15.23	13.44	9.13	6.86
		Oxygenated Sesquiterpenes %				9.66	35.48	6.97	2.03
		Oxygenated Diterpenes %				**-**	**3.65**	**0.13**	**-**
		Phenylpropanoids %				**0.52**	**1.92**	**7.69**	**0.86**
		Others %				37.01	26.15	10.57	27.04
Total %						63.22	85.41	96.46	58.26

RI: (retention index or Kovats index) calculated with respect to n-alkanes (C6-C24). %: Relative percentage obtained from the area of the peak and electronic integration measurements using a selective mass detector. Iv-S: I. viscosa L./Summer, Iv-A: I. viscosa L./Autumn, Ig-S: I. graveolens L./Summer, Ig-A: I. graveolens L./Autumn

Hydro-distillation is a simple and fast method for extracting the essential oils from plants. It has a good yield, good recovery of essential oil constituents and less labor-intensive[32].

According to the GC/MS analysis of the essential oil samples, as presented in Table 1, 3,5-di-tert-butyl-4-hydroxybenzaldehyde (10.48%) (hydroxybenzaldehyde) and caryophyllene oxide (33.2%) (Oxygenated sesquiterpene) were the main compounds in *I. viscosa* L. essential oils of the summer and autumn harvests respectively. 3,5-di-tert-butyl-4-hydroxybenzaldehyde has been used

as an intermediate compound for synthesizing pharmaceutical products that have a strong anti-oxidant properties[33]. In addition, caryophyllene oxide has strong anticancer activities against breast cancer cell lines (MCF7 and T47D)[34], colon cancer (HCT 116 and HT29), pancreatic cancer (PANC-1), as well as having antioxidant, antimicrobial and antifungal properties[35].

I. graveolens L. essential oils were found to have a large amount of oxygenated monoterpenes (35.21% of borneol and 22.86% of bornyl acetate, in the summer harvest, and 16.13% bornyl acetate in the autumn harvest). Essential oils of plants that contain bornyl acetate and borneol were reported to have antimicrobial, antioxidant and insecticidal properties[20].

Studies similar to this one were carried out on the same plant species. In those studies the main constituents of the essential oils varied depending on plant parts, harvest regions and extraction methods[36]. In this study, the composition of *I. viscosa* L. essential oils from the autumn harvest showed some similarities to its counterpart in other studies in Syria[37], Algeria[15], Jordan[38] and Morocco[20], particularly concerning the content of caryophyllene oxide. However, *I. viscosa* L. essential oil compositions from the summer harvest did not show any similarities to the other studies. On the other hand, the composition of *I. graveolens* L. essential oils from the autumn and summer harvest showed some similarities with studies from Turkey[22,39], Algeria[40], Iran[23], Tunisia[26], and Jordan[38], particularly concerning the content of bornyl acetate and borneol. The amounts of compounds may increase, decrease or even disappear altogether depending of the harvest season. For example, in the essential oils of *I. graveolens* L., the largest amount of bornyl acetate, a main compound, was recorded in July while it decreased in January. Furthermore, β-selinene was only found in July, whereas s-cadinol was found in October and, then, dramatically decreased in January[26].

The major compounds of the essential oils of *I. viscosa* L. and *I. graveolens* L. from different areas in Syria and abroad are shown in Table 2 and Table 3.

According to the results in Table 2 and Table 3, it can be concluded that the chemical compositions of the plants vary according to various factors. Some of these factors include genetic factors, environmental factors, seasonal change, plant parts, developmental stage and extraction methods[26,31]. Therefore, the chemical composition of *I. viscosa* and *I. graveolens* from different periods is an important factor in determining their bioactivity.

Table 2. Major components found in the essential oils of *I. viscosa* L. obtained from different areas

Major compounds of *I. viscosa* L. essential oil	Area % Syria (AL-Qardaha region)		Area % Syria (Al-Qadmous region)[36]	Area % Algeria (Sidi Rezine village, South)[8]	Area% Algeria (Sidi Rezine village, South)[8]	Area% Algeria (Northwest)[15]	Area % Jordan (Irbid)[38]	Area % Morocco (Fez)[20]
	PP: L EM:HD HS:S	PP: L EM:HD HS:A	PP: L EM:HD	PP: L EM:HD	PP: L EM:SD	PP: L EM:HD	PP: AP EM:HD	PP: L EM:HD
3,5-Di-tert-butyl-4-hydroxybenzaldehyde	10.48	-	-	-	-	-	-	-
2,6-Di-tert-butyl-4-ethylphenol	7.76	-	-	-	-	-	-	-
Methyl-Cyclopentane	7.01	-	-	-	-	-	-	-
Bornyl acetate	-	-	-	-	-	-	-	41
Borneol	-	-	-	-	-	-	-	9.3
12-Carboxyeudesma-3,11 (13) diene	-	-	-	28.88	56.81	-	-	-
Linolenic acid	-	-	-	7.80	-	-	-	-
Pentacosane	-	-	4.36	5.43	2.31	-	-	-
Heneicosane	-	6.29	-	-	-	-	-	-
n-Hexadecanoic acid	-	-	-	5.38	1.91	-	-	-
Caryophyllene oxide	-	33.2	7.83	-	-	10.4	2.57	5.7
Heptacosane	-	-	-	4.82	2.09	-	-	-
Butyl hydroxy toluene	-	-	-	4.11	2.63	-	-	-
Fokienol	-	-	-	3.37	1.89	9.6	20.87	-
2,3-Didehydrocostic acid	-	-	-	-	3.25	-	-	-
α-Eudesmol	-	-	-	-	-	7.6	2.68	-
Trans-Nerolidol	-	-	13.64	-	-	7.0	19.75	-
γ-Eudesmol	-	-	-	-	-	6.2	-	-
β–Eudesm-6-en-4α-ol	-	-	-	-	-	-	5.64	-

Major compounds of *I. viscosa* L. essential oil	Area % Syria (AL-Qardaha region)		Area % Syria (Al-Qadmous region)[36]	Area % Algeria (Sidi Rezine village, South)[8]	Area% Algeria (Sidi Rezine village, South)[8]	Area% Algeria (Northwest)[15]	Area % Jordan (Irbid)[38]	Area % Morocco (Fez)[20]
α-Vetivone	-	-	-	-	-	-	3.60	-
Selin-11-en-4α-ol	-	-	-	-	-	-	2.18	-
Selina-6-en-4-ol	-	-	4.46	-	-	-	-	-
α-Selinene	5.61	-	-	-	-	-	-	-
8-Cedren-13-ol	-	-	3.10	-	-	-	-	-
Cedren-14-olacteate	-	-	-	-	-	-	2.0	-
Dodecanoic acid	-	3.21	-	-	-	-	-	-
Khusimol	-	-	-	-	-	-	1.80	-
E-Farnesene epoxide	-	-	16.55	-	-	-	-	-
Alpha. copaene-11-ol	-	-	3.03	-	-	-	-	-
α-amorphene	-	-	-	-	-	-	-	6.6
Aromadendrene	-	5.6	-	-	-	-	-	-

PP: Plant Part, L: Leaves, AP: Arial Parts, EM: Extraction Method, HD: Hydro-Distillation, SD: Steam DistillatioN, HS: Harvest Season, S: Summer, A: Autumn

Table 3. Major components of essential oils of *I. graveolens* L. obtained from different areas

Major components of I.graveolens L. essential oil	Area % Syria (AL-Qardaha region)		Area % Turkey (Bingol University Campus)[38]	Area % Turkey (Gaziantep/ Karatas highway (steppe land)[22]	Area% Algeria (Sidi Rezine village, South)[8]	Area% Iran (Shush)[23]	Area % Tunisia (Chebba salt marsh)[26]	Area % Jordan (Al-Jubeiha region)[33]
	PP: L EM:HD HS:S	PP: L EM:HD HS:A	PP: AP EM:HD	PP: L EM:HD	PP: AP EM:SD	PP: AP EM:HD	PP: AP EM:HD	PP: AP EM:HD
Bornyl acetate	22.86	16.13	-	68.5	-	-	45.34	70.58
Borneol	35.21	3.77	20.4	7.7	18.3	5.44	37.29	-
Isobornylacetate	-	-	-	-	50.8	-	-	-
Anethol	5.57	-	-	-	-	-	-	-
Thymol	-	-	-	-	-	-	4.62	-
Camphene	-	-	-	4.6	-	-	3.20	1.97
Adamantane	3.88	4.4	-	-	-	-	-	-
β-Cubebene	-	5.25	-	-	-	-	-	-
1,8-Cineole	-	-	22.4	-	-	54.89	-	-
α-Cadinol	-	-	11.8	-	-	-	-	-
P-Cymen	-	-	-	-	-	16.2	-	-
β-Pinene	-	-	-	-	-	6.94	-	-
ι-Cadinol	-	-	-	-	6.2	-	6.09	-
α-Terpineol	-	-	-	-	-	-	1.71	-
(2E,6E)-Farnesol	-	-	-	-	-	-	1.37	-
Caryophyllene oxide	-	-	-	-	-	-	1.30	1.82
β-Caryophyllene	-	-	-	-	-	-	1.22	-
Epi-a-cadinol	-	-	-	4.0	-	-	-	-
Eicosane	-	-	-	3.2	-	-	-	-

PP: Plant Part, L: Leaves, AP: Arial Parts, EM: Extraction Method, HD: Hydro-Distillation, SD: Steam Distillation
HS: Harvest Season, S: Summer, A: Autumn

Total phenolic and flavonoid contents

Based on the equation curves, the total amounts of phenols and flavonoids in the dry extracts of the inula plant leaves were calculated. The results showed that *I. viscosa* L. contained greater amounts of phenols and flavonoids when compared to *I. graveolens* L. (before and after blooming). The amounts of phenols and flavonoids in *I. viscosa* L. before blooming is greater than those after blooming. However, the amounts of phenols and flavonoids in *I. graveolens* L. after blooming is greater than those before blooming (Table 4).

Table 4. The total amounts of phenols and flavonoids in *I. viscosa* L. and *I. graveolens* L. dry extracts

Plant	n	Phenols content		Flavonoids contents	
		Yield(mean) ± SD mg Gallic acid. E / g D.E	CV%	Yield(mean) ± SD mg Rutin.E / g D.E	CV%
I. viscosa L. (S)	3	920 ± 1.573	0.002	42.587 ± 0.4343	0.0102
I. viscosa L. (A)	3	724.565 ± 2.627	0.0036	37.390 ± 0.442	0.012
I. graveolens L. (S)	3	516 ± 0.563	0.0011	24.532 ± 0.515	0.021
I. graveolens L. (A)	3	678.04 ± 1.766	0.0026	30.419 ± 0.363	0.012

n: number of repetitions, SD: standard deviation, CV%: the coefficient of variation in percent, S: for summer season, A: for autumn season

Phenols and flavonoids, the most common groups of secondary metabolites, are important in plants for normal growth development and defense against infections. Flavonoids are phenolic compounds and are very important pigments for flower coloration as they produce yellow or red/blue pigmentation in petals. They also protect plants from attacks by microbes and insects. They also show anti-allergic, anti-inflammatory, anti-microbial, anticancer, antioxidant[30] and free radical scavenging properties[41] as well as antidiabetic and weight loss effects[42].

Among different spectrophotometric techniques, The ultraviolet-visible (UV/Vis) spectrophotometry appears to be suitable for the quantification of phenolic and flavonoid contents in the plants extracts[3] due to its operational simplicity, speed, low cost of implementation, and wide availability in control laboratories[43]. Therefore, this technique is more accessible methods than analytical chromatography techniques, such as high performance liquid chromatography (HPLC)[3].

The present study revealed the phenolic contents in the extracts of *I. viscosa* L. and *I. graveolens* L. dry leaves. The amounts of phenolic compounds were found to be 920 and 724.56 mg gallic acid equivalent/g dry extracts in *I. viscosa* L. regarding the summer and autumn harvest respectively; the corresponding amounts for *I. graveolens* L. were 516 and 678 mg gallic acid equivalent/g dry extract. As for the flavonoids contents, they were found to be 42.6 and 37.4 mg rutin equivalent/g dry extract in *I. viscosa* L. in summer and autumn harvest respectively; the corresponding amounts for *I. graveolens* L. were 24.53 and 30.42 mg rutin equivalent/g dry extract.

Different studies have reported identifying many phenolic and flavonoid compounds in *I. viscosa* L. Examples of such compounds include: chlorogenic acid, hyperoside, protocathuic acid, apigenin, 7-o-methylaromadendrin, inuviscolide[6,44,45], hispidulin hexoside, patuletin, spinacetin[46] and kaempferol[47]. Gökbulut et al. from Turkey, identified the phenolic contents in methanolic extract of the whole *I. viscosa* L. plant as 176.9 ± 7.8 mg gallic acid equivalent / g extract[48]. In addition, an Algerian study by Amrouche et al. reported that the methanolic extracts of *I. viscosa* L. leaves had high content of polyphenols (106.34 ± 1.49 mg gallic acid equivalent.gdw^{1} and 125.73 µg quercetin equivalent.gdw^{-1})[49]. In a second Algerian study, Mouas et al. reported that the ethanolic extracts of aerial parts of the plant had 138.30 ± 0.00 mg EGA/mg DE and 34.57 ± 0.04 mg QE/mg DE of polyphenols and flavonoids respectively[7]. Another study from Turkey, performed by Bayar and Genc, reported that the total phenolic and flavonoid contents of organic extracts (hexane, ethyl acetate and methanol extracts) of the whole *I. viscosa* L. plant were determined to be in the ranges of 11.38-136.18 mg GAE / g extract and 14.38-28.83 mg QE/g extract[44] respectively.

Large amounts of phenolic compounds and flavonoids (1.63%, calculated as gallic acid, for phenols and 0.52% calculated as quercetin, for flavonoids, equivalents per 100 g of dry mass) were identified in the methanolic extracts of *I. graveolens* L. grown in Iraq[50]. Chlorogenic acid, quinic acid, hyperoside, protocatechuic acid and quercetin were the major phenolic compounds found in the methanolic extracts of *I. graveolens* L. leaves grown in Turkey, with 845 ± 41 µg/kg of gallic acid and 51 ± 3 µg/kg of rutin[51].

This study provides information about the major constituents of essential oils of *I. viscosa* L. and *I. graveolens* L. from Wadi Al-Janayin region in Syria. It confirms that the methanolic extracts of *I. viscosa* L. and *I. graveolens* L. have the largest phenolics and flavonoids contents when compared to the above-mentioned studies. This contrasts with some of the findings reported in the literature regarding both species (for both essential oils and extracts) in other

countries, which may be attributed to the differences in soil contents and the environment.

STATEMENT OF ETHICS

Ethical approval was not required to perform this study as no human participants or experimental animals were involved.

CONFLICT OF INTEREST STATEMENT

All authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION

All authors contributed to data analysis and interpretation as well as revising the article. They also gave final approval of the version to be published and agreed to be accountable for all aspects of the work.

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