



Chemical Composition and Biological Activities of The Essential Oil Of *Pulicaria Crispa* In The Middle East

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Abstract

Pulicaria crispa, family Compositae is an annual herb, locally known as gethghath. The plant was reported to be used in Saudi Arabia on bruises, skin infections and gastrointestinal disturbances due to its essential oil content. It has been used from ancient times in the treatment of sinusitis and respiratory tract infections in the traditional medicine system in southern Iran. Many studies have been conducted on *P. crispa* essential oil in many countries in the middle east; the essential oil of *P. crispa* was found to be characterized by changes in the chemotype due to various factors including for example: the part of the plant used, stage of plant development, genetic factors, the environmental conditions, the harvest period and the nature of the soil. The oil showed antibacterial, sedative, antioxidant activity and cytotoxic activity. Moreover, the oil was reported to possess a good anticancer efficacy against human colorectal adenocarcinoma (Caco-2) and a slightly lower anticancer efficacy against hepatocellular cancer cell line (HepG-2).

Keywords: *Pulicaria crispa*, essential oil, composition, GC-MS, biological activity.

Introduction

Pulicaria crispa (Forsskal) Benth. ex D. Oliver, Family: Asteraceae,⁽¹⁾⁽²⁾. Many synonyms were proposed for *P. crispa*, among them are the followings; as reported by Boulos (1995): Firstly, *Aster crispus* Forssk., in Grant, Trans. Linn. Soc. London 29: 96 (1775). Secondly, *Inula crispa* (Forssk.)Pers., Fl. Aegypt. Arab. 150 (1807). Thirdly, *Francoeuria crispa* (Forssk.) Cass., Dict.Sci. Nat. 34: 44 (1825). Fourthly, *Inula undulata* L., Mant. 115 (1767). Fifthly, *Pulicaria undulata* (L.)C. A. Mey., Verz. Pfl.Casp. Meer. 79 (1831). Sixthly, *Francoeuria undulata* (L.) Lack in Rech. F.,Fl. Iran. 145 :120 (1980). Chaudhary (2000) and Mandaville (1990) cited the name *Pulicaria undulata* as the main name and *Pulicaria crispa* as a synonym, according to the international association for

plant taxonomy (IAPT) nomenclature committee decision, as cited by Mandaville (1990), not to reject using the name *Pulicaria undulata* as the main name⁽³⁾.

The genus *Pulicaria*, belonging to the tribe Inuleae of the Compositae family, consists of about 100 species with a distribution from Europe to North Africa and Asia, particularly around the Mediterranean⁽⁴⁾. Most of these species have been reported as traditional medicines. *Pulicaria arabica* is reported for the treatment of digestive disorders, *P. crispa* is used to treat inflammation and as an insect repellent, *Pulicaria incisa* is used for the treatment of heart disease and as a hypoglycemic agent⁽²⁾. Due to this traditional medicinal uses, various *Pulicaria* species have been investigated both phytochemically and biologically.

Phytochemical studies on *Pulicaria* species have yielded some flavonoids and terpenoids, including sesquiterpenoids and diterpenoids (4).

Pulicaria crisa - locally known as gethath- is an annual herb or sometimes a perennial shrub; ascending and often with hemispherical appearance, 12-75 cm high, intricately and densely branched from the base with stems closely white-wooly tomentose. Leaves are sessile, somewhat amplexicaul, narrowly linear, acute to obtuse, undulate to rarely toothed, wooly tomentose on both sides or glabrescent, 0.5 – 3 × 0.15 – 0.3 cm and much smaller on the upper stems. Heads are solitary-terminal, hemispherical, heterogamous, radiate, and golden-yellow to orange, 0.5-1 cm across. Bracts are many-seriate, imbricate, linear-lanceolate, acuminate, and glabrous to ciliate. Ray florets; in a single marginal row, small, as long as disc florets. Achenes are 0.5-0.9 mm, brown and glabrous. Pappus contains 7-10 bristles; it is 2-4 mm long, fused at the base (5, 6).



Figure 1: *Pulicaria crisa* at the site of collection in the Egyptian Western desert.

Pulicaria crisa is distributed in Saudi Arabia, Kuwait, Iran, Iraq, Egypt, Afghanistan, Pakistan, India and parts of north and west tropical Africa (7). *Pulicaria crisa* is a medicinal plant used by people of southern Egypt and Saudi Arabia to treat inflammation and as an insect repellent and is also used as an herbal tea (8). It was

reported to be used in Saudi Arabia on bruises⁽⁹⁾, skin infections and gastrointestinal disturbances⁽¹⁰⁾. Due to the strong smell of the essential oil in the aerial parts of this medicinal plant, it has been used from ancient times in the treatment of sinusitis and respiratory tract infections in the traditional medicine system in southern Iran (11).

Phytochemical studies of this herb have identified it to be a rich source of sesquiterpene lactones of the guaianolide, eudesmanolide and xanthanolide classes as well as kaurane diterpenes (8). Sesquiterpene lactones were isolated from *P. crisa* in Egypt in addition to xantholides pulicariolide (12). *P. crisa* in Saudi Arabia revealed the presence of B-sitosterol, B-amyrin and a neutral triterpene, choline (13). In Qatar *P. crisa* contains triterpene, sesquiterpene lactones, tannins and alkaloids (14). Isolation of a new compound from sesquiterpene lactones known as 1 β ,4 β -dihydroxy-5 α (H)-guaia-10 (14),11(13)-dien 8 α ,12-olide was possible from *P. crisa* in Algeria (15).

Many studies have been conducted on *Pulicaria crisa* essential oil in many countries in the middle east; the essential oil of *Pulicaria crisa* is characterized by changes in the chemotype due to various factors including for example: the part of the plant used, stage of plant development (16), genetic factors (17), the environmental conditions, the harvest period and the nature of the soil (18).

Chemical composition of the essential oil of *Pulicaria crisa* from different geographical regions:

1- Yemen

Ali, N., et al. (2012) (19) had studied the essential oil of *Pulicaria undulate* collected in the early morning from Zingibar Outskirt, Abyan province, Yemen, in April, 2010.

Dried leaves from *P. undulata* were hydro-distilled for three hours in a Clevenger type apparatus; the obtained oil was subsequently dried over anhydrous Na₂SO₄ and kept at 4°C until analysis.

Analysis of the essential oil of *Pulicaria undulata* using Gas chromatographic – mass spectral analysis revealed that the essential oil is characterized by high content of carvotanacetone. The composition of the essential oil of *Pulicaria undulata* is summarized in (Table 1).

2- Iran

Nematollahi, F., et al. (2006) ⁽²⁰⁾ had studied the essential oil of *Pulicaria undulata* collected in August from Darabad, province of Tehran, Iran. The plant material had been subjected to hydrodistillation for three hours and yielded 0.32 % (V/W) of the essential oil.

The GC/MS analysis of the oil showed the presence of twenty-eight components representing 91.6% of the total composition of the essential oil of *Pulicaria undulata*. The oil consists of about 88.5% monoterpenes, including α -pinene (45.7%), 1,8-cineole (27.1%), and about 3.1% sesquiterpenes, with aromadendrene (0.5%) and γ -cadinene (0.5%) as the main components. The results of the GC/MS analysis performed by Nematollahi, F., et al. (2006) are summarized in (Table 2).

Ravandeh, M., et al. (2011) ⁽²¹⁾ had studied the essential oil of *Pulicaria undulata* collected in November from Saravan area of Sistan and Baluchestan province in Iran. The finely dried powdered aerial parts (40 gm) were subjected for two hours to hydrodistillation using a Clevenger-type apparatus. The yield of volatile oil of *Pulicaria undulata* obtained by hydrodistillation of the finely powdered aerial parts was 0.5% (V/W). The oil was light yellow and with a perfumery odor.

The GC/MS analysis of the oil revealed the presence of monoterpenes (14.51%), oxygenated monoterpenes (54.41%), sesquiterpenes (29.20%), oxygenated sesquiterpenes (1.14%) and (1.41%) as other compounds. The main monoterpene component was 4-terpineole (20.12%), α -terpinene (4.02%), γ -terpinene (7.0%), cis-sabinene hydrate (8.29%), linalool (5.60%). The main sesquiterpene component was junipene (8.66%) and 1S-Cis-calamenene (13.37%). The high content of oxygenated compounds might explain the characteristic and fragrant odor of the oil. The percentage composition of the essential oil of *Pulicaria undulata* cultivated in Iran and analyzed by Ravandeh M., et al. are expressed in (Table 3).

Javadinamin, A., et al. (2014) ⁽²²⁾ had studied the essential oil of *Francoeuria undulata* collected in November 2012 from Pardi Mountain, Bastak County, West of Hormozgan Province, Iran: (27° 11' 53" N 54° 22' 7" E, 1200 m). Freshly collected aerial parts (100 g) were immediately hydrodistilled in a Clevenger-type apparatus for three hours. The hydrodistillation of aerial parts of *F.undulata* gave yellow oil with pleasant odor and yield of 0.7 % (v/w). The collected oil was dried with anhydrous Na₂SO₄, measured, and transferred to glass flasks and kept at a temperature of -18°C until analysis.

From the GC/MS data, forty two components were identified in this oil which presented about 96.9 % of the total composition of the oil. The compounds identified through GC/MS are presented in (Table 4). The major constituents of the oil were α -bisabolol (17.5 %), chrysanthenone (12.5 %), 1,8-cineol (10.7 %), trans-thujone (9.7 %) and linalool (6.6 %). The essential oil of *F. undulata* comprised four hydrocarbons (3.7 %), one phenylpropanoid (0.5 %), nineteen monoterpenoids (58.6 %)

seventeen sesquiterpenoids (33.5 %) and one diterpenoid (0.6 %).

3- Algeria

Boumaraf, M., et al. (2016) ⁽²³⁾ studied the essential oil of *pulicaria undulata* where the aerial parts were collected in April from the area of Djanet (Tassili) wilaya of Illizi in Algerian Sahara. The aerial parts (200g) were subjected to steam distillation in a Kaiser Lang apparatus for three hours. The yield of the oil was calculated in relation to the dry weight of the plant. The steam distillation of the aerial parts of *P. undulata* yielded 1.2 % (W/W) of yellowish oil with a perfumery odor.

Thirty-one constituents representing about 68.4% of the total essential oil of the plant were identified and illustrated in (Table 5), among which 74.3% of oxygenated compounds. The major constituents were carvotanacetone (14.8%) followed by δ -cadinene (8.2%), α -cadinol (4.7%), thujanol (4.7%), epi- α -cadinene (3.4%), carvacrol (3.14%) and 14-hydroxy α -murolene (3.1%).

4- Sudan

EL-Kamali, H.H., et al. (2009) ⁽²⁴⁾ had studied the essential oil of *pulicaria undulata* where The aerial parts were collected in November, from EL-Fiteehab region, University City Campus of Omdurman Islamic University in Omdurman , South Sudan. The finely dried powdered aerial parts (385 grams) were subjected to steam distillation using steam distillation apparatus. The obtained oil (2ml) was collected. The yield of volatile oil of *Pulicaria undulata* obtained by steam distillation of the finely powdered aerial parts was 2.5 %. The oil was light yellow and with a perfumery odor.

The GC/MS chromatogram of the oil - presented in (Table 6) - revealed the presence of 70.97 % monoterpenes and 10.0 % sesquiterpenes. The major components of the essential oil were carvotanacetone (55.87

%), beta-linalool (4.55 %), thymol (3.01 %), beta-caryophyllene (2.99 %), ethyl cinnamate (2.78 %), isobornyl formate (2.7 %) and cyclododecyl-1-ethanon (2.0 %).

5- Egypt

Ross, S.A., et al. (1997) ⁽²⁵⁾ had studied the essential oil of *Francoeuria crispa* collected in January from the South-Eastern coast of Egypt. The plant material had been steam distilled for 3 hours to yield 0.8% W/V of the volatile oil.

Analysis of *F. crispa* oil showed the presence of 26 components, 16 of which were identified. The GC/MS data of the major component is (Abundance: 93.0%, R_t: 20.15 minutes) and the compound was not identified through the GC/MS library used by Ross, S. A. et al. (2007). The major compound was isolated from the oil through TLC and identified via 1-D and 2-D NMR to be S-Carvotanacetone.

The GC/MS data for the essential oil of *F. crispa* provided by Ross, S. A. et al. is illustrated in (Table 7).

Dekinash M.F., et al. (2017) ⁽²⁶⁾ studied the essential oil of *Pulicaria crispa* cultivated from the western Egyptian desert, El Menoufia governorate, El Sadat city. The exact location of collection is 30.38182°N, 30.51159°E. The aerial parts were air-dried in shade at room temperature for 7 days, and then pulverized to fine pieces.

The air-dried finely ground aerial parts of *P. crispa* (200 gm.) were subjected to hydro-distillation using a Clevenger type distillation apparatus for three hours to yield 1.2 ml of sweet odor, greenish yellow color and lighter than water essential oil. The essential oil yield from *P. crispa* collected from the western Egyptian desert is 0.6 % (V/W) based on the dry weight of the plant.

The GC/MS analysis of the essential oil of *P. crispa* exhibited eight major components illustrated in (Table 8).

Gas chromatogram of the essential oil of *Pulicaria crispa* showing eight peaks

representing the major components of the essential oil is presented as **Figure 2**.

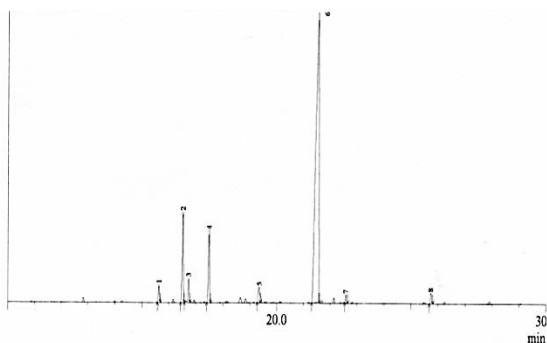


Figure 2: Gas chromatogram of the essential oil of *Pulicaria crispera*.

All the identified compounds in the oil were oxygenated monoterpenes; the major compound is carvotanacetone representing 81.99%. This result is in a good agreement with previous studies on essential oil of *P. crispera* collected from the Middle East region (11, 19, 23-25) except those from Iran. The essential oils of *P. undulata* cultivated in Iran did not contain carvotanacetone at all (20-22). The oil also contained chrysanthenone (6.87%), β -linalool (5.34%), 4-oxatricyclo (4.3.1.1(3,8))undecan-5-one (2.06%), isothujole (1.6%), α -methyl- α -(4-methyl-3-pentenyl)oxiranemethanol (1.1%), 2-(1-methyl-2-oxopropyl)cyclohexanone (0.69%) and cis-jasmone (0.35%). The study by Ross et al. (1997) on *Francoeuria crispera* from Egypt showed some qualitative and quantitative differences in the oil composition compared to what obtained in our study. This was attributed to the difference in geographical origin of the plant which was collected from Elba Mountain in the Southeastern Egyptian coast in case of Ross et al. study and from the western Egyptian desert in the recent study (26).

Carvotanacetone is also accumulated as major component in other *Pulicaria* species such as *P. inuloides* and *P. jaubertii* from Yemen (47.3 and 63.9% respectively),

P. mauritanica from Morocco (87.3%) and *P. jaubertii* from Saudi Arabia (98.6%) (23).

In-vitro biological activities of *Pulicaria crispera* essential oil:

An aromatic tea of *Pulicaria undulata* is used in the central Sahara to treat chills, diabetes, cardiac disorders, skin diseases, and abscesses, and in Egypt to treat inflammation, as an insect repellent, and an herbal tea. *Pulicaria undulata* oil has shown antibacterial, sedative and insecticidal activities (27).

1- Biological activities of the essential oil of *Pulicaria undulata* from Yemen:

The biological activities of the essential oil obtained from the leaves of *Pulicaria undulata* collected from Yemen was studied by Ali N. A., et al. (2012) (19).

The antimicrobial activity of the essential oil was evaluated against six microorganisms *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *Bacillus subtilis*, and *Candida albicans*, using disc diffusion and broth microdilution methods.

The oil exhibited antibacterial activity against *Staphylococcus aureus* with minimum bactericidal concentration (MBC) of 3.12 μ l/ml for *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus*. The MBC against *Candida albicans* and *Bacillus subtilis* was 6.25 μ L/mL, whereas MBC against *Escherichia coli* was 12.5 μ l/ml. The diameters of the zones of inhibition, minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the essential oil for the microorganisms tested are shown in (Table 9).

Staphylococcus aureus was the most sensitive microorganism tested, with the strongest inhibition zone (32 mm), and *Pseudomonas aeruginosa* was the most

resistant strain. The lowest MBC was 3.12 $\mu\text{L}/\text{mL}$ for *S. aureus* and MRSA ⁽¹⁹⁾.

The essential oil of *P. undulata* from Yemen was screened for antifungal activity against *Cladosporium cucumerinum* using a bioautographic technique. At a concentration of 400 $\mu\text{g}/\text{mL}$, marked antifungal activity was observed, with inhibition zones of 18 mm ⁽¹⁹⁾.

The inhibitory effect of the essential oil of *P. undulata* from Yemen on acetylcholine esterase (AChE) was detected by TLC assay. The active band was isolated and characterized by ESIMS, LC-MS, ¹H NMR, NMR and HMBC as carvotanacetone ⁽¹⁹⁾.

The anticancer activity of the essential oil of *P. undulata* from Yemen was assessed against MCF-7 breast tumor cells. The essential oil showed moderate cytotoxic activity against MCF-7 breast tumor cells with an IC_{50} of $64.6 \pm 13.7 \text{ Mcg}/\text{ml}$ ⁽¹⁹⁾.

2- Biological activities of the essential oil of *Pulicaria crispa* from Egypt:

Ross, S.A., et al. (1997) ⁽²⁵⁾ had studied the essential oil of *Francoeuria crispa* collected in January from the South-Eastern coast of Egypt. The main compound was not identified through the GC/MS library in the study by Ross, S. A. et al. (2007). The major compound was isolated from the oil through TLC and identified via 1-D and 2-D NMR to be S-Carvotanacetone. S-Carvotanacetone did not show antimicrobial activity against *Candida albicans* B31 1 and *Cryptococcus neoformans* (50 μl , 1mg/ml), as compared to amphotericin B. No cytotoxicity against K562 cells, human chronic myelogenous leukemia and KB cells, human oral epidermoid carcinoma or antiviral activity (Parainfluenza virus in Hep 2 cell host) was found ⁽²⁵⁾.

Dekinash M., et al. (2017) had studied the antioxidant activity and the in-vitro cytotoxic activity of the essential oil of

Pulicaria crispa cultivated from the western Egyptian desert, El Menoufia governorate, El Sadat city ⁽²⁶⁾.

The results of in-vitro cytotoxic activity of *P. crispa* oil on human peripheral blood mononuclear cells were expressed in terms of maximum safe concentration that keeps 100% cell viability (EC_{100}), which was found to be 12.8 $\mu\text{l}/\text{ml}$. However, the effective concentration that kills 50 % of the cells (EC_{50}) was 38.61 $\mu\text{l}/\text{ml}$. The essential oils of *P. crispa* showed stimulation indices lower than 1.5 for all the tested concentrations. Accordingly, the essential oil of *P. crispa* cultivated in Egypt proved to exhibit no immune-stimulant activity ⁽²⁶⁾.

The percent reduction in the absorbance of the DPPH radical based on the highest safe dose (EC_{100}) is computed to be 66.193% which indicates a powerful antioxidant capacity. The percent scavenging of DPPH radical is then expressed as "trolox equivalent antioxidant capacity" (TEAC) and found to be about 9.220086546 $\mu\text{g}/\text{ml}$. This means that the highest percentage scavenging activity obtained by the oil was found to be equivalent to the antioxidant potential of 9.22008654 $\mu\text{g}/\text{ml}$ of trolox. This finding indicates that the essential oil of *P. crispa* can be regarded as a powerful antioxidant agent ⁽²⁶⁾.

The essential oil of *P. crispa* showed high anticancer efficacy against human colorectal adenocarcinoma (Caco-2) with high IC_{50} value of 4.73 $\mu\text{l}/\text{ml}$; whereas a slightly lower anticancer efficacy against hepatocellular cancer cell line (HepG-2) was observed with IC_{50} values of 20.11 $\mu\text{l}/\text{ml}$. Moreover, the percentage inhibition of the maximum safe concentration ($\text{EC}_{100}=12.8 \mu\text{l}/\text{ml}$) of *P. crispa* essential oil on Caco-2 and HepG-2 cell lines were computed to be 97.224 and 33.3022 %, respectively ⁽²⁶⁾.

P. crispa oil was found to have safety index (SI) of 8.163 against colorectal adenocarcinoma, which is higher than that against hepatocellular carcinoma (SI=1.92). Accordingly, this oil may provide a more sufficient therapeutic activity with a comfortable safety margin in the treatment of colorectal cancer than hepatocellular carcinoma. Accordingly, the P. crispa oil can be considered to be a potential anticancer candidate against colorectal cancer. Future investigation on the use of the oil as an enema for the treatment of colorectal cancer in rats is worth experimentation ⁽²⁶⁾

Due to the variation in chemotype of the essential crispa from different regions, we suggest that each chemotype would have its own characteristic biological activities.

Table (1): Chemical composition of *Pulicaria undulata* essential oil from Yemen ⁽¹⁹⁾.

Compound	RI	%
α -Pinene	939	0.1
1,8-Cineole	1030	0.1
Linalool	1101	0.1
Camphor	1144	0.3
Borneol	1165	0.7
Dill ether	1185	0.2
cis-p-Menthan-2-one	1196	0.2
trans-p-Menthan-2-one	1200	0.3
cis-Carvotanacetol	1214	0.2
Carvotanacetone	1247	91.4
Carvenone	1261	0.3
Thymol	1289	0.2
Carvacrol	1299	0.6
Methyl eugenol	1405	0.3
(E)-Caryophyllene	1418	0.1
2,5-Dimethoxy-p-cymene	1425	2.6
Unidentified aromatic	1486	0.7
Neryl isobutanoate	1493	0.4
4-Isopropylveratrole	1495	0.2
Neryl isovalerate	1579	0.2
Caryophyllene oxide	1584	0.4
τ -Cadinol	1641	0.1

Table (2): Percentage composition of the oil of *Pulicaria undulata* (20).

Compound	Retention indices	Percentage
α -thujene	931	0.6
α -pinene	940	45.7
camphene	953	0.2
verbenene	967	0.1
sabinene	976	0.2
β -pinene	980	1.2
α -phellandrene	1005	0.2
α -terpinene	1018	1.0
p-cymene	1026	0.9
1,8-cineole	1033	27.1
γ -terpinene	1062	1.6
cis-sabinene hydrate	1068	0.2
terpinolene	1088	0.2
trans-sabinene hydrate	1097	0.6
α -campholenal	1125	0.6
terpinen-4-ol	1177	4.4
α -terpineol	1189	2.0
Myrtenol	1194	0.4
trans-carveol	1217	0.2
thymol	1290	0.5
α -copaene	1376	0.2
α -gurjunene	1409	0.3
aromadendrene	1461	0.5
β -selinene	1485	0.4
α -muurolene	1499	0.2
γ -cadinene	1513	0.5
δ -cadinene	1524	1.0

Table (3): Chemical composition of the essential oil of the aerial parts of *P. undulata* (21).

NO	Components	RT(Min)	(%)
1	□-thujene	10.37	0.14
2	□-pinene	10.68	0.52
3	Sabinene	12.61	0.21
4	β-myrcene	13.56	0.07
5	1-phellandrene	14.16	0.08
6	□-terpinene	14.96	4.02
7	1,8-Cineole	15.55	1.00
8	γ-terpinene	17.25	7.00
9	Trans-sabinene hydrate	17.65	2.71
10	□-terpinolene	18.58	2.14
11	Cis-sabinene hydrate	19.41	8.29
12	1,3,8-para-menthatriene	19.68	0.22
13	Isolimonenol	21.34	2.37
14	4-terpineole	23.71	20.12
15	□-terpineol	24.08	2.45
16	Myrtenol	24.50	5.77
17	z -citral	26.19	1.00
18	Linalool	27.25	5.60
19	E-citral	27.59	1.32
20	Thymol	28.70	0.48
21	□-fenchene	28.95	0.11
22	Myrtenyl acetate	29.90	0.94
23	Copaene	32.04	0.09
24	Geranyl acetate	32.35	0.15
25	Trans-caryophyllene	33.88	0.13
26	□-amorphene	36.26	0.13
27	□-gurjunene	36.82	0.05
28	□-muurolene	37.24	0.26
29	γ -cadinene	37.80	0.29
30	□-cadinene	38.21	1.03
31	Trans-gamma-bisabolene	38.48	0.61
32	Citronellyl valerate	40.22	0.29
33	Caryophyllene oxide	40.55	0.62

34	Fenenol	42.37	0.30
35	Junipene	43.01	8.66
36	Trans-calamenene	43.55	2.66
37	Valerenol	43.91	0.23
38	Cadalene	44.05	1.92
39	1S-cis-calamenene	45.12	13.37
40	Neryl acetate	59.09	0.32

Table (4): GC/MS analysis of the essential oil of *F. undulata* aerial parts ⁽²²⁾.

No	Compound ^a	KI ^b	KI ^c	Percentage
1	Cyclopentanol	792	790	0.4
2	Hexanol	870	871	1.3
3	α -Pinene	937	939	1.5
4	α -Terpinene	1016	1017	0.4
5	1,8-Cineol	1030	1031	10.7
6	m-Cymene	1082	1085	0.5
7	γ -Terpinene	1061	1060	0.8
8	<i>cis</i> -Sabinene hydrate	1072	1070	0.6
9	m-Cymenene	1082	1085	0.6
10	Linalool	1099	1097	6.6
11	<i>trans</i> -Thujone	1116	1114	9.7
12	Chrysanthenone	1130	1128	12.5
13	<i>cis</i> -Verbenol	1140	1141	0.9
14	4-Terpineol	1180	1177	1.9
15	ρ -Cymene-8-ol	1183	1183	0.5
16	α -Terpineol	1190	1189	2.2
17	Nerol	1228	1230	1.7
18	Thymol methyl ether	1232	1235	0.6
19	Neral	1235	1238	1.2

20	Geraniol	1254	1253	4.6
21	Geranial	1269	1267	1.1
22	Thymol	1292	1290	0.5
23	β -Longipinene	1400	1401	1.1
24	α -Gurjunene	1409	1410	0.5
25	Aromadendrene	1439	1441	0.6
26	Dehydro Aromadendrene	1465	1463	0.7
27	α -Amorphene	1488	1485	0.9
28	δ -Cadinene	1520	1523	1.8
29	Longipinanol	1571	1569	0.6
30	Germecrene D-4-ol	1576	1576	0.9
31	<i>epi</i> - α -Cadinol	1643	1640	1.7
32	α -Cadinol	1653	1654	2.4
33	Bisabolone oxide	1683	1685	1.7
34	α -Bisabolol	1685	1686	17.5
35	Germacrone	1692	1694	1.2
36	Z-E, Farnesol	1721	1718	0.8
37	Santalol	1738	1740	0.3
38	E-Z, Farnesol	1744	1746	0.4
39	E- β -Santalol acetate	1871	1869	0.4
40	Cembrene A	1966	1967	0.6
41	Eicosene	1986	1988	0.5
42	n-Eicosane	2000	2000	1.5
Total				96.9

^a Compounds listed in order of elution

^b KI (Kovats index) measured relative to n-alkanes (C9-C28) on the non-polar DB-5 column under condition listed in the Materials and Methods section

^c KI, (Kovats index) from literature

Table (5): Retention times, Retention indices and percentage composition of the essential oil of *Pulicaria undulata* (23).

Peak N°	RT	^b RI	^a Compound	%
1.	8.753	1099	Linalol	2.4
2.	8.839	1103	Thujol	0.9
3.	9.556	1142	camphor	0.2
4.	10.016	1166	borneol	0.8
5.	10.214	1177	terpinen-4-ol	2.8
6.	10.379	1186	thujanol	4.7
7.	10.491	1192	cis mentan-2-one	0.7
8.	10.626	1199	trans menthan-2-one	0.6
9.	10.805	1209	transpiperitol	1.5
10.	11.546	1252	carvotanacetone	14.8
11.	12.438	1303	carvacrol	3.1
12.	14.082	1404	2,5-dimethoxy-p-cymene	0.8
13.	14.238	1414	β-caryophyllene	0.8
14.	15.111	1472	γ-muurolene	0.9
15.	15.266	1482	amorpha-4,7(11)-diene	1.0
16.	15.345	1487	epi-cubebol	1.1
17.	15.469	1495	α-muurolene	2.1
18.	15.676	1509	γ-cadinene	1.9
19.	15.811	1518	δ-cadinene	8.2
20.	16.103	1539	α-cadinene	0.4
21.	16.204	1546	α-calacorene	0.2
22.	16.561	1570	palustrol	0.3
23.	16.628	1575	epi-globulol	0.7
24.	16.783	1586	caryophyllene oxyde	1.2
25.	17.051	1605	oplopenone	0.8
26.	17.127	1610	Humuleneepoxide II	1.3
27.	17.381	1630	cadina-4,1(10)-diene-7--ol	0.8
28.	17.548	1642	epi-α-cadinol	3.4
29.	17.571	1644	epi-α-muurolol	2.2
30.	17.753	1658	α-cadinol	4.7
31.	19.707	1804	14-hydroxy-α-muurolene	3.1

^aCompounds listed in order of their RI

^bRI (retention index) measured relative to n-alkanes (C8-C20) using HP-5 ms

Table (6): Composition of the essential oil of *Pulicaria undulata* in Sudan ⁽²⁴⁾

No.	Compound	%
1	2-Butanol	0.05
2	alpha-pinene	0.42
3	alpha-phellandrene	0.32
4	cymene	1.20
5	(+)-limonene	0.21
6	Eucalyptol	0.14
7	unidentified	0.08
8	Beta-linalool	4.55
9	Nonanal	0.10
10	1-methyl-4-(1-methylethyl)- 2-cyclohexen-1-ol	0.09
11	(+)-camphor	0.20
12	1,3-dimethyl cyclohexene	0.06
13	Borneol	0.56
14	Isobornyl formate	2.70
15	alpha-terpineol	0.38
16	unidentified	0.10
17	1-Decanal	0.06
18	unidentified	0.27
19	Thymol methyl ether	0.26
20	Sabiny acetate	0.44
21	(+)-carvotanacetone	55.87
22	unidentified	1.40
23	Bornyl acetate	0.08
24	Cyclododecyl-1-ethanone	2.00
25	1-methyl carvacrol	0.76
26	3-methyl-4-iso-propyl	0.65
27	4-carene	0.34

28	unidentified	0.7
29	1,4-Ethanonaphthalene	0.25
30	unidentified	1.60
31	2-tert-butyl-1,4-dimethoxy-benzene	2.20
32	Beta-caryophyllene	2.99
33	Ethyl cinnamate	2.78
34	Thymol	3.01
35	alpha-curcumene	0.13
36	2,6-octadien-1-ol	0.17
37	alpha-Guaiene	0.40
38	Gamma-cadinene	0.38
39	Spathulenol	0.84
40	unidentified	1.62
41	unidentified	1.66
42	unidentified	3.60
43	unidentified	1.76

Table (7): Composition of *Francoeuria crispa* essential oil from Egypt (25).

t _R	RI	Component	%
4.85	842	Hexenal<2->	t
6.72	919	Tricyclene	t
7.17	935	a-Pinene	0.1
8.96	987	Unknown(M 138,109,93,81*, 69, 53)	t
9.15	992	Carene<2->	t
10.22	1022	ortho-Cymene	0.3
10.43	1028	Limonene	0.1
10.50	1030	1,8-Cineole	0.1

12.13	1071	Linaloloxide<cis->	0.2
13.00	1090	Linaloloxide<trans>	t
13.25	1096	Linalool	3.5
17.01	1182	Isomenthol	0.2
17.35	1188	α -Terpineol	0.2
17.65	1194	1,4-Cineole	t
17.80	1197	Menthol<L->	t
18.13	1204	Unknown (M*, 154, 136, 121 111,93, 83, 77, 69, 55, 51,43*)	0.2
20.15	1251	S-Carvotanacetone	93.0
21.12	1273	Unknown(M 168,153,135,125,111,97, 83,69,55 43*)	0.2
21.81	1287	Unknown (M 179, 151, 135, 125, 111,97, 83,69,5543*)	0.4
22.00	1290	Unknown(M164,149,135,122,110,91, 79, 65, 5544*)	t
26.90	1400	cis-Jasmone	0.3
27.81	1422	Unknown(M 194,179*, 164,149,136,117, 105, 91,77, 65, 51)	0.6
27.89	1423	β -Caryophyllene	0.1
28.90	1448	Unknown (M 218, 203,189,175,162,148, 133,115,105,91,77,51,43*)	t
30.44	1483	Unknown (M* 220, 165, 150, 135, 105,91, 77, 51,43*)	0.3
30.60	1488	Unknown (M' 165, 150, 136, 121, 105.93*, 79, 67, 53)	t

t_R = Retention time in minutes (GC/FID).

RI = Retention index.

trace = (< 0.001 %).

* Base peak.

Table (8): Chemical composition of *P. crisper* essential oil using GC/MS⁽²⁶⁾.

No.	Compounds ^a	R _t (min.) [*]	Peak area	Relative area percentage (%)	RI ^{**}
1	α -Methyl- α -(4-methyl-3-pentenyl) oxiranemethanol	15.636	721618	1.10%	1182
2	β -linalool	16.518	3496847	5.34%	1082
3	4-Oxatricyclo (4.3.1.1(3,8))undecan-5-one	16.733	1346750	2.06%	1349
4	Chrysanthenone	17.485	4497837	6.87%	1119
5	Isothujol	19.348	1050913	1.60%	1079
6	Carvotanacetone	21.563	5369117	81.99%	1158
7	2-(1-methyl-2-oxopropyl) cyclohexanone	22.595	450603	0.69%	1322
8	cis-Jasmone	25.744	229037	0.35%	1338

Notes:

Compounds ^a: Compounds listed in order of elution

R_t(min.)^{*}: retention time in minutes.

RI^{**}: Kovats retention indices calculated relative to homologous series of n-alkanes determined by GC-MS QP2010 on a TR5- CPSIL- 5CB column.

Table (9): Antimicrobial activity of *P. undulata* oil ⁽¹⁹⁾.

Test microorganisms	Essential oil		Antibiotics Inhibition zone (mm)		
	Inhibition zone (mm) 10 μ L/disc	MIC (MBC) (μ L/ml)	Ampicillin (10 μ g/disc)	Gentamycin (10 μ g/disc)	Nystatin (100 units/disc)
<i>E. coli</i>	16 (\pm 1.2)	6.25 (12.5)	17 (\pm 1.2)	25 (\pm 0.9)	nt
<i>Ps. Aeruginosa</i>	not active	not active	not active	26 (\pm 1.1)	nt
<i>S. aureus</i>	32 (\pm 1.5)	3.12 (3.12)	28 (\pm 1.4)	23 (\pm 1.3)	nt
MRSA	28 (\pm 1.7)	3.12 (3.12)	22 (\pm 1.6)		
<i>B. subtilis</i>	16 (\pm 1.4)	6.25 (6.25)	29 (\pm 1.1)	28 (\pm 1.8)	nt
<i>C. albicans</i>	26 (\pm 2.5)	3.12 (6.25)	nt	nt	23(\pm 1.4)

^a Inhibition zone diameter (mm), including 6 mm diameter of sterile disk; values are given as mean + SD.

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