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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL OF *ORIGANUM VULGARE* L.

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ABSTRACT

The essential oil of the dried flowering aerial parts of *Origanum vulgare* L (Labiatae) collected from the suburb of Nour, Mazandaran Province (North of Iran) was isolated by hydrodistillation and analyzed by means of GC and GC/MS. The major components of *O. vulgare* oil were elemol (9.3%), sabinene (8.7%), β-eudesmol (7.7%), terpinen-4-ol (7.6%), and β-caryophyllene (6.7%). The *O. vulgare* oil exhibited a pronounced antibacterial effect on some bacteria tested. The essential oil did not show antifungal activity.

Keywords: *Origanum vulgare*, Labiatae, Essential oil composition, Elemol, Sabinene, β-Eudesmol, Terpinen-4-ol, β-Caryophyllene, Antimicrobial activity.

INTRODUCTION

The genus *Origanum* is reported by one species in Iran (Mozaffarian, 1996). *Origanum vulgare* grows wild in Azerbaijan and Mazandaran Provinces of Iran (Rechinger, 1982). *O. vulgare* (oregano, origanum or wild marjoram) is a strong aromatic herb used in Mediterranean food (Prieto *et al*., 2007). In Iranian traditional medicine, this plant has been used as tonic, diuretic, laxative, analgesic and emmenagogue (Zargari, 1993). A literature survey has shown some reports on the analysis and antimicrobial activity of the oil of *O. vulgare* (Afsharypour *et al*., 1997; Ivask *et al*., 2005; Bozin *et al*., 2006; D'Antuono *et al*., 2000; Mockute *et al*., 2001; Mockute *et al*., 2004; Radusiene *et al*., 2005; Pedro *et al*., 2005; Rodrigues *et al*., 2004; Romero *et al*., 2005; Santoyo *et al*., 2006; Sartoratto *et al*., 2004; Tian and Lai, 2006, Veres *et al*., 2003).

O. vulgare is characterized by a wide range of volatile compounds. Since the climate of Nour in Mazandaran Province is very wet and different from mentioned areas in previous researches; for comparison purposes, we decided to investigate the chemical constituents of the oil of the dried flowering aerial parts of *O. vulgare* growing in the suburb of Nour, Mazandaran Province of Iran and its antimicrobial activity.

MATERIALS AND METHODS

Plant material

The aerial parts of *O. vulgare* were collected in May 2006 from the suburb of Nour, Mazandaran Province, North of Iran and identified by Mohammad Akbarzadeh (Department of Botany, Research Center of Natural Resources of Mazandaran). Voucher specimen (herbarium No. 124) was deposited in the Herbarium of the Department of Botany, Research Center of Natural Resources of Mazandaran.

Essential oil extraction and analysis

The air-dried flowering aerial parts of *O. vulgare* were subjected to hydrodistillation using a Clevenger-type apparatus for 3 h to yield 0.9% (v/w) of oil. The oil was analyzed by GC and GC/MS. Gas chromatographic analysis was carried out on a Perkin-Elmer 8500 gas chromatograph with FID detector and a DB-5 capillary column (30 m × 0.25 mm; film thickness 0.25 μm). The operating conditions were as follows: carrier gas helium with a flow rate of 2 ml/min, split ratio was 1:30, the oven temperature was programmed 4 min. Isothermal at 60 °C and then 60°-220 °C at 4 °C/min., injector and detector temperatures were set at 240 °C. Gas Chromatography-Mass Spectrometry (GC/MS) was carried out on Hewlett Packard 6890 series, using a DB-5 capillary column (30 m × 0.25 mm, film thickness 0.25 μm) which was programmed as follows: 60 °C for 5min and then up to 220 °C at 4 °C/min. The carrier gas was helium at a flow rate of 2 ml/min; split ratio, 1: 40; ionization energy, 70 eV; scan time, 1 s; acquisition mass range, *m/z* 40-400. The components of the oil were identified by their retention time, retention indices relative to C_9-C_{28} n-alkanes, computer matching with the WILEY275.L library and as well as by comparison of their mass spectra with those of authentic samples or with data already available in the literature (Adams, 2001; Davies, 1990).

Antimicrobial assay

Bacillus subtilis PTCC 1023, *Staphylococcus aureus* PTCC 1112, *Escherichia coli* PTCC 1330, *Salmonella typhi* PTCC 1639, *Aspergilus niger* PTCC 5011 and *Candida albicans* PTCC 5027 were used for testing the antimicrobial activity. Diffusion method using filter paper disk (6 mm) was used for the screening of oil antibacterial and antifungal activities (Dehghan *et al*., 2007). Bacterial and fungal strains were tested on Muller-Hinton agar and Sabouraud dextrose agar, respectively. Sterilized paper disks were loaded with different amounts of *O. vulgare* oil (250, 500, 1000, 2000, 4000, 8000 and 16000 μg /disk) and applied on the surface of agar plates. All plates were incubated at 37°C for 24 h for bacteria; at 25°C for 24 h for *C. albicans*; and at 25°C for 3 days for *A. niger*. Inhibition zone diameters were measured after conventional incubation period. Gentamycin (50 μg/disk), Amikacin (3 μg/disk) and Amphotericin B (100 μg/disk) (obtained from Sigma) were used as positive reference standards.

The estimation of the minimal inhibitory concentration (MIC) was carried out by the broth dilution method. Dilutions of essential oil from 16 to 0.25 mg/ml were used. MIC values were taken as the lowest essential oil concentration that prevents visible microbe growth after the incubation period (described above) (Hernandez *et al*., 2007). Gentamycin, Amikacin and Amphotericin B (128, 64, 32, 16, 8, 4 and 2 μg/mL) were used as positive reference standards and control with no essential oil was used. Each experiment was made three times.

RESULTS AND DISCUSSION

Chemical analyses of essential oil

As shown in Table I, 137 compounds were identified in the oil of *O. vulgare*, which presented about 99.6% of the total composition of the oil; the major components of this oil were elemol (9.3%), sabinene (8.7%), *β*-eudesmol (7.7%), terpinen-4-ol (7.6%), and *β*-caryophyllene (6.7%). The oil of *O. vulgare* comprised 57 monoterpenoids (39.7%), 48 sesquiterpenoids (54.5%), 2 diterpenoids (0.4%) and 30 non-terpenoids (5.0%). The essential oil of the flowering aerial parts of *O. vulgare* is rich in sesquiterpenoids.

In 1997, linalyl acetate, sabinene, *γ*-terpinene, *trans*-ocimene and *cis*-ocimene were reported as the major compounds of *O. vulgare* ssp. *viride* collected from Kodjour in Iran (Afsharypour *et al*., 1997). In 2000, *O. vulgare* ssp. *vulgare* from Emilia region of northern Italy was characterized by the presence of abundant sesquiterpenes (D'Antuono *et al*., 2000). In 2001, *β*-ocimene (14.9-21.6%), germacrene D (10.0-16.2%), *β*-caryophyllene ((10.8-15.7%) and sabinene (6.6-4.2%) were reported as the main components of *O. vulgare* ssp. *vulgare* collected from 8 localities in Lithuania (Mockute *et al*., 2001). In 2003, *p*-cymene (22.3%), caryophyllene oxide ((10.2%), sabinene (7.9%), *γ*-terpinene (5.1%) were reported as the main components of *O. vulgare* ssp. *vulgare* cultivated in Hungary (Veres *et al*., 2003). In 2004, fourteen essential oils from inflorescences and leaves of cultivated *O. vulgare* L. were analyzed by GC and GC/MS; the main constituents in 6 inflorescence oils out of 7 and in 2 samples of 7 leaf oils were *β*-caryophyllene (15.4-24.9%), sabinene (6.2-19.5%) and germacrene D (11.4-14.6%) and in the 4 leaf oils, *β*-caryophyllene (17.2-21.3%), germacrene D (12.7-15.7%) and caryophyllene oxide (7.6-11.1%) (Mockute *et al*., 2004). Sartoratto et al. (2004) reported thymol (38.0%) and terpin-4-ol (33.3%) as the main compounds of the oil of *O. vulgare*. In 2004, the chromatographic analysis permitted the identification of thymol and *cis*-sabinene hydrate as the most prominent compounds present in commercial oregano sample and carvacrol and *cis*-sbinene hydrate in the cultivated *O. vulgare* (Rodrigues *et al*., 2004). In 2005, linalool (0.3-20.6), *β*-caryophyllene (1.3-45.0%), germacrene D (0.7-21.6%), caryophyllene oxide (1.5-31.3%) and spathulenol (0.9-10.1%) were reported as the major compounds of the oils of *O. vulgare* ssp. *vulgare* gathered from different regions and cultivated in Estonia (Ivask *et al*., 2005). Radusiene et al. (2005) reported mono- and sesquiterpene hydrocarbons were dominant compounds accounting for 49.8-76.8% of total essential oil in inflorescences and for 41.9-714% in leaves of *O. vulgare* collected from Lithuania; the content of phenols (thymol and carvacrol) was up to 5%. Tian & Lai (2006) reported thymol and carvacrol as the main compounds of *O. vulgare* oil. Santoyo *et al*. (2006) reported carvacrol, *trans*-sabinene hydrate, *cis*-piperitol, borneol, terpinen-4-ol and linalool as the main components of *O. vulgare* oil.

Antimicrobial activity

The preliminary screening for antimicrobial activity using the paper disk diffusion method showed that the oil exhibited a pronounced antibacterial effect on some bacteria tested. The essential oil did not show antifungal activity against fungi. Table 2 and 3 show the antimicrobial activity (inhibition zone and MIC) of *O. vulgare* oil against microorganisms tested.

Table 1. Chemical composition of the oils of *Origanum vulgare*

No.	Components	KI	GC area (%)	Method of identification[a]
1	2-Ethyl furan	746	trace[b]	MS, KI
2	Hexanal	804	0.1	MS, KI
3	2*E*-Hexenal	856	0.1	MS, KI
4	2,5-Diethyltetrahydrofuran	903	Trace	MS, KI
5	α-Thujene	932	1.0	MS, KI, CoI
6	α-Pinene	940	0.6	MS, KI, CoI
7	Camphene	956	0.1	MS, KI
8	Benzaldehyde	962	trace	MS, KI, CoI
9	Sabinene	976	8.7	MS, KI
10	1-Octen-3-ol	981	0.1	MS, KI
11	3-Octanone	984	1.7	MS, KI
12	Myrcene	992	0.4	MS, KI
13	3-Octanol	993	0.3	MS, KI
14	α-Phellandrene	1004	0.1	MS, KI
15	*p*-Mentha-1(7),8-diene	1005	0.1	MS, KI
16	2,4-Heptadienal	1012	trace	MS, KI
17	α-Terpinene	1019	1.5	MS, KI
18	*p*-Cymene	1027	2.6	MS, KI, CoI
19	Limonene	1030	0.4	MS, KI, CoI
20	β-Phellandrene	1031	1.0	MS, KI
21	1,8-Cineole	1032	1.2	MS, KI
22	Phenylacetaldehyde	1043	0.2	MS, KI
23	γ-Terpinene	1062	3.7	MS, KI
24	*cis*-Sabinene hydrate	1072	0.5	MS, KI
25	*trans*-Linalool oxide	1075	0.1	MS, KI
26	3-Nonanone	1084	trace	MS, KI
27	Terpinolene	1090	0.6	MS, KI
28	Linalool	1099	1.9	MS, KI
29	*trans*-Sabinene hydrate	1100	1.0	MS, KI
30	β-Thujone	1116	trace	MS, KI
31	*cis*-*p*-Mentha-2-en-1-ol	1124	0.5	MS, KI
32	α-Campholenal	1127	trace	MS, KI
33	1-Undecyne	1128	0.1	MS, KI
34	1-terpineol	1136	0.3	MS, KI
35	*cis*-*p*-Mentha-2,8-dien-1-ol	1139	trace	MS, KI
36	*trans*-Pinocarveol	1141	0.1	MS, KI
37	*cis*-Chrysanthenol	1166	0.1	MS, KI
38	Sabina ketone	1160	0.1	MS, KI
39	Borneol	1171	0.3	MS, KI
40	*trans*-*p*-Mentha-1(7),8-dien-2-ol	1190	0.1	MS, KI
41	Terpinen-4-ol	1179	7.6	MS, KI
42	*p*-Cymen-8-ol	1185	0.1	MS, KI
43	Dill ether	1189	0.1	MS, KI
44	α-Terpineol	1191	1.2	MS, KI
45	Dihydrocarveol	1196	0.1	MS, KI
46	*cis*-Piperitol	1197	0.1	MS, KI
47	*trans*-Dihydrocarvone	1202	0.1	MS, KI
48	*trans*-Piperitol	1209	0.2	MS, KI
49	*trans*-Carveol	1217	0.1	MS, KI
50	Pulegone	1239	0.1	MS, KI
51	Neral	1240	0.1	MS, KI
52	Cuminal	1244	trace	MS, KI
53	Carvacrol methyl ether	1246	0.1	MS, KI
54	Geraniol	1254	0.1	MS, KI
55	Piperitone	1255	0.1	MS, KI
56	Geranial	1268	0.1	MS, KI

57	Perilla aldehyde	1274	trace	MS, KI
58	*p*-Menth-1-en-7-al	1277	0.1	MS, KI
59	Thymol	1291	trace	MS, KI, CoI
60	*p*-Cymen-7-Ol	1292	0.1	MS, KI
61	Perilla alcohol	1297	trace	MS, KI
62	Carvacrol	1300	0.1	MS, KI, CoI
63	2*E*,4*E*-Decadienal	1319	0.1	MS, KI
64	Methyl geranate	1327	0.6	MS, KI
65	δ-Elemene	1337	trace	MS, KI
66	Eugenol	1360	0.7	MS, KI, CoI
67	(*Z*)-β-Damascenone	1366	trace	MS, KI
68	α-Copaene	1379	0.2	MS, KI
69	Geranyl acetate	1383	0.5	MS, KI
70	(*E*)-β-Damascenone	1387	0.1	MS, KI
71	β-Bourbonene	1389	0.4	MS, KI
72	β-Elemene	1393	0.2	MS, KI
73	β-Caryophyllene	1420	6.7	MS, KI
74	β-Gurjunene	1436	0.1	MS, KI
75	Neryl acetone	1438	0.1	MS, KI
76	(*Z*)-β-Farnesene	1445	0.1	MS, KI
77	α-Humulene	1456	1.2	MS, KI
78	Alloaromadendrene	1461	0.1	MS, KI
79	9- *epi*-(*E*)-Caryophyllene	1467	0.1	MS, KI
80	α-Amorphene	1486	0.1	MS, KI
81	Germacrene D	1487	1.5	MS, KI
82	*trans*-β-Ionone	1490	0.2	MS, KI
83	β-Selinene	1492	0.1	MS, KI
84	Ledene	1494	0.1	MS, KI
85	*n*-Pentadecanone	1500	0.1	MS, KI
86	Bicyclogermacrene	1501	2.7	MS, KI
87	(*E*,*E*)-α-Farnesene	1507	0.6	MS, KI
88	γ-Cadinene	1515	0.1	MS, KI
89	β-Sesquiphellandrene	1524	0.1	MS, KI
90	δ-Cadinene	1525	0.4	MS, KI
91	(*E*)-γ-Bisabolene	1532	1.7	MS, KI
92	(*Z*)-Nerolidol	1534	0.1	MS, KI
93	Elemol	1552	9.3	MS, KI
94	Silphiperfol-5-en-3-ol	1562	0.3	MS, KI
95	Germacrene B	1563	0.3	MS, KI
96	(*E*)-Nerolidol	1564	0.2	MS, KI
97	Germacrene D-4-ol	1577	0.2	MS, KI
98	Spathulenol	1579	3.9	MS, KI
99	Caryophyllene oxide	1584	3.1	MS, KI
100	Globulol	1586	0.1	MS, KI
101	Persilphiperfolan-8-ol	1587	0.4	MS, KI
102	*cis*-β-Elemenone	1591	0.5	MS, KI
103	Viridiflorol	1594	0.2	MS, KI
104	5-*epi*-7-*epi*-α-eudesmol	1609	0.2	MS, KI
105	Humulene epoxide II	1610	0.5	MS, KI
106	10- *epi*-γ-Eudesmol	1625	0.3	MS, KI
107	1- *epi*-Cubenol	1631	0.1	MS, KI
108	γ-Eudesmol	1633	4.2	MS, KI
109	Hinesol	1644	0.5	MS, KI
110	α-Muurolol	1647	0.5	MS, KI
111	β-Eudesmol	1653	7.7	MS, KI
112	α-Eudesmol	1655	4.6	MS, KI
113	14-Hydroxy-9-*epi*-(*E*)-caryophyllene	1672	0.1	MS, KI
114	*epi*-α-Bisabolol	1686	0.1	MS, KI
115	Eudesma-7(11)-en-4-ol	1702	0.2	MS, KI
116	14-Hydroxy-α-muurolene	1782	0.2	MS, KI

117	8-α-Acetoxyelemol	1794	trace	MS, KI
118	Hexadecanal	1811	0.1	MS, KI
119	(2*Z*,6*E*)-Farnesyl acetate	1823	0.1	MS, KI
120	6,10,14-Trimethyl-2-pentadecanone	1845	0.5	MS, KI
121	(2*E*,6*E*)-Farnesyl acetate	1848	0.1	MS, KI
122	*n*-Hexadecanol	1877	trace	MS, KI
123	Phytol	1944	0.1	MS, KI
124	Isophytol	1949	0.3	MS, KI
125	Hexadecanoic acid	1984	0.9	MS, KI
126	*n*-Heneicosane	2000	0.1	MS, KI
127	*n*-Octadecanol	2079	trace	MS, KI
128	(*Z*,*Z*,*Z*)-9,12,15-Octadecatrienoic acid methyl ester	2089	0.1	MS, KI
129	*n*-Eicosane	2100	trace	MS, KI
130	*n*-Docosane	2200	0.1	MS, KI
131	*n*-Tricosane	2300	0.1	MS, KI
132	*n*-Tetracosane	2400	0.1	MS, KI
133	*n*-Pentacosane	2500	trace	MS, KI
134	*n*-Hexacosane	2600	trace	MS, KI
135	*n*-Heptacosane	2700	trace	MS, KI
136	*n*-Octacosane	2800	0.1	MS, KI
137	*n*-Nonacosane	2900	0.1	MS, KI
		Total	99.6	

[a]KI, Kovats index on DB-5 column. MS, mass spectroscopy. CoI, co-injection; [b] trace= less than 0.05%.

O. vulgare oil showed strong activity against *Enterococcus faecium* and moderate activity against *Salmonella choleraesuis*, *Staphylococcus aureus* and *Bacillus subtilis* (Sartoratto *et al*., 2004). Pedro et al. (2005) reported use of *O. vulgare* oil can constitute a powerful tool in the control of *Listeria monocytogenes* in food and other industries. *O. vulgare* oil has also showed the antimicrobial activity against two strains *Escherichia coli* and four strains of *Salmonella* (Romero *et al*., 2005). Radusiene *et al*. (2005) reported leaf oils were more active than inflorescence oils against several microorganisms tested. Bozin *et al*. (2006) reported antimicrobial activity of *O. vulgare* oil against 13 bacterial strains, even on multiresistant strains of *Pseudomonas aeroginosa* and *Escherichia coli* and six fungi. In other research, all of the supercritical fluid extraction fractions obtained from *O. vulgare* showed antimicrobial activity against many of the microorganisms tested (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeroginosa*, *Aspergillus niger*, *Candida albicans*) (Santoyo *et al*., 2006).

It is known that many factors influence the chemical composition of essential oils. The differences in the quantity or quality of oils composition in present study and previous researches may be because of the collection time, chemotypes, drying conditions, mode of distillation, geographic and climatic factors. Since the antimicrobial activity of oil depends on its chemical composition, the differences in antimicrobial effects of *O. vulgare* oil of Iranian origin, in comparison to other researches, may be attributable to the differences in its chemical composition.

Table 2. Antimicrobial activity of the essential oil of *Origanum vulgare*

Sample	Conc. (μg/disc)	Diameter of zone of inhibition (mm)					
		Bacteria				Fungi	
		Bacillus subtilis (G+)	*Staphylococcus aureus* (G +)	*Escherichie coli* (G -)	*Salmonella typhi* (G -)	*Candida albicans*	*Aspergilus niger*
Origanum vulgare Essential oil	250	-	-	-	-	-	-
	500	-	-	-	-	-	-
	1000	-	-	-	7.95 ± 0.58	-	-
	2000	7.67 ± 0.58	-	-	8.33 ± 0.58	-	-
	4000	8.0 ± 0.0	-	-	10.0 ± 0.82	-	-
	8000	8.9 ± 0.82	7.33 ± 0.58	-	10.67 ± 0.58	-	-
	16000	11.0 ± 2.0	9.0 ± 1.0	-	11.67 ± 2.89	-	-
Gentamycin	50	29.8 ± 1.9	37.3 ± 2.5	31.6 ± 3.2	29.0 ± 2.5	-	-
Amikacin	3	21.8 ± 1.55	24.9 ± 3.1	23.8 ± 2.5	16.8 ± 3.1	-	-
Amphotericin B	100	-	-	-	-	22.3 ± 2	22.7 ± 2.1

Table 3. Minimal inhibitory concentration (MIC) of essential oil of *Origanum vulgare*

Strains	MIC (mg/ml)			
	Essential oil	Gentamycin	Amikacin	Amphotericin B
Bacillus subtilis (G+)	2	32×10^{-3}	4×10^{-3}	ND
Staphylococcus aureus (G +)	4	8×10^{-3}	4×10^{-3}	ND
Escherichia coli (G -)	-	16×10^{-3}	2×10^{-3}	ND
Salmonella typhi (G -)	1	32×10^{-3}	8×10^{-3}	ND
Candida albicans	-	ND	ND	64×10^{-3}
Aspergilus niger	-	ND	ND	32×10^{-3}

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