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Original article

Chemical composition and antioxidant activity of *Origanum vulgare* subsp. *vulgare* essential oil from Iran

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Abstract

Background and Objectives: Essential oils are very complex mixture of components and their composition may vary in different species or varieties or even within the same variety. *Origanum vulgare* L. subsp. *vulgare* is one of the most distributed subspecies within the genus *Origanum* and has been found to be a poor-oil, categorized in cymyl, bornane or sabinyl chemotypes with higher proportion of sesquiterpenes. In this experiment, the Iranian sample was studied for the chemical composition of the oil and evaluation of its antioxidant activity. **Methods:** Essential oil was obtained by hydro-distillation and analyzed by GC/MS for determination of components. Antioxidant activity was evaluated by radical scavenging ability (DPPH method) and reducing power (FRAP assay). **Results:** The sample belonged to "thymol" chemotype with the main components as thymol (37.13%), gama-terpinene (9.67%), carvacrol (9.57%), carvacrol methyl ether (6.88), *cis*-alpha-bisabolene (6.80%), eucalyptol (3.82%), *p*-cymene (3.58%) and elemol (2.04%). The oil of plant showed very strong antioxidant activity (IC_{50}=2.5 µg/mL in DPPH method), which was stronger than the standard antioxidants (Vit E and BHA, $p<0.05$) and it demonstrated good reducing power (467.25 µmole Eq $FeSO_4.7H_2O$/mg of the oil in FRAP assay). **Conclusion:** The data suggests the plant as a good potential natural antioxidant preservative.

Keywords: antioxidant, DPPH, essential oil, FRAP, *Origanum vulgare* L. subsp. *vulgare*

Introduction

Essential oils are very complex mixtures of components, while their composition may vary between different species or varieties or even within the same variety, related to different cultivation, origin, vegetative stage and growing seasons of the sources [1-6]. Beside therapeutic values of many of the oils, some of them are considered antioxidant and antimicrobial agents for preservation of drugs and foods [1].

The genus *Origanum* (Lamiaceae), with about 40 known species, is native to Mediterranean, Euro-Siberian and Irano-Siberian regions [2]. The species have been used since ancient times for treating various ailments such as digestive disorders, menstrual problems, spasmodic conditions, whooping and convulsive cough, etc. [2]. Beside the extensive usage of the herb in pharmaceutical and cosmetic industries, the intra-

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specific essential oil variability of the species has been observed in a number of publications [7].
The two main species growing in Iran are *O. vulgare* L. and *O. marjorana* L. [8]. Searching the literature for biological activities of these species, antimicrobial and antioxidant activities were found to be the most evaluated effects [9-18] with some other effects like improving spatial learning [19], lowering blood pressure, anti-diabetic, anti-inflammatory, anti-mutagenic, cytotoxic [8] and anticancer in the next rates [20]. *Origanum vulgare* ssp. *vulgare* is one of the most widely distributed subspecies [2] with some reports about its chemical composition and pharmacological effects in different parts of the world [7,21,22]. Based on previous studies, it was concluded that the species from the northern hemisphere (including *O. vulgare* L. subsp. *vulgare*) are poor sources of volatiles (compared to southern subspecies) and are often composed of cymyl-compounds, bornane type (e.g. borneol, camphor, camphene), acyclic (mainly linalool and linalyl acetate) and sabinyl compounds (e.g. sabinene) with a larger contribution of sesquiterpenes [2,7,21]. In the present study, the chemical composition of *O. vulgare* L. subsp. *vulgare* has been analyzed by means of GC/MS to more broaden the information about chemical pattern of the species. In addition, the antioxidant activity has been measured by DPPH and FRAP methods.

Experimental

Plant material

The aerial parts of *O. vulgare* L. subsp. *vulgare* were collected from Noshahr, Mazandaran province (Iran) in July, 2012. They were then dried in shade at room temperature and were cut just before extraction of the essential oil. Voucher specimen (TEH-6760) was prepared for authentication and a sample was deposited at the Herbarium of Department of Pharmacognosy, Tehran University of Medical Sciences, Tehran, Iran.

Essential oil extraction and analysis

The crashed sample was extracted for essential oil by Clevenger type apparatus and separation and detection of chemical components was performed using an Agilent 6890 GC apparatus, equipped with a HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm) and an Agilent 5973 mass detector. The temperature of the oven column was 50 ^{o}C (isothermal for 5 min), then at rate of 10 ^{o}C/min raised to 240 ^{o}C with a final hold time of 3 min. Carrier gas was helium at a flow of 0.8 mL/min. The injector was set at 290 °C. Ionization energy was 70 eV and mass scan range between 50-550 amu. Characterization was achieved based on calculating kovats index using a homologous series of *n*-alkanes (C8-C25) and by comparing the mass spectra with those in Wiley library and the literature data [23].

Evaluation of antioxidant activity by DPPH and FRAP methods

Free radical scavenging capacity was evaluated by DPPH method, as previously described in literature with minor modifications [24]. Based on a pre-examination, four concentrations of 0.5, 5, 10 and 50 μL/mL of the essential oil were prepared in methanol. Vitamin E (129 μg/mL) and BHA (220 μg/mL) were used for comparison as standard antioxidants, respectively, all in triplicate. Different prepared solutions (1 mL of each) were mixed with a freshly prepared 40 μg/mL (methanol) DPPH and after 30 min the absorbance were read at 517 nm against proper blank. The percent of inhibitions were calculated as:

$$I\ \% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

Where A_{blank} is the absorption of control. The concentration in which 50% of inhibition was achieved (by calculations based on inhibition percentage against concentration of sample) was considered as IC_{50} and reported as means ± SD.
For determination of the reducing power, 1.5 mL of FRAP reagent (5 mL of TPTZ + 5 mL $FeCl_3$ + 50 mL of acetate buffer with a pH of 3), which is a labile solution and should be prepared just before the experiment, was added to the tubes. The mixture was heated at 37 ^{o}C for 5 min. Fifty μL of the prepared samples including a concentration of 0.19 mg/mL of the essential oil and five concentrations of aqueous solutions of

$FeSO_4.7H_2O$ (125, 250, 500, 750 and 1000 µmol/L (for plotting the calibration curve) were added to the mixture. The change in the absorbance of the sample (in triplicate) was recorded after 30 min at 593 nm against proper blank (in which FRAP reagent was absent). The results were expressed as µmol $FeSO_4.7H_2O$ equivalents per mg of the sample [25].

Statistical analysis was performed by ANOVA followed by Tukey post-hoc test for multiple comparisons of means ($p \leq 0.05$).

Results and Discussion

Essential oil composition

The yield of the oil was 0.5% (v/w) with a pale yellow color and pungent odor. As shown in table 1, analysis of the essential oil resulted in identification of 37 compounds, representing 95.84% of the total oil, while thymol (37.13%), gama-terpinene (9.67%), carvacrol (9.57%), carvacrol methyl ether (6.88), *cis*-alpha-bisabolene (6.80%), eucalyptol (3.82%), *p*-cymene (3.58%) and elemol (2.04%) were the predominant components. The most abundant chemical structure within components was oxygenated monoterpenes (59.25%), followed by monoterpene hydrocarbons (18.71%), oxygenated sesquiterpenes (12.86%) and sesquiterpene hydrocarbons (5.02%).

In another study, focusing on variation in chemical composition of the essential oil of *O. vulgare* L. subsp. *vulgare*, collected from Chaloos (Mazandaran province, Iran) in flowering and seeding stages, linalyl acetate (27.2%), gamma-terpinene (16.5%), 3-octanone (10.9%), beta-pinene (8.4%) and carvacrol (6.4%) were the main components of the flowering stage and carvacrol (23.2%), α-pinene (15.8%), beta-pinene (10.7%) and trans-caryophyllene (5.3%) were the main compounds of seeding stage. This study relates this subspecies to cymyl and acyclic types [26]. Among four chemotypes found for *O. vulgare* subs. *vulgare* by Italian scientists (i.e. *p*-cymene, terpinene-4-ol, thymol and *β*-caryophyllene) [27], the plant could be placed to thymol chemotype and seems different from most found chemotypes

Table 1. Chemical composition of the essential oil from aerial parts of *Origanum vulgare* subsp. *vulgrare*.

Number	compound	RI_S	RI_R	RT	(%)
1	alpha-Thujene	918	931	10.818	0.431
2	alpha-Pinene	925	939	11.135	0.409
3	Sabinene	967	976	13.214	0.774
4	beta-Pinene	969	980	13.314	0.225
5	unknown	980	978	13.827	0.453
6	unknown	984	965	14.018	0.378
7	beta-Myrcene	987	991	14.208	0.628
8	alpha-Terpinene	1012	1018	15.456	1.018
9	*p*-Cymene	1025	1026	16.117	3.583
10	Eucalyptol (1,8 Cineole)	1032	1033	16.434	3.823
11	*cis*-beta-Ocimene	1038	1040	16.747	1.037
12	*trans*-beta-Ocimene	1048	1050	17.254	0.314
13	gama-Terpinene	1065	1062	18.122	9.668
14	unknown	1068	-	18.280	0.257
15	alpha-Terpinolene	1086	1088	19.169	0.181
16	Linalool	1099	1098	19.846	0.322
17	E,Z-Alloocimene	1127	1129	21.231	0.181
18	Geijerene	1139	1150	21.802	0.262
19	Borneol	1163	1165	23.003	0.067
20	Terpinene-4-ol	1175	1177	23.606	0.261
21	alpha-Terpineol	1189	1189	26.076	1.200
22	Carvacrol, methyl ether	1251	1241	27.207	6.880
23	Thymol	1319	1289	30.301	37.129
24	Carvacrol	1332	1298	30.883	9.573
25	alpha-Copaene	1380	1374	33.020	0.237
26	beta-Bourbonene	1390	1387	33.437	0.315
27	*trans*-Caryophyllene	1427	1417	35.013	1.948
28	unknown	1433	-	35.267	0.161
29	*trans*-alpha-Bergamotene	1438	1432	35.484	0.153
30	*trans*-beta-Farnesene	1459	1454	36.341	0.416
31	Germacrene D	1486	1484	37.483	1.055
32	unknown	1500	-	38.028	0.465
33	unknown	1513	-	38.541	0.678
34	gama-Cadinene	1519	1513	38.774	0.293
35	delta-Cadinene	1523	1522	39.117	0.603
36	*cis*-alpha-bisabolene	1556	1506	40.233	6.805
37	Elemol	1563	1548	40.524	2.041
38	Spathulenol	1587	1577	41.471	0.554
39	Caryophyllene oxide	1592	1582	41.651	0.668
40	Muurolol	1650	1644	43.851	1.750
41	alpha-Eudesmol	1661	1652	44.253	0.364
42	Intermedeol	1666	1665	44.443	0.376
43	alpha-Bisabolol	1687	1685	45.236	0.298
	Identified 95.842				
	Unknown 2.392				
Total	Oxygenated monoterpenes 59.25%				
	Hydrocarbon monoteroenes 18.71%				
	Oxygenated sesquiterpenes 12.86%				
	Hydrocarbon sesquiterpenes 5.02%				

RI_S: Retention index of sample; RI_R: Retention index of reference; RT: Retention Time.

in other parts of the world including Lithuania [22,27], Turkey [2], Italy [28], Corsica and Austria [7], which have been reported to be *β*-caryophyllene or cymyl-sabinyl types, rich in *β*-caryophyllene, sabinene, spathulenol and germacrene D. The oil of the plant was also rich in phenolic compounds, which comprised about 53% of the total oil.

The different results of the present study from those in other parts of the native regions and also in similar regions of Iran seemed similar to what had been previously observed for Sicilian Oregano (which has been concluded to be *O. vulgare* L. subsp. *hirtum*) [5]. Regarding the previously published data about chemical composition of *O. vulgrare* L. subsp. *vulgare*, it seemed that despite being a poor-oil like other northern chemotypes, the mentioned *O. vulgare* L. subsp. *vulgare* belongs to a totally different chemotype (thymol) which has not been identified before within the same subspecies, with higher percentage of monoterpenes (77.96%) and the dominant oxygenated terpenoids.

Antioxidant activity

The essential oil demonstrated radical scavenging ability with IC_{50} 2.5 µg/mL.

The Antioxidant Activity Index which has been introduced by Scherer & Godoy [29] and has been measured considering the following equation:

$$AAI = \frac{\text{DPPH stock concentration (µg /mL)}}{IC_{50}\text{ (µg /mL)}} = 16$$

This above result introduced the species as showing very strong antioxidant activity for radical scavenging ability which could be attributed to the high phenolic content of the oil.

Comparison of the IC_{50} values for the sample and standard antioxidants revealed that the sample has shown stronger activity than BHA and Vitamin E in scavenging DPPH radicals (p <0.05).

By measuring the absorbance values of different concentrations of $FeSO_4.7H_2O$, the calibration curve was plotted (R^2=0.9961). The obtained results were subsequently calculated as 467.25 µmole Eq $FeSO_4$.7H2O/mg of the oil.

It has been found that the reducing power of dietary plants depends mostly on sample's content in polyphenols, anthocyanins and ascorbic acid [25], while high reducing power of the oil could also be related to the high phenolic content.

It could be concluded that with a different chemical composition of the essential oil, Iranian *O. vulgare* subsp. L. *vulgare* may be categorized in "thymol" chemotype and by its very strong antioxidant activity, it could be considered as a good potential as a suitable natural antioxidant preservative.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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