

# Variability of Essential Oil Composition of *Origanum vulgare* L. ssp. *vulgare* Populations from Iran

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## ABSTRACT

In this study, the chemical composition of the essential oils (EOs) extracted from aerial parts of native populations of *Origanum vulgare* ssp. *vulgare* from Iran was compared by GC and GC/MS. Plants were collected at bloom stage from their natural habitats located in Kaleybar, Sabalan, Meshkin Shahr and Chalus regions, respectively from East Azerbaijan, Ardabil, Ardabil and Mazandaran provinces. Forty identified compounds accounted for 96.2-99.8% of the EO composition. According to the GC/MS analysis, four chemotypes consisting of carvacrol, sabinene, caryophyllene oxide and linalyl acetate were identified in the populations. Nonetheless, the monoterpenes were included the greatest EO fraction in all the populations. Of the monoterpenes, hydrocarbon compounds accounted for more than the oxygenated ones. This paper represents the first study on the EO composition of Iranian specimens of this plant and can be very useful for future breeding programs and medicinal purposes.

**Keywords:** chemotypes, future breeding programs, GC/MS analysis, monoterpenes

## INTRODUCTION

The genus *Origanum* (tribe Mentheae, Labiatae family) is characterized by a high morphological and chemical diversity (Kokkini 1997). The morphological diversity within the genus causes in the classification of 10 sections including 42 species or 49 taxa (Ietswaart 1980; Carlström 1984; Danin 1990; Danin and Künne 1996). The genus is an annual, perennial and shrubby herb and its species grow abundantly on stony slopes and in rocky mountain areas at a wide range of altitudes (Snogerup 1971; Aligiannis *et al.* 2001). The species are widely used all over the world as a very popular spice, under the vernacular name 'oregano'. In addition, as recent studies have pointed out, oregano is used in many other ways as their essential oils (EOs) have antimicrobial, cytotoxic and antioxidant activity (Sivropoulou *et al.* 1996; Ozkan *et al.* 2007). *Origanum* species are traditionally used as sedative, diuretic, degasifer, sweater and antiseptic, and also in the treatment of gastrointestinal diseases and constipation (Kordali *et al.* 2008). A number of studies have shown that variation among the populations of *Origanum* species such as *Origanum vulgare* may occur with regard to morphological and phytochemical features (Chalchat and Pasquier 1998; D'Antuono *et al.* 2000). To optimally manage genetic resources for improvement of the cultivars, and to maintain and restore biodiversity, knowledge of chemical diversity within species is indispensable (Azizi *et al.* 2009a). The genus contains only one species (*O. vulgare*) and three subspecies (ssp. *viride*, ssp. *vulgare* and ssp. *gracile*) in North line of Iran. *O. vulgare* ssp. *viride* is widely spread in the country, whereas the other two subspecies are found only in few localities (Rechinger 1982). The seeds of *O. vulgare* ssp. *vulgare* from 4 locations were studied in Italy and four chemotypes were identified:  $\beta$ -caryophyllene, thymol, terpinen-4-ol and *p*-cymene- $\beta$ -caryophyllene (Melegari *et al.* 1995). In France, the EOs related to many different locations from the latter subspecies were classified into six groups: sabinene, germacrene D, germacrene D- $\beta$ -caryophyllene, *cis*-sabinene hydrate, terpinen-

4-ol and  $\beta$ -ocimene (Chalchat and Pasquier 1998). Moreover, the composition of EOs of *O. vulgare* ssp. *vulgare* was carefully analyzed in Lithuania. In this country,  $\beta$ -ocimene, germacrene D,  $\beta$ -caryophyllene and sabinene were reported as major constituents (Mockute *et al.* 2001, 2003). The other subspecies of *O. vulgare* formed several different chemotypes based on EO such as the thymol and carvacrol chemotypes in *O. vulgare* ssp. *hirtum* (Melegari *et al.* 1995; Sivropoulou *et al.* 1996; Kokkini *et al.* 1997; Bocchini *et al.* 1998; Skoula *et al.* 1999), the carvacrol chemotype in *O. vulgare* ssp. *glandulosum* (Melegari *et al.* 1995), the thymol (Melegari *et al.* 1995) and sabinene-germacrene D (Leto *et al.* 1994) chemotypes in *O. vulgare* ssp. *gracile*, the germacrene D-sabinene or  $\gamma$ -terpinene (Chalchat and Pasquier 1999), linalool- $\delta$ -elemene (Alves-Pereira and Fernandes-Ferreira 1998), linalool- $\beta$ -caryophyllene, linalool- $\alpha$ -terpineol, linalool-terpinen-4-ol, terpineol-linalool and terpineol-carvacrol (Melegari *et al.* 1995) chemotypes in *O. vulgare* ssp. *viridens* and the linalyl acetate- $\beta$ -caryophyllene-sabinene chemotype in *O. vulgare* ssp. *viride* (Afsharypuor 1997). This paper presents the EO chemical composition from aerial parts of *O. vulgare* ssp. *vulgare* in four different geographical locations as the first report from Iran for (a) presentation new chemotypes, (b) future breeding programs and conservation and exploitation of genetic resources.

## MATERIALS AND METHODS

### Plant material

The aerial parts of *O. vulgare* ssp. *vulgare* were collected at bloom stage from four different geographical locations including Kaleybar, Sabalan, Meshkin Shahr and Chalus from East Azerbaijan, Ardabil, Ardabil and Mazandaran provinces in Iran, respectively. The plants identification was done by a connoisseur plant taxonomist and according to the given information in Flora Iranica (Rechinger 1982). A voucher specimen of each population has been deposited in the Herbarium of Horticulture Department, Fac-

ulty of Agriculture and Natural Resources, University of Tehran, Karaj, Iran.

## Isolation of EOs

The EOs of the air-dried and ground aerial parts of the plants were extracted for 4 h with water-distillation using a Clevenger-type apparatus. The EO yield was 0.1% (v/w) for Chalus population and 0.2% (v/w) for the others. The extracted EOs were dried over anhydrous sodium sulphate and, after filtration, stored at 4°C until analyzed.

## Gas chromatography (GC) and gas chromatography-mass spectroscopy (GC-MS) analysis

GC analysis was performed using a thermoquest gas chromatograph with a flame ionization detector (FID) (Varian CP 3800, Japan). The analysis was carried out on fused silica capillary DB-5 column (60 m × 0.25 mm i.d.; film thickness 0.25 µm). The injector and detector temperatures were kept at 250 and 300°C, respectively. Nitrogen was used as the carrier gas at a flow rate of 1.1 mL/min; oven temperature program was 60-250°C at the rate of 4°C/min and finally held isothermally for 10 min; split ratio was 1:50. GC-MS analysis was carried out by use of Thermoquest-Finnigan gas chromatograph equipped with fused silica capillary DB-5 column (60 m × 0.25 mm i.d.; film thickness 0.25 µm) coupled with a TRACE mass (Manchester, UK). Helium was used as carrier gas with ionization voltage of 70 eV. Ion source and interface temperatures were 200 and 250°C, respectively. Mass range was from 43 to 456 amu. Oven temperature program was the same as mentioned above for the GC.

## Identification of compounds

The constituents of the EOs were identified by calculating their retention indices under temperature-programmed conditions for *n*-alkanes (C<sub>6</sub>-C<sub>24</sub>) and the EO on a DB-5 column under the same chromatographic conditions. Identification of individual compounds was made by comparing their mass spectra with those of the internal reference mass spectra library (Adams and Wiley 7.0) or with authentic compounds and confirmed by comparing their retention indices with authentic compounds or with those of reported in the literature (Adams 2007). For quantification purpose, relative area percentages obtained by FID were used without the use of correction factors.

## RESULTS AND DISCUSSION

The retention indices and percent composition of EOs separated from aerial parts of *O. vulgare* ssp. *vulgare* from four different geographical localities of Iran have been shown in **Table 1**. Forty identified components accounted for 96.2-99.8% of the EO composition. According to our findings four chemotypes were identified: (I) carvacrol, (II) sabinene, (III) caryophyllene oxide and (IV) linalyl acetate, respectively from Kaleybar, Sabalan, Meshkin Shahr and Chalus populations. Chemotype I was characterized by high content of carvacrol (21.3%),  $\gamma$ -terpinene (17.5%), *trans*-caryophyllene (11.3%),  $\alpha$ -pinene (5.7%), 3-octanone (4.7%),  $\alpha$ -humulene (4.6%), camphene (3.9%) and  $\beta$ -bourbonene (3.6%) were the other important components in the chemotype. Carvacrol has been reported as the major constituent of the EOs of *O. onites*, *O. minutiflorum* and *O. vulgare* (Arslan and Dervis 2010), *O. syriacum* (Al-Kalaldehy *et al.* 2010), *O. vulgare* var. *creticum*, *O. vulgare* ssp. *hirtum* and *O. vulgare* var. *samothrake* (Azizi *et al.* 2009b), *O. vulgare* (Bisht *et al.* 2009), *O. vulgare* ssp. *viride* (D'Antuono *et al.* 2000), *O. vulgare* ssp. *hirtum* (Melegari *et al.* 1995; Sivropoulou *et al.* 1996; Kokkini *et al.* 1997; Bocchini *et al.* 1998; Skoula *et al.* 1999), *O. vulgare* ssp. *glandulosum* and *O. vulgare* ssp. *virens* (Melegari *et al.* 1995) and *O. onites* (Skoula *et al.* 1999). Chemotype II was found to be rich in sabinene (20.8%), the other main components belonging to this chemotype were carvacrol (17.8%), (*E*)- $\beta$ -ocimene

(10.4%),  $\alpha$ -pinene (8.8%),  $\gamma$ -terpinene (7.1%), *trans*-caryophyllene (6.1%),  $\alpha$ -humulene (4.5%),  $\beta$ -pinene (4.1%),  $\alpha$ -cadinene (3.7%) and geranyl acetate (3.4%). Sabinene was one of the main constituents identified in the EOs of *O. vulgare* ssp. *vulgare* (Chalchat and Pasquier 1998; Mockute *et al.* 2001, 2003), *O. vulgare* ssp. *gracile* (Leto *et al.* 1994), *O. vulgare* ssp. *virens* (Chalchat and Pasquier 1999) and *O. vulgare* ssp. *viride* (Afsharypuor *et al.* 1997). Chemotype III was resulted by great percentage of caryophyllene oxide (21.0%), followed by  $\gamma$ -terpinene (12.3%), dihydrocarvone (11.2%),  $\beta$ -pinene (10.5%), carvacrol (8.0%), (*E*)- $\beta$ -ocimene (6.2%), 3-octanone (6.0%) and myrcene (5.4%). Some previous studies on the EO composition of *O. vulgare* have shown the  $\beta$ -caryophyllene chemotype (Melegari *et al.* 1995; Chalchat and Pasquier 1998; Mockute *et al.* 2001, 2003). Chemotype IV was consisted of linalyl acetate (27.2%) as the most prominent component, an agreement with literatures (Baser *et al.* 1995; Afsharypuor *et al.* 1997). The other important components related to the latter chemotype were  $\gamma$ -terpinene (16.5%), 3-octanone (10.9%),  $\beta$ -pinene (8.4%), carvacrol (6.4%) and  $\alpha$ -terpinene (4.7%). However, the monoterpenes were the most predominant fraction of the EOs in all the chemotypes. The obtained result was in contrast with the others that the sesquiterpenes had been identified as one of the main fraction of the EOs of this plant (Lawrence 1984; Melegari *et al.* 1995; Chalchat and Pasquier 1998; Mockute *et al.* 2001, 2003). As a result, the EOs from these locations may be attributed to new chemotypes. Of the monoterpenes, hydrocarbon compounds were more than that of oxygenated ones. The monoterpene components made up 62.1%, 75.6%, 62.1% and 78.7% of the EOs in Kaleybar, Sabalan, Meshkin Shahr and Chalus populations, respectively. The highest and lowest of sesquiterpene compounds content were found in Kaleybar (30.5%) and Chalus (3.5%), respectively. In all of the chemotypes, sesquiterpene hydrocarbons were more in comparison with oxygenated sesquiterpenes except for chemotype III, that was inverse (4.4% < 22.7%). The percentage of the other components of the EOs was 6.6, 3.2, 10.6 and 16.8% in the populations, respectively. Among these populations, Meshkin Shahr was different from the others due to the lower monoterpenes percentage and identification of an oxygenated sesquiterpene namely caryophyllene oxide as the most predominant component. It might be due to specific ecological condition for this population because these plants were collected in a higher altitude and ecologically clean places (far from towns and roads). As reported previously, this shows the high effectiveness of geographical and climate condition of collection sites on EO composition of *O. vulgare* ssp. *vulgare* (Pande and Mathela 2000; Kumar *et al.* 2007; Economou *et al.* 2011). In *O. vulgare* ssp. *vulgare* populations from the origins of Chalus, Sabalan and Meshkin Shahr, thymol constituent was not detected and also in Kaleybar, it was trace, whereas carvacrol was one of the main constituents identified in EOs composition. With respect to a previous work from Iran (Afsharypuor *et al.* 1997), analysis of EO composition of *O. vulgare* ssp. *viride* showed low percentages of phenolic monoterpenoids (thymol and carvacrol). Apparently, the Iranian specimens of *O. vulgare* are poor in thymol amount, whereas the present work suggests the higher measurement of carvacrol in ssp. *vulgare* than that of ssp. *viride*. Apart from Sabalan population that  $\gamma$ -terpinene was found as the fifth major component, it was identified as the second major component in the other three populations.  $\beta$ -ocimene exists in two forms of *cis* (*Z*) and *trans* (*E*) and in this study, the ratio of *trans/cis* isomers was high. The oils of Sabalan and Meshkin Shahr populations of Ardabil province were rich in (*E*)- $\beta$ -ocimene. Dihydrocarvone was the third major constituent in Meshkin Shahr region, whereas it was not detected in Chalus and Kaleybar and was identified trace in Sabalan. *O. vulgare* ssp. *vulgare* samples originated from Kaleybar and Sabalan were consisted of the more percentage of *trans*-caryophyllene in comparison with the others. In addition, pinene isomers including  $\alpha$  and  $\beta$  were one of the major components iden-

**Table 1** Composition of the essential oils of four populations of *Origanum vulgare* L. ssp. *vulgare* (%) growing wild in Iran.

No.	RI <sup>a</sup>	Identified components <sup>b</sup>	Kaleybar	Sabalan	Meshkin Shahr	Chalus
1.	848	(E)-2-Hexenal	-	-	-	1.4*
2.	931	$\alpha$ -Thujene	-	-	-	1.3
3.	941	$\alpha$ -Pinene	5.7	8.8	1.6	3.4
4.	958	Camphene	3.9	t	-	1.9
5.	976	1-Octen-3-ol	-	3.2	2.5	1.9
6.	980	Sabinene	0.9	20.8	2.2	3.2
7.	984	3-Octanone	4.7	t	6.0	10.9
8.	986	$\beta$ -Pinene	t	4.1	10.5	8.4
9.	992	Myrcene	0.9	-	5.4	1.3
10.	1011	$\alpha$ -Phellandrene	2.0	t	-	-
11.	1022	$\alpha$ -Terpinene	1.8	-	3.3	4.7
12.	1030	<i>p</i> -Cymene	-	t	t	-
13.	1036	(Z)- $\beta$ -Ocimene	2.5	-	t	-
14.	1038	1,8-Cineole	-	t	1.4	2.8
15.	1041	(E)- $\beta$ -Ocimene	2.6	10.4	6.2	1.6
16.	1057	$\gamma$ -Terpinene	17.5	7.1	12.3	16.5
17.	1100	1-Octen-3-yl acetate	1.9	t	2.1	2.6
18.	1178	Terpinen-4-ol	-	t	-	-
19.	1198	Dihydrocarvone	-	t	11.2	-
20.	1249	Linalyl acetate	1.3	-	-	27.2
21.	1284	Thymol	t	-	-	-
22.	1293	Carvacrol	21.3	17.8	8.0	6.4
23.	1354	Neryl acetate	-	3.2	-	-
24.	1373	Geranyl acetate	1.7	3.4	-	-
25.	1397	$\beta$ -Bourbonene	3.6	-	-	-
26.	1431	<i>trans</i> -Caryophyllene	11.3	6.1	1.2	1.4
27.	1450	(E)- $\beta$ -Farnesene	1.1	-	-	1.0
28.	1465	$\alpha$ -Humulene	4.6	4.5	1.9	-
29.	1491	Germacrene D	1.5	-	-	-
30.	1501	(E, E)- $\alpha$ -Farnesene	-	t	-	-
31.	1509	Bicyclgermacrene	-	-	-	1.1
32.	1533	$\beta$ -Sesquiphellandrene	t	-	-	-
33.	1536	$\alpha$ -Cadinene	1.3	3.7	-	-
34.	1549	(E)- $\gamma$ -Bisabolene	2.5	t	1.3	-
35.	1567	(E)-Nerolidol	1.9	-	-	-
36.	1597	Caryophyllene oxide	2.7	3.1	21.0	-
37.	1650	$\alpha$ -Muurolol	-	-	t	-
38.	1664	$\alpha$ -Cadinol	-	t	1.7	-
39.	1667	$\alpha$ -Eudesmol	-	-	t	-
40.	1676	Intermedeol	-	-	t	-
		Sum	99.2	96.2	99.8	99.0
		Hydrocarbon compounds	63.7	65.5	45.9	45.8
		Monoterpene hydrocarbons	37.8	51.2	41.5	42.3
		Oxygenated monoterpenes	24.3	24.4	20.6	36.4
		Sesquiterpene hydrocarbons	25.9	14.3	4.4	3.5
		Oxygenated sesquiterpenes	4.6	3.1	22.7	-
		Other compounds	6.6	3.2	10.6	16.8

(a): List of published retention indices; (b): Components in the order of elution on the DB-5 column; (\*): Calculated from response on FID; (-): not detected; (t): trace &lt; 0.1%

tified in present study. Nonetheless, the major components identified in this study were different than those in Lithuania (Mockute *et al.* 2001, 2003), Italy (Melegari *et al.* 1995), France (Chalchat and Pasquier 1998) and Turkey (Şahin *et al.* 2004). It can be due to the differences of the climatic and geographic conditions of collection sites among these countries. The main components reported from *O. vulgare* ssp. *vulgare* plants in this study and *O. vulgare* ssp. *viride* in a previous work from Iran are different, and it can be due to genetic differences between two subspecies. Notably, there were significant differences in EOs composition of *O. vulgare* ssp. *vulgare* in all of the collection sites that can suggest the role of extrinsic and intrinsic genetic factors in determining oil components. In this study, four chemotypes of carvacrol, sabinene, caryophyllene oxide and linalyl acetate in four localities of *O. vulgare* ssp. *vulgare* were introduced as the first report from Iran.

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