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The essential oils compositions of Iranian Oregano (*Origanum vulgare*L.) populations in field and provenance from Piranshahr district, West Azarbaijan province, Iran.

Mohamad Pirigharnaei[1*], Samad Zare[1], Reza Heidary[1], Jalil Khara[1], Reza EmamaliSabzi[2], Farshad Kheiry[2]

Abstract

Objective: *Origanum vulgare* L. is rarely cultivated in Iran but it is the only species of the *Origanum* genus growing wild in this country. *O.vulgare* L. is widely spread all over the country. In this study, parameters of content, composition and antioxidant activity of the essential oils of domestic and wild Iranian Oregano populations were compared with one another.

Materials and Methods: The extractions were performed using a clevenger-type apparatus and the essential oils in the Oregano plants were obtained by hydro-distillation and analyzed by GC/MS.

Results: The essential oils were obtained in the Oregano field at yield of (0.80%) and the 4 provenance Oregano (ranging from 0.93% to 1.66% v/w). In cultivated plant 22 constituents, representing 94.02% and in 4 provenance plants (O.v-w_1--- O.v-w_4), 21, 25, 22, 20 constituents, representing 96.55%, 95.66%, 95.8%, 94.48% of the oils, respectively, were identified. The two major constituents of the essential oils, carvacrol ranging from 23.54 to 67.09% and, γ-terpinene ranging from 7.71 to 20.94% were present in relatively equal amounts in all five samples from different localities. Five chemotypes of essential oils were identified. The main chemotype was carvacrol-γ-terpinene. Furthermore, the antioxidant activity of essential oils of *O.vulgare* using DPPH radical scavenging was determined. All extracts exhibited almost the same pattern of antioxidant activity as ascorbic acid (vit C).

Conclusion: Our findings demonstrated that the chemical composition of the essential oils of *O. vulgare* L. varies considering geographical location of collection site, climate and other ecological conditions which suggest both intrinsic/genetic and extrinsic/environmental factors such as: altitude, edaphic, temperature, humidity and climate, may play important roles in determining the oils composition.

KeyWords: Essential oil, Iranian Oregano (*Origanumvulgare*L.), Carvacrol ,γ-terpinene.

1- Biology Department ,Urmia University, Urmia, I. R. Iran
2- Chemistry Department and Institute of Biotechnology of Urmia University, Urmia, I. R. Iran
∗**Corresponding author**: Tel: 09141670213; fax: +984444232412.
E-mail: mpirigharnaei@yahoo.com / m.pirigharna@gmail.com.

Introduction

The genus *Origanum* belongs to the family of *Labiatae* and includes many species that are commonly found as wild plants in the Mediterranean areas, Euro-Siberian and Irano-Siberian regions (Skoula et al., 2002; Aligiannis et al., 2001). A total of 38 *Origanum* species are recognized in the world. Most of the *Origanum* species, over 75%, are growing in the east Mediterranean sub-region of which 16 species are considered as endemic to the flora of Turkey (Guner et al., 2000; Sahin et al., 2004). *Origanum vulgare* L. is the only species of the *Origanum* genus growing wild in Iran. *O.vulgare* L. is widely spread all over the country, particularly Gilan, Mazandaran and West Azarbaijan provinces. (SalehiSurmaghi, 2010).

Origanum species grow abundantly on stony slopes and in rocky mountain areas at a wide range of altitudes (0 – 4000 m) (Sahin et al., 2004; Snogerop, 1971). Due to the variability in chemical and aroma characteristics, *Origanum* plants belonging to different species and ecotypes (biotypes) are widely used in agriculture as well as in pharmaceutical and cosmetic industries as a culinary herb, flavouring substances of food products, alcoholic beverages and perfumery for their spicy fragrance (Aligiannis et al., 2001; Snogerop, 1971; Sivropoulou et al., 1996).

The essential oil of oregano is composed of carvacrol and/or thymol as dominant components, followed by γ-terpinene, p-cymene, linalool, terpinen-4-ol and sabinene hydrate (Skoula et al., 2002; D,antuono et al., 2000). Results of various studies indicated that the antioxidant effects of oregano might be related to the dominant components including, carvacrol and thymol present in, its essential oil (Lagouri et al., 1999).

The quality of oregano is determined mainly by the essential oil content and the its composition. Both parameters may vary considerably depending on genotypes, climate conditions and nutrient supply during the cultivation (D'antuono et al., 2000). For example, nitrogen fertilization affected the composition of the essential oils by increasing the percentage of thymol and carvacrolwith a simultaneous decrease of γ-terpinene and p-cymene in *Origanum syriacum* (Omer, 1999). Studies on oregano plants in Greece showed that *O. vulgare* ssp. *hirtum*, contained a high amount of essential oil. The content of essential oil as high as 8% with carvacrol as dominant component (95%) was reported for this subspecies (Kokkini and Vokou, 1989). Because of its high essential oil content with high percentage of carvacrol, this subspecies which is systematically and widely cultivated in Greece is known as "Greek oregano"(Chatzopoulou et al., 2004). In Mediterranean countries, *O. vulgare* var. *creticum* was found to contain essential oil with a varying percentages of carvacrol ranging from 3% to 68% (Berna'th, 1997).

The composition of essential oils of *O.vulgare* L. spp. *vulgare* was carefully analyzed in Italy (Melgari et al., 1995) and France (Chalchat and Pasquier, 1998). The seeds from four localities were studied in Italy and 4 chemotypes of essential oils were identified. The seeds of 70 clones cultivated in France were collected from different locations in France and other European countries and the essential oils were classified into six chemotypes (Chalchat and Pasquier, 1998).

In various studies, it has been demonstrated that the essential oils and extracts composition of medicinal plants such as *Origanum* species showing antimicrobial, antioxidant and other biological activities may change according to the differences in cultivation, origin, vegetative stage and growing seasons of the plants (Deans et al.,1996; Kustrak et al.,1996; Milos et al., 2000; Muller-Ribebau et al.,1995).

Also, previous studies showed that the other subspecies of *O. vulgare* L. constitute several different chemotypes based on their essential oil compositions. The thymol and carvacrol chemotypes were identified in *O.vulgare*L. spp. *hirtum* (Sivropoulou et al.,1996; Melgari et al., 1995; Kokkini et al.,1997; Skoula et al.,1999).

Furthermore, the carvacrol chemotype of essential oil is characteristic of *O.vulgare*L. ssp. *glandulosum* while thymol, sabinene-germacrene D chemotypes for *O. vulgare* L. spp. *gracile* (Melgari et al., 1995; Leto et al., 1994). The *O.vulgare* L. spp. *viride* growing in Iran, produced linalyl acetate-β-caryophyllene-sabinene chemotype of essential oil (Afsharypour et al., 1997), while carvacrol, γ- terpinene and p-cymene were characteristics of plants cultivated in Kishenev botanical garden in Russia (Bodrug et al., 1990). The other species of the *Origanum* genus mainly formed the similar chemotypes as *O. vulgare* L. The major constituents of the essential oil of *O. laevigatum* Boiss. were bicyclogermacrene, germacrene D and β-caryophyllene (Tucker and Maciarelle, 1992; Baser et al.,1996). The essential oil from *O. vulgare*L. is a complex mixture containing lipophilic monoterpenes, of which carvacrol and thymol are believed to be responsible for its antimicrobial properties (Lambert et al., 2001).

O. vulgare L. is rarely cultivated in Iran but it is the only species of the *Origanum* genus growing wild in the country. *O.vulgare* L. is widely found all over the country, particularly in Gilan, Mazandaran and West Azarbaijan provinces (SalehiSurmaghi, 2010).

So far, there is no report on the comparison of the yield and composition of the essential oil for cultivated and wild species of Iranian Oregano populations in the literatures. Therefore, in the present study, the aerial parts of *Origanum vulgare* L. were collected in the field and 4 habitats in mountains of Piranshahr district as well as their valley in southwestern of west Azarbaijan. Five chemotypes of essential oils were identified. The essential oils of the Carvacrol-γ-terpinene main chemotype were found in Origanum growing wild and cultivated populations. All essential oil obtained form these species showed almost the same pattern of antioxidant activity as ascorbic acid (vit C).

Materials and Methods

Plant materials

O. vulgare L. species were collected from five different geographical locations in June 2010 pre-flowering stage. Locations and taxonomical assignments were: (I) Field (1200m) O.v-F; (II) Gerdrahmat valley (2200m) O.v-W_1; (III) Khezr Abad forest(1750m) O.v-W_2; (IV) Perdanan mountains (2650m) O.v-W_3; (V) Gaderan highlands (2800m) O.v-W_4, in Piranshahr district, west Azarbaijan province, Iran.

The taxonomic identification of plant materials was confirmed by a senior plant taxonomist (Miss Mozhgan Larti), in Agricultural Research Center, Urmia, Iran. Collected plant materials were dried in shadow at room temperature for 5 days, and the air-dried aerial parts of the plant (the leaves with young stems) were powdered in a grinder with a 2 mm diameter mesh. A voucher specimen of each population has been deposited at the Herbarium of the Agricultural Research Center, Urmia, Iran.

Isolation of the essential oils

The oregano samples of at least 30 g of the air-dried aerial parts of the plants were hydro-distilled for 2h (3 times) using a clevenger-type apparatus (Britania pharma-copeia model), (yield ranging from 0.8% to 1.66% v/w). The obtained essential oils (EO) were dehydrated over anhydrous sodium

sulphate and after filtration, stored at 4°C until tested. (Sahin et al., 2004).

GC–MS analysis conditions

The analysis of the essential oil was performed using a Thermo Finnigan Trace MS2000 GC- MS, equipped with a HP-5 MS capillary column (30 m, 0.25 mm i.d, film thickness 0.25µm). For GC–MS detection an electron ionization system with ionization energy of 70 eV was used. Helium was the carrier gas, at a flow rate of 35 ml/min. Injector and detector temperatures were 200 and 250°C, respectively. Column temperature was initially kept at 120°C for 5 min, then gradually increased to 260°C at a 10°C /min rate. The components were identified based on the comparison of their relative retention time and retention indices with Wily library data of the GC–MS system and literature data (Adams, 2001).

Antioxidant activity (DPPH assay)

The hydrogen atoms or electrons donation ability of the essential oils were measured from the bleaching of purple coloured methanol solution of DPPH (Figure1). DPPH radical scavenging activity was determined as described by Zijia Zhang, et al.,2009, with a slight modification. Fifty microliter of various concentrations of the extracts in methanol was added to 5 ml of a 0.004% methanol solution of DPPH. After gentle mixing and 30 min incubation period at room temperature, the absorbance of the resulting solutions was measured at 517 nm using a Biowave S2100 spectrophotometer. The percent of DPPH inhibition by each oil sample was calculated using the following equation:

$$\%I = [Ao-(A_s-A_1)] / Ao \times 100_{Ao}$$

(Control)=50µl (methanol) +5ml (DPPH) A_s (Extract sample)=50µl (extract) +5ml (DPPH)
A_1 (Blank)=50µl (extract) + 5ml (methanol)

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. Synthetic antioxidant reagent, ascorbic acid (Vit C) was used as positive control and all tests were carried out in triplicate.

Results

Chemical composition of oregano essential oils

Compositions of the essential oils isolated from all the five localities are reported in Table 1. The extractions were performed in a clevenger apparatus for 120 min (3 time) and the essential oils in the field (O.v-F) and the 4 provenance oregano plants (O.v-W_1--- O.v-W_4) were obtained by hydro-distillation at yield of (0.80%) and (1.26%, 1.66%, 0.93%, 1.36%) (based on v/w), respectively. The GC/MS analysis of *Origanum vulgare* L. aerial parts showed the presence of both mono- and sesqui terpenes. 32 components were identified. In the cultivated plant (O.v-F), 22 constituents, representing 94.02% and in the 4 provenance plants (O.v-W_1--- O.v-W_4), 21, 25, 22, 20 constituents representing 96.55%, 95.66%, 95.8%, and 94.48% of the oils were identified, respectively (Table 1). The main components of the essential oils from cultivated plants (O.v-F) were carvacrol (29.85%), γ-terpinene (20.94%), α-himachalene (12.17%), β-pinene (11.67%) and from 4 localities were. (I) - O.v-W_1: carvacrol (23.54%), γ-terpinene (20.50%), thymol (15.41%), germacreneD-4-ol (9.26%), β-pinene (6.28%), (II)-O.v-W_2: carvacrol (59.37%), γ-terpinene (18.36%), cedrene (6.65%), (III)-O.v-W_3: carvacrol (58.51%), humulene (11.46%), γ-terpinene (9.56%), (IV)-O.v-W_4: carvacrol (67.09%), γ-terpinene (7.71%), humulene (7.67%). Five chemotypes of essential oils were identified. The main chemotype was carvacrol-γ-terpinene.(Table 2).

Table1. The composition of the essential oils (%) of Iranian Oregano(*O.vulgare* L.) plants*, a field & wild(4 various localities) in Piranshahr district, Iran.

NO	Composition	RI	Components(%)				
			Field	Provenance(Wild types)			
			O.v-F	O.v-W_1	O.v-W_2	O.v-W_3	O.v-W_4
1	**α-Pinene**	948		1.53	0.61	0.70	1.86
02	**βPinene -**	976	11.67	6.28	0.77	1.72	0.92
3	**γ-Terpinene**	1066	20.94	20.50	18.36	9.56	7.71
4	**cis-β-Terpineol**	1158	0.31	2.10		0.44	0.21
5	**2-Isopropyl-1-methoxy-4-methylbenzene**	1231	5.18	1.63	1.24	2.57	2.47
6	**Thymol**	1302		15.41			
7	**Carvacrol**	1304	29.85	23.54	59.37	58.51	67.09
8	**1-methoxymethyl- Decalin**	1338	0.47	0.47	0.20		
9	**Cedrene**	1398			6.65		
10	**β-Caryophyllene**	1418	2.28	5.10		3.71	
11	**γ-Muurolene**	1476		1.22	0.39	0.66	0.81
12	**α-Himachalene**	1494	12.17	3.38	0.41	0.87	
13	**Isocaryophyllene**	1494	0.49		1.49	0.21	1.87
14	**Ent-Spathulenol**	1536	2.35		0.75	1.98	0.55
15	**Limonen-6-ol, pivalate**	1560	0.09		0.12	0.05	
16	**Germacrene D-4-ol**	1567	3.46	9.26	0.09		0.14
17	**Caryophyllene oxide**	1581		2.11		0.33	0.39
18	**Humulene**	1579		0.49		11.46	7.67
19	**Isoaromadendrene epoxide**	1583		0.30	0.12		
20	**Carotol**	1596			0.74	0.21	0.65
21	**Bisabolol**	1625		0.45	0.71	0.33	0.24
22	**Cubenol**	1641	0.19		0.10		
23	**tau-Muurolol**	1642	0.79	1.01	0.90	1.11	0.87
24	**α-Cadinol**	1653	1.00	0.96	1.17	1.01	0.28
25	**Tetradecanoic acid**	1767	0.08		0.19		
26	**Hexahydrofarnesyl acetone**	1846	0.30		0.07		0.11
27	**3-Deoxyestradiol**	1949				0.08	0.15
28	**n-Hexadecanoic acid**	1983	0.39	0.29	0.35		
29	**Phytol**	2045	0.81		0.30	0.18	0.29
30	**Heneicosane**	2100	0.23				0.20
31	**Linolenic acid**	2191	0.85	0.33	0.33		
32	**n-Heptacosane**	2705	0.09	0.19	0.18	0.11	
	Total		**94.02**	**96.55**	**95.66**	**95.8**	**94.48**
	Monoterpene hydrocarbons		33.45	28.31	19.74	11.98	10.49
	Oxygenated monoterpenes		35.34	42.68	60.31	61.52	69.77
	Sesquiterpene hydrocarbons		15.53	10.68	9.10	16.91	10.35
	Oxygenated sesquiterpene		8.82	15.19	5.67	5.10	3.23
	Others		0.88	0.27	0.84	0.29	0.64

*The plants were collected before flowering in the 5 localities: O.v-F (cultivated), O.v-W1 (Gerdrahmat valleys), O.v-W2 (Khezr Abad forest), O.v-W3 (Perdanan mountains), O.v-W4 (Gaderan highlands).

Antioxidant activity of oregano essential oils

In the present study, the antioxidant activity of *O. vulgare* L. essential oil using the DPPH radical scavenging assay was determined. DPPH (2,2-diphenyl-1-picryl hydrazyl) is a nitrogen-centered free radical (Figure1). In the essential oils, the reactions followed a concentration dependent pattern. As shown in Figure 2, the DPPH radical scavenging activities of the essential oils and the positive control (Vit C) were significant. IC_{50} values for DPPH radical-scavenging activity for the essential oils in the provenance (O.v-W_1--- O.v-W_4) and field Oregano (O.v-F) were obtained at 5.3, 5.27, 5.26, 5.25 and 5.3 μg ml^{-1}, respectively which were comparable with vitamin C (5.05

μg ml-1). All of the extracts exhibited almost the same pattern of antioxidant activity as ascorbic acid. Regarding free radical scavenging activity, superiority of the essential oils could be attributed to the presence of oxygenated monoterpens e.g. carvacrol and other chemicals such as γ-terpinene, β - pinene and thymol as they comprise major constituents of the oils (Table 2).

Table2. The main chemotypes and their components of the known essential oils of *O.vulgare* L. plants under study in Iran.

percentage	Chemotypes	plants [a]
29.85	Carvacrol	**(I)- O.v-F**
20.94	γ-Terpinene	
12.17	α-Himachalene	
11.67	β - pinene	
23.54	Carvacrol	**(II)- O.v-W_1**
20.50	γ-Terpinene	
15.41	Thymol	
9.26	Germacrene D-4-ol	
6.28	β - pinene	
59.37	Carvacrol	**(III)- O.v-W_2**
18.36	γ-Terpinene	
6.65	Cedrene	
58.51	Carvacrol	**(IV)- O.v-W_3**
11.46	Humulene	
9.56	γ-Terpinene	
67.09	Carvacrol	**(V)- O.v-W_4**
7.71	γ-Terpinene	
7.67	Humulen	

[a] - The growing localities are shown in the footnotes of the Table-1.

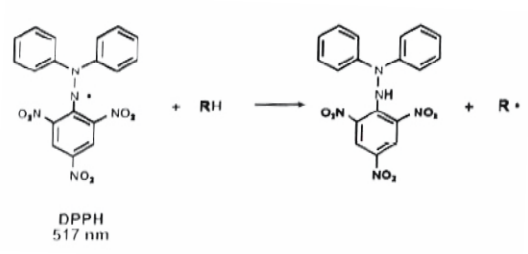

Figure1. DPPH radical scavenging reaction

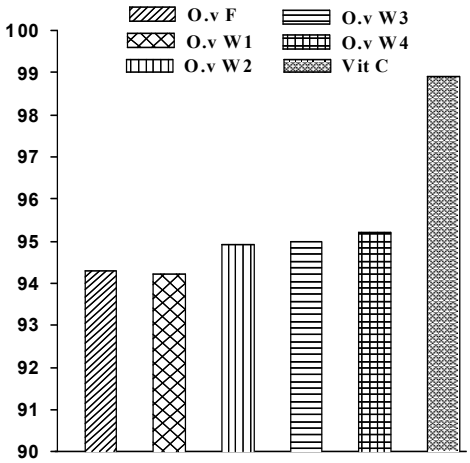

Figure 2. Antioxidant activity of *O. vulgare* L. essential oils defined as inhibition percentage in DPPH radical scavenging assay.

Discussion

The essential oils from *Origanum vulgare* L. plants collected in 5 localities in piranshahr, west Azarbaijan (Iran) contained carvacrol (ranging from 23.54% to 67.09%) as the main constituent (Table1,2). The plants with a relative high percentage of carvacrol in essential oil were collected in 3 locations: O.v-W_2 (59.37%), O.v-W_3 (58.51%), O.v-W_4 (67.09%). The two major constituents (carvacrol, γ-terpinene) of essential oils were the same in all samples from 5 localities (Table1,2). The oxygenated and hydrocarbon monoterpenes contributed 35.34% and 33.45% in oil sample collected from field or cultivated plants (O.v-F) followed by sesquiterpene hydrocarbons and oxygenated sesquiterpenes (15.53% and 8.82%), respectively. Carvacrol (29.85%) was the major oxygenated monoterpenoid and γ-terpinene (20.94%) and β-pinene (11.67%) were the major monoterpene hydrocarbons. Of the 24.35% sesquiterpenes, α-himachalene (12.17%), was the major component of this fraction. In the sample

population of Gerdrahmat valley (O.v-W_1), monoterpenes fraction consisted the highest proportion (70.99%) of the oil, of which oxygenated monoterpenes accounted for the 42.68%, with carvacrol (23.54%) and thymol (15.41%), being the major components of this fraction. Also γ-terpinene (20.50%), β-pinene (6.28%) and germacriene D-4-ol (9.26%) were the main components of monoterpenehydrocarbons and oxygenated sesquiterpenoids, respectively. In the Khezrabad forest collection (O.v-W_2), the mono- and sesquiterpenoids accounted for 80.05% and 14.77%, respectively. Carvacrol was recognized as the main oil component (59.37%) together with γ-terpinene (18.36%). Similarly, the major sesquiterpenoid was cedrene (6.65%). Monoterpenes constituted the main fraction of essential oil of Perdanan mountains (O.v-W_3), reaching 73.5% of the oil. Hydrocarbons and oxygenated components accounted for 11.98% and 61.52%, respectively. γ-terpinene (9.56%) was the major component of monoterpene hydrocarbon fraction and carvacrol (58.51%) was the most abundant oxygenated monoterpene. In the sample population of Gaderan highlands(O.v-W_4), monoterpenes consisted the highest proportion (80.26%) to the essential oil, of which oxygenated monoterpenes accounted for 69.77%, carvacrol (67.09%) being the main component of this fraction. The results of this study on oregano plants collected from forenamed localities showed that O.v-W_1 and O.v-F contained the highest and the least amount of essential oil (1.66% & 0.8% based on v/w), respectively and were found to contain carvacrol with varying concentrations ranging from 23.54% to 67.09% in O.v-W_1 and O.v-W_4, respectively (Table2).

In previous studies, it has been demonstrated that the chemical composition of the essential oil of *O. vulgare* L. varies with geographical location of the collection site, climate and other ecological factors (Melgari et al., 1995; Chalchat and Pasquier, 1998; Mockute et al., 2001). Our findings on the chemical composition of the essential oils of this specimen in various habitats are in accordance with previous reports.

DPPH radical scavenging activity of the oils was very high, and this was obviously related to its chemical composition. In several reports, thymol and carvacrol, in particular, were found to be the main antioxidant constituents of the oils isolated from several *Origanum* species (Milos et al., 2000; Barrata et al., 1998; Ruberto et al., 2002). The results of the current study on radical scavenging activity of the essential oil of *Origanum* species are in accordance with these reports as, the percentage of carvacrol was remarkably high (23.54% - 67.09%) in all of *O.vulgare* L. essential oils. These results showed that variation of ecological conditions such as altitude, edaphic, temperature, humidity, slope percentage and climate can affect not only the quantity and the quality of the essential oil components but also the DPPH scavenging activity.

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References

Adams R P. (2001).Identification of essential oils components by gas Chromatography/ quadrupole mass spectroscopy. Illinois, USA: AlluredPublishing Corporation.

Aligiannis N, Kalpoutzakis E, Mitaku S, &Chinou I B. (2001).Composition and antimicrobial activity of the essential oils of two *Origanum* species. Journal of Agriculture and Food Chemistry, 49: 4168–4170.

Afsharypuor S, Sajjadi E S, Erfan- Manesh, M. 1997.Volatile constituents of *O. vulgar e*ssp*viride* (syn. *O.heracleoticum*) from Iran.PlantaMedica, 63: 179-180.

Barrata M D S, Dorman H J D, Deans S G, Figueiredo A C,Barroso J G,Ruberto G. (1998). Chemical composition,antimicrobial and antioxidative activity of laurel, sage, rosemary, oregano and coriander essential oils. Journal of Essential Oil Research, 10: 618–627.

Baser K H C, Ozek T, Kurkcouglu M, Tumen G.1996. essential oil of *Origanum laevigatum* Boiss., Journal of Essential OilResearch, 8:185-186.

Berna'th J. 1997.Some scientific and practical aspects ofproduction and utilization of oregano in central Europe. In:Padulosi, S. (Ed.), Oregano, 14. Proceedings of the IPGRI International Workshop. Italy, Rome, pp: 75–92.

Bodrug M V, Dragalin I P,Vlad P F. 1990. Composition of essential oil of *Origanum heracleoticum* L. at vegetation phases (in Russian). Thesis of Simpheropol Conference, Ukrina, p:161.

Chalchat J C, Pasquier B. 1998. Morphological and chemical studies of *Origanum* clones: *Origanum vulgare* L. ssp. *vulgare*, Journal of Essential Oil Research, 10: 119–125.

Chatzopoulou PS, Koutsos TV, Katsiotis ST, Determination of the essential oil content and composition ofsome *Origanum* species cultivated in Greece, 5–8 Sep. 2004, Proceedings of 3rd conference on medicinal and aromatic plants of southeast European countries, Nitra,Slovak Republic, pp: 55–56.

D'Antuono L F, Galleti G C, Bocchini P. 2000. Variability ofessential oil content and composition of *Origanum vulgare* L. Populations from a North Mediterranean Area (Liguria Region,Northern Italy). Ann. Bot.-Lond, 86: 471–478.

Deans SG, Svoboda K, Gundidza M, Brechany E Y. 1992. Essential oil profiles of severe temperate and tropical aromatic plants: their antimicrobial and antioxidant activities. Acta Horticulture, 306: 229–232.

Guner A, Ozhatay N, Ekim T, Baser K H C. 2000. Flora of Turkey and the East Aegean Islands (Vol. 11 (supplement-II)).Edinburgh: Edinburgh University Press.

Kokkini S, Karousou R, Dardioti A, Krigas N, Lanaras T.1997. Autumn essential oils of Greek oregano.Phytochemistry, 44:883-886.

Kokkini S, Vokou D. 1989. Carvacrol-rich plants in Greece.Flavour Frag. J. 4: 1–7.

Kustrak D, Kuftinec J, Blazevic N. Maffei M.1996. Composition of the essential oil composition of two subspecies of *Satureja montana*. Journal of Essential Oil Research, 8:7-13.

Lagouri V, Blekas G, Tsimidou M, Kokkini S, Boskou D.1993. Composition and antioxidant activity of essential oils fromoregano plants grown wild in Greece. Z. Lebensm. Unters.Forsch. 197: 20–23.

Lambert RJW, Skandamis PN, Coote P, Nychas GJE. 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol, and carvacrol. Journal of Applied Microbiology, 91: 453–462.

Leto C, Carrubba A, Trapani P. 1994.Taxonomy, ecology, properties and uses of genus *Origanum*. Proceedings of the International Conference. Cultivation and Improvement of Medicinal Plants, Trento, Italy, pp:343-355.

Melegari M, Severi F, Bertoldi M, Benvenuti S, Circetta G, Morone FI, Bianchi A, Leto C, Carrubba A. 1995. Chemical characterization of essential oils of some *Origanum vulgare* L. subspecies of various origin. Rivista Italiana EPPOS,16: 21–29.

Milos M, Mastelic J, Jerkovic I. 2000. Chemical composition and antioxidant effect of glycosidically bound volatile compounds from oregano (*Origanum vulgare* L. ssp. *hirtum*. Food Chemistry, 71: 79–83.

Mockute D, Bernotiene G, Judzentiene A. 2001. The essential oil of *Origanum vulgare* L. ssp. *vulgare* growing wild in Vilnius district (Lithuania).Phytochemistry, 57: 65–69.

Muller-Riebau F, Berger B, Yegen O. 1995. Chemical composition and fungitoxic properties to phytopathogenic fungi of essential oils of selected aromatic plants growing wild in Turkey. Journal of Agriculture and Food Chemistry, 43(8): 2262–2266.

Omer EA. 1999. Response of wild Egyptian oregano to nitrogenfertilization in a sandy soil. Journal Plant Nutrient., 22: 103–114.

Ruberto G, Barrata M T, Sari M, Kaabexhe M. 2002.Chemical composition and antioxidant activity of essential oilsfrom Algerian *Origanum glandulosum* Desf.Flavour and FragranceJournal, 17: 251–254.

Sahin F, Gulluce M, Daferera D, SokmeneA, Sokmen M, Polissiou M, Agar G, Ozer H. 2004. Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp. *vulgare* in the eastern Anatolia region of Turkey. Food Control, 15: 549-557.

Salehi Surmaghi MH. 2010. Medicinal plants and phytotherapy. Nutrition world publications , Tehran, Iran, Vol:3,pp:368-371.

Skoula M, Gotsiou P, Naxakis G, Johnson BC. 1999. A chemosystematic investigation on the mono-and sesquiterpenoids in the genus *Origanum* (*Labiatae*). Phytochemistry, 52: 649-657.

Skoula M, Harborne JB. 2002. Taxonomy and chemistry. In:Kintzios, S.E. (Ed.), Oregano: The Genera *Origanum* and *Lippia*. Medicinal and Aromatic Plants—Industrial Profiles, 25. Taylor & Francis/CRC Press, USA, pp: 67–108.

Snogerup S. 1971.Evolutionary and plant geographical aspects of Chasmophyti communities. In P. H. Davis, P. C. Harper, & I. C.Hedge (Eds.), Plant life of South–West Asia. Edinburgh: The Botanical Society, pp: 157–170

Sivropoulou A, Papanikolaou E, Nikolaou C, Kokkini S, Lanaras T, Arsenakis M. 1996. Antimicrobial and cytotoxic activities of *Origanum* essential oils. Journal of Agriculture Food Chemistry, 44: 1202–1205.

Tucker O, Maciarelle M.1992. The essential oils *Origanum laevigatum,* Boiss. (*Labiatae*). Journal of Essential Oil Research, 4: 419-420.

Zijia Z, Liping L, Jeffrey M, Tao W, Zhengtao W. 2009. Antioxidant phenolic compounds from walnut kernels (*Juglansregia* L.). Food Chemistry., 113: 160–165.