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ARTICLE

Comparative ontogenetic survey of the essential oil composition in *Origanum vulgare* L., and *Origanum majorana* L.

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ABSTRACT The aim of this study was to evaluate the influences of the different harvest stages on the value and components of the essential oil (EO) of wild oregano (*Origanum vulgare* L.) and European marjoram (*Origanum majorana* L.). Two species were collected during five stages of vegetative and reproductive growth period with four replications. The content of EO in the dried aerial parts was determined by hydro-distillation of herbs, and its constituents determined by gas chromatography (GC) and gas chromatography mass spectrometry (GC/MS). The analysis showed that the amount of EO in *O. majorana* was more than *O. vulgare*. The highest EO content for both species were obtained in the full flowering stage. 78 and 39 components were identified in the EO of *O. vulgare* and *O. majorana*, respectively. The main components were germacrene D, (*trans*)caryophyllene, terpinene-4-ol, and α-terpinene in the EO of *O. vulgare*, and terpinene-4-ol, γ-terpinene, α-terpinene, and α-terpineol in the EO of *O. majorana*. In general, sesquiterpene compounds had maximum amounts in the EO of *O. vulgare* in five stages of growth, especially in the beginning of flowering stage. In contrast, monoterpene compounds had maximum value in the EO of *O. majorana* in the full flowering stage.
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KEY WORDS
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Introduction

Wild oregano (*Origanum vulgare* L.) or Mediterranean oregano is perennial herb with a wide distribution in the Mediterranean region, Irano-Sybryn, and Euro-Sybryn that grows on the rocky slopes in a wide range of altitudes (0-4000 meter) (Kordali 2008). Oregano grows in the Northern areas (Chalous, Rasht and Gorgan), Western (Azerbaijan, Ardebil, and Kurdistan), and Eastern (Ahar and Bojnoord) of Iran (Rechinger 1982). This species growing to 100 cm tall, shoot straight, hairy and reddish-green, oval leaves, dark green and covered with trichome, which has covered the surface of the lower lamina (Afsharypuor et al. 1997). In addition to the traditional use for the treatment of diseases of the stomach, intestines, constipation and respiratory problems (asthma), the aerial parts and the leaves of this species are being used throughout the world as a very pleasant and aromatic spices. Besides, it also has been used as a disinfectant and healer in many applications (Kordali 2008). Prevalence studies have shown the antifungal activity, antibacterial and antioxidant of the species' EO from genus *Origanum* (due to thymol, and carvacrol in them) (Muller 1989; Gouladis et al. 2003). In a study at the National Botanical Garden of Iran, the dominant components of EO in the *O. vulgare* were found to be β-caryophyllene, germacrene D, and (*cis*)sabinene hydrate (Barazandeh 2000). In another relevant study, the main components of EO in the *O. vulgare* ssp. *vulgare* in four regions of Italy were recognized to be β-caryophyllene, thymol, terpinene-4-ol, and (*para*)cymene (Melegari et al. 1995).

Marjoram (*Origanum majorana* L.) is a perennial plant, with opposite leaves, elliptical, toothless and small white flowers covered with 4 rows of whitey bracts. These bracts cause the appearance of globular flowers (Afsharypuor et al. 1997). Biochemical compounds of this species are the EO and tannins. Its essence is obtained by hydro-distillation and has a greenish yellow color and mild odor and taste, 40% terpene especially terpinene, terpinolene and sabinene (Naghdi Badi et al. 2004). Some studies indicate that it has antimicrobial and antifungal properties (Oliveira et al. 2009). This type of majoram has been used in flavoring foods and has some important properties such as calmative effect, energy provider, diuretic, sudatory and stomach tonic (Afsharypuor et al.

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1997). Research at the National Botanical Garden was conducted on this species. So that, sabinee and linalil acetate was the most important compounds of EO (Barazandeh 2000).

So far, many studies have been done on quantitative and qualitative changes in the components of EO and their composition during the period of growth. These changes depending on the type of plant were varied, as in some species the most EO were in pre-flowering stage, the others were in flowering stage and in some of them also the maximum amount of EO identified after flowering stage (Wang et al. 2009). According to previous studies, a scientific research was not performed on quantitative and qualitative changes in EO of oregano and marjoram cultivated in Iran, as yet. Therefore, study the effects of various harvest stages on the quantitative and qualitative of EO in both species was the aim of the present experiment. Additionally, the scientific results can be applied to the use of these herbs in the pharmaceutical and food industries.

Materials and Methods

Plant material and experimental field profile

In this study, seedlings of two species of oregano (*O. vulgare*) and marjoram (*O. majorana*) with registration codes MPISB-259, and MPISB-158, respectively, were prepared from the Germplasm Bank at Medicinal Plants Institute (MPI) of Academic Center for Education, Culture and Research (ACECR). In early April 2013, seedlings were planted at the experimental farm of medicinal plants institute located in the Karaj region for evaluation the influences of the different harvest stages during growth cycle on the value and components of the oregano and marjoram EO in the same climate conditions. The geographical location of the station was 35º 54' 17" N and 50º 53' 7" E with about 1461 m (elevation) altitude above the mean sea level. The soil was loam-silt with 0.071% N, 48.9 mg/kg phosphorous, 33.6 mg/kg potassium, EC 2.71 dS/m, and pH 8.3. During the experimental periods, the mean weekly temperature varied from 24.5 to 43.7 ºC in day and 9.1 to 23.5 ºC in night for growth season. The average weekly rainfall and weekly relative humidity were 1.4 mm and 50.34%, respectively. Each experimental plot was 5 m long and 2 m wide which prepared after tillage operations. There was 1 m space between the plots and 1.5 meters between the replications. In May, June and July 2013, the aerial parts of plants were collected in five growth stages (two stages in the vegetative phase and three stages in the reproductive phase) between 12:00 and 13:30 P.M. Conditions on the day of collection were clear and sunny for each of the five stages. The harvested plant materials were air-dried in a shaded place at a convenient temperature (22 ± 2 ºC) and in an air-flow during 6 days. The samples were transferred to the phytochemical analysis laboratory for measuring the percentage of essential oils and their components. For the sake of accuracy and reducing the possible errors, samples were randomly picked from separate experimental plot with four replications.

Extraction of EO and identification of its components

The content of EO in dried samples was determined by the hydro-distillation of 100 g herb in a Clevenger-type apparatus during 4 h. The oils were dried over anhydrous sodium sulphate and kept at 4 ºC until it was analyzed (British Pharmacopoeia 1988; Sefidkon and Abbasi 2006). The extracted essential oils were also identified by gas chromatography (GC) and gas chromatography mass spectrometry (GC/MS) analysis. Besides, the volatile constituents were analyzed using an Agilent instrument coupled with a 6890 mass system equipped with flame ionization detector (FID) equipped with BPX5 capillary columns (30 m × 0.25 mm, i.d. 0.25 µm film thicknesses). The carrier gas was helium, at a flow rate of 0.5 ml per min. The column temperature was gradually increased from 60 to 220 ºC with a rate of 2 ºC per min for the polar column and from 60 to 240 ºC with a rate of 3 ºC per min for the non-polar column. The injection volume was set to be 1 µL. The column temperature was considered to be the primary oven temperature 50 ºC and 5 minutes at this temperature was stopped, thermal gradient was 3 degrees per minute, increasing the temperature to 240 ºC and then at a rate of 15 degrees per minute, increasing the temperature to 300 ºC and 3 minutes at this temperature was stopped. Split injector was used at 290 ºC in a split mode at a ratio of 1:50. GC/MS analyses were performed using an Agilent 5973 mass spectrogram in the electron impact ionization mode at 70 eV, the mass range was m/z 40-500. Injector temperature was 220 ºC. The identity of the components was assigned by comparing their Kováts retention indices (KI) relative to C_8-C_{32} n-alkanes (Sigma Chemical Co., St. Louis, MO), obtained on non-polar DB-5 column with those provided in literature. Tentative identification of the compounds based on the comparison of their relative retention time and mass spectra with those of the NIST-98 and Wiley-275 library data of the GC/MS system and the literature data (Adams 2007) was carried out. Each extraction and the compound percentages were replicated four times.

Statistical analysis

All the obtained data were used in statistical analysis (Proc GLM) based on a one-way Analysis of Variance (ANOVA) with four replications, which was performed using the program of SAS software. The probabilities of significance were

Table 1. Means comparison* of chemical composition based on percentages in the EO of *O.vulgare* and *O.majorana* at different growth and developmental stages.

No.	Compound	KI	Beginning of vegetative growth		Full vegetative growth		Beginning of flowering		Mid-bloom		Full flowering	
			O. vulgare	*O. majorana*	*O. vulgare*	*O. majorana*	*O. vulgare*	*O. majorana*	*O. vulgare*	*O. majorana*	*O. vulgare*	*O. majorana*
1	(*2E*)Hexenal	855	1.07^{b}	0.51^{cd}	0.42^{d}	1.42^{a}	0.12^{f}	1.13^{b}	0.59^{c}	0.24^{ef}	0.28^{e}	0.12^{f}
2	Sabinene	975	4.56^{c}	5.87^{a}	4.66^{c}	5.03^{bc}	1.49^{e}	4.70^{c}	2.42^{d}	5.40^{ab}	2.14^{d}	5.34^{ab}
3	(1)Octen-3-ol	979	4.36^{b}	-	5.32^{a}	-	2.07^{d}	-	4.73^{b}	-	3.62^{c}	-
4	Myrcene	991	1.79^{b}	2.01^{ab}	1.66^{b}	1.62^{b}	0.58^{c}	0.76^{c}	0.90^{c}	2.09^{ab}	0.94^{c}	2.47^{a}
5	α-Terpinene	1017	7.32^{abcd}	10.39^{a}	6.13^{bcd}	10.10^{a}	2.64^{e}	8.11^{abc}	4.37^{de}	8.96^{ab}	5.57^{cde}	8.76^{ab}
6	(*ortho*)Cymene	1026	0.76^{b}	0.61^{b}	0.75^{b}	0.62^{b}	0.54^{b}	0.53^{b}	0.78^{b}	1.60^{a}	0.83^{b}	1.75^{a}
7	(δ-*3*)Carene	1030	-	1.19^{b}	-	1.29^{a}	-	1.00^{c}	-	1.26^{a}	-	1.29^{a}
8	β-Phellandrene	1030	1.87^{a}	-	0.54^{c}	-	0.37^{d}	-	0.64^{c}	-	0.87^{b}	-
9	(*Z*-β)Ocimene	1037	1.13^{d}	3.81^{a}	0.12^{e}	3.15^{b}	0.10^{e}	2.36^{c}	0.20^{e}	2.99^{b}	0.22^{e}	3.12^{b}
10	(*E*-β)Ocimene	1050	0.24^{c}	3.42^{a}	0.20^{c}	3.10^{a}	0.16^{c}	2.34^{b}	0.29^{c}	3.41^{a}	0.31^{c}	3.53^{a}
11	γ-Terpinene	1060	3.23^{f}	16.22^{a}	3.12^{f}	15.61^{ab}	1.59^{g}	13.94^{d}	4.32^{e}	14.40^{cd}	4.19^{e}	15.26^{bc}
12	(*cis*)Sabinene hydrate	1070	-	0.72^{b}	-	0.69^{b}	-	0.57^{c}	-	0.82^{a}	0.09^{d}	0.83^{a}
13	Terpinolene	1089	2.47^{c}	3.72^{a}	2.40^{c}	3.50^{ab}	0.31^{e}	2.59^{c}	0.45^{de}	3.09^{b}	0.79^{d}	3.41^{ab}
14	Linalool	1097	1.69^{c}	4.06^{a}	0.89^{de}	3.67^{ab}	0.82^{e}	3.33^{b}	1.09^{de}	3.81^{ab}	1.46^{cd}	4.08^{a}
15	(*trans*)Sabinene hydrate	1098	-	2.73^{bc}	-	2.37^{c}	-	2.36^{c}	-	3.00^{ab}	-	3.40^{a}
16	(*cis-para*)Menth-2-en-1-ol	1122	-	1.95^{b}	-	1.92^{b}	-	1.89^{b}	-	2.63^{a}	-	2.82^{a}
17	(*trans-para*)Menth-2-en-1-ol	1141	-	1.36^{b}	-	1.36^{b}	-	1.25^{b}	-	2.14^{a}	0.34^{c}	2.09^{a}
18	Terpinene-4-ol	1177	6.27^{c}	25.65^{a}	0.57^{c}	23.09^{a}	5.15^{c}	23.11^{a}	7.05^{c}	22.15^{a}	12.99^{b}	21.81^{a}
19	α-Terpineol	1189	0.47^{e}	5.13^{b}	0.42^{e}	4.53^{c}	0.37^{e}	4.65^{bc}	0.60^{e}	4.99^{bc}	1.56^{d}	6.39^{a}
20	β-Bourbonene	1388	0.91^{c}	-	1.33^{b}	-	1.95^{a}	-	1.47^{b}	-	1.35^{b}	-
21	(*trans*)Caryophyllene	1419	11.51^{d}	1.95^{f}	11.71^{d}	1.98^{f}	21.46^{a}	7.02^{e}	18.45^{b}	3.69^{f}	15.09^{c}	1.05^{f}
22	β-Gurjunene	1434	0.38^{c}	-	0.39^{c}	-	0.57^{a}	-	0.54^{ab}	-	0.51^{b}	-
23	Aromadendren	1441	-	-	-	-	0.42^{a}	-	0.40^{a}	-	0.27^{b}	-
24	α-Humulene	1455	2.72^{bc}	0.09^{d}	3.04^{b}	0.09^{d}	3.62^{a}	0.19^{d}	2.74^{bc}	0.15^{d}	2.54^{c}	0.12^{d}
25	Germacrene D	1485	16.31^{bc}	-	17.36^{b}	-	18.99^{a}	-	17.26^{b}	-	15.73^{c}	-
26	epi-Bicyclogermacrene	1499	2.22^{a}	-	2.10^{b}	-	-	-	-	-	-	-
27	Bicyclogermacrene	1500	3.10^{bc}	2.10^{d}	3.74^{ab}	2.26^{d}	4.00^{a}	4.06^{a}	3.78^{ab}	2.67^{cd}	3.75^{ab}	1.19^{e}
28	(*E,E*-α) Farnesene	1506	6.53^{b}	-	6.13^{b}	-	9.12^{a}	-	6.47^{b}	-	2.48^{c}	-
29	δ-Cadinene	1523	2.09^{b}	-	2.08^{b}	-	2.69^{a}	-	2.67^{a}	-	2.20^{b}	-
30	Spathulenol	1578	1.64^{ab}	0.16^{e}	1.94^{a}	1.21^{bc}	1.93^{a}	1.72^{a}	1.02^{cd}	0.14^{e}	0.56^{de}	0.11^{e}
31	Caryophyllene oxide	1583	1.45^{ab}	-	1.50^{ab}	-	1.60^{a}	1.81^{a}	1.16^{b}	0.09^{d}	0.66^{c}	0.08^{d}
32	α-Cadinol	1654	2.06^{b}	-	2.84^{a}	-	2.37^{b}	-	1.42^{c}	-	1.25^{c}	-
33	EO	-	0.14^{f}	1.25^{b}	0.14^{f}	1.23^{b}	0.13^{f}	1.09^{c}	0.34^{e}	1.38^{a}	0.67^{d}	1.50^{a}

*Means in each row followed by the same letter (a-g) are not significantly different according to Duncan's multiple range test at the 5% level of probability. The obtained values were expressed as mean from three replications.

used for testing the significance among the species during five growth stages. Then, the differences between means were compared by Duncan's multiple range test at 95% confidence interval.

Results

Seasonal variations of the isolated chemical composition in EO from *O. vulgare* and *O. majorana* were analyzed by GC and GC/MS, and the results were shown in Table 1. Analysis of variance for the essential oil content and composition demonstrated that there were statistically significant differences between the both species ($p \leq 0.01$) and different stages of harvest ($p \leq 0.01$, and $p \leq 0.05$). The results showed that 88 different compounds were detected in total of both species. In respect of comparative evaluation in the essential oil composition, only 10 and 49 compounds were in the *O. majorana* and *O. vulgare*, respectively, while 29 compounds were common in both species (Table 1).

Overall, 78 active components were known in EO of the

Table 2. Means comparison* of compound groups based on percentages in the EO of *O.vulgare* and *O.majorana* at different growth and development stages.

No.	Compound	Beginning of vegetative growth		Full vegetative growth		Beginning of flowering		Mid-bloom		Full flowering	
		O. vulgare	*O. majorana*	*O. vulgare*	*O. majorana*	*O. vulgare*	*O. majorana*	*O. vulgare*	*O. majorana*	*O. vulgare*	*O. majorana*
1	Monoterpene hydrocarbons	25.39^{c}	54.43^{a}	21.44^{cd}	50.53^{a}	9.33^{f}	42.53^{b}	16.28^{e}	52.33^{a}	18.51^{de}	55.08^{a}
2	Oxygenated monoterpenes	8.63^{d}	37.39^{ab}	8.03^{d}	34.72^{b}	6.44^{d}	34.10^{b}	8.74^{d}	35.62^{ab}	20.03^{c}	38.56^{a}
3	Sesquiterpene hydrocarbons	48.16^{c}	4.96^{e}	51.03^{c}	5.25^{e}	69.18^{a}	14.60^{d}	60.06^{b}	7.64^{e}	50.46^{c}	3.54^{e}
4	Oxygenated sesquiterpenes	7.99^{b}	0.16^{e}	9.53^{a}	1.21^{e}	9.94^{a}	3.53^{d}	5.50^{c}	0.23^{e}	3.07^{d}	0.19^{e}
5	Others	7.07^{a}	1.91^{d}	7.32^{a}	5.85^{b}	3.26^{c}	3.30^{c}	6.95^{a}	1.53^{d}	5.52^{b}	1.10^{d}

*Means in each row followed by the same letter (a-g) are not significantly different according to Duncan's multiple range test at the 5% level of probability. The obtained values were expressed as mean from three replications.

O. vulgare. Many components were separately identified in the beginning of vegetative growth (52), full vegetative growth (50), beginning flowering (55), mid-bloom (50), and full flowering stage (69) which these components were 97.24, 97.36, 98.16, 97.53, and 97.59% of EO in the O. *vulgare*, respectively. The major components in the EO were germacrene D and *(trans)*caryophyllene in the beginning of vegetative growth, full vegetative growth, and full flowering stage and vice versa, (trans)caryophyllene and germacrene D in the beginning flowering, and mid-bloom stage (Table 1). Overall, 39 active components were recognized in EO of the *O. majorana*. Several components were separately indicated in the beginning of vegetative growth (37), full vegetative growth (37), beginning flowering (37), mid-bloom (38), and full flowering stage (39) that these components were 98.84, 97.85, 89.09, 97.36, and 98.46% of EO in the *O. majorana*, respectively. The main components in the EO were terpinene-4-ol and γ-terpinene in all growth stages, respectively (Table 1).

Analysis of variance on the categories of the compounds revealed that there were statistically significant ($p \leq 0.01$) differences between the both species in different stages of harvest. According to the comparison of the means, the highest content of compounds were obtained for sesquiterpene hydrocarbons and oxygenated sesquiterpenes in the beginning of flowering stage, and also oxygenated monoterpenes in the full flowering stage in both species. The maximum amount of monoterpene hydrocarbons in EO of *O. vulgare* and *O. majorana* were observed in the beginning of vegetative growth and full flowering stage, respectively (Table 2). Maximum amount of total monoterpenes with values of 38.54% (*O. vulgare*) and 93.64% (*O. majorana*) and total sesquiterpenes with values of 79.12% (*O. vulgare*) and 18.13% (*O. majorana*) was respectively related to the full flowering and beginning flowering stage. Ratio of total monoterpenes/total sesquiterpenes (0.72 and 25.10) was also associated to the full flowering stage in both species (Table 2). With a general account, sesquiterpene compounds had the maximum amounts in EO of *O. vulgare* in five stages of growth especially in the beginning of flowering stage. In contrast, monoterpene compounds had maximum value in EO of *O. majorana* (Table 2).

Discussion

Comparison of the obtained EO from aerial parts of *O. vulgare* and *O. majorana* in different stages showed that the maximum value of EO (0.67%, and 1.5%, respectively) reached in the final harvesting time. In this regard, our results are consistent with the findings of Baranauskien et al. (2013). They studied the effect of harvesting time on yield and EO components in two subspecies of the *O. vulgare* growing in Lithuania which the highest value of EO (0.56%) obtained in the full flowering stage. Our analysis showed that the maximum EO being reached during full flowering stage in both species. Furthermore, Selami et al. (2009) studied the EO content of *O. majorana* growing in the Tunisia and determined the maximum of EO being during full flowering stage. Many studies were done on aromatic plants and all of them indicate that the maximum content of EO being during full flowering stage and end of flowering stage such as Ozguven and Tansi (1998) on *Thymus vulgaris*, Mallavarapu et al. (1999) on *Artemisia pallens*, Sefidkon et al. (2007) on *Satureja rechingeri*, Kizil et al. (2008) on *Origanum onites*, and Verdian (2008) on *Laurus nobilis*. Decrease in the EO in vegetative growth stage can be caused by low speed biosynthesis of volatile compounds during this stage which may be due to the relative inactivity of enzymes required for the synthesis of certain compounds.

Naghdi Badi et al. (2004), and Oliveira et al. (2005) reported that yields of EO during the development stages increasing and reaches its maximum in the flowering stage.

Comparison of the chemical composition in the EO of *O. vulgare* and *O. majorana* in five different development stages showed significant differences in major components. Galoburda et al. (2008) studied the compounds in EO of *O. vulgare* in development stages and determined that sabinene, (*trans*)caryophyllene, germacrene D, and (*Z*-β)-ocimene been the major constituents of the EO, while in our study germacrene D, (*trans*)caryophyllene, terpinene-4-ol, α-terpinene, and γ-terpinene were major constituents. Content and compounds of the EO varied with phenological changes that matched with other studies on Lamiaceae family species such as *Salvia officinalis*, *Salvia fruicosa*, *O. vulgare*, and species from other aromatic families such as *Pelargonium graveolens* (Putievsky et al. 1986; Ravid and Dudai 1988; Ravid and Putievsky 1984). Raina and Negi (2012) studied on the two EO components of *O. vulgare* and *O. majorana* in India and determined that thymol, carvacrol, γ-terpinene and *p*-cymene were the major components of the *O. vulgare*, which thymol and carvacrol made up 40% of EO while these compounds were quietly absent in the *O. majorana.* But in present study, germacrene D, (*trans*)caryophyllene, terpinene-4-ol, and α-terpinene in the *O. vulgare* and terpinene-4-ol, γ-terpinene, α-terpinene, and α-terpineol in the *O.majorana* were the main constituents. Comparing the results with previous research (Rohloff et al. 2005; Mirjalili et al. 2006; Telci and His 2008) suggesting that physiological processes and harvesting time significantly affected the EO components. Komaitis (1992) determined that terpinene-4-ol was the main compound of EO in the Greece (37%) and then was *p*-cymene (12.05%). Trevino and Johnson (2000) also achieved a similar result about the *O. majorana*. Vera and Chane (1999) studied the EO of *O. majorana* grown in the Reunion Island and determined that the main components included terpinene-4-ol (38.4%), (*cis*)sabinene hydrate (15.0%), *p*-cymene (7.0%), and γ-terpinene (6.9%). The analyzed EO of *O. majorana* grown in the Argentina by Branchio et al. (2008) had general similarity to the present EO, terpinene-4-ol (55.09%), (*trans*)sabinene hydrate (13.2%), α-terpineol (9.09%), and (*cis*)sabinene hydrate (8.37%). Diversity in the EO compounds of *O. majorana* at different regions can be caused by interaction of genetic and environmental factors. Ricci et al. (2005) determined that the EO compounds of aromatic plants in particular were influenced by environmental factors. Also, Sangwan et al. (2001); Kim and Lee (2004) believed that the EO compounds were influenced by many factors, including genetic, development stages, extraction method and assay conditions. Baranski et al. (2005) determined that the subspecies of *O. vulgare* grown in the Lithuania were related to chemotype sabinian, β-ocimene, β-caryophyllene and germacrene D. This opinion was resembled with present results and also with previous studies (Radusien et al. 2005; Mockute et al. 2001).

Comparison of the classified compounds in EO of *O. vulgare* and *O. majorana* in five different development stages showed significant differences in the compounds groups. In the beginning of flowering, the level of monoterpenes hydrocarbons drastically reduced and sesquiterpenes hydrocarbons had the maximum amount of EO and then in flowering stage, monoterpenes hydrocarbons were increased again. Researchers like Sarir et al. (1982); Jolivet et al. (1971); Baser et al. (1993); Sellami et al. (2009) stated that *O. majorana* species studied in the different regions of world. There were two main chemotype: a chemotype mostly monoterpenes alcohol and other included complex of phenols. In the first category terpinene-4-ol with other monoterpenes alcohol, such as (*cis* and *trans*)sabinene hydrate formed the major component of EO and the second group, the main component consisted mainly of thymol and/or carvacrol. However, our results showed that over 90% of EO components in the *O. majorana* were monoterpenes and unlike to previous research reports. The amounts of phenolic compounds were very low that matched with studies on the *O. majorana* which cultivated in the Finland (Vera and Chane 1999). Precursors of the phenolic compounds such as *p*-cymene, and γ-terpinene in the *O. vulgare* were the greater than *O. majorana*, while sabinian, (*cis and trans*)sabinene hydrate, and α-terpineol were the higher in *O. majorana.* Also, amount of the oxygenated monoterpenes were much more in the *O. majorana*. It seems the high content of terpinene-4-ol due to rearrangements occurs during the distillation process (Sangwan et al. 2001; Kim and Lee 2004).

Conclusions

Since, all samples of both species in this study were cultured in the same climatic conditions. Therefore, the changes of content and composition in the EO were relevant to the genetic differences between species in dealing with seasonal changes. By focusing on the EO and their compounds, our results showed that maximum amount of EO of *O. vulgare* and *O. majorana* were obtained in the full flowering stage. In the EO of *O. vulgare*, germacrene D was the major component that its maximum amount was detected in the beginning flowering stage. In the *O. majorana*, terpinene-4-ol was the major component of EO that its maximum amount was identified in the beginning of the vegetative growth. Sesquiterpene compounds had the maximum amounts in EO of *O. vulgare* in five stages of growth, especially in the beginning of flowering stage. In contrast, monoterpene compounds had the maximum value in EO of *O. majorana* in the full flowering stage. *O. majorana* is good source of EO, especially in full bloom.

The EO of *O. vulgare* is also important as it contains important bioactive compounds such germacrene D, and (*trans*) caryophyllene that would be harvest in a good time to achieve maximum concentration of these compounds in the EO and being exploited in pharmaceutical and food industries.

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