



The Impact of Macro Environmental Factors on Essential Oils of *Oliveria decumbens* Vent. from Different Regions of Iran

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Received 2017 August 05; Revised 2018 December 18; Accepted 2018 January 22.

Abstract

Background: Environmental factors may affect qualitative and quantitative properties of essential oil in medicinal plants.

Objectives: The aim of the present study was to determine the effect of macro environmental factors on essential oils content and composition of Iranian populations of *Oliveria decumbens*.

Methods: The aerial parts of 25 wild populations of *Oliveria decumbens* Vent. were collected from various natural habitats in the southwest of Iran. The essential oil of samples was analyzed by gas chromatography (GC) and gas chromatography-mass spectroscopy (GC-MS). Therefore, macro environmental factors were determined by the PCA method. The correlations between the factors were calculated based on the Pearson correlation coefficient.

Results: The major compounds of essential oils were thymol, carvacrol, p-cymene, and γ -terpinene, respectively, with an average of 28.77%, 25.31%, 14.02%, and 8.12%. The results showed that the altitude, mean evaporative per month (MEM) and Mean Day Temperature (MDT) were effective macro environmental factors on essential oil, thymol, and carvacrol content.

Conclusions: The highest content of essential oil, thymol, and carvacrol was related to warm and semi-humid regions.

Keywords: *Oliveria decumbens* Vent., Essential Oil, Environmental Factors, Thymol, Population

1. Background

Oliveria decumbens Vent., a member of the Umbelliferae family, is a shrub commonly found in the south-east of Iran. In traditional medicine, its aerial part was used for treating indigestion, diarrhea, abdominal pains, and fever (1). According to recent studies, it can be used as an antibacterial (2), antifungal (3), antioxidant (4), and antimicrobial agent (1). These properties are influenced by quantity and quality of their secondary metabolites, which are usually produced under the influence of special environmental and climatic conditions. Therefore, the identification of the best environmental and climatic conditions is necessary.

A study on *Thymus vulgaris* showed that environmental conditions influenced essential oil composition (5). The study on chemical composition of essential oils and methanolic extracts of *Thymus mastichina* populations indicated that the total phenol contents of the methanolic extracts varied between 2.90 and 9.15 mg GAE/g extract. Also, the oil yields varied between 2.27 and 6.48 mL/100 g

DM. This difference seems to be related to the different environmental conditions (6). The investigation on *Lavandula × intermedia* from ten regions of Zagros regions of Iran showed significant differences for essential oil content and composition due to differences in environmental conditions (7). In another study, the essential oil content of *Thymus pubescens* had a significant positive relationship with soil pH and also a significant negative relationship with altitude (7). The major environmental factors had a significant effect on essential oil of *Verbascum songaricum*. It's essential oil content was increased under semi-arid cold climate, uplands, higher precipitation, relatively light texture, and fertile soil (8).

2. Objectives

Climate variation and altitude changes affect essential oil biosynthesis and accumulation in medicinal herbs. Therefore, evaluation of the effects of different climatic conditions on plants is important. Since, there is no published and comprehensive research related to the effect of

different climatic conditions on *O. decumbens*, the present study was carried out to explore the effects of macro environmental factors on phytochemical changes of *O. decumbens*.

3. Methods

3.1. Geographical Characteristics of Habitats

According to a previous study, natural habitats of *Olive-ria decumbens* were identified (9). The locations characteristics are shown in Table 1.

3.2. Metrological Characteristics of Habitats

The climatic characteristics were determined using the nearest meteorological stations. The type of climate classification of each region was indicated according to the coefficient of temperance method (Temoenatenus Index) (Table 1).

3.3. Soil Characteristics of Habitats

The soil samples were taken from the root depth of plants and their physicochemical properties were determined (Table 2). The total nitrogen (N), phosphorus (P), and potassium (K) were determined using the Kjeldahl procedure, Olsen method, and a flame photometer, respectively. The total neutralizing value (TNV %) by neutralization with HCl and the organic matter (OM%) by titration method were measured (10).

3.4. Plant Collection

In all habitats, area 1 parts of *O. decumbens* in the flowering stage were taken by a random-systematic method along with the located transects. This plant was identified and recorded by Seed Bank of Medicinal Plants Institute, ACECR (1364-MPISB). Four plants were collected from each transect between mid-June to mid-August, 2015. The samples were then dried in shaded areas at room temperature.

3.5. Essential Oils Extraction

The Essential Oil (EO) was obtained by hydrodistillation method for three hours, using Clevenger apparatus, according to the pharmacopoeia (11). Then, essential oil content (%) was measured based on mass of essential oil (g) extracted from 50 g mass of dried aerial part (g) using the following formula (12).

$$EO \text{ content } \% = \frac{\text{mass of EO obtain (g)}}{\text{mass of dry matter (g)}} \times 100 \quad (1)$$

3.6. Gas Chromatography

Analytical gas chromatography (GC) was performed using a Younglin Acme6000 gas chromatograph equipped with BP-5 column 30 m, 0.25 mm internal diameter, and 0.25 μm film thickness. The column temperature program was set as follows: Oven temperature was held at 50°C for five minutes, from 50 to 240°C at a rate of 3°C/min and to 300°C at a rate of 15°C/min and held at this temperature for three minutes. Injection and detection temperature was 290°C and 300°C, respectively. Helium was used as carrier gas with a linear velocity of 0.8 mL/min. All samples were diluted 1:100 by the N-Hexane solvent. One microliter of the diluted sample was injected manually in the split mode 1:25 (13).

3.7. Gas Chromatography-Mass Spectroscopy

The gas chromatography-mass spectroscopy (GC-MS) analyses were carried out with the Agilent 6890 system equipped with a Mass-Spectrometer Agilent 5973, as a detector and a BPX5 column (30 m, 0.25 mm internal diameter, and 0.25 μm film thicknesses). The carrier gas was helium at a flow rate of 0.5 mL/min. The column temperature was held at 50°C for five minutes, and was gradually increased from 50 to 240°C with a rate of 3°C/min and to 300°C with a rate of 15°C/min and held at this temperature for three minutes. For detection, an electron ionization system was used with ionization energy of 70 Electron Volt. The injector and ionization source temperatures were 290 and 220°C, respectively. One microliter of the sample was injected manually in the split mode 1:25 (13).

3.8. Identification of Components

The identification of the constituents was based on the comparison of the Kovats Indices (KI) to n-alkanes ($\text{C}_8\text{-C}_{20}$) and ($\text{C}_{20}\text{-C}_{40}$) and mass spectra of authentic samples with library data of the GC-MS system and the literature data. Relative percentages of the identified compounds were obtained by the electronic integration of the FID peak areas.

3.9. Statistical Analysis

At first, the macro environmental factors were indicated by Principal Components Analysis (PCA), and correlations between these factors were calculated based on Pearson correlation coefficient by the Minitab software. As a result, the most important environmental factors and how they influenced the production of main essential oil component were identified.

Table 1. Geographical Origins and Climate Characteristics of *O. decumbens* Habitats

Population No.	Province	Region	Latitude, N	Longitude, E	Altitude, m	MDT, °C	MDRH, %	MPM, mm	MEM, mm	MSM, h	Climate
1	Kermanshah	Soomar	33°55'03.6"N	45°47'18.3"E	500	25.63	41	20.36	219	256	Warm and dry
2	Kermanshah	Qasr-e Shirin	34°29'02.9"N	45°34'27.4"E	345	24.59	40	30.25	218	259	Warm and dry
3	Kermanshah	SarpolZahab	34°26'29.8"N	45°53'45.6"E	609	20.39	47	35.48	184	247	Warm and dry
4	Ilam	Bedreh	33°16'27.5"N	47°09'23.3"E	896	22.58	43	17.41	132	242	Warm and dry
5	Ilam	Dehloran	32°46'39.7"N	47°02'09.5"E	365	26.76	44	22.50	194	245	Warm and dry
6	Ilam	Abdanan	33°00'39.2"N	47°23'45.9"E	981	23.18	41	45.71	142	243	Warm and dry
7	Ilam	DarrehShahr	33°04'40.0"N	47°30'07.0"E	655	22.58	41	34.25	141	241	Warm and dry
8	Khuzestan	Dezful	32°21'56.6"N	47°57'59.0"E	196	24.41	45	32.27	217	258	Warm and semi-humid
9	Khuzestan	Bebbahan	30°39'19.9"N	50°12'45.5"E	302	25.00	43	34.25	221	261	Warm and semi-humid
10	Khuzestan	Fedelak	32°05'25.5"N	48°53'24.9"E	236	27.88	44	25.06	209	256	Warm and semi-humid
11	Khuzestan	Perchestan	32°03'13.6"N	48°55'13.7"E	165	27.88	44	25.06	209	256	Warm and semi-humid
12	Khuzestan	Lali	32°19'07.1"N	49°05'24.5"E	359	26.73	41	32.75	219	252	Warm and semi-humid
13	Khuzestan	MasjedSoleyman	32°00'56.0"N	49°13'37.5"E	275	25.73	39	35.50	218	255	Warm and semi-humid
14	Khuzestan	KuhZar	32°05'14.3"N	48°57'12.5"E	109	27.07	43	22.79	207	257	Warm and semi-humid
15	Fars	Jahrom1	28°29'39.8"N	53°35'59.0"E	1104	23.42	47	19.40	219	279	Warm and dry
16	Fars	Jahrom 2	28°38'20.0"N	53°37'26.5"E	1021	23.42	47	19.40	219	279	Warm and dry
17	Fars	Kazerun 1	29°36'24.2"N	51°34'32.7"E	874	24.38	42	29.46	255	267	Warm and semi-humid
18	Fars	kazerun 2	29°39'46.1"N	51°37'00.3"E	895	24.38	42	29.46	255	267	Warm and semi-humid
19	Fars	DoshmanZiari	30°06'51.0"N	52°21'39.6"E	1699	18.31	40	39.33	172	274	Warm and semi-humid
20	Fars	Nourabad	30°04'49.6"N	51°32'48.9"E	981	23.10	48	39.49	196	251	Warm and semi-humid
21	Fars	Ghaemiyeh	29°51'45.6"N	51°34'56.7"E	913	21.96	46	26.95	211	257	Warm and semi-humid
22	Lorestan	Kuhdasht	33°33'06.6"N	47°32'34.8"E	1260	16.62	48	31.39	157	245	Moderate and dry
23	ChaharMahal VaBakhtiari	Lordegan	31°31'14.8"N	50°46'00.3"E	1556	16.36	51	45.96	132	264	Moderate and dry
24	Bushehr	Borazjan	29°21'20.2"N	51°16'56.8"E	146	26.66	43	19.02	246	257	Warm and humid
25	Hormozgan	Geno	27°24'00.6"N	56°14'05.9"E	653	27.32	53	14.51	259	270	Warm and humid

Abbreviations: MDT, mean day temperature; MDRH, mean day relative humidity; MEM, mean evaporative per month; MPM, mean precipitation per month; MSM, mean sun hour per month.

4. Results

4.1. Essential Oil Content and Composition

The essential oil content in dry plant materials ranged between 2.25% and 6%. The minimum and maximum content of essential oil of *O. decumbens* populations were obtained in kazerun 2 (2.25%) and Fedelak (6%) populations, respectively. The results obtained from GC and GC-MS analyses of the oils showed that the main compounds were thymol, carvacrol, p-cymene, and γ -terpinene. The highest and the lowest values of thymol were observed in Kuhdasht (43.9%) and Fedelak (0%) populations, respectively. However, carvacrol content was differentially influenced by these two populations and its highest and lowest values were detected in Fedelak (35.98%) and Kuhdasht (0%) populations, respectively (Table 3).

4.2. Important Environmental Factors Based on PCA

Based on Principal Components Analysis (PCA), Latitude (N), Longitude (E), Altitude, Mean Day Temperature (MDT), mean evaporative per month of Class A pan evaporation (MEM), soil pH, and clay (%) were major environmental factors that had significant effects on essential oil content and composition (Figure 1).

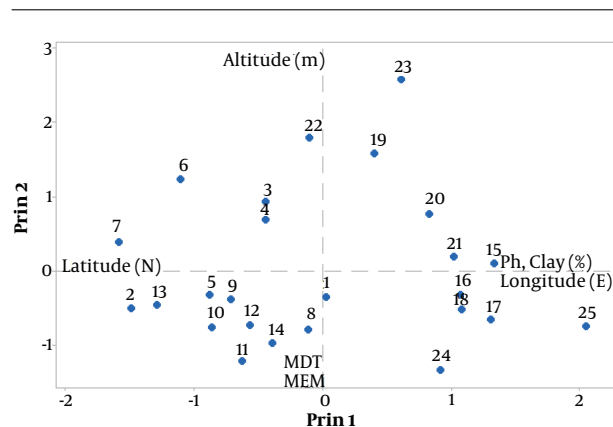


Figure 1. Principle Component Analysis (PCA) based on environmental parameters

4.3. Correlation Between Environmental Factors and Essential Oil Composition

The significant correlation between two variables were determined by P values greater than 0.05. Among the principal factors, only altitude showed a significant relationship with essential oil content, however, it was negative (Ta-

Table 2. Soil Physicochemical Characteristics of Different Habitats of *O. decumbens* Populations

Population No	P (Av.), ppm	K (Av.), ppm	N, %	OM, %	TNV, %	EC, dS/m	pH	Sand, %	Clay, %	Silt, %
1	3.4	141	0.17	0.41	41.1	2.1	8.2	33	30	37
2	24	548	0.73	0.87	7.7	0.48	6.9	22	33	45
3	9.7	280	0.15	1.3	48	0.52	7.3	25	30	45
4	13.4	400	0.25	0.46	15	0.63	7.42	52.3	20.7	27
5	14.3	435	0.3	1.3	18	0.6	7.28	25.4	38.7	35.9
6	12.2	324	0.21	0.21	10	0.59	7	20.3	39.7	40
7	27	650	0.84	1.6	8	0.5	7.1	17	35	48
8	11	107	0.74	1.12	11	5.27	7.1	58.16	13.48	28.36
9	2	231	0.27	1.1	8	0.43	6.2	3	47	50
10	7	106	0.4	0.61	8.3	0.92	6.8	5	43.5	51.5
11	9	342	0.78	2.3	9	0.63	7.1	30.2	31	37.8
12	11	112	0.41	0.84	7	1.4	6.9	37.4	20	42.6
13	5	320	0.25	1.11	6	0.21	5.95	5	49	46
14	8.4	121	0.38	0.73	8	0.86	6.9	41.9	17.2	40.9
15	6.8	345	0.42	0.2	21.3	0.16	7.7	54.1	10.6	35.3
16	6	261	0.62	1.7	31	1.78	7.8	19	9	72
17	3	374	0.48	2.5	34	5.13	8.9	46.7	20.6	32.7
18	6	275	0.57	0.35	32	3.78	7.9	52	10.8	37.2
19	5.6	311	0.53	0.8	37	0.05	7.8	8.7	51.3	40
20	6.1	342	0.46	0.53	42	0.1	7.9	51.5	11.8	36.7
21	12.4	321	0.62	1	56	0.2	7.7	58.2	3.8	38
22	6.107	215	0.11	0.34	9	0.48	7.3	40.3	30.5	29.2
23	8.8	288	0.102	0.89	30.75	0.222	7.95	24.13	31.2	44.67
24	6.6	135	0.91	0.43	59	4.9	7.9	54.7	12.6	32.7
25	5	175	0.21	1.51	37	0.52	8.32	61	7	32

Abbreviations: EC, electrical conductivity; K, potassium; N, total nitrogen; OM, organic matter; P, phosphorus; pH, potential of hydrogen; TNV%, total neutralizing value.

ble 4). Therefore, essential oil content of *O. decumbens* increased in some regions by decreasing the elevation. Correlation analysis showed that thymol content had not significant correlation with selected environmental factors. In contrast, significant correlations between carvacrol content with MDT (0.788) and MEM (0.454) were observed. Carvacrol content had a significant negative correlation with altitude (-0.632).

5. Discussion

5.1. Essential Oil Content

According to the current results, there was a significant negative correlation between essential oil content and altitude (Table 4). The findings revealed that the essential oil content of *O. decumbens* was increased by decreasing

altitude. Therefore, the highest amounts of essential oils were observed in Khuzestan province populations, where the altitude was lower than others. At higher altitudes, decreased CO₂ partial pressure negatively influenced net photosynthetic rate (14), which could influence essential oil content. High altitudes induced UV-B radiation stress, which mainly influences the production of phenolic compounds to absorption and/or dissipation of solar energy (15). Furthermore, these differences in EO may be the result of either uncontrolled biotic and abiotic elicitation or of inherent genetic differences. The current results are in line with the results of previous studies on different medicinal plants (7, 16).

Table 3. Essential oil Content and Composition of *O. decumbens* Populations

Component	KI Book	KI Cal- culated	Population No.																								
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
<i>α</i>-Pinene	939	935	0.13	0.06	0.12	0.12	0.11	0.28	0.28	0.38	0.21	0.07	0.18	0.13	0.19	0.21	0.20	0	0.20	0.21	0.16	0	1.42	12	0.18	3.53	3.11
<i>p</i>-Cymene	1025	1031	15.3	14.8	15	13.71	14.2	12.16	14.4	11.9	13.08	14.50	15.02	16.29	12.35	17.60	9.02	7.81	14.44	12.8	18.67	12.3	13.2	11	18.81	17.52	14.48
Limonene	1029	1034	3.12	1.08	3.11	3	0	3.08	2.86	2.99	2.39	1.30	2.88	3.21	4.06	0	3.70	0.65	2.81	0	2.84	1.64	3.1	3.4	2.67	4.88	4.11
<i>γ</i>-Terpinene	1060	1063	19.8	16.21	18.2	0	16.33	0	0	12.8	0	0	0	0	4.92	0	0	16.34	0	8.99	21.8	13.68	6.78	22.9	24.1	0	0
Thymol	1290	1295	28.3	30.64	28	27.18	32.9	28.15	28	36.9	29.67	0	28.00	29.45	28.03	24.56	31.48	39.82	27.92	26.9	21.4	38.7	34.7	43.9	22.01	25.3	27.22
Carvacrol	1299	1305	28.8	29	28.8	25.72	31.7	26.45	28.7	23.2	27.88	35.98	26.89	27.87	26.27	24.14	29.31	28.56	26.25	27.54	10.21	25.23	26.1	0	11	28.9	28.11
Myristicin	1519	1539	3.61	3.67	3.23	3.87	0.6	3.72	3.78	11.38	3.21	3.961	5.62	5.64	17.99	9.05	6.09	0.63	7.3019	7.13	20.9	5.72	6.36	2.9	19.9	8.4	7.57
Essential oil, %	-	-	4.1	3.4	3.9	3.45	4.3	3.76	3.35	5.21	3.9	6	5.53	5.93	2.95	4.65	3.62	3.45	3.2	2.25	4.1	3.78	3.8	3.31	3.66	3.2	3.54

5.2. Essential Oil Compounds

In this study, there was a non-significant correlation between some environmental factors and thymol content. The study on *Thymus kotschyanus* revealed that thymol content was not influenced by changes in altitude (17). Variations in the relative amounts of these compounds under different environmental conditions indicate their adaptively ecological value. According to the current results, it seems that thymol is more stable in different geographical conditions and more affected by genetic factors.

Carvacrol content is positively correlated with MDT and MEM, yet there was a strong negative relationship between carvacrol and location altitude. In this study, content of carvacrol was reduced with increasing altitude. Therefore, differences in the relative quantities of carvacrol in *O. decumbens* populations originated from locations with high MDT, and MEM may be a defense mechanism to extreme temperatures.

Previous studies indicated that meteorological conditions could inhibit or stimulate the synthesis of carvacrol (18-21). The carvacrol content is positively correlated with aridity in *Thymus piperella* chemotype (18), while a progressive increase of temperature and decrease of humidity was accompanied by a progressive decrease in carvacrol and thymol in *T. numidus*. Also, temperature differentially influenced percentages of monoterpenic phenols in *Origanum vulgare*, *Origanum majorana*, and *Origanum syriacum*. The increase of temperature was accompanied by an increase of carvacrol, while decrease of temperature was associated with an increase of thymol (20). Similar results were also reported by Vaiculyte et al. (21) on *Thymus pulegioides* plants. Homer et al. (22) suggested that a plant's ability to synthesize phytochemicals may be the result of selective pressure and further variability in chemotypes may reflect different selective pressure in different areas.

Previously, the study on *Thymus vulgaris* chymotypes indicated that carvacrol content increased with decreasing altitude (5). In accordance with the current results, Jamshidi et al. (17) found that carvacrol content of *Thymus kotschyanus* was reduced by increasing altitude. The relationship between the essential oil components and habitat altitude of *Thymus pubescens* in East Azarbaijan province revealed that the carvacrol content had a negative correlation with altitude yet it was not significant (7).

In this study, there was a positive correlation between MEM and carvacrol. In a study on chemotype of wild oregano (*Origanum vulgare*), it was found that they accumulated more essential oil under lower rainfall (23). However, the dry weather increased the essential oils (24).

Table 4. Correlations Between Macro Environmental Factors and Essential Oils Parameter from *O. decumbens* in 25 Regions of Iran

Variables	MDT, C	MEM, mm	Latitude, N	Longitude, E	Altitude, m	pH	Clay, %	Thymol, %	Carvacrol, %
MEM, mm	0.634 ^a								
Latitude, N	-0.115	-0.544 ^a							
Longitude, E	0.026	0.437 ^a	-0.972 ^a						
Altitude, m	-0.830 ^a	-0.449 ^a	-0.265	0.355					
pH	-0.206	0.215	-0.497 ^a	0.452 ^a	0.487 ^a				
Clay, %	-0.121	-0.405 ^a	0.522 ^a	-0.457 ^a	-0.031	-0.547 ^a			
Thymol, %	-0.269	-0.005	-0.057	0.025	0.175	0.110	-0.391		
Carvacrol, %	0.788 ^a	0.454 ^a	-0.079	-0.033	-0.632 ^a	-0.125	-0.146	-0.302	
Essential oil, %	0.368	-0.020	0.266	-0.232	-0.412 ^a	-0.312	0.155	-0.341	0.181

Abbreviations: MDT, mean day temperature; MEM, mean evaporative per month; pH, potential of hydrogen.

^a Values are significant ($\alpha < 0.05$).

5.3. Conclusions

This study indicated that phytochemical traits of plants are greatly influenced by different climatic conditions. Also, environmental conditions had a significant role on essential oil quantity and quality. The altitude, mean evaporative per month (MEM) and Mean Day Temperature (MDT) were effective macro environmental factors on essential oil, thymol, and carvacrol content. In general, the regions with warm and semi-humid climate were the best locations for essential oil production with high amount of thymol and carvacrol.

Footnotes

Conflict of Interests: In this study, there were no conflicts with other researchers and institutes.

Funding/Support: In this study, research grants were received from Institute of Medicinal Plants, ACECR. Also, this study was supported by Science and Research Branch, Islamic Azad University in respect to funding.

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