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Original Article

Effect of Geographical Location on Yield and Chemical Composition of *Teucrium orientale* L. Essential Oils Collected from Eleven Different Localities in Iran

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ABSTRACT

Essential oils of the air-dried aerial parts of *Teucrium orientale* L. collected from 11 different localities which obtained by hydro-distillation were investigated. The essential oils were analyzed by GC-FID and GC-MS. The oil yields were 0.03–0.20% (w/w), based on dry weight. Forty-four components, representing 91.0–99.5% of the oils, were identified. The results showed that there is a significant difference in yields, chemical compositions and concentrations of essential oils obtained from different geographical regions. However, there are main and common compounds such as caryophyllene oxide (3.72 to 23.60%), E-caryophyllene (1.03 to 39.36%), germacrene D (0.95 to 29.7%), spathulenol (1.98 to 11.11%), β-cubebene (1.31 to 24.64%), δ-cadinene (1.14 to 4.90%), hexadecanoic acid (0.47 to 32.60%), α-cubebene (0.53 to 11.37%) and hexahydrofarnesyl acetone (0.34 to 10.56%) that are found in all essential oils. According to the cluster analysis, the populations were placed in three clusters. Percentages of hexahydrofarnesyl acetone (10.6%) and humulene epoxide II (6.8%) were considerably higher in cluster 1 compared to other clusters. Percentages α-cubebene, β-cubebene and hexadecanoic acid were considerably highest in cluster 2 with means of 7.2%, 11.9% and 20.4% respectively. Populations of cluster 3 were rich sources of E-caryophyllene (29.4%), germacrene D (16.1%) and bicyclogermacrene (3.4%) compared to other clusters.

INTRODUCTION

According to The Plant List, till now 971 scientific plant names of *Teucrium* genus are present. Of this list, 341 plant names including species, subspecies, varieties, forms and hybrids are accepted. *Teucrium* is a cosmopolitan genus of flowering plants in the family Lamiaceae [1]. In Iran, the genus *Teucrium* is represented in 12 species [2]. *T. polium* L. is the well-known member of this genus which has been used as one of the main antidiabetic agents in Iranian herbal medicine [3]. Among the *Teucrium* species, *T. orientale* L. is distributed in many parts of Iran and has three Synonyms including *Melosmon orientale* (L.) Raf., *T. nivale* Boiss. and *T. orientale* subsp. *orientale.*

The *Teucrium* species are a source of pharmacologically active compounds such as terpenoids, diterpenoids, steroids, phenols and flavonoids [4-8] which has different biological properties such as antimicrobial [9,10], antioxidant [11,12], antiproliferative [13], proapoptotic [13] and anti-diabetic [14] activities. This genus shows specified similarity in essential oil composition, for example sesquiterpene hydrocarbons were found as the major compounds in many of species of *Teucrium* [15-19]. The essential oil of *T. alyssifolium* Stapf is consist of sesquiterpene hydrocarbons such as trans-β-caryophyllene (16.87%), ar-curcumene (11.43%) and bisabolene (11.06%) as the most important components [20]. Morteza-Semnani *et al*. (2005) showed major

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constituents of *T. chamaedrys* L. essential oil is germacrene D (16.5%), (Z)-β-farnesene (12.2%), β-caryophyllene (10.5%), α-pinene (9.1%) and δ-cadinene (7.4%) [21]. The study of Essential oil from *T. arduini* L'Hér. indicates the presence of a high percentage of sesquiterpene hydrocarbons (48.76%), of which the main constituents were germacrene D (16.98%) and β -caryophyllene (14.98%) [22].

A literature survey has shown that there are few reports on the volatile constituents of *T. orientale* subspecies [23-25] and in all of these articles, only one sample of one locality has been studied. Since yield and chemical composition of the essential oils is dependent on the plant species, varieties and geographical region [26,27], samples of *T. orientale* L. were collected from 11 different localities and their essential oils yield and chemical composition were studied in this article.

Experimental

Plant Material

Plant Materials were collected in May 2019 from Hormozgan province (2 localities), June 2019 from Hormozgan province (1 locality), June 2019, Kermanshah province (3 localities), May 2020 from Ardabil province (1 locality), June 2020 from Ilam province (1 locality), June 2020 from Hamedan province (2 localities) and June 2020 from Gilan province (1 locality) of Iran, and identified by the herbarium of the Research Institute of Forests and Rangelands (TARI).

Isolation of Essential oil

Analysis of the oil: The aerial parts of *T. orientale* L. were air-dried at ambient temperature in the shade. Essential oils were collected using a Clevenger-type apparatus for 3 hours by hydrodistilation method. The yields of oils were 0.03-0.20% (w/w). The yellow essential oils were dried over anhydrous sodium sulphate and stored in refrigerator until analysis.

Gas Chromatography (GC)

Analytical GC was carried out on an Agilent 7890A equipped with a Flame Ionization Detector (FID) and a DB-5 column (methyl phenyl siloxane, 30 m x 0.25 mm, 0.25 μm film thickness). Nitrogen was used as the carrier gas with 0.7 ml/min flow rate. Temperature programming was performed from 60 °C and increased to 220 °C by 4 °C/min rate, then increased to 260 °C by 20 °C/min rate, and hold at this temperature for 10 min. The injector and detector temperatures were 260 °C. The split ratio was 1:40.

Gas Chromatography/Mass Spectrometry (GC)

Gas Chromatography/Mass spectrometry was carried out on an Agilent 7890A equipped with a 5975C Mass Detector and a DB-5 ms column (methyl phenyl siloxane, 30 m × 0.25 mm, 0.25 μm film thickness). Helium was used as the carrier gas with 0.7 ml/min flow rate. The temperature program was exactly the same as the GC program. Quadrupole mass spectrometer was operated at 70 eV ionization energy. EIMS spectra were obtained in scan mode in 40-340 m/z range.

Quantification and Identification of Components

The components of the essential oil were identified based on the basis of their retention indices. Identification confirmation was by comparison of their mass spectra with published spectra (Adams, 1989) and those of reference compounds from Adams Library. Also, their identification was confirmed by co-injection of available compounds. The number of identified compounds was computed from the GC peak area without any correction factor.

RESULTS

Yield of Essential Oils

The percentage yield of essential oil obtained from the water distillation process of the air-dried aerial part of *T. orientale* L. ranged from 0.03-0.20% (w/w). The sample from 'Ardabil province' had the highest oil yield, and the sample from 'Ilam province' had the lowest oil yield (Table 1).

Chemical Composition

The total number of compounds identified in the essential oils of the air-dried aerial part of *T. orientale* L. were 15 to 24 compounds, representing 91.0% to 99.5% of the total oils (Figure 1 and Table 2). The main and common constituents of essential oils were caryophyllene oxide (3.72 to 23.60%), E-caryophyllene (1.03 to 39.36%), germacrene D (0.95 to 29.7%), spathulenol (1.98 to 11.11%), β-cubebene (1.31 to 24.64%) and δ-cadinene (1.14 to 4.90%). Although compounds such as hexadecanoic acid (0.47 to 32.60%), α-cubebene (0.53 to 11.37%) and hexahydrofarnesyl acetone (0.34 to 10.56%) were also present in all samples of this species, but they don't have high amounts in some samples.

Cluster Analysis

Cluster analysis, based on the essential oil components, divided the populations to three clusters (Fig. 2). E-caryophyllene, germacrene D and caryophyllene oxide, were the reported compounds in oils of all populations. Some compounds such as *cis*-calamenene, dodecanoic acid and tetradecanoic acid were only detected in cluster 1. Furthermore, percentages of hexahydrofarnesyl acetone (10.6%) and humulene epoxide II (6.8%) were considerably higher in cluster 1 compared to clusters 2 and 3.

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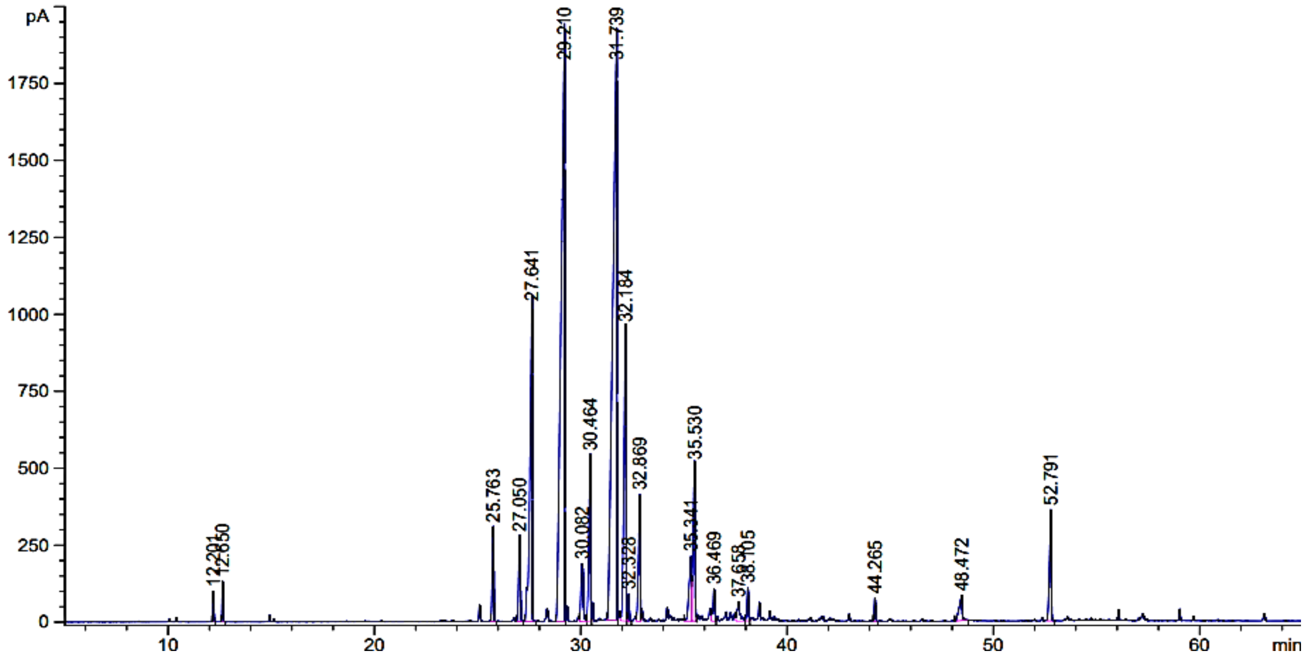

Fig. 1 Gas chromatogram of essential oil of *T. orientale* L. collected from Gilan province

Table 1 Essential oil percent (w/w) of *T. orientale* L. collected from different localities

Locality	Altitude above sea level (m)	Geographic coordinates		Essential oil percent (w/w)
		Longitude	Latitude	
Kermanshah province 1st sample	1650-1700	46° 31′ 49″ E	34° 51′ 00″ N	0.07
Kermanshah province 2nd sample	1550	47° 27′ 13″ E	34° 42′ 01″ N	0.16
Kermanshah province 3rd sample	1850	47° 35′ 39″ E	34° 45′ 27″ N	0.05
Hormozgan province 1st sample	1000	55° 57′ 50″ E	27° 55′ 27″ N	0.07
Hormozgan province 2nd sample	800	56° 10′ 64″ E	38° 01′ 65″ N	0.04
Hormozgan province 3rd sample	430	54° 23′ 10″ E	27° 14′ 05″ N	0.06
Hamedan province 1st sample	1800-2020	48° 35′ 30″ E	34° 45′ 32″ N	0.07
Hamedan province 2nd sample	2200-2421	48° 40′ 45″ E	34° 36′ 46″ N	0.07
Ilam province	1485	46° 41′ 00″ E	33° 23′ 26″ N	0.03
Ardabil province	1050	48° 35′ 08″ E	37° 15′ 25″ N	0.20
Gilan province	155	49° 41′ 35″ E	36° 39′ 08″ N	0.19

Table 2 Percentage composition of *T. orientale* L. Essential oil in different localities of Iran

Chemical compound	RI	Percentage composition of different localities										
		Kermanshah			Hormozgan			Hamedan		Ilam	Ardabil	Gilan
		1st sample	2nd sample	3rd sample	1st sample	2nd sample	3rd sample	1st sample	2nd sample	-	-	-
1-octen-3-ol	980	-	0.21	-	-	-	-	-	-	1.19	-	-
z-β-ocimene	1037	0.70	0.19	-	-	-	-	-	-	0.67	-	0.39
E-β-ocimene	1047	-	-	-	-	-	-	-	-	-	-	0.52
linalool	1099	4.36	-	1.79	-	-	1.90	3.91	0.75	2.33	-	-
n-nonanal	1102	-	-	-	-	-	1.70	-	-	-	-	-
α-terpineol	1189	0.41	-	0.25	-	-	-	-	-	-	-	-
Thymol	1290	1.54	-	0.87	-	7.50	-	-	-	-	-	-
carvacrol	1300	-	-	1.24	-	1.30	1.90	3.68	1.14	-	-	-
α-cubebene	1351	0.61	0.61	0.53	11.37	4.20	6.90	2.25	2.97	1.39	3.36	1.75
α-copaene	1376	2.46	1.79	1.17	6.64	1.34	3.10	1.11	0.82	2.58	-	1.89
β-cubebene	1380	1.89	0.37	1.29	-	-	-	-	-	1.70	-	-
β-bourbonene	1386	1.57	1.31	1.40	24.64	3.97	8.80	3.87	6.00	6.44	2.11	9.75
α-gurjunene	1408	0.70	0.71	-	1.58	-	-	-	-	-	-	-
α-cedrene	1412	1.67	-	0.97	-	-	-	-	-	-	-	-
E-caryophyllene	1420	28.47	39.36	28.05	8.70	1.03	1.70	28.22	35.16	20.06	2.21	26.27
α-humulene	1453	4.13	6.28	4.14	-	-	-	1.24	1.64	1.54	1.16	1.78
(E)-β-farnesene	1455	-	-	-	1.12	-	-	3.48	4.75	3.26	-	3.72
germacrene D	1480	26.30	6.69	19.43	0.95	5.28	3.60	7.84	5.53	17.43	6.14	29.71
bicyclogermacrene	1494	3.76	0.67	0.96	-	-	-	2.59	2.21	5.39	2.66	8.35
β-bisabolene	1508	-	-	-	-	-	-	5.75	2.92	-	4.64	-
γ-cadinene	1513	-	-	-	2.83	4.49	2.50	-	-	-	-	0.51
δ-cadinene	1523	4.33	1.14	3.13	3.78	2.20	4.90	2.36	2.65	3.16	3.50	2.92
cis-calamenene	1532	-	-	-	-	1.04	-	-	-	-	-	-
Elemol	1547	0.52	1.13	1.43	-	-	-	1.12	1.36	1.08	-	-
Dodecanoic acid	1562	-	-	-	-	2.96	-	-	-	-	-	-
Spathulenol	1576	4.40	7.52	6.57	2.01	4.79	2.90	2.73	2.80	11.11	5.66	1.98
Caryophyllene oxide	1580	6.98	20.27	14.59	3.72	17.93	23.60	19.62	21.68	7.68	6.53	4.35
Guaiol	1597	-	-	-	-	-	-	-	-	-	3.20	-
Humulene epoxide II	1604	0.59	2.21	1.62	-	6.83	4.40	2.42	2.30	2.55	-	-
1-epi-cubenol	1628	-	-	-	-	2.44	2.40	-	-	-	-	-
Selina-3,11-dien-6α-ol	1638	-	-	-	-	-	1.00	-	-	-	-	-
τ-murrolol	1640	-	-	-	-	1.72	1.10	-	-	-	-	-
Cedr-8(15)-en-9-α-ol	1644	-	-	-	-	-	1.90	-	-	-	-	-
2-pentadecanone	1699	-	-	-	1.00	1.63	-	-	-	-	-	-

Chemical compound	RI	Percentage composition of different localities										
n-pentadecanol	1761	-	-	-	3.00	-	-	-	-	-	-	-
Tetradecanoic acid	1768	-	-	-	-	3.52	-	-	-	-	-	-
Hexahydrofarnesyl Acetone	1844	0.80	0.34	0.49	3.07	10.56	7.00	2.25	1.05	1.44	2.78	1.03
Hexadecanoic acid	1968	0.77	0.47	1.83	19.97	10.32	8.50	-	-	3.17	32.60	-
Heneicosane	2100	-	-	-	-	-	-	2.31	1.01	-	12.58	-
E-phytol acetate	2218	-	-	-	-	-	-	-	-	-	-	2.57
n-tricosane	2300	-	-	0.21	-	-	-	-	-	0.38	-	-
n-pentacosane	2500	0.35	0.26	0.49	-	-	1.23	1.30	-	0.42	3.39	-
Hexacosane	2600	2.18	1.27	5.17	2.89	-	-	-	-	2.29	1.16	-
Heptacosane	2700	-	-	-	-	-	-	-	-	-	3.24	-

Table 3 Correlation of percentage composition of *T. orientale* L. essential oil in different localities of Iran

	linalool	thymol	Carvacrol	α-cubebene	α-copaene	β-cubebene	E-caryophyllene	α-humulene	(E)-β-farnesene	germacrene D	bicyclogermacrene	δ-cadinene	elemol	spathulenol	caryophyllene oxide	humulene epoxide II	hexahydrofarnesyl acetone	hexadecanoic acid
Linalool	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thymol	- 0.14^{ns}	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carvacrol	0.43^{ns}	0.09^{ns}	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
α-cubebene	-0.33^{ns}	0.00^{ns}	0.01^{ns}	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
α-copaene	-0.05^{ns}	-0.14^{ns}	-0.25^{ns}	0.74**	1	-	-	-	-	-	-	-	-	-	-	-	-	-
β-cubebene	-0.31^{ns}	-0.19^{ns}	-0.17^{ns}	0.86**	0.87**	1	-	-	-	-	-	-	-	-	-	-	-	-
E-caryophyllene	0.26^{ns}	-0.38^{ns}	-0.00^{ns}	-0.63*	-0.23^{ns}	-0.32^{ns}	1	-	-	-	-	-	-	-	-	-	-	-
α-humulene	0.15^{ns}	-0.21^{ns}	-0.28^{ns}	-0.69**	-0.26^{ns}	-0.54*	0.76**	1	-	-	-	-	-	-	-	-	-	-
(E)-β-farnesene	0.07^{ns}	-0.34^{ns}	0.20^{ns}	-0.12^{ns}	-0.10^{ns}	0.18^{ns}	0.42^{ns}	-0.22^{ns}	1	-	-	-	-	-	-	-	-	-
Germacrene D	0.34^{ns}	-0.08^{ns}	-0.30^{ns}	-0.62*	-0.17^{ns}	-0.28^{ns}	0.41^{ns}	0.40^{ns}	0.16^{ns}	1	-	-	-	-	-	-	-	-
Bicyclogermacrene	0.14^{ns}	-0.29^{ns}	-0.29^{ns}	-0.46^{ns}	-0.20^{ns}	-0.08^{ns}	0.31^{ns}	0.07^{ns}	0.57*	0.78**	1	-	-	-	-	-	-	-
δ-cadinene	0.34^{ns}	-0.20^{ns}	-0.07^{ns}	0.41^{ns}	0.39^{ns}	0.32^{ns}	-0.49^{ns}	-0.40^{ns}	-0.22^{ns}	0.12^{ns}	0.01^{ns}	1	-	-	-	-	-	-
Elemol	0.36^{ns}	-0.28^{ns}	0.25^{ns}	-0.58*	-0.34^{ns}	-0.44^{ns}	0.77**	0.57*	0.34^{ns}	0.10^{ns}	-0.01^{ns}	-0.44^{ns}	1	-	-	-	-	-
Spathulenol	0.07^{ns}	0.01^{ns}	-0.33^{ns}	-0.49^{ns}	-0.21^{ns}	-0.43^{ns}	0.12^{ns}	0.40^{ns}	-0.17^{ns}	0.14^{ns}	0.07^{ns}	-0.25^{ns}	0.42^{ns}	1	-	-	-	-
Caryophyllene oxide	0.09^{ns}	0.15^{ns}	0.66*	-0.10^{ns}	-0.29^{ns}	-0.37^{ns}	0.15^{ns}	0.07^{ns}	-0.01^{ns}	-0.48^{ns}	-0.54*	-0.29^{ns}	0.39^{ns}	-0.07^{ns}	1	-	-	-
Humulene epoxide II	-0.01^{ns}	0.70*	0.46^{ns}	0.03^{ns}	-0.17^{ns}	-0.24^{ns}	-0.31^{ns}	-0.28^{ns}	-0.16^{ns}	-0.38^{ns}	-0.45^{ns}	-0.20^{ns}	-0.00^{ns}	0.12^{ns}	0.69**	1	-	-
Hexahydrofarnesyl Acetone	-0.22^{ns}	0.74**	0.29^{ns}	0.46^{ns}	0.07^{ns}	0.12^{ns}	-0.78**	-0.65*	-0.34^{ns}	-0.47^{ns}	-0.46^{ns}	0.13^{ns}	-0.58*	-0.19^{ns}	0.30^{ns}	0.78**	1	-
Hexadecanoic acid	-0.44^{ns}	0.04^{ns}	-0.27^{ns}	0.55*	0.13^{ns}	0.29^{ns}	-0.75**	-0.46^{ns}	-0.41^{ns}	-0.47^{ns}	-0.28^{ns}	0.30^{ns}	-0.61*	-0.05^{ns}	-0.37^{ns}	-0.18^{ns}	0.32^{ns}	1

^{ns}: Not Significant, *and**: Significant at P=0.05 and P=0.01 levels of probability, respectively.

Table 4 Correlation of essential oil percent and geographic conditions in different localities of Iran

-	Essential oil percent
Altitude	0.296 ns
Latitude	0.290 ns
Longitude	0.494 ns

ns: Not Significant

One of fundamental differences between populations of cluster 2 with other clusters was the percentages α-cubebene, β-cubebene and hexadecanoic acid whose amounts were considerably highest in cluster 2 with means of 7.2%, 11.9% and 20.4% respectively. Populations of cluster 3 were rich sources of E-caryophyllene (29.4%), germacrene D (16.1%) and bicyclogermacrene (3.4%) compared to other clusters.

Correlation between essential oil percent and geographic conditions in different localities of Iran was indicated in Table 4. No Significant relationships were observed in studied populations.

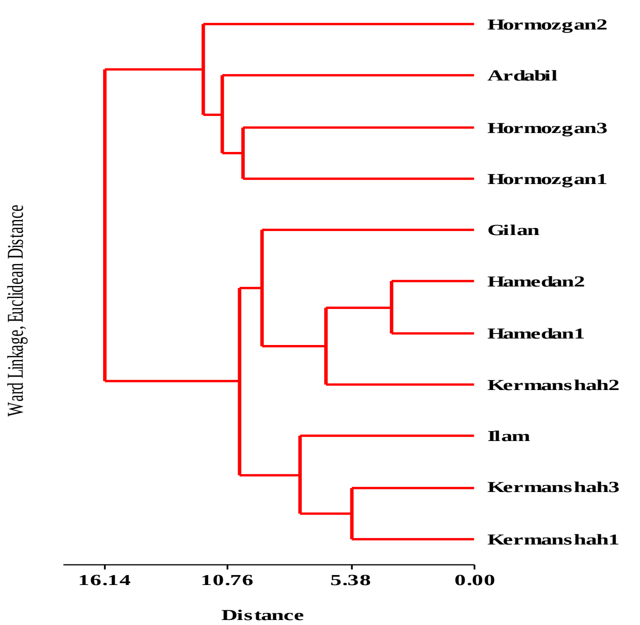

Fig. 2 Dandrogram of cluster analysis by Ward method according to the amounts of oil components in 11 populations of *T. orientale* L.

Correlation between percentage of different composition of *T. orientale* L. essential oil in different localities of Iran was indicated in Table 3. Results showed that α-copaene has a positive correlation with α-cubebene. β-Cubebene has a positive correlation with α-cubebene and α-copaene. E-Caryophyllene has a negative correlation with α-cubebene. α-Humulene has a negative correlation with α-cubebene and β-cubebene and positive correlation with E-caryophyllene. Germacrene D has a negative correlation with α-cubebene. Bicyclogermacrene has a positive correlation with (E)-β-farnesene and germacrene D. Elemol has a negative correlation with α-cubebene and positive correlation with E-caryophyllene and α-humulene. Caryophyllene oxide has a positive correlation with carvacrol and negative correlation with bicyclogermacrene. Humulene epoxide II has a positive correlation with thymol and caryophyllene oxide. Hexahydrofarnesyl acetone has a positive correlation with thymol and Humulene epoxide II, and negative correlation with E-caryophyllene, α-humulene and elemol. Hexadecanoic acid has a positive correlation with α-cubebene and negative correlation with E-caryophyllene and elemol

DISCUSSION

There is a significant difference in yields, chemical compositions and concentrations of essential oils obtained from different geographical regions. This is in agreement with earlier reports which revealed considerable difference in chemical composition of essential oils with respect to geographical locations. Results show that some sesquiterpene compounds are present in all samples, including caryophyllene oxide (3.72 to 23.60%), E-caryophyllene (1.03 to 39.36%), germacrene D (0.95 to 29.7%), spathulenol (1.98 to 11.11%), β-cubebene (1.31 to 24.64%), α-cubebene (0.53 to 11.37%) and δ-cadinene (1.14 to 4.90%) which is in good agreement with previously published articles on *Teucrium* essential oils [15-17].

Other compounds such as hexadecanoic acid (0.47 to 32.60%), hexahydrofarnesyl acetone (0.34 to 10.56%) and long chain hydrocarbons (0.00 to 20.37%) are also present in all specimens which don't have sesquiterpene skeleton. Due to the fact that *T. orientale* L. compared to other high content essential oil species of this genus such as *T. polium*, has less essential oil, perhaps this difference in the amount of essential oil and its composition, is because of low content of volatile compounds. To the extent that, heavy normal alkanes such as hexacosane and heptacosane have been extracted by Clevenger due to the lack of volatile compounds in this species.

CONCLUSION

The chemical composition of *T. orientale* L. essential oils collected from different localities of Iran vary within geographical regions. There is no regular relationship between essential oil content and regions altitude. There is also no regular relationship between concentrations of chemical compounds and geographical regions. However, some major compounds are common in all samples, including caryophyllene oxide (3.72 to 23.60%), E-caryophyllene (1.03 to 39.36%), germacrene D (0.95 to 29.7%), spathulenol (1.98 to 11.11%), β-cubebene (1.31 to 24.64%), δ-cadinene (1.14 to 4.90%), hexadecanoic acid (0.47 to 32.60%), α-cubebene (0.53 to 11.37%) and hexahydrofarnesyl acetone (0.34 to 10.56%) which represent 60.3 to 78.5% of the total oils. Analysis of the essential oils of different populations showed three clusters with different amounts of E-caryophyllene, germacrene D, hexahydrofarnesyl acetone, humulene epoxide II α-cubebene, β-cubebene and hexadecanoic acid, bicyclogermacrene and other compounds.

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