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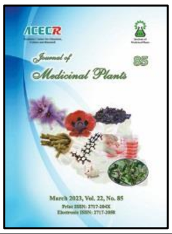

Research Article

Evaluation of essential oil diversity of different populations of *Thymus fallax* Fisch. & C.A.Mey. collected from some of its habitats in Iran

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ABSTRACT

Background: *Thymus fallax* Fisch. & C.A.Mey. is one of the medicinal plants of the Lamiaceae family, which aerial part contains valuable phytochemical compounds such as thymol and carvacrol. **Objective**: This study was performed for assessing the essential oil diversity of different populations of *Thymus fallax* in its habitats. **Methods:** The populations of *Thymus fallax* were collected from its natural habitats in Alborz, Tehran, Zanjan, Mazandaran, and North Khorasan provinces. Their essential oils were extracted by hydro-distillation method and the compounds were identified using GC and GC-MS. **Results:** The result indicated that the essential oil content of populations was on average 1.71 %, the highest percentage of which was observed in the population of Migoun (2.58 %), Chelcheshmeh (2.35 %), and Ghormehdareh (2.25 %), and the lowest was obtained in Dizin (1.05 %) and Ira (1.15 %). Overall, 30 essential oil compounds were identified, comprising 95 % of the total essential oil, of which carvacrol, thymol, *p*-cymene, and geraniol were the major constituents, accounting for 75 % of the essential oil. Five groups of thyme population were identified using cluster analysis, including the first group with high content of carvacrol, the second group with high content of geraniol, the third group with high content of thymol and carvacrol, the fourth group with high content of thymol, and the fifth group with high content of thymol, carvacrol and geraniol. **Conclusion:** The high amounts of essential oil, carvacrol and thymol in some populations were valuable results that can be considered in future and breeding studies.

1. Introduction

Medicinal plants are considered as one of the most valuable resources in Iran's wide range of natural resources [1], which have secondary metabolites with numerous biological properties [2].

Thyme is one of the most important genus of the mint family (*Lamiaceae*), of which 14 species

Abbreviations: GC, Gac Chromatography; GC-MS, Gac Chromatography-Mass Spectroscopy

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were identified as native to Iran [2]. Nowadays, thyme is widely used for anti-flatulence, digestion, anti-spasm, anti-inflammatory, as well as expectorant effects [3].

Thymus fallax Fisch. & C.A.Mey. is an upright plant with branched stems 8-30 cm high and pale red and rarely purple flowers, which flowers around summer. This species is exclusive to Iran and Turkey, which grows in Lorestan, Hamedan, Azerbaijan, Kurdistan, Tehran, Markazi, and Isfahan provinces in Iran at an altitude range of 1400-3600 meters above sea level [3]. The most important components of essential oil of this species were reported differently in different regions. Barazandeh [4] reported that thymol (65.9 %), γ -terpinene (10.8 %), and carvacrol (2.6 %) are the main components of this plant in Hamedan region. Moreover, Tumen et al. [5] in Turkey indicated that the major compounds of this plant include carvacrol (68.1 %), thymol (5.4 %), *p*-cymene (4.8 %), γ-terpinene (3.6 %), α-pinene (2.8 %), and β-caryophyllene (3.8 %). However, amount of thymol and carvacrol is the most important factor for determining the chemotype of thyme, which showed a high correlation with the geographical origin [6].

The yield of medicinal plants are influenced by internal factors (genetic structure of the species and population) and external factors (environmental factors such as temperature, light, precipitation, height above sea level, latitude, soil conditions, and agricultural management) [7]. Munoz et al. [8] in a study on seven populations of *Lavandula latifolia* indicated that the yield of essential oil was significantly different among the regions and populations. A study on *Salvia officinalis* in 18 regions of southern Italy showed that the percentage of essential oil compounds varied in different regions [9]. Different species of thyme were collected from 12 regions with two desert (warm Mediterranean) and semi-Mediterranean climates in the southeast of Spain and assessed in respect of the essential oil content and composition. Based on the results, the populations in dry and low-altitude habitats had lower flavonoid percentage and accumulated more essential oil, anthocyanin, and terpenoid [10]. The effect of ecological conditions was evaluated on yield and quality traits of *Mentha piperita* in four regions in Turkey. The main components of the essential oil were menthol and menthone in the regions with a warmer climate. However, a higher percentage of menthol and a lower percentage of menthone were observed in the temperate regions [11].

Altitude above sea level is one of the environmental factors that affects the essential oil content, and lower altitudes lead to an increase in marjoram essential oil content [6]. Habibi et al. [12] in a study on effect of the altitude on essential oil content and composition of *Thymus kotschyanus* indicated that the highest (2.56 %) and lowest (1.31 %) percentage of essential oil were related to the altitudes of 1800 and 2800 meters, respectively. Jamshidi et al. [13] evaluated the thyme (*Thymus kotschyanus*) in Damavand region and reported that the percentage of essential oil and carvacrol decreased by increasing altitude. However, increasing the altitude led to an increase in the percentage of thymol. Further, increasing the altitude and low air temperature may lead to an increase in the accumulation of essential oil, which was reported for *Thymus daenensis* Celak and *Mentha piperita* L. in Shahrekord climate and Turkey, respectively [6-11]. In a study on *Thymus fallax* in Lorestan region, increasing the height above the sea level led to a significant increase in percentage of carvacrol and thymol [14].

Soil characteristics are considered as one of the most important factors in plant growth and the production of secondary metabolites [15]. The significant impact of the physical and chemical properties of soil on essential oil content and composition of of Greek oregano [16], climates and soils on essential oil content and composition of different Yarrow species [17], and soil fertility on the yield of Mentha piperita L. essential oil [18] were reported. Also, Mohammadian et al. [14] reported that some physical and chemical properties of the soil of plant habitats had a significant correlation with the compounds in the essential oil of *Thymus fallax* [14].

However, quantity and quality of medicinal plants in the ecosystem and natural habitats are affected by various factors, and this study aims to evaluate the different populations of *Thymus fallax* species quantitatively and qualitatively, identify the superior ecotype, and determine their phytochemical diversity.

2. Materials and Methods

In this study, 19 populations of *Thymus fallax* were identified in different regions of Alborz, Tehran, Mazandaran, Zanjan and Khorasan provinces using information from Flora Iranica and Flora of Iran [3]. The geographic coordinates of the habitat were recorded after confirming and identifying the species in the area. The characteristics of habitats including geographical location, soil characteristics, and meteorological information were collected and recorded (Table 1).

2.1. Plant materials

Identification and description key of the desired species in the samples of virtual herbarium such as Edinburgh and Vienna were carefully evaluated before starting the field studies [19]. Also, the authentication of plant specimens were done in Herbarium of Department of Pharmacognosy, School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. The herbarium codes of the populations were from 2346 to 2364. Narrow and elongated leaves, lying and dense trichomes on the stem and non-prominent side veins of the leaf are the diagnostic characteristics of this species. A 40X hand magnifier was used for identifying the plant in the field accurately since some of these characteristics, especially trichomes, are somewhat microscopic characteristics.

2.2. Phytochemical evaluation

To determine the essential oil amount in the plant, 50 g of dry leaf from each population was poured into a one-liter flask after grinding, and 300 mL of water was added. Then, the essential oil was extracted by hydrodistillation method using a clevenger apparatus for 4 hours. The essential oil content was determined based on the plant dry weight. It was dried using sodium sulfate [20].

The amount and type of compounds of essential oil were evaluated using instruments including Agilent 6890 Gas Chromatography (GC) and Agilent 5973 Gas Chromatography-Mass Spectrometry (GC-MS). The analysis of GC/MS was done on an Agilent instrument coupled with a 5973 Mass system equipped with flame ionization detector (FID) and a BPX5 capillary column (30 m × 0.25 mm; 0.25 µm film thicknesses). In temperature program, oven temperature remained for 2 min at 50 °C and was enhanced to 130 °C with rate of 2 °C per min. Then, temperature incerment was planned up to 270 °C as rate of 5 °C per min and this temperature remained for 3 min.

Other operating conditions were: gas of carrier was He with a flow rate of 1 ml/min;

temperatures of detector and injector were 280 °C, and split ratio, 1:10. Mass spectra were taken at 70 eV. The compounds were identified using different parameters such as time and Retention index. Moreover, mass spectra were studied and these spectra were compared with the standard compounds and information available in the library of the computer of GC-MS [21]. The relative percentage of each of the compounds forming the essential oil were determined based on the area under its curve in the gas chromatogram using area normalization method and ignoring the response factors.

2.3. Statistical analysis

The data were analyzed based on the completely randomized design with three replications using statistical softwares (SAS version 9.1 and SPSS version 19 software). The least significant difference (LSD) test in a probability level of 5 % was used for comparing the average of experimental treatments.

Table 1. Geographical positions, soil characteristics and average temperature and average precipitation (2015-2021) of the studied habitats

Province	**Region**	**Geographic positions**		
		Altitude (m)	**Latitude (N)**	**Longitude (E)**
Mazandaran	Tizkooh	2370	52°00'12.8" E	35°53'56.2" N
Mazandaran	Donbk	2523	51°59'24.9" E	35°54'57.2" N
Mazandaran	Ira	2620	51°52'22" E	35°50'05" N
Mazandaran	Chelcheshmeh	2700	51°54'09" E	35°55'28" N
Mazandaran	Ghormehdareh	3120	51°57'02" E	35°52'08" N
Mazandaran	Koohpahnak	3280	51°55'23" E	35°22'24" N
Mazandaran	Siahplas	2700	51°52'03" E	35°52'31" N
Alborz	Polkhab	1957	51°08'49.1" E	36°00'43.8" N
Alborz	Badmoroud	2143	51°09'58" E	36°00'08" N
Alborz	Moroud	2332	51°12'03.5" E	36°00'07.6" N
Alborz	Nashtrood	2007	51°98'08.4" E	36°03'39.3" N
Alborz	Dizin	2571	51°24'24.2" E	36°03'32.9" N
Alborz	Varangrood	2533	51°22'18" N	36°07'44" N
Alborz	Taleghan	1930	50°41'18" E	36°09'21" N
Tehran	Ahar	2130	51°28'32.5" E	35°55'22" N
Tehran	Migoun	2270	51°30'44" E	35°57'03" N
Tehran	Oshan	1990	35°54'57" N	35°54'57" N
Zanjan	Zanjan	1780	35°54'57" N	35°54'57" N
North Khorasan	Shirvan	1590	57°54'53.7" E	37°35'54" N

Table 1. Geographical positions, soil characteristics and average temperature and average precipitation (2015-2021) of the studied habitats (Continued)

		Soil properties							Climatic data	
Province	**Region**	**Soil Texture**	**EC (dS/m)**	**pH**	**Total N (%)**	**Available P (mg/kg)**	**Available K (mg/kg)**	**Organic carbon (%)**	**Temperature (°C)**	**Precipitation (mm)**
Mazandaran	Tizkooh	Loam	2.2	7.49	0.08	17.3	240	0.88	12.7	349.86
Mazandaran	Donbk	Loam	0.4	7.65	0.08	14.2	308	0.81	12.7	349.86
Mazandaran	Ira	Loam-Silt	0.45	7.55	0.04	4.3	159	0.48	12.7	349.86
Mazandaran	Chelcheshmeh	Loam-Silt	0.51	7.31	0.14	15.6	460	1.45	12.7	349.86
Mazandaran	Ghormehdareh	Loam-Silt	0.49	7.05	0.14	37	390	1.4	12.7	349.86
Mazandaran	Koohpahnak	Loam-Silt	0.38	7.72	0.05	6.3	121	0.67	12.7	349.86
Mazandaran	Siahplas	Loam-Silt	0.4	7.6	0.05	7.2	140	0.65	12.7	349.86
Alborz	Polkhab	Loam	0.38	7.73	0.04	4.5	140	0.4	11.1	557.33
Alborz	Badmoroud	Silt-Loam	0.96	7.03	0.09	10.3	240	0.9	11.1	557.33
Alborz	Moroud	Sand-Loam	0.74	7.51	0.01	3.8	95	0.19	11.1	557.33
Alborz	Nashtrood	Loam	0.56	7.56	0.03	10.7	149	0.38	11.1	557.33
Alborz	Dizin	Loam	0.56	7.62	0.08	11	198	0.86	11.1	557.33
Alborz	Varangrood	Sand-Loam	0.45	7.21	0.06	3.6	86	0.68	11.1	557.33
Alborz	Taleghan	Loam	0.67	7.62	0.07	7.5	168	0.71	12.33	419.6
Tehran	Ahar	Loam	0.4	7.66	0.09	10.4	240	0.92	16.33	432.37
Tehran	Migoun	Loam	0.4	7.56	0.08	11.6	280	0.98	16.33	432.37
Tehran	Oshan	Loam	0.5	7.8	0.08	8	238	0.87	16.33	432.37
Zanjan	Zanjan	Silt-Loam	0.49	7.52	0.11	26.5	331	1.1	18.71	256.56
North Khorasan	Shirvan	Loam	0.37	7.67	0.06	8.8	460	0.65	13.79	181.63

3. Results

The results showed that there were significant ($P \leq 0.01$) differences among the populations in terms of essential oil content (Table 2) and the essential oil content of populations varied in the range of 1.05-2.58 %. The highest content of essential oil was related to the population of Migun (2.58 %), Chalcheshmeh (2.35 %) and Ghormehdareh (2.25 %). Also, the lowest content of essential oil was related to Dizin population (1.05 %) and Ira (1.15 %) (Fig. 1).

As Fig. 2 shows, changes in the amount of essential oil with the increase in altitude from the sea level were not a clear and uniform trend, and with the increase in altitude, the amount of essential oil decreased in some cases and increased in others.

Table 2. ANOVA of main essential oil compounds of *T. fallax* populations

So.V	df	Mean of squares						
		Essential oil	**Carvacrol**	***α*-Pinene**	**Camphene**	**Thymol**	**Myrcene**	**Borneol**
Population	18	0.0641**	1892.82**	1.78**	0.659**	1300.14**	0.829**	2.82**
Error	38	0.017	3.62	0.013	0.011	1.124	0.011	0.05
CV (%)		7.62	4.74	9.11	13.15	4.58	7.96	10.81

ns, * and ** show non-significant and significant at 5 and 1% level, respectively.

Table 2. ANOVA of main essential oil compounds of *T. fallax* populations (Continued)

So.V	df	Mean of squares				
		***α*-Terpinene**	***p*-Cymene**	**Geraniol**	***γ*-Terpinene**	**Eucalyptol**
Population	18	0.417**	9.17**	633.4**	10.3**	0.979**
Error	38	0.0086	0.17	0.822	0.155	0.01
CV (%)		10.74	9.69	10.55	11.4	9.98

ns, * and ** show non-significant and significant at 5 and 1% level, respectively.

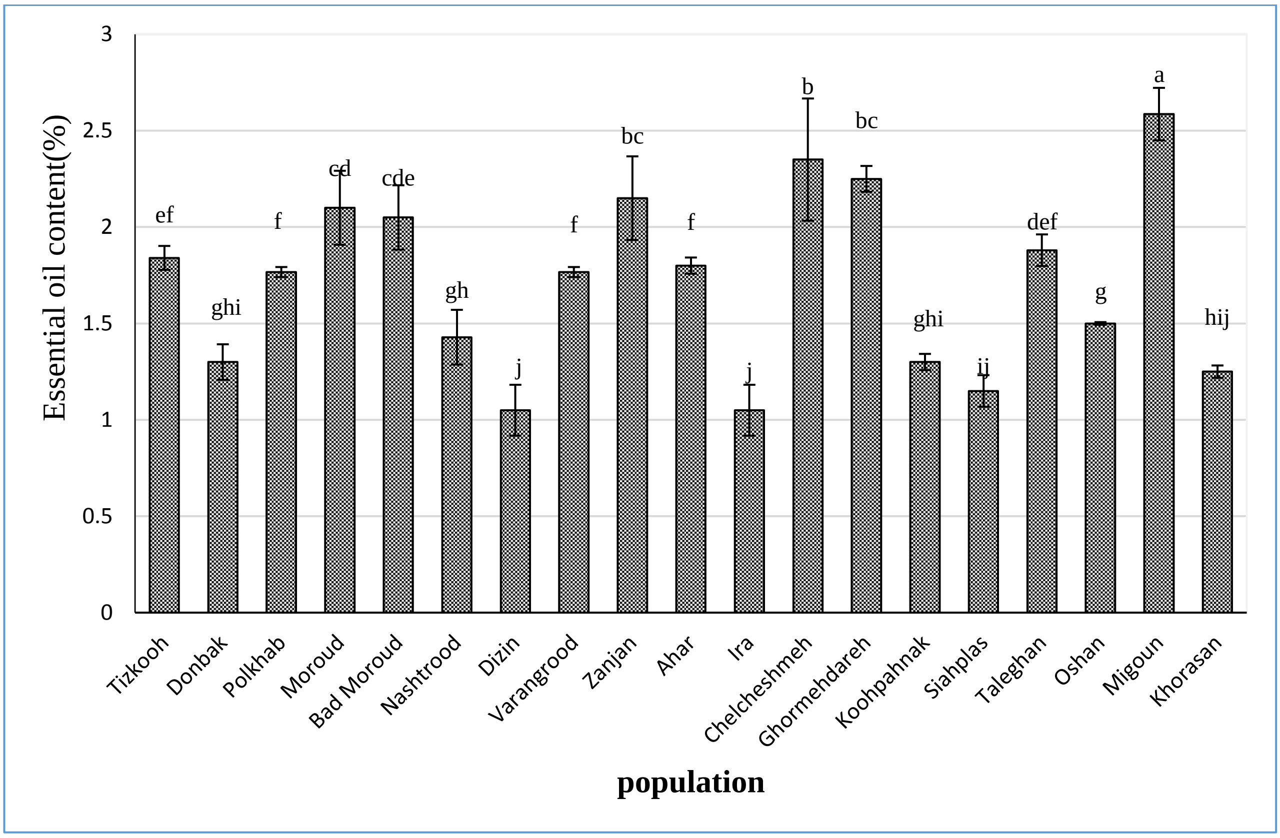

Fig. 1. Essential oil content of *T. fallax* populations

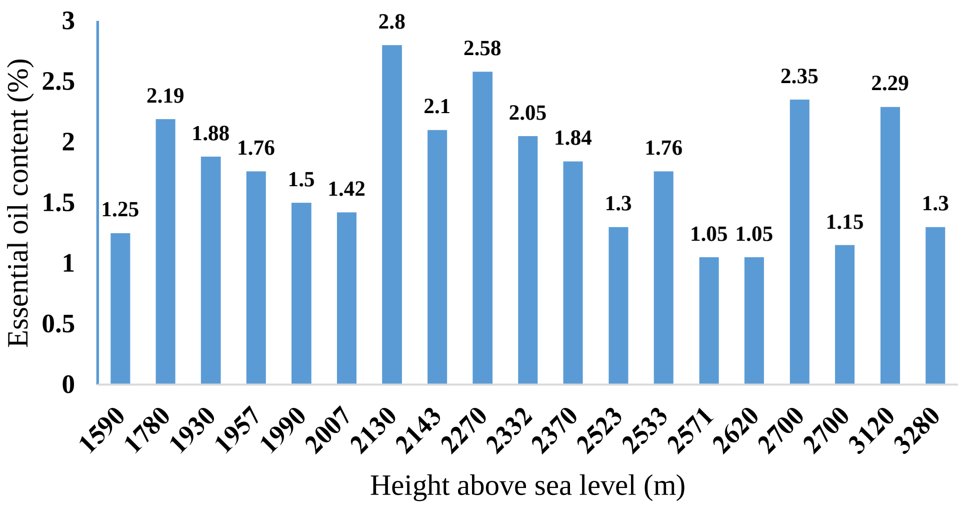

Fig. 2. Changes in essential oil content of *T. fallax* populations at different altitudes above sea level

Analysis of the essential oil of different populations showed that 30 compounds were identified in them, which constituted 95 % of the total essential oil. The main compounds of the essential oil included 12 compounds including thymol, carvacrol, geraniol, borneol, *γ*-terpinene, *p*-cymene, myrcene, *α*-terpinene, camphene, *trans*-sabinene hydrate, eucalyptol, and *α*-pinene, which included about 87% of the total essential oil compounds (Table 3). On average, four compounds including carvacrol (40.09 %), thymol (23.13 %), *p*-cymene (4.26 %), and geraniol (8.56 %) with more than 75 % of essential oil were the main compounds (Table 3). Based on the results of ANOVA in the dominant compounds of thyme essential oil, the effect of different regions was significant on the components of the essential oil (Table 2). Comparing the average of the dominant essential oil compounds in different regions indicated that the changes in essential oil components were significant in response to the change of regions. For example, thymol, carvacrol, borneol, *γ*-terpinene, *p*-cymene, myrcene, *α*-terpinene, *trans*-sabinene hydrate, and eucalyptol compounds were observed in the essential oils of all the populations. However, *α*-pinene, camphene, and geraniol were not found in the essential oil compounds in some populations (Table 4). The greatest amount of thymol was observed in Meygoun (72.67 %), Khorasan (58.93 %), and Chehelcheshme (40.45 %) populations. The lowest value belonged to Oshan (1.53 %), Nashtrood, and Ghormehdareh (2.53 %) populations (Table 4).

The highest amount of carvacrol was obtained in Ghormehdareh (76.34 %), Donbak (67.85 %), and Koohkharabeh (66.97 %) populations. The lowest amount was observed in Dizin (2.54 %) and Meygoun (2.24 %) populations (Table 4). The greatest amount of *p*-cymene belonged to Khorasan (7.99 %), Taleghan (6.3 %), and Ira (6.8 %) populations. Ahar (0.62 %) and Oshan (2.61 %) populations showed the lowest value (Table 4). The amount of geraniol of essential oil was the highest value in the populations of Dizin (43.65 %), Nashtrood (37.7 %), and Oshan (30 %) and was the minimum in the populations of Donbak (1.34 %) and Khorasan (0.15 %). This compound was not detected in 10 populations such as Polkhab. The highest and lowest amounts of *α*-pinene were observed in the populations of

Zanjan (3.68 %) and Oshan (0.43 %), respectively. However, *α*-pinene was not found in Nashtrood population. The greatest amount of camphene was observed in the population of Tizkooh (1.85 %) and Taleghan (1.65 %). The population of Dizin (0.36 %) and Ahar (0.4 %) showed the lowest percentage. However, camphene was found in all the populations except Nashtrood. The percentage of *α*-terpinene was the highest amount in Ahar population (1.48 %), and the lowest value was determined in Nashtrood (0.15 %) and Dizin (0.22 %) populations. The maximum and minimum amount of sabinene hydrate was obtained in Khorasan (2.6 %) and Dizin (0.23 %) populations, respectively. The population of Khorasan and Nashtrood had the highest (6.88 %) and lowest (0.38 %) value of *γ*-terpinene, respectively. The highest (2.7 %) and lowest (0.38 %) amount of eucalyptol was found in Taleghan and Khorasan populations, respectively. The maximum (6.88 %) and minimum (0.38 %) value of myrcene belonged to the population of Dizin and Nashtrood, respectively. The highest amount of borneol was observed in the population of thyme of Tizkooh (3.65 %) and Nashtrood (3.16 %), and the lowest was obtained in the Varangrood population (0.11 %).

Table 3. The average amount of *T. fallax* essential oil compounds in the studied populations

No.	Chemical Composition	KI (Kovats index)	Percentage
1	*α*-Thujene	927	0.68
2	*α*-Pinene	934	1.26
3	Camphene	952	0.81
4	*β*-Pinene	981	0.26
5	Myrcene	992	1.35
6	*α*-Phellandrene	1011	0.15
7	*α*-Terpinen	1021	0.86
8	*p*-Cymene	1031	4.26
9	Limonene	1034	0.46
10	Eucalyptol	1037	3.45
11	*γ*-Terpinene	1063	1.04
12	*trans*-Sabinene hydrate	1078	0.99
13	Linalool	1107	0.39
14	Camphor	1160	0.24
15	Borneol	1185	2.07
16	Terpinen-4-ol	1191	0.48
17	Nerol	1234	0.62
18	Thymol methyl ether	1239	0.18
19	Carvacrol methyle ether	1248	0.73
20	Neral	1250	0.52
21	Geraniol	1261	8.56
22	Geranial	1280	0.60
23	*trans*-Bornyl acetate	1292	0.04
24	Thymol	1307	23.13
25	Carvacrol	1315	40.09
26	Geranyl acetate	1385	1.39
27	Caryophyllene	1427	0.78
28	Germacrene D	1490	0.08
29	*β*-Bisabolene	1514	0.26
30	*γ*-Cadinene	1523	0.10
Total			95.98

Table 4. Comparison of the average percentage of main compounds in the essential oil of *T. fallax* populations

Origin	Components of essential oil					
	Thymol	**Carvacrol**	***p*-Cymene**	***α*-Pinene**	**Camphene**	**Geraniol**
Tizkooh	33.9 ± 1.71^{e}	16.11 ± 1.94^{j}	0.85 ± 0.07^{de}	1.57 ± 0.125^{cd}	1.85 ± 0.19^{a}	16.45 ± 0.788^{e}
Donbk	6.2 ± 0.96^{l}	67.85 ± 3.51^{b}	0.89 ± 0.05^{de}	0.83 ± 0.042^{i}	0.44 ± 0.1^{gh}	1.34 ± 0.27^{h}
Polkhab	12.02 ± 0.29^{ij}	63.97 ± 0.61^{cde}	0.86 ± 0.07^{de}	0.91 ± 0.1^{hi}	0.63 ± 0.11^{efg}	-
Moroud	39.38 ± 1.38^{d}	28.43 ± 1.89^{i}	1.36 ± 0.17^{c}	1.43 ± 0.05^{de}	1.31 ± 0.07^{c}	4.67 ± 0.436^{f}
Badmoroud	31.43 ± 1.4^{f}	37.03 ± 1.59^{h}	1.65 ± 0.12^{b}	1.69 ± 0.08^{c}	1.35 ± 0.04^{c}	-
Nashtrood	2.53 ± 0.36^{m}	28.23 ± 3.11^{i}	0.9 ± 0.05^{de}	-	-	37.72 ± 1.84^{b}
Dizin	11.17 ± 0.27^{jk}	2.54 ± 0.36^{kl}	0.23 ± 0.02^{i}	0.8 ± 0.104^{i}	0.36 ± 0.04^{h}	43.65 ± 1.18^{a}
Varangrood	43.36 ± 0.94^{c}	4.27 ± 0.28^{kl}	0.45 ± 0.01^{h}	0.86 ± 0.22^{00}	0.55 ± 0.1^{efgh}	25.72 ± 0.845^{d}
Zanjan	1.53 ± 0.07^{m}	65.84 ± 1.17^{bcd}	0.79 ± 0.009^{ef}	3.68 ± 0.18^{a}	0.96 ± 0.25^{d}	3.01 ± 0.538^{g}
Ahar	13.23 ± 0.79^{i}	63.01 ± 1.6^{de}	0.62 ± 0.02^{g}	0.91 ± 0.07^{hi}	0.4 ± 0.05^{h}	-
Ira	27.75 ± 1.26^{g}	43.62 ± 2.29^{g}	6.82 ± 0.21^{b}	2.21 ± 0.014^{b}	0.66 ± 0.1^{ef}	-
Chelcheshmeh	40.45 ± 2.26^{d}	35.64 ± 2.28^{h}	4.13 ± 0.05^{efg}	1.67 ± 0.17^{c}	1.04 ± 0.09^{d}	-
Ghormehdareh	2.53 ± 0.38^{m}	76.34 ± 1.56^{a}	3.25 ± 0.49^{hi}	0.89 ± 0.11^{hi}	0.61 ± 0.02^{efg}	-
Koohpahnak	9.86 ± 0.87^{k}	66.97 ± 3.87^{bc}	4.11 ± 0.07^{efg}	1.29 ± 0.01^{ef}	0.67 ± 0.09^{ef}	-
Siahplas	24.49 ± 0.44^{h}	51.72 ± 0.65^{f}	5.47 ± 0.38^{c}	1.08 ± 0.11^{gh}	0.47 ± 0.007^{fgh}	-
Taleghan	6.53 ± 1.26^{i}	61.43 ± 1.1^{e}	6.3 ± 0.75^{b}	1.61 ± 0.014^{cd}	1.65 ± 0.09^{b}	-
Oshan	1.53 ± 0.21^{m}	40.77 ± 1.58^{g}	2.06 ± 0.21^{j}	0.45 ± 0.03^{j}	0.63 ± 0.11^{efg}	30.007 ± 2.98^{c}
Migoun	72.67 ± 0.75^{a}	2.24 ± 0.24^{i}	4.75 ± 0.25^{cde}	0.96 ± 0.07^{hi}	0.72 ± 0.07^{e}	-
Khorasan	58.93 ± 1.3^{b}	5.74 ± 0.27^{k}	7.99 ± 0.87^{a}	1.17 ± 0.07^{fg}	1.04 ± 0.09^{d}	0.153 ± 0.023^{h}

Values within the same column followed by the same letter are not significantly different based on the LSD test ($p \leq 0.05$)

Table 4. Comparison of the average percentage and important compounds of the essential oil of different populations of *T. fallax* (Continued)

Region	Components of essential oil					
	α-Terpinene	trans-Sabinene hydrate	γ-Terpinene	Eucalyptol	Myrcene	Borneol
Tizkooh	0.89 ± 0.01^{efgh}	0.85 ± 0.07^{de}	3.62 ± 0.07^{de}	1.01 ± 0.02^{ef}	1.19 ± 0.08^{fghi}	3.65 ± 0.23^{b}
Donbk	1.15 ± 0.14^{cd}	0.89 ± 0.05^{de}	6.17 ± 0.34^{b}	0.75 ± 0.04^{h}	1.27 ± 0.03^{efgh}	1.53 ± 0.09^{hig}
Polkhab	0.8 ± 0.02^{ghi}	0.86 ± 0.07^{de}	3.13 ± 0.17^{efg}	1.21 ± 0.07^{d}	0.9 ± 0.12^{j}	2.01 ± 0.05^{fg}
Moroud	0.96 ± 0.05^{efg}	1.36 ± 0.17^{c}	3.35 ± 0.06^{ef}	0.78 ± 0.14^{gh}	1.44 ± 0.04^{cde}	2.8 ± 0.14^{cd}
Badmoroud	1.29 ± 0.02^{bc}	1.65 ± 0.12^{b}	5.17 ± 0.84^{c}	1.15 ± 0.04^{de}	1.61 ± 0.12^{bc}	2.68 ± 0.29^{d}
Nashtrood	0.15 ± 0.01^{j}	0.9 ± 0.05^{de}	0.38 ± 0.52^{k}	0.49 ± 0.08^{i}	0.27 ± 0.02^{k}	3.16 ± 0.12^{c}
Dizin	0.22 ± 0.01^{j}	0.23 ± 0.02^{i}	1.15 ± 0.12^{j}	0.13 ± 0.01^{j}	3.11 ± 0.27^{a}	1.02 ± 0.14^{k}
Varangrood	0.77 ± 0.04^{hi}	0.45 ± 0.01^{h}	2.63 ± 0.42^{gh}	0.88 ± 0.09^{fgh}	1.34 ± 0.005^{defg}	0.11 ± 0.01^{i}
Zanjan	0.81 ± 0.02^{ghi}	0.79 ± 0.009^{ef}	4.23 ± 0.37^{d}	0.95 ± 0.05^{fg}	1.32 ± 0.01^{defg}	2.83 ± 0.26^{cd}
Ahar	1.48 ± 0.03^{a}	0.62 ± 0.02^{g}	6.76 ± 0.64^{ab}	0.77 ± 0.04^{gh}	1.49 ± 0.07^{bcd}	1.14 ± 0.08^{jk}
Ira	1 ± 0.06^{def}	1.28 ± 0.03^{c}	2.76 ± 0.35^{fgh}	2.01 ± 0.17^{b}	1.35 ± 0.001^{def}	1.6 ± 0.08^{hi}
Chelcheshmeh	1.03 ± 0.08^{de}	0.69 ± 0.04^{fg}	3.68 ± 0.1^{de}	0.86 ± 0.1^{fgh}	1.49 ± 0.06^{bcd}	2.18 ± 0.03^{ef}
Ghormehdareh	0.06 ± 0.98^{def}	0.03 ± 0.59^{g}	0.07 ± 3.62^{de}	1.19 ± 0.06^{de}	1.44 ± 0.04^{cde}	1.89 ± 0.11^{fgh}
Koohpahnak	0.85 ± 0.005^{fghi}	0.82 ± 0.03^{def}	3.44 ± 0.01^{ef}	0.87 ± 0.02^{fgh}	1.11 ± 0.12^{hi}	1.73 ± 0.12^{ghi}
Siahplas	1.02 ± 0.07^{def}	0.98 ± 0.01^{d}	3.81 ± 0.16^{de}	1.5 ± 0.01^{c}	1.15 ± 0.1^{ghi}	1.32 ± 0.09^{ijk}
Taleghan	0.68 ± 0.09^{i}	1.3 ± 0.09^{c}	2.31 ± 0.58^{hi}	2.7 ± 0.32^{a}	1.11 ± 0.12^{hi}	4.08 ± 0.7^{a}
Oshan	0.16 ± 0.02^{j}	0.6 ± 0.08^{g}	0.58 ± 0.02^{jk}	1.04 ± 0.01^{def}	1.05 ± 0.15^{ij}	2.51 ± 0.19^{de}
Migoun	0.7 ± 0.07^{i}	1.42 ± 0.07^{c}	1.89 ± 0.19^{i}	1.19 ± 0.06^{de}	1.67 ± 0.15^{b}	1.58 ± 0.26^{hi}
Khorasan	1.39 ± 0.06^{ab}	2.6 ± 0.2^{a}	6.88 ± 0.7^{a}	0.38 ± 0.05^{i}	1.38 ± 0.01^{def}	1.51 ± 0.1^{hij}

Values within the same column followed by the same letter are not significantly different based on the LSD test ($P \leq 0.05$)

Assessing the correlation between essential oil components indicated that there was a negative and significant correlation between thymol and carvacrol. However, a positive and significant correlation was observed between thymol and *α*-pinene, *trans*-sabinene hydrate, and camphene (Table 5). Moreover, there was a positive and significant correlation between carvacrol and *γ*-terpinene and eucalyptol. However, a negative and significant correlation was found between carvacrol and geraniol and myrcene. There was a negative correlation between geraniol and all of the features except myrcene (Table 5).

The cluster analysis of the essential oil compounds identified five groups of thyme populations including first group with high carvacrol content, second group with high geraniol content, third group with high thymol and carvacrol content, fourth group with high thymol content, and fifth group with high content of thymol, carvacrol, and geraniol (Fig. 3). First group included seven populations of Polkhab, Ahar, donbak, Koohpahnak, Taleghan, Zanjan, and Ghormehdareh. Second group consisted of three populations of Nashtrood, Oshan, and Dizin. Third group is five populations of Moroud, Chelcheshmeh, Badmoroud, Ira, and Siahplas. Fourth group is considered as two populations of Meygoun and Khorasan. Fifth group included two populations of Tizkooh and Varangrood.

Table 5. Correlation between main compounds of the essential oil in different populations of *T. fallax*

	***α*-Pinene**	**Camphene**	**Myrcene**	***α*-Terpinen**	***p*-Cymene**	***γ*-Terpinene**	**Eucalyptol**	***trans*-Sabinene hydrate**	**Borneol**	**Geraniol**	**Thymol**	**Carvacrol**
α-Pinene	-											
Camphene	0.49**	-										
Myrcene	0.106	0.02	-									
α-Terpinene	0.304*	0.275*	0.02	-								
p-Cymene	0.333*	0.514**	-0.127	0.582**	-							
γ-Terpinene	0.27*	0.2	0.051	0.915**	0.451**	-						
Eucalyptol	0.297*	0.365**	-0.276*	0.067	0.491**	-0.134	-					
trans-Sabinene hydrate	0.161	0.389**	-0.15	0.434**	0.752**	0.389**	0.131	-				
Borneol	0.262*	0.602**	0.397**	-0.209	0.116	-0.206	0.358**	0.195	-			
Geraniol	-0.445**	-0.341**	0.171	-0.769**	-0.697**	-0.638**	-0.447**	-0.454**	-0.039	-		
Thymol	0.026	0.29*	0.197	0.333*	0.468**	0.171	-0.092	0.551**	0.252	-0.23	-	
Carvacrol	0.221	-0.094	0.359**	0.236	0.019	0.267*	0.383**	-0.233	0.186	0.506**	0.705**	-

ns, * and ** show non-significant and significant at 5 and 1% level, respectively.

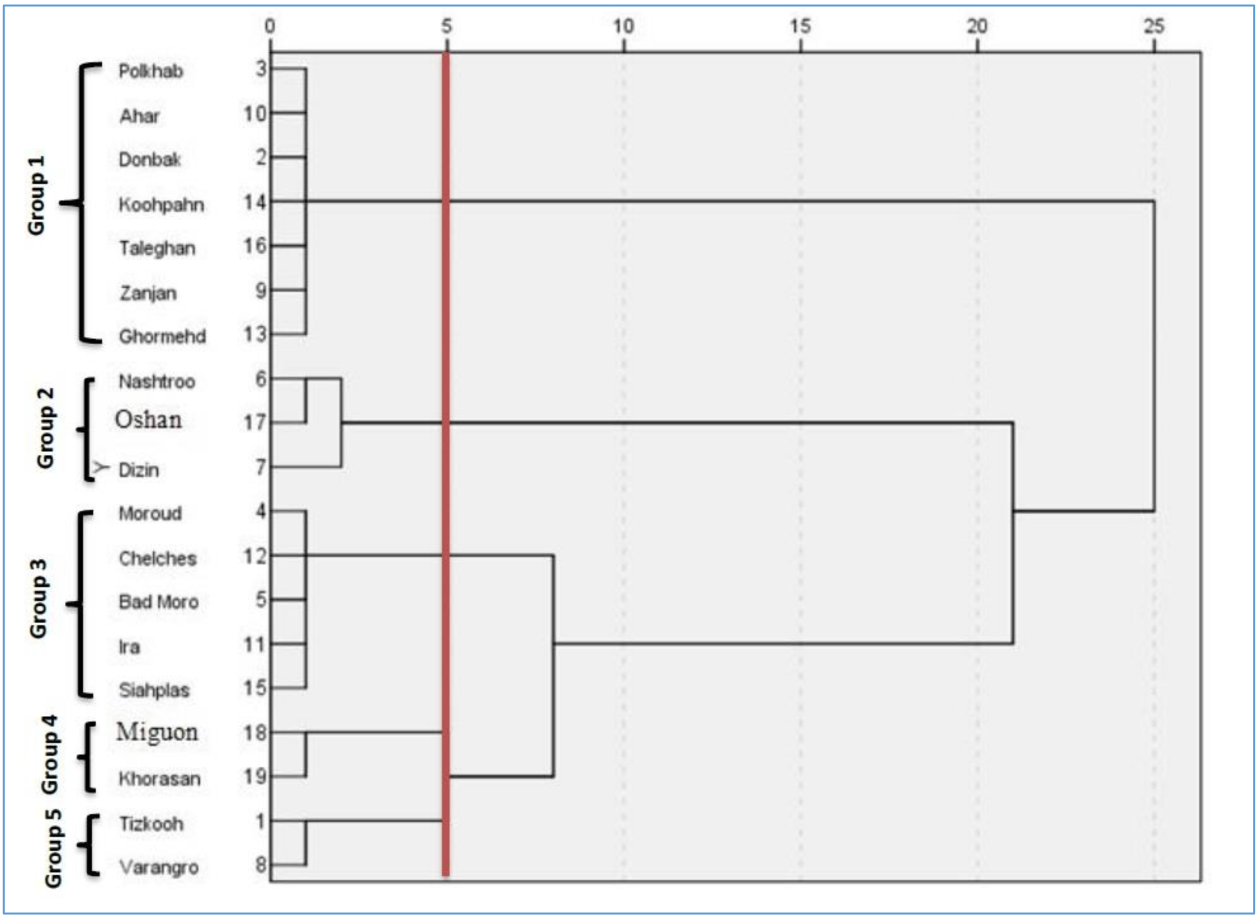

Fig. 3. Cluster analysis of 19 populations of *T. fallax* based on essential oil compounds

4. Discussion

As Fig. 1 shows, the essential oil content of the populations varies from 1.05 to 2.6 %, which shows the high diversity of the populations in terms of the amount of essential oil. In previous studies, different amounts of essential oil have been reported for thyme species. A maximum of 2.9 % in the flowering stage and a minimum of 0.7 % in the seed ripening stage were reported for *T. fedtschenkoi* [22]. Hezit et al. [23] indicated that the amount of essential oil of *T. pallescens* was in the range of 2.4-4.6 % in complete flowering stage and 0.9-1.3 % in the stage of beginning of vegetative growth. The essential oil percentage of *T. fallax* in Lorestan (Rimaleh) region was reported 2.8 % [14].

The amount of essential oil can be mainly affected by environmental and genetic factors [24]. In our study, assessing the amount of essential oil in the different populations showed no significant relationship between the changes in the amount of essential oil and soil and climate characteristics (temperature and precipitation) and changes in the altitude above sea level (Fig. 2 and Table 6). Although, altitude above sea level is one of the environmental factors affecting the content of the essential oil and it is reported that the lower altitudes led to an increase in the essential oil content in Marjoram [6]. Habibi et al. [12] on *Thymus kotschyanus* indicated that the amount of essential oil decreased by increasing the altitude. The highest (2.56 %) and lowest (1.31 %) percentage of essential oil was related to the altitude of 1800 m and 2800 meters, respectively. Jamshidi et al. [13] evaluated thyme in Damavand region and reported that the essential oil content, total compounds, and carvacrol percentage decreased by increasing altitude. However, increasing the altitude resulted in increasing the percentage of thymol. Another study assessed the effect of altitude on the percentage of essential oil of Satureja khuzestanica in its natural habitat, northern region of Khuzestan, and reported that the essential oil from the altitudes of 1200, 1050, 850, 750, and 650 m was 2.08, 2.55, 3.36, 3.85, and 4.91 % respectively. The highest and lowest of which belongs to the altitude of 650 and 1200 m, respectively [25]. In a study on *Satureja khuzestanica* and *Satureja Rechingeri* showed that the higher percentage of essential oil was observed in the areas where the altitude was lower than the sea level [26]. Also, in a study on seven populations of Lavandula Latifolia in different geographical regions of Spain indicated that the yield of essential oil was different among populations and regions, and warmer regions had a higher percentage of leaf essential oil compared to the other regions [8]. However, the review of previous studies regarding the effect of altitude on medicinal plants shows that altitude is an influencing factor on the amount of essential oil, our study showed that with the increase in altitude, the amount of thyme essential oil did not have a clear trend (Fig. 2) and was not a key factor.

Table 6. Correlation between altitude, soil and climate characteristics with the amount of main compound of *T. fallax* essential oil

Soil and climatic properties	Amount of essential oil	*p*-Cymene	Geraniol	Thymol	Carvacrol
EC	0.19	0.08	0.13	0.16	-0.28
pH	-0.37	0.21	-0.05	-0.02	0.01
N	0.49	-0.24	-0.12	-0.05	0.16
P	0.37	-0.31	-0.05	-0.34	0.34
K	0.27	0.08	-0.22	0.13	0.02
Sand	-0.01	-0.25	0.47	0.1	-0.44
Silt	0.1	0.05	-0.4	-0.2	0.49
Clay	-0.15	0.49	-0.33	0.15	0.08
OC	0.45	-0.28	-0.07	-0.04	0.14
altitude	-0.17	-0.24	-0.02	-0.21	0.27
Temperature	0.269	-0.47	-0.26	-0.27	0.181
Precipitation	0.09	-0.44	0.479	-0.08	-0.236

Our study showed that thymol, carvacrol, geraniol, borneol, *γ*-terpinene, *p*-cymene, myrcene, *α*-terpinene, camphene, *trans*-sabinene hydrate, eucalyptol, and *α*-pinene were the main compounds of the essential oil, which included about 87 % of the total essential oil compounds (Table 3). It has already been reported that the main compounds in the essential oils of *Thymus* species include thymol, carvacrol, borneol, *p*-cymene, *γ*-terpinene, *α*-terpinene, linalool, linalool acetate, geraniol, and 1,8-cineole [3]. In a study, carvacrol, thymol, *γ*-terpinene, *p*-cymene, camphene and geraniol were reported as the dominant compounds of *Thymus fallax* in Lorestan region [14]. The qualitative and quantitative analysis of *Thymus kotschyanus* essential oil in the area of Tar Lake, Damavand based on the three altitude areas indicated that the percentage of essential oil was between 0.95-1.87 % and 37 essential oil compounds were identified, the most important effective contens being carvacrol (60.82-82.05 %) and thymol (1.56-13.94 %) [13]. In a study in Hamedan showed that the carvacrol (69.2 %), *p*-cymene (15.4 %), thymol (5.3 %), and terpinene (4.5 %) were the main component of *Thymus fallax* [22]. In a study in Turkey, 32 different compounds were identified in the essential oil of *Thymus fallax* collected from natural habitats, and the most important of them was carvacrol (68.1 %) [5].

In our study, no special relationship exist between soil and climate characteristics, and the essential oils content and composition (Table 6), which is consistent with the results of some studies. For example, Khorshidi et al. [27] in a study on ecotypes of *Thymus daenensis* reported that thymol was the dominant component of the essential oil in all the ecotypes, which was not significantly influenced by climatic and soil conditions. Therefore, these changes can be probably related to the genetic differences, since there was no significant difference based on the climatic and soil characteristics between the vegetation areas of Hamadan province.

Carvacrol was low in the ecotypes with high amount of thymol, and vice versa, which is completely normal because thymol and carvacrol are isomers of each other and are formed from similar precursors, which is consistent with the results of the studies conducted by Khorshidi et al. [27], Tohidi et al. [2], and Nabavi et al. [28] on thyme. However, different chemotypes of thyme species were reported from different regions of the world, for example, chemotype of *Thymus vulgaris* with high thymol in southern Italy [29] and or chemotypes of thymol and carvacrol in *Thymus daenensis* from different regions of Fars province [30].

5. Conclusion

Overall, different percentage and various types of the essential oil compounds were observed in different populations of *Thymus fallax*. The presence of higher amounts of thymol (72 %) with 2.5 % essential oil in Migoun populations, and higher values of carvacrol (76 %) with 2.25 % essential oil in the population of Ghormehdareh were the interest points compared to the other populations, which can be considered in further and breeding studies. Evaluating all populations under the same cultivation conditions is suggested for achieving more accurate results and identifying the superior population based on the quantitative and qualitative yield.

Author contributions

K. R.R.: Investigation, Formal analysis, Resources, Methodology, Software, and Writing- Original draft. H. N.B. and M. A.: Conceptualization, Project administration, Supervision, Methodology, Validation, Data curation, and Writing-review & editing. A. K: Supervision, data curation, Validation, reviewing and editing the manuscript.

Conflicts of interest

The authors confirm that there are no conflicts of interest.

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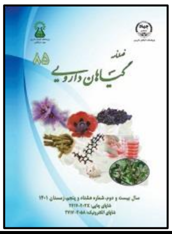

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مقاله تحقیقاتی

ارزیابی تنوع اسانس جمعیت‌های مختلف آویشن آناتولی جمع‌آوری شده از برخی از رویشگاه‌های آن در ایران

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آویشن آناتولی

چکیده

مقدمه: آویشن آناتولی یکی از گیاهان دارویی خانواده نعناعیان است که قسمت هوایی آن حاوی ترکیبات فیتوشیمیایی ارزشمندی مانند تیمول و کارواکرول است. **هدف**: این مطالعه به منظور بررسی تنوع فیتوشیمیایی اسانس جمعیت‌های مختلف آویشن آناتولی در رویشگاه‌های آن انجام شد. **روش بررسی**: جمعیت‌های آویشن آناتولی از رویشگاه‌های طبیعی آن در استان‌های البرز، تهران، زنجان، مازندران و خراسان شمالی جمع‌آوری شدند. اسانس آنها به روش تقطیر با آب استخراج و ترکیبات آنها با استفاده از GC و GC-MS شناسایی شد. **نتایج**: نتایج نشان داد که میزان اسانس جمعیت‌ها به‌طور میانگین ۱/۷۱ درصد بوده و جمعیت میگون (۲/۵۸ درصد)، چلچشمه (۲/۳۵ درصد) و قرمه‌دره (۲/۲۵ درصد) دارای بیشترین میزان اسانس بودند. کمترین میزان اسانس مربوط به دیزین (۱/۰۵ درصد) و ایرا (۱/۱۵ درصد) بود. در مجموع، ۳۰ ترکیب در اسانس که شامل ۹۵ درصد از کل اسانس بود شناسایی شد که کارواکرول، تیمول، پی سیمن و ژرانیول مهمترین آنها بودند که ۷۵ درصد اسانس را تشکیل می‌دادند. پنج گروه از جمعیت آویشن با استفاده از تجزیه و تحلیل خوشه‌ای شناسایی شدند و گروه اول با میزان بالای کارواکرول، گروه دوم با میزان بالای ژرانیول، گروه سوم با میزان بالای تیمول و کارواکرول، گروه چهارم با میزان بالای تیمول و گروه پنجم با میزان بالای تیمول، کارواکرول و ژرانیول بودند. **نتیجه‌گیری**: مقادیر بالای اسانس، کارواکرول و تیمول در برخی جمعیت‌ها نتایج ارزشمندی بود که می‌تواند در مطالعات آتی و اصلاحی مورد توجه قرار گیرد.

مخفف‌ها: GC، کروماتوگرافی گازی؛ GC-MS، کروماتوگرافی گازی متصل به طیف‌سنج جرمی

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