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COMPARATIVE ESSENTIAL OIL COMPOSITION OF AERIAL PARTS OF *MICROMERIA PERSICA* POPULATIONS FROM FOUR REGIONS IN FARS PROVINCE, IRAN.

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Abstract*:There are three species of the Micromeria plant in Iran, one of which is Micromeria persica. Different species of Micromeria has been used in traditional medicine. The current study deals with identifying chemical compounds of Micromeria persica populations in four regions of Fars province in Iran. These regions are Kuh-e Zireh, Firuzabad, Bezyn defile in Darab and Ghir to Firuzabad. First, the aerial parts of Micromeria persica collected as samples were dried. Then, the essence of the dried samples was extracted by water distillation in the Clevenger machine, and identification of compounds was made using the GC/MS machine. In Kuh-e Zireh, Firuzabad, Bezyn defile in Darab and Ghir to Firuzabad regions, the numbers of recognized compounds were 30, 45, 50 and 25 respectively. The main essence compounds of the four examined populations were Germacrene D, Bicyclogermacrene, spathulenol, and δ-cadinene. Geographical position and ecological parameters of habitat, such as height, annual rainfall, and climate, can change the quality and quantity of the essential oil's compounds in Micromeria persica.*

Key words*:* Micromeria Persica, Phytochemical, essential oil composition GC-MS, Iran

1. Introduction

There are three known species of the *Micromeria* genus in Iran: *Micromeria persica*, *Micromeria hedgei*, and *Micromeria myrtifolia*. The first two species are endemic [1,2]. Some *Micromeria* species are used in folk medicine for different purposes. The aerial flowering parts of the plants are locally used for treatment of cold. Several *Micromeria* species have been reported as antiseptic, abortifacient, antirheumatic, CNS stimulant, and tonic [3]. They are also used for treatment of heart disorders, indigestion, and headaches and as topical anaesthetic for toothache and wounds, inflamed eyes, skin infections and chest pains [4,5]. Some *Micromeria* species have also shown antioxidant and

antimicrobial properties [6-8]. *M. biflora* and *M. graeca* species in Spain are used for treating disorders of the digestive tract and stomach pains, respectively; *M. fruticose* is used in Turkey to relieve headache; in the Canary Islands *M. herpyllomorpha* and *M. varia* are used as a capillary tonic [9].

Sefidkon et al. [10] studied *M. Persica* in Hamadan province and analyzed the essence extracted from aerial parts of the plant before flowering and full flowering stages. They concluded that Thymol, limonene, γ-terpinene, p-cymene and 1,8-cineole are the main constituents of the essence of the plant. Subsequently, essential oil isolated by hydrodistillation from the aerial parts of *M. Persica* Boiss from Persepolis, Province of Fars (Iran) during the flowering stage were analyzed. The main constituents were linalool, a-pinene and (E)-nerolidol [11]. In studies of Kazemi Zadeh et al. [12] on chemical compositions of the essential oil of the two populations of Teucrium hyrcanicum, it was showed that the qualitative and quantitative differences in the essence composition of these two populations may be due to differences in the ecological properties of growth areas such as temperature, humidity, height from sea and other soil and geographical factors.

The current study deals with identifying chemical compounds of *Micromeria persica* populations in four regions of Fars province in Iran. It also investigates the effects of different parameters, such as height, annual rainfall, climate, and location on constituents of the essence.

2. Material and Methods

2.1.1 Plant Material

The aerial parts of *Micromeria persica* were collected in mid-spring (in May and June) from altitude of Kuh-e Zireh, Firuzabad, Bezyn defile in Darab and 14 kilometers after Ghir to Firuzabad in Fars Province in the flowering stage. Geography and climate of the sampling regions are given in Tab. 1. Herbarium plant was identified by the Agriculture and Natural Resources Research Center of Fars province.

Table 1. Environmental Factors of the Sampling Regions.

Region	Location	Height	Climate	Annual Rainfall
Ghir to Firuzabad	southwestern of Shiraz	2500	dry moderate	356
Kuh-e Zireh	80 kilometers south-east of Shiraz	1832	dry moderate	340
Firuzabad	30 kilometers west of Shiraz	2125	dry moderate	315.7
Bezyn defile in Darab	70 kilometers east of Shiraz	1692	dry moderate	237

2.1.2 Isolation Procedure

The air-dried parts of *M. persica* were separately subjected to hydrodistillation using a Clevenger-type apparatus for 3 h. The essential oils were obtained in 3.00% (w/w) yield. The oils were dried over sodium sulfate and stored in sealed vials at low temperature before analysis. Identification of the constituents of each of the oils was made using the Gas Chromatography/Mass Spectrometry (GC/MS) machine.

2.1.3 Gas Chromatography

GC analyses were performed using an Agilent 7890A gas chromatograph equipped with a HP-5 column (30 m × 0.32 mm i.d., film thickness 0.25 μm) and connected to flame ionization detector (FID). Nitrogen was selected as the carrier gas with a flow rate of 1 ml/min. The injector temperature was 280°C, and detector temperature was 290°C, while column temperature was linearly programmed from 60 to 210°C (at rate of 3°/min) and then held for 7 min at 210°C.

2.1.4 Gas Chromatography-Mass Spectrum

GC analyses were performed using an Agilent 5975A gas chromatograph equipped with a HP-5MS column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Nitrogen was selected as the carrier gas with a flow rate of 1 ml/min. column temperature was linearly programmed from 60 °C to 210 °C (at rate of 3°/min) and then 210 °C to 240 °C (at rate of 20 °C/min). MS were taken at 70 eV.

3. Results

3.1. The Composition of the Oils of *M. Persica*

The composition of the oils of the aerial parts of *M. persica* in Kuh-e Zireh, Firuzabad, Bezyn defile in Darab and Ghir to Firuzabad regions are listed in Tables 2, 3, 4 and 5 respectively, in which the percentage and retention indices of components are given.

3.1.1 The Composition of the Oil of M. Persica in Kuh-e Zireh

Thirty compounds were identified in the essential oil of *M. persica* Kuh-e Zireh, representing more than 99.99% of the oil. The major components were found to be Spathulenol (30.25%), Bicyclogermacrene (18.89%), Germacrene D (19.37%). One compound was unknown.

The chemical composition of the essential oil of *M. persica* in Kuh-e Zireh can be seen in Tab. 2.

Table 2. Percentage Composition of the Oils of *Micromeria persica* in Kuh-e Zireh

No	Compound (P920365)	RI	% of compound
1	α-Thujene	925	0.156
2	α-Pinene	932	2.82
3	Sabinene	971	6.228
4	β-Pinene	976	0.959
5	Myrcene	989	1.35
6	p-Cymene	1023	0.259
7	Limonene	1026	1.912
8	1,8-Cineole	1029	0.854
9	(Z)–β-Ocimene	1034	0.949
10	(E)-β-Ocimene	1045	0.493
11	γ-Terpinene	1056	0.442
12	Linalool	1098	0.224

13	Terpinene-4-ol	1175	0.929
14	α-Terpineol	1188	1.115
15	Bornyl acetate	1283	0.274
16	n-Tridecane	1297	1.173
17	δ-Elemene	1334	0.808
18	α-Terpinyl acetate	1347	1.58
19	α-Copaene	1373	0.367
20	β-Bourbonene	1382	1.781
21	β-Elemene	1389	0.401
22	Germacrene D	1478	19.361
23	Bicyclogermacrene	1493	18.882
24	δ-Cadinene	1520	0.534
25	Spathulenol	1574	30.247
26	Caryophyllene oxide	1579	1.085
27	γ-Eudesmol	1634	1.253
28	epi-α-Muurolol	1650	1.317
29	Khusinol	1682	1.681
30	Unknown	1686	0.566

3.1.2 The Composition of the Oil of M. Persica in Firuzabad

There are 45 compounds in *M.Persica* essential oil in Firuzabad region, that is a total of 99.4 percent. The major components of the oil were Germacrene D (35.62%), Bicyclogermacrene (15.72%), Phytol (7%), Spathulenol (6.47%).

The chemical composition of the essential oil of *M. persica* in Firuzabad can be seen in Tab. 3.

Table 3. Percentage Composition of the Oils of *Micromeria persica* in Firuzabad

No	Compound (P920363)	RI	% of compound
1	□-Pinene	932	0.697
2	Sabinene	972	0.186
3	□-Pinene	976	0.719
4	Myrcene	989	0.66
5	□-Phellandrene	1005	0.131
6	□-3-Carene	1010	0.701
7	□-Terpinene	1016	0.052
8	p-Cymene	1023	0.283
9	Limonene	1027	1.273
10	1,8-Cineole	1030	0.163
11	(Z)□□-Ocimene	1035	5.04
12	(E)-□-Ocimene	1045	0.655
13	□-Terpinene	1056	0.519

14	cis-Sabinene hydrate	1064	0.077
15	Terpinolene	1087	0.435
16	Linalool	1098	0.386
17	n-Nonanal	1103	0.127
18	allo-Ocimene	1127	0.275
19	Terpinene-4-ol	1175	0.28
20	□-Terpineol	1188	0.215
21	Carvone	1241	0.466
22	Bornyl acetate	1283	0.102
23	Thymol	1289	0.279
24	Carvacrol	1298	0.201
25	□-Elemene	1335	1.562
26	□-Copaene	1374	4.093
27	□-Bourbonene	1382	0.68
28	□-Elemene	1390	1.351
29	(E)-Caryophyllene	1417	1.983
30	□-Copaene	1426	0.474
31	□-Humulene	1451	0.604
32	allo-Aromadendrene	1458	0.476
33	Germacrene D	1481	35.619
34	Bicyclogermacrene	1496	15.714
35	□-Cadinene	1521	5.221
36	(E)-□-Bisabolene	1529	0.169
37	Spathulenol	1575	6.469
38	Viridiflorol	1588	1.008
39	□-Cadinol	1650	0.513
40	6,10,14-trimethyl-2-pentadecanone	1840	0.32
41	Diisobutyl phthalate	1861	0.364
42	Dibutyl phthalate	1959	0.465
43	epi-13-Manool	2055	1.417
44	Phytol	2116	7.006
45	n-Tricosane	2297	0.57

3.1.3 The Composition of the Oil of M. Persica in Bezyn defile in Darab

50 components in the oil of *M. Persica* in Bezyn defile in Darab, which represented about 100% of the total oil, were identified. The oil of *M. Persica* consisted of GermacreneD (22.1%), Bicyclogermacrene (17.30%), Spathulenol (10.9%), δ -Cadinene (8.7%), α -Copaene (5.9%).

The chemical composition of the essential oil of *M. persica* in Bezyn defile in Darab can be seen in Tab. 4.

Table 4. Percentage Composition of the Oils of *Micromeria persica* in Bezyn Defile in Darab

No	Compound (P920363)	RI	% of compound
1	α-Thujene	925	0.012
2	α-Pinene	932	0.647
3	Camphene	947	0.014
4	Sabinene	971	0.203
5	β-Pinene	975	0.418
6	Myrcene	989	0.341
7	α-Phellandrene	1004	0.056
8	δ-3-Carene	1009	0.051
9	α-Terpinene	1015	0.028
10	p-Cymene	1023	0.125
11	Limonene	1027	1.143
12	1,8-Cineole	1029	0.798
13	(Z)–β-Ocimene	1035	1.668
14	(E)-β-Ocimene	1045	0.287
15	γ-Terpinene	1056	0.239
16	Terpinolene	1086	0.114
17	Linalool	1098	0.775
18	n-Nonanal	1102	0.136
19	cis-p-Menth-2-en-1-ol	1119	0.096
20	trans-p-Menth-2-en-1-ol	1136	0.131
21	Camphor	1142	0.165
22	Borneol	1163	0.1
23	Terpinene-4-ol	1175	1.595
24	α-Terpineol	1189	2.025
25	Carvone	1242	3.136
26	Bornyl acetate	1283	0.14
27	Thymol	1289	0.258
28	Carvacrol	1298	0.958
29	δ-Elemene	1335	1.679
30	α-Terpinyl acetate	1347	0.844
31	α-Copaene	1374	5.879
32	β-Bourbonene	1382	1.292
33	β-Elemene	1390	1.184
34	(E)-Caryophyllene	1416	1.287
35	α-Humulene	1451	0.51
36	allo-Aromadendrene	1458	0.676
37	Germacrene D	1482	22.034
38	Bicyclogermacrene	1497	17.297
39	γ-Cadinene	1512	0.846
40	δ-Cadinene	1522	8.689

41	Spathulenol	1577	10.817
42	Caryophyllene oxide	1581	1.665
43	Salvial-4(14)-en-1-one	1591	0.556
44	epi-α-Cadinol	1639	1.983
45	β-Eudesmol	1647	1.828
46	Khusinol	1683	2.449
47	6,10,14-trimethyl-2-pentadecanone	1840	0.254
48	n-Hexadecanoic acid	1963	1.038
49	Phytol	2114	1.061
50	n-Tricosane	2297	0.473

3.1.4 The Composition of the Oil of M. Persica in Ghir to Firuzabad

In the region of 14 km after Ghir to Firuzabad, there are 25 compounds in the essential of *M. Persica*, which are 100 percent of essential oil. In the oil n-Hexadecanoic acid (16.77), Germacrene D (12.60%), Spathulenol (8.89%), 1-β-ol Eudesma-4 (15) 7-dine- (7.18%), α -Cadinol (5.45%) are major componenets.

The chemical composition of the essential oil of *M. persica* in Ghir to Firuzabad can be seen in Tab. 5.

Table 5. Percentage Composition of the Oils of *Micromeria persica* in Ghir to Firuzabad

No	Compound (P920365)	RI	% of compound
1	Linalyl acetate	1254	0.228
2	□-Bourbonene	1382	0.191
3	□-Elemene	1390	0.549
4	(E)-Caryophyllene	1417	1.617
5	Germacrene D	1479	12.595
6	Bicyclogermacrene	1494	4.294
7	□-Cadinene	1521	0.613
8	Spathulenol	1575	8.898
9	□-Atlantol	1608	2.416
10	epi-□-Cadinol	1639	2.042
11	epoxy-allo-Aromadendrene	1643	1.926
12	□-Cadinol	1650	5.445
13	Eudesma-4(15),7-dien-1-□-ol	1683	7.177
14	6,10,14-trimethyl-2-pentadecanone	1840	3.202
15	Diisobutyl phthalate	1861	1.628
16	Dibutyl phthalate	1959	5.29
17	n-Hexadecanoic acid	1967	16.769
18	Neryl phenylacetate	2018	4.963
19	n-Heneicosane	2101	1.445
20	Phytol	2114	2.186
21	(E,E,E)-Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tetramethyl	2138	6.932

22	Unknown	2162	4.868
23	n-Docosane	2198	0.589
24	n-Tricosane	2297	2.096
25	n-Pentacosane	2498	2.043

3.2. Comparison of Essential Oil Components of *M. persica* in Four Regions Based on Height

In essence of Micromaria, most frequent compounds are Germacrene D, Bicyclogermacrene, Spathulenol, δ- Cadinene in different regions. Height is one of the important environmental factors which has a significant impact on the amount of active ingredient. Fig. 1 of shows comparing the compounds based on the height of the regions, Germacrene D has the most values at the height of 2125 meters (Firuzabad region), Bicyclogermacrene has the most values at the height of 1832 meters (Kuh-e Zireh), Spathulenol has the most values at the height of 1832 meters (Kuh-e Zireh) and δ - Cadinene has the most values at the height of 1,692 meters (Bezyn defile in Darab), respectively.

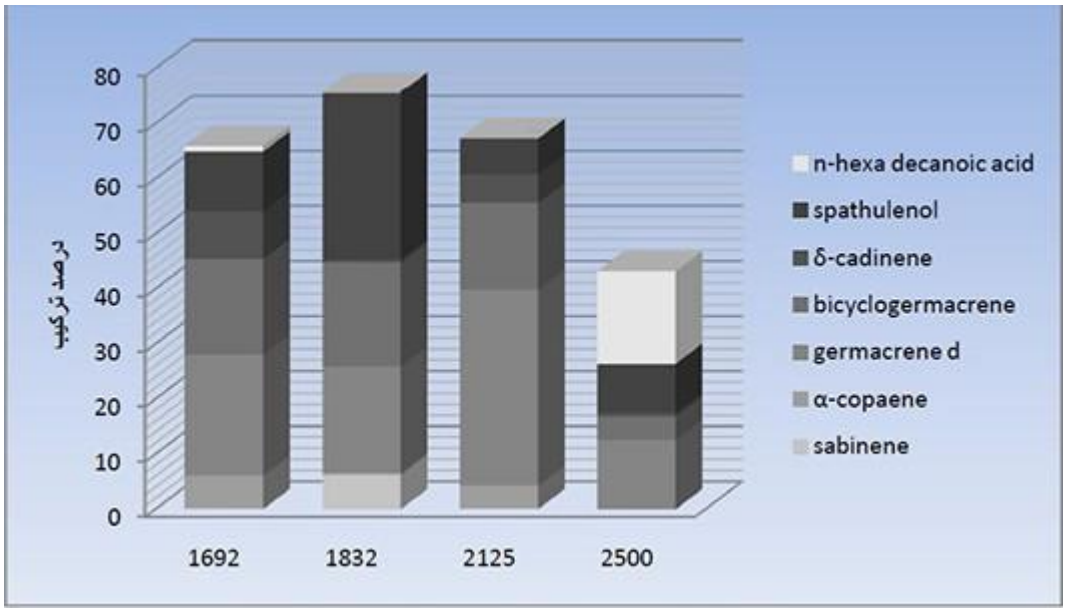

Fig 1. Comparison of Essential Oil Components of *M. persica* in Four Regions Based on Height

4. Conclusion

Changing height can have a direct impact on the temperature and moisture content, so it is an important factor. Increasing the height is along with decreasing temperature, increasing light intensity and increasing wind intensity. These changes along with decreasing temperature affect the moisture content. In addition to changes in climatic factors, height also changes the light quality. Increasing UV created at high altitudes interferes with plant growth. The direction of slope of earth is one of the factors that affect significantly on the amount of light received by ecosystem. This effect is especially evident in medium and high altitudes that sun angle decreases especially in the winter [13].

The growth location of medicinal plants, in terms of height from sea, slope and latitude and the impact of these factors on temperature, light, and relative humidity is very important in the medicinal

plants' metabolism and synthesis of their active ingredients. Height and climate are two important environmental factors in determining the chemical composition of medicinal plants essence. [14]

The results showed that differences in characteristics of the growth location such as height, slope, and direction of slope, cover and other climate conditions has a considerable impact on essence compounds. Differences in the quality and quantity of essence compounds of four populations of Micromaria in Fars province are due to the differences in ecological characteristics of growth areas such as temperature, humidity, altitude or other soil and geographical factors.

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