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Original Article

Comparative Analysis of Intra-and Inter Populational Heterogeneity of the Essential Oils in White Savory Plants

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Abstract

White savory (*Satureja mutica* Fisch & C.A.Mey.) is one of the most widely used medicinal plants in food processing, pharmaceutical and cosmetic industry due to the strongly scented and presence of phenolic compounds such as carvacrol and thymol. This experiment was carried out to evaluate the levels of inter and intra-populations variability of essential oil compositions of *S. mutica* grown in north of Iran. The essential oil was extracted by hydro-distillation method and analyzed using GC-FID and GC-MS apparatus. The results showed a high level of variation among individual plants of the studied populations based upon their essential oil production. The essential oil content ranged from 0.5 to 4.2%. thymol (6.5-74.6%), carvacrol (0.9-70.4%), borneol (0.1-38.0%), *p*-cymene (0.30-14.2%), and γ-terpinene (0.1-9.9%) were recognized as the major components of the all tested individual plants. Therefore, the variability identified here, might be considered as characterizing the large gene pool for breeding programs to comply the requirements of pharmaceutical and food industries.

Key words: Lamiaceae, Satureja, Essential oils, Population, Individuals, Metabolic variation

Introduction

The genus *Satureja* L., with the common persian name of "Marzeh" belonging to the Lamiaceae family and the Nepetoideae subfamily, has over 30 species that some of them are annual and most of species are perennial. These species are mostly native to the eastern Mediterranean region and west Asia, and generally grow in humid areas with deep soil and areas with dry, sunny and rocky soils. More than fourteen wild species are growing in the north, northwest, northeast, southwest and central parts of Iran. *Satureja mutica* Fisch. & C.A.Mey., is a highly aromatic species growing on calcareous rocks in the northeastern parts of Iran [1].

Like some other savory species, *S. mutica* has been traditionally used as muscle pain reliever, tonic and carminative in treating stomach and intestinal disorders such as cramps, nausea, indigestion and diarrhea [2]. The main phytochemical components of *S. mutica* are phenols, carvacrol, thymol, and flavonoids [3].

The use of secondary metabolites in plant taxonomy is well recognized, as these compounds can sometimes help in taxonomical classification.

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Also, essential oil analysis has been used with success on the study of intra-specific diversity and geographic patterns of variation in several plant species, such as Lamiaceae family: *Salvia fruticosa* Mill., *Ocimum gratissimum* L., *Thymus* L. species, *Cunila incisa* Benth., *Cunila galioides* Benth and Cunila spicata Benth., among the others [3-5].

The analysis of the essential oil composition of several *Satureja* species indicates that they contains phenolic components such as carvacrol, thymol, γ-terpinene, *p*-cymene, β-caryophyllene, linalool and other terpenoids, but chemical composition and the amount of components are distinctive among different *Satureja* species oils [4-6].

The selection of medicinal plants is a conscious process, which has led to medicinal plants being used by the numerous cultures of the world. Understanding of natural variability has been one of the most important issues of the exploration of medicinal plants. The characterization of essential oil diversity is of great commercial importance and offers opportunity to choose plant essential oils with preferential compound for pharmaceutical, perfume and food industries.

It is well known that the chemical constituents of medicinal and aromatic plants, also their biological activities are influenced by genetic and environmental factors [3]. It has been reported that different *Satureja* species represent great variability in the phytochemical constituents [3,6,7]. The aim of the present research was to investigate the chemotypic variability among and within different *S. mutica* populations of Iran.

Material and Methods

Populations and plant materials

The present study was undertaken in order to study the level of essential oil variation among and within populations of *S. mutica*. The studied populations of *S. mutica* consisted of seven sampling areas mostly distributed in the north and northeast of Iran (Fig. 1), including Darkesh, Keshanak, Garmabdasht, Manjil, Namnik, Pono and Tangegol. In each site, the individual plants were selected depending on the population size with a minimum distance of 100 m. The aerial parts of plants were harvested when the plants were flowering. The samples were dried in the shade. Geological information and altitude of each sampling area were recorded using a global positioning system (GPS).

Essential oil extraction and analysis procedure

The essential oil of each individual sample was extracted by hydro-distillation for 3 h, using a Clevenger-type apparatus according to the method recommended in British Pharmacopoeia [8]. The isolated oils were dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4 ºC until analysis. GC analysis was performed using a Thermoquest-Finnigan Trace gas chromatograph with a flame ionization detector (FID). The analysis was carried out on fused silica capillary DB-5 column (30 m × 0.25 mm i.d.; film thickness 0.25 µm). The injector and detector temperature were kept at 250 ºC and 300 ºC, respectively. N_2 was used as the carrier gas at a flow rate of 1.1 ml/min; oven temperature program was 60-250 ºC at the rate of 4 ºC /min and finally held isothermally for 10 min; split ratio was 1:50. GC–MS analysis was carried out by use of Thermoquest-Finnigan Trace GC equipped with fused silica capillary DB-5 column (60 m×0.25 mm i.d.; film thickness 0.25µm) coupled with a TRACE mass (Manchester, UK). Helium was used as carrier gas with ionization voltage of 70 eV. Ion source and interface temperature were 250 ºC and 200 ºC, respectively. Mass range was from 35 to 456 amu. The oven temperature program was the same as mentioned above for the GC. The constituents of the essential oils were identified by calculating their retention indices under temperature-programmed conditions for *n*-alkanes (C6-C24) and the oil on a DB-5 column under the same chromatographic conditions. Identification of individual compounds was made by comparing their mass spectra with those of the internal reference mass spectra library (Wiley 7.0) or with authentic compounds and confirmed by comparing their retention indices with authentic compounds or with those of reported by Adams [9]. For quantification purpose, relative area percentages obtained by FID were used without the use of correction factors.

Statistical analysis

The significance of differences in studied parameters between these populations was analyzed using one-way ANOVA with the Fisher's least significance difference (LSD) at the 0.05 probability level.

Results

Phytochemical heterogeneity

Considrable differences were observed for the essential oil content (w/w, based on dry weight) within and among *S. mutica* populations. Oil content ranged from 2.4 to 4.61% within Darkesh individuals, 1.61 to 2.59% within Pono individuals, 3.48 to 5% within Keshanak individuals, 0.9 to 2.54% within Garmabdasht individuals, 1.93 to 3.7% within Tangegol individuals, 1.2 to 3% within Namnik individuals and from 0.17 to 1.14% within Manjil individuals (Tables 1-7). Variation of oil content within individuals of the same population could be the consequence of genetic variation within populations [10]. Among populations, the highest (4.22%) and the lowest (0.55%) mean oil content were found in populations of Keshanak and Manjil, respectively.

Essential oil constituents

The variability of the essential oil compositions of the fifty-eight individuals from seven populations of *S. mutica* were separately presented in tables 1-7. Considerable variation was observed among and within populations for the main components of the essential oils (thymol; 6.51-74.64%, carvacrol; 0.9-70.4%), borneol; 0.1-38.0%, *p*-cymene; 0.3-14.2% and γ-terpinene; 0.0-9.9%). Although, the highest amount of thymol was obtained in the Pono population, the wide range of its variability was observed in individuals of Namnik. Similarly, individuals of Manjil population showed a high variation of carvacrol (1.5-57.6%), however, the maximum mean value (70.4%) of this phenol was observed in Tangegol population. Chemical structure of some major constituents of *S. mutica* essential oils are presentd in Fig. 2.

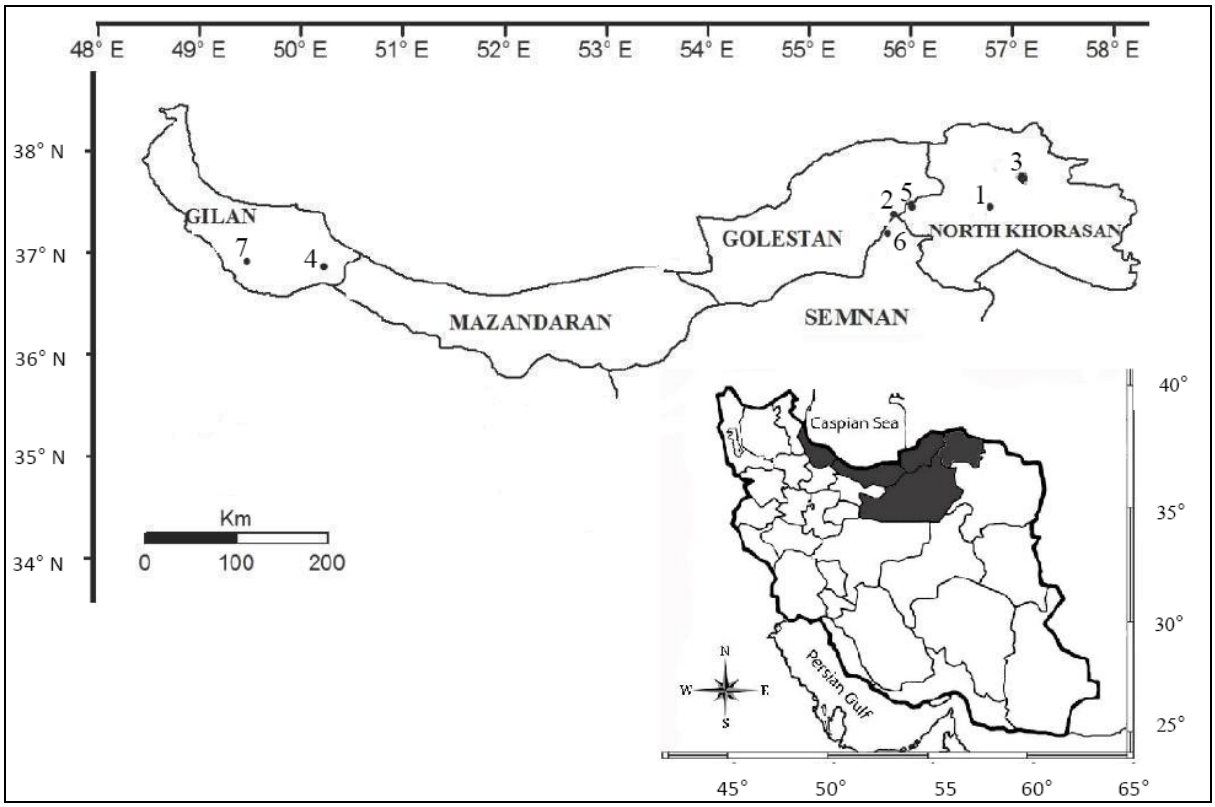

Fig. 1 Map of distribution of the studied *Satureja mutica* Fisch & C.A.Mey. populations. 1, 2, 3, 4, 5, 6 and 7 on the map are the populations of Darkesh; Pono; Keshanak; Garmabdasht; Tangegol; Namnik and Manjil, respectively.

Table 1 Essential oil constituents of *Satureja mutica* Fisch & C.A.Mey. individuals from Darkesh population.

No	Compound		Individuals										
		RI[a]	*Dar1*	*Dar2*	*Dar3*	*Dar4*	*Dar5*	*Dar6*	*Dar7*	*Dar8*	*Dar9*	*Dar10*	Mean±SD[c]
1	α-Thujune	931	-[b]	0.4	0.6	0.2	0.9	0.9	0.6	0.3	0.4	0.3	0.5±0.3
2	α-Pinene	941	-	0.3	0.4	0.3	0.6	0.7	0.5	0.3	0.3	0.4	0.4±0.2
3	Camphene	957	-	0.1	0.1	-	-	0.1	0.1	0.1	-	1.0	0.2±0.3
4	β-pienene	986	-	1	1.1	0.9	1.7	2.1	1.3	0.8	0.8	-	1.0±0.7
5	Myrcene	992	-	0.1	0.1	-	0.2	0.2	0.1	0.1	-	-	0.1±0.1
6	α-Terpinene	1022	-	0.8	1.1	0.8	1.5	1.7	1	0.9	0.8	1.1	1.0±0.5
7	ρ-Cymene	1029	0.2	13.4	12.8	11.8	16.2	24.9	21.9	11.4	14.7	14.9	14.2±6.6
8	Limonene	1035	-	-	0.1	-	0.4	-	-	0.2	-	-	0.1±0.1
9	γ-Terpinene	1063	9.4	4.5	12.9	4.7	12.6	13.8	10.2	6.2	5.8	8.5	8.9±3.5
10	*trans*-Sabinene hydrate	1071	0.3	1.1	0.8	0.7	0.7	1.0	0.8	0.9	1.1	1.0	0.8±0.2
11	Terpinolene	1093	0.6	0.1	-	-	0.2	0.2	0.1	0.1	0.1	0.1	0.2±0.2
12	Linalool	1099	0.7	0.1	0.2	0.3	0.2	0.1	0.1	0.1	0.1	-	0.2±0.2
13	Terpin-4-ol	1184	-	-	0.2	0.4	0.1	0.1	0.1	-	-	-	0.1±0.1
14	α-Terpineol	1201	-	0.1	0.2	-	0.3	-	0.4	0.3	0.2	0.3	0.2±0.1
15	*cis*-Dihydrocarvone	1206	0.2	-	0.1	-	-	-	-	-	-	-	0.0±0.1
16	Carvacrol methyl ether	1245	0.2	0.4	0.3	0.3	0.5	1.9	0.3	0.8	0.4	0.5	0.6±0.5
17	2,4-diethyl phenol	1282	0.7	0.1	-	-	0.2	-	0.1	-	0.1	0.1	0.1±0.2
18	Thymol	1290	41.4	25.2	24.2	37.8	22	44.6	28.7	37.6	30.6	55.8	34.8±10.6
19	Carvacrol	1304	38.6	48.4	42.2	37.2	34.1	3.7	30.7	37.3	42.3	10.9	32.5±14.2
20	Ascaridole	1316	0.1	0.5	0.8	0.3	0.8	0.6	0.5	0.4	0.5	0.9	0.5±0.2
21	E-Jasmone	1333	0.2	-	-	-	-	0.2	0.1	-	-	0.3	0.1±0.1
22	Thymol acetate	1355	0.2	-	-	0.3	0.3	0.2	0.2	0.1	-	-	0.1±0.1
23	Carvacrol acetate	1374	0.4	0.3	0.5	0.7	0.6	0.5	0.6	0.6	0.7	1.0	0.6±0.2
24	β-Caryophyllene	1437	0.5	-	-	-	-	-	-	-	-	0.1	0.1±0.2
25	β-Bisabolene	1515	0.8	-	-	-	0.3	0.1	0.2	0.1	-	0.1	0.2±0.2
26	*Delta*-Cadinene	1535	0.2	0.5	0.4	0.3	0.3	0.6	0.3	0.2	0.4	0.3	0.4±0.1
27	Spathulenol	1596	0.3	-	-	0.5	0.5	0.2	0.2	0.1	-	0.2	0.2±0.2
28	Caryophyllene oxide	1603	1.6	0.1	0.2	-	0.2	0.2	0.2	0.2	0.3	0.2	0.3±0.5
29	α-Terpinyl butanoate	1914	-	0.2	-	-	-	-	-	-	-	-	0.0±0.1
30	*trans*-β-Terpinyl Butanoate	1943	-	0.3	0.2	0.8	1.1	-	-	0.1	-	0.3	0.3±0.4
	Total	-	96.6	98.0	99.5	98.3	96.5	98.6	99.3	99.2	99.6	98.3	98.4
	Oil content (%)	-	3.8	4.6	3.4	3.1	4.0	3.3	3.7	2.4	3.8	3.6	3.6±0.6

a). RI, retention indices relative to C6–C25 *n-alkanes* on the *DB-5* column. b) t, trace <0.1%. c) SD: standard deviation

Table 2 Essential oil constituents of *Satureja mutica* Fisch & C.A.Mey. individuals from Pono population.

No	Compound	RI[a]	Individuals								
			Pono1	*Pono2*	*Pono3*	*Pono4*	*Pono5*	*Pono6*	*Pono7*	*Pono8*	Mean±SD
1	α-Thujune	931	0.5	0.3	0.4	0.6	0.2	0.7	-	0.3	0.4±0.2
2	α-Pinene	941	0.3	0.2	0.3	0.4	0.2	0.5	-	0.2	0.3±0.2
3	Sabinene	976	0.2	-	0.6	0.9	0.16	-	-	0.6	0.3±0.3
4	Myrcene	992	0.8	0.3	0.5	0.8	0.3	0.6	1.6	0.6	0.7±0.4
5	ρ-Cymene	1029	11.0	9.3	9.0	18.4	6.0	15.0	-	5.5	9.3±5.7
6	Limonene	1035	5.9	1.9	3.9	9.2	-	5.4	0.6	5.1	4.0±3.1
7	γ-Terpinene	1063	1.6	0.7	0.7	1.3	2.7	0.5	1.0	1	1.2±0.7
8	*trans*-Sabinene hydrate	1071	-	-	-	0.1	0.7	-	-	-	0.1±0.2
9	Linalool	1099	-	-	0.2	0.1	-	-	-	-	0.1±0.1
10	Borneol	1174	-	0.2	-	-	0.1	-	-	-	0.0±0.1
11	Terpin-4-ol	1184	3.0	-	1.6	0.7	1.4	4.1	1.4	1.7	1.7±1.3
12	*cis*-Dihydrocarvone	1206	-	-	-	0.2	-	-	0.3	-	0.1±0.1
13	Carvacrol methyl ether	1245	3.8	1.9	1.9	1	-	0.2	1.8	1.2	1.5±1.2
14	Thymol	1290	69.2	81.9	58.7	62.9	87.0	69.2	86.4	81.8	74.6±11.0
15	Carvacrol	1304	0.9	0.1	0.5	0.7	0.5	1.5	2.6	0.7	0.9±0.8
16	α-Thujaplicine	1330	0.2	0.6	0.3	0.1	-	0.2	0.3	0.2	0.2±0.2
17	β-Caryophyllene	1437	-	0.1	-	0.8	-	-	1.1	-	0.3±0.4
18	β-Bisabolene	1515	0.6	0.5	0.6	-	0.2	0.5	-	0.7	0.4±0.3
19	γ-Cadinene	1527	0.3	-	-	0.1	-	0.2	-	-	0.1±0.1
20	*Delta*-Cadinene	1535	-	0.2	0.2	-	-	0.1	1.5	-	0.3±0.5
21	Spathulenol	1596	0.7	-	11.6	0.7	0.4	0.7	-	0.3	1.8±4.0
22	α-Terpinyl butanoate	1914	0.1	1.1	3.1	0.2	-	0.2	0.4	-	0.6±1.1
23	*trans*-β-Terpinyl Butanoate	1943	-	-	5.3	-	-	-	-	-	0.7±1.9
	Total	-	99.1	99.3	99.4	99.2	99.9	99.6	99.0	99.9	99.5
	Oil content (%)	-	2.2	1.6	2.0	2.3	2.6	2.0	1.9	2.1	2.1±0.3

a). for abbreviations see Table 2.

Table 3 Essential oil constituents of *Satureja mutica* Fisch & C.A.Mey. individuals from Keshanak population.

No	Compound	RI[a]	Individuals										
			Kesh1	*Kesh2*	*Kesh3*	*Kesh4*	*Kesh5*	*Kesh6*	*Kesh7*	*Kesh8*	*Kesh9*	*Kesh10*	Mean±SD
1	α-Thujune	931	-	0.3	-	-	-	1.2	0.9	0.6	-	-	0.3±0.4
2	α-Pinene	941	-	0.3	0.3	-	-	0.7	0.7	0.6	0.5	-	0.3±0.3
3	Sabinene	976	-	0.7	0.3	0.4	-	0.1	0.1	-	0.4	0.7	0.3±0.3
4	β-pienene	986	0.4	0.8	0.7	0.3	0.5	0.1	-	-	-	0.5	0.3±0.3
5	Myrcene	992	0.3	-	0.5	0.9	0.4	2.4	1.9	-	1.0	1.3	0.9±0.8
6	α-Phellandrene	1010	0.9	-	-	1.0	1.0	0.2	0.2	1.6	0.9	0.2	0.6±0.5
7	α-Terpinene	1022	1.0	-	-	-	1.0	1.7	1.3	1.2	-	1.4	0.8±0.7
8	ρ-Cymene	1029	9.4	6.0	12.7	9.4	13.5	20.6	16.7	11.7	14.2	11.1	12.5±4.1
9	γ-Terpinene	1063	8.5	6.4	6.3	8.5	9.1	17.8	12.7	8.5	6.7	14.8	9.9±3.9
10	*trans*-Sabinene hydrate	1071	0.7	1.1	0.5	0.7	0.7	1.8	0.8	0.8	-	0.9	0.8±0.5
11	Terpinolene	1093	0.1	-	0.2	0.1	-	0.2	-	0.3	1.6	-	0.3±0.5
12	*cis*-Sabinene hydrate	1102	0.3	0.3	0.3	0.3	-	0.1	-	-	-	0.2	0.2±0.1
13	Borneol	1174	0.3	0.4	0.4	0.3	-	0.2	0.2	0.7	0.1	0.4	0.3±0.2
14	Carvacrol methyl ether	1245	0.6	-	0.2	0.6	-	-	0.4	-	0.1	-	0.2±0.3
15	2,4-diethyl phenol	1282	0.2	-	-	0.2	0.4	2.1	0.6	-	1.8	-	0.5±0.8
16	Thymol	1290	24.9	39.3	27.8	24.9	60.0	7.5	31.8	37.1	20.3	24.1	29.8±13.9
17	Carvacrol	1304	50.4	41.5	47.0	50.4	7.1	39.5	29.5	34.2	49.9	41.8	39.1±13.3
18	β-Caryophyllene	1437	-	-	-	-	-	0.1	-	-	-	-	0.0±0.0
19	β-Bisabolene	1515	-	-	-	-	-	0.6	1.1	0.9	0.4	-	0.3±0.4
20	Spathulenol	1596	-	0.7	0.4	-	-	0.3	-	-	0.1	-	0.2±0.2
21	Caryophyllene oxide	1603	-	-	0.2	-	1.7	-	-	-	-	1.1	0.3±0.6
	Total	-	98.0	97.8	97.8	98.0	95.4	97.2	98.9	98.2	98.0	98.5	97.8
	Oil content (%)	-	4.9	4.9	3.9	4.3	3.5	3.6	3.8	4.0	5.0	4.4	4.2±0.6

a). for abbreviations see table 2.

Table 4 Essential oil constituents of *Satureja mutica* Fisch & C.A.Mey. individuals from Garmabdasht population.

No	Compound	RI[a]	Individuals							
			Gar1	*Gar2*	*Gar3*	*Gar4*	*Gar5*	*Gar6*	*Gar7*	Mean±SD
1	ρ-Cymene	1029	5.3	9.5	12	-	13.6	5.7	4.3	7.2±4.8
2	*trans*-Sabinene hydrate	1071	-	9.9	-	0.5	-	0.6	0.3	1.6±3.7
3	*cis*-Sabinene hydrate	1102	3.5	0.5	-	1.7	6.4	1.4	1.0	2.1±2.2
4	Borneol	1174	0.2	-	1.0	0.2	-	0.1	0.4	0.3±0.3
5	Terpin-4-ol	1184	0.2	-	0.9	0.4	-	0.2	1.7	0.5±0.6
6	ρ-Cymene-8-ol	1188	2.1	-	-	0.6	-	3.2	1.8	1.1±1.3
7	Thymol methyl ether	1244	0.1	0.6	0.7	-	-	-	0.2	0.2±0.3
8	Carvacrol methyl ether	1245	1.4	-	3.4	2.8	2.4	1.0	3.5	2.1±1.3
9	Thymol	1290	81.9	68.5	63.5	77.8	57.5	79.7	57.2	69.4±10.5
10	Carvacrol	1304	0.9	1.5	7.8	7.8	4.9	1.4	18.2	6.1±6.1
11	β-Caryophyllene	1437	-	1.1	-	1.1	0.1	-	2.4	0.7±0.9
12	β-Bisabolene	1515	-	0.1	0.9	0.4	3.5	0.1	-	0.7±1.3
13	*Delta*-Cadinene	1535	-	-	-	0.2	0.2	-	-	0.1±0.1
14	Spathulenol	1596	0.1	0.3	0.8	-	0.2	-	-	0.2±0.3
15	Caryophyllene oxide	1603	0.5	1.1	-	0.2	0.4	0.3	2.1	0.7±0.7
	Total	-	96.2	93.1	91.0	93.7	89.2	93.7	93.1	92.9
	Oil content (%)	-	2.5	1.2	1.7	0.9	0.9	1.3	2.1	1.5±0.6

a). for abbreviations see table 2.

Table 5 Essential oil constituents of *Satureja mutica* Fisch & C.A.Mey. individuals from Tangegol population.

No	Compound	RI[a]	Individuals									
			Tange1	*Tange2*	*Tange3*	*Tange4*	*Tange5*	*Tange6*	*Tange7*	*Tange8*	*Tange9*	Mean±SD
1	β-pienene	986	-	0.2	-	0.2	-	-	-	0.3	-	0.1±0.1
2	ρ-Cymene	1029	0.1	0.2	-	-	-	-	-	0.6	-	0.1±0.2
3	β-Phellandrene	1037	0.2	-	-	-	1.8	0.2	-	0.3	-	0.3±0.6
4	*Trans*-Sabinene Hydrate	1071	0.2	0.4	0.3	0.4	1.1	-	-	0.9	-	0.4±0.4
5	Linalool	1099	-	-	0.2	-	-	-	0.5	-	0.4	0.1±0.2
6	*cis*-Sabinene Hydrate	1102	0.2	0.5	0.3	0.5	1.1	-	0.6	-	0.4	0.4±0.3
7	Borneol	1174	6.7	9.8	7.2	12.0	38.0	2.1	-	0.1	11.0	9.7±11.5
8	Terpin-4-ol	1184	-	-	-	-	-	-	-	0.2	-	0.0±0.1
9	ρ-Cymene-8-ol	1188	0.9	4.2	1.4	3.5	4.0	0.7	11.0	-	2.0	3.1±3.3
10	Linalyl Propionate	1196	1.0	1.1	1.0	1.0	8.8	1.3	3.8	0.4	1.0	2.2±2.7
11	P-menth-1-en-8-ol	1199	-	-	0.4	-	-	0.3	1.4	-	-	0.2±0.5
12	*cis*-Dihydrocarvone	1206	0.1	0.1	0.3	-	-	0.4	-	-	0.4	0.1±0.2
13	Dihydrocarvone	1211	0.1	0.3	0.3	-	-	0.3	-	-	0.3	0.1±0.2
14	Carvacrol methyl Ether	1245	0.2	0.2	0.6	1.4	3	0.3	1.4	-	1.8	1.0±1.0

15	Thymol	1290	0.9	0.3	8.6	19.8	2.3	4.8	2.9	12.7	6.3	6.5±6.4
16	Carvacrol	1304	86.4	80.1	77.3	57	22.7	84.5	73.6	78.9	73.5	70.4±19.8
17	Carvacrol acetate	1374	0.2	0.3	0.6	0.3	2.6	0.5	0.5	0.5	0.5	0.7±0.7
18	β-Caryophyllene	1437	0.1	-	-	-	-	-	-	0.5	-	0.1±0.2
19	γ-muurolene	1489	0.1	0.2	-	0.3	-	-	0.2	0.1	-	0.1±0.1
20	β-Bisabolene	1515	-	0.8	0.7	1.0	-	1	0.2	0.6	-	0.5±0.4
21	*Delta*-Cadinene	1535	0.9	-	-	-	4.5	-	-	-	0.6	0.7±1.5
22	Spathulenol	1596	0.2	0.2	-	-		0.2	1.3	0.1	-	0.2±0.4
23	Caryophyllene Oxide	1603	0.1	0.3	0.3	-	1.2	0.4	0.3	0.1	-	0.3±0.4
24	*trans*-β-Terpinyl Butanoate	1943	0.6	0.1	0.4	0.3	6.3	0.4	0.6	0.1	0.9	1.1±2.0
	Total		99.2	99.3	99.9	97.7	97.4	97.4	98.3	96.4	99.1	98.3
	Oil content (%)		2.5	2.9	2.5	3.7	2.2	2.7	2.8	1.9	2.3	2.6±0.5

a). for abbreviations see table 2.

Table 6 Essential oil constituents of *Satureja mutica* Fisch & C.A.Mey. individuals from Namnik population.

No	Compound		Individuals						
		RI[a]	*Nam1*	*Nam2*	*Nam3*	*Nam4*	*Nam5*	*Nam6*	Mean±SD
1	Sabinene	976	0.5	-	-	-	0.4	-	0.2±0.2
2	Myrcene	992	0.4	-	-	-	0.3	-	0.1±0.2
3	ρ-Cymene	1029	21.3	-	3.3	1.1	18.4	-	7.4±9.8
4	*trans*-Sabinene hydrate	1071	1.0	-	0.8	-	1.5	-	0.6±0.6
5	Linalool	1099	0.1	-	-	-	-	0.6	0.1±0.2
6	*cis*-Sabinene hydrate	1102	-	-	0.3	-	-	-	0.1±0.1
7	Terpin-4-ol	1184	0.1	-	1.0	-	-	-	0.2±0.4
8	Thymol methyl ether	1244	-	-	-	-	-	0.5	0.1±0.2
9	Carvacrol methyl ether	1245	2.3	1.4	3.5	-	4.7	0.3	2.0±1.8
10	2,4-diethyl phenol	1282	0.1	-	-	-	1.2	1.4	0.5±0.7
11	Thymol	1290	29.7	47.1	22.3	3.0	14.0	0.3	19.4±17.6
12	Carvacrol	1304	34.3	31.4	49.9	62.0	50.0	86.3	52.3±20.1
13	Ascaridole	1316	0.9	-	1.8	1	1.1	6.0	1.8±2.1
14	1,8-Cineole	1037	4.9	-	0.3	-	4.8	1.1	1.9±2.4
15	α-Thujaplicine	1330	-	-	1.8	1.4	-	-	0.5±0.8
16	β-Caryophyllene	1437	0.2	-	-	2.2	-	0.3	0.5±0.9
17	β-Bisabolene	1515	0.8	-	0.7	1	-	-	0.4±0.5
18	Spathulenol	1596	0.1	-	-	1.7	0.9	0.4	0.5±0.7
19	Caryophyllene oxide	1603	0.9	-	4.1	2.2	-	-	1.2±1.7
20	*trans*-β-Terpinyl Butanoate	1943	0.2	6.8	7.3	0.7	1.2	1.5	3.0±3.2
	Total	-	97.8	86.7	97.1	76.3	98.5	98.7	92.6
	Oil content (%)	-	2.1	1.3	1.3	1.2	2.6	3.0	1.9±0.8

a). for abbreviations see table 2.

Table 7 Essential oil constituents of *Satureja mutica* Fisch & C.A.Mey. individuals from Manjil population.

No	Compound	RI[a]	Individuals								
			Man1	*Man2*	*Man3*	*Man4*	*Man5*	*Man6*	*Man7*	*Man8*	Mean±SD
1	α-Thujune	931	-	0.7	-	-	-	-	-	-	0.1±0.2
2	α-Pinene	941	-	0.5	-	-	-	-	-	-	0.1±0.2
3	Myrcene	992	-	0.8	-	-	-	-	-	-	0.1±0.3
4	ρ-Cymene	1029	3.0	39.5	1.2	2.0	3.0	0.8	3.7	-	6.7±13.3
5	γ-Terpinene	1063	0.7	6.4	-	6.7	6.7	0.8	-	-	2.7±3.3
6	Terpin-4-ol	1184	0.5	1.6	0.8	1.2	-	1.3	0.9	5.2	1.4±1.6
7	P-menth-1-en-8-ol	1199	-	-	3.5	1.0	2.0	-	0.7	-	0.9±1.3
8	Carvacrol methyl ether	1245	-	-	-	5.0	-	-	6.1	-	1.3±2.6
9	Thymol	1290	71.2	42.9	48.7	47.6	41.5	77	37.1	25.9	49.0±17.1
10	Carvacrol	1304	16.5	1.5	38.2	27.5	36.7	9.6	42.9	57.6	28.8±18.7
11	Carvacrol acetate	1374	-	-	-	-	-	-	1.3	0.75	0.3±0.5
12	β-Caryophyllene	1437	0.4	1.0	-	-	-	-	-	-	0.2±0.4
13	Germacrene D	1497	0.7	0.5	-	-	-	-	-	-	0.2±0.3
14	β-Bisabolene	1515	-	0.8	1.4	-	0.6	-	-	-	0.4±0.5
15	Spathulenol	1596	-	0.8	-	-	-	-	-	-	0.1±0.3
16	Caryophyllene oxide	1603	-	2.2	1.3	0.7	-	1.8	-	1	0.9±0.9
	Total	-	93.0	99.2	95.1	91.7	90.5	91.3	92.7	90.5	93.0
	Oil content (%)	-	0.3	1.1	0.6	1.0	0.3	0.6	0.4	0.2	0.5±0.4

a). for abbreviations see table 2.

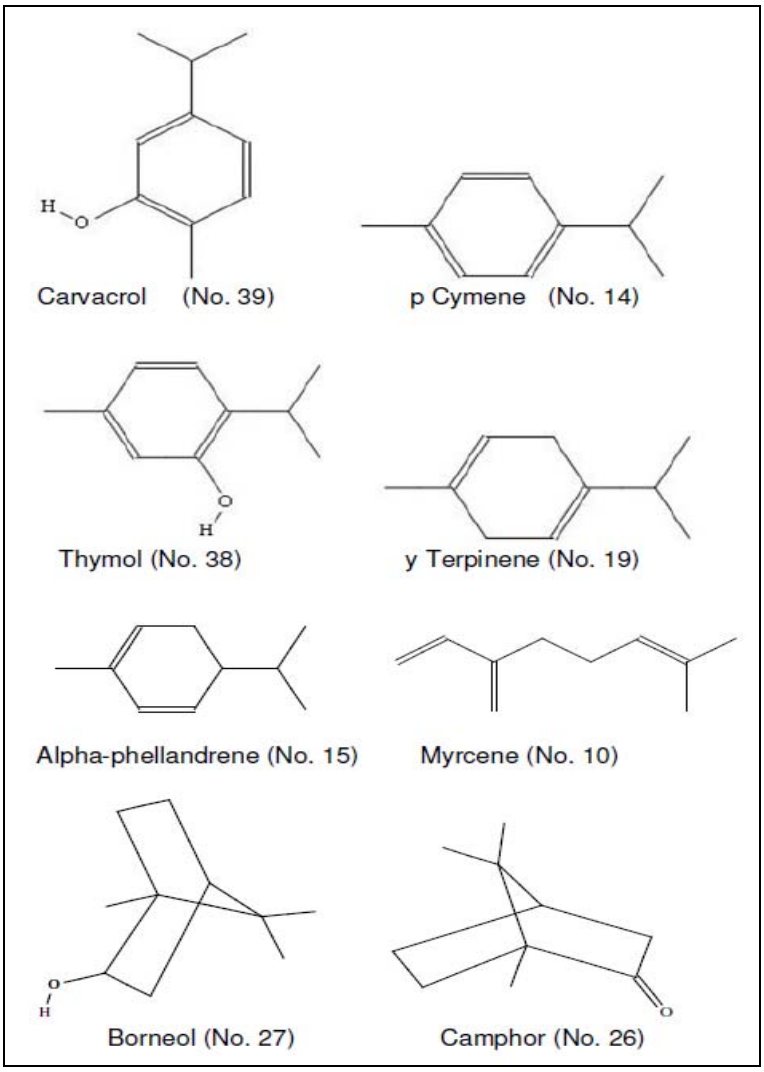

Fig. 2 Chemical structure of some major constituents of essential oils in *Satureja mutica* Fisch & C.A.Mey..

Discussion

Different *Satureja* (Labiatae) species are present in mountainous areas in Iran, and have been used in traditional medicine as antimicrobial, spasmolytic, analgesic, cicatrizing and diuretic agents [2]. Literature review showed variation between chemical yield and compositions of different *Satureja* species oils [3,4,7,10].

Essential oil biosynthesis and phytochemical variability is widely affected by a number of factors such as environmental conditions, physiological and geographical variations, genetic factors and evolution [6]. As previously reported by Sefidkon and Jamzad [4], the oil content of *S. mutica* was 2.31% in samples collected from an eastern part of Iran (Khorasan province). Among examined populations, Keshanak and Darkesh were from the rangeland habitats, while the other five populations were of the forest origin. The highest essential oil content of Keshanak and Darkesh populations could be mainly due to the differences of habitats. According to Hadian *et al*. [10] altitude and rainfall are the main factors affecting the essential oil accumulations of *S. khuzistanica*. In the same manner, Karousou *et al*. [11] concluded that the essential oil accumulation in *Coridothymus capitatus* and *S. thymbra* is correlated to the water deficiency. Chemical variations among populations can be attributed to both genetic divergence and/or environmental factors [6]. Also, relations between geographic distribution and biochemical composition have been previously reported for some other medicinal plants [12-16]. Chemical composition of the essential oil of *S. mutica*, collected from Khorasan proviance, has been reported to be carvacrol (30.9%), thymol (26.5%), γ-terpinene (14.9%), and *p*-cymene (10.3%) [4].

Also, Gohari *et al*. [5] reported thymol (62.6%), *p*-cymene (9.4%), and carvacrol (6.6%) as the main components of *S. mutica* from Guilan province. Various species of the genus *Satureja* have been investigated for the essential oil variability. In an investigation on thirty accessions of *S. hortensis*, twenty nine components were identified in the oils of them carvacrol (42.0- 83.3%), γ-terpinene (0.5-28.5%), and *p*-cymene (1.0-17.1%) were the major components [6]. The main constituents of the essential oils of eight populations of *S. sahendica* were thymol (19.6-41.7%), *p*-cymene (32.5-54.9%) and γ-terpinene (1.0-12.8%) [3]. Analysis of the oils of 69 sampled individuals from seven different populations of *S. khuzistanica* showed that all have the high percentage of carvacrol (89.59-95.41%) as main component [10]. The ratios of the constituents determine the activity of the essential oils. Valero and Giner [17] reported that carvacrol shows more antibacterial activity than thymol. However, the combination of thymol and *p*-cymene shows more antibacterial activity than the single compound [18, 19]. On the contrary, the antioxidant activity of thymol in lipid system is stronger than carvacrol [20]. It can be concluded that different chemical profiles, identified among *S. mutica* populations, may possess different biological activities.

From the results obtained, it can be concluded that high level of phytochemical variability exists among and within *S. mutica* populations. In contrast to the most of *Saturjea* species in which one of the phenols, carvacrol or thymol, are dominant, in *S. mutica* a special harmony exist between the two compounds. Among the studied populations, the plants of Keshanak and Darkesh populations had moderate level of both carvacrol and thymol. The populations of Pono and Garmabdasht were rich in thymol and the population of Tangegol was rich in carvacrol. Three different identified chemical profiles, could be used in various food and pharmaceutical industries when respective biological activity is needed.

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