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ANALYSIS OF THE ESSENTIAL OILS OF *THYMUS KOTSCHYANUS* L. (10 POPULATIONS) FROM IRAN

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ABSTRACT. *Thymus* species are well known as medicinal plants because of their biological and pharmacological properties. *Thymus kotschyanus* seeds (10 populations) were collected from various provinces of Iran and transferred into a new uniform environment. This study was carried out at Badiei Research Station (Qom Province, Iran) in 2008, under field conditions. The experiment was carried out as a randomized complete block design with three replicates. The content of essential oils of this species was assessed during the flowering stage, and the chemical composition of the essential oils were obtained by hydrodistillation and analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC-MS). According to populations, 17 components representing 84.57-97.08% of the total components, were identified. Oxygenated monoterpenes were the main group of constituents in all samples (87.14-98.93%). thymol (2.45-78.65%), carvacrol (1.84-49.38%), α-terpineol (1.79-17.1%), borneol (.68-3.8%), linalool (.5-39.05%), 1,8cineole (.53- 8.39%), p-cymene (.38 - 7.74%) represented the mager compounds. The highest oil yields were obtained from Mazandaran 2 (2.5%) and Rudbar (2.3%) populations and lowest oil yields were obtained from Avan (1.1%) and Alamut (1.09%) populations. The highest level of thymol was obtained from Piranshahr (78.65%) and Semnan (60.80%) populations and lowest level of it obtained from Mazandaran 2 (2.45%), Siahkal (3.95%) populations. The highest level of carvacrol was exist in Mazandaran 2 (49.38%) and Rudbar (39.68%) populations. In this study, the linalool and α-terpineol were found as the main constituents of essential oil.

Key words: *Thymus kotschyanus*; Gas chromatography; Gas chromatography/ mass spectrometry; Medicinal plants; Oil yield.

INTRODUCTION

Thymus species are well known as medicinal plants because of their biological and pharmacological properties. The genus *Thymus* L. (*Labiatae*) consists of about 215

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species of herbaceous perennials and subshrubs. This genus is represented in Iranian flora by 14 species, four of which (*T. carmanicus, T. daenensis* subsp. *daenensis* and *T. daenensis* subsp. *lancifolius*, *T. persicus* and *T. trautvetteri*) are endemic (Rechinger, 1982). In Iran, *T. daenensis* Celak., *T. kotschyanus* Boiss. and Hohen. are more widely used as herbal tea, flavoring agents (condiment and spice) and medicinal plants. Infusion and decoction of aerial parts of *Thymus* species are used as tonic, carminative, digestive, antispasmodic, anti-inflammatory, antitussive, expectorant and for the treatment of colds in Iranian traditional medicine (Zargari, 1990). Thyme is a robust and dense shrub distributed in a wide range of mountainous rangelands as dominant companion species. Having aboundant stems relatively short and woody which gives a pulvinate crown to this species along with robust and dense roots play a key role in soil stabilization and also prevent water erosion in mountainous and sharp slope regions (Moghimi, 2005).

The medicinal properties of thyme come mainly from its essential oil which is extracted through steam distillation of fresh flowers and leaves. The chief constituents of its essential oil are Alpha Thujone, Alpha Pinene, Camphene, Beta Pinene, Para Cymene, Alpha Terpinene, Linalool, Borneol, Beta Caryophyllene, Thymol and Carvacrol. Chemical polymorphism of essential oils is characteristic to species of *Thymus*. It is supposed that environmental abiotic factors (temperature, moisture, chemical composition of soil) influence chemical polymorphism of *Thymus* species and chemical composition of cenopopulations in the course of time (Guillén and Manzanos, 1999). Essential oils are produced by special secretory structures that are not evenly distributed over the aerial parts of the plant (sometimes the flowers posses a higher number of different types of trichomes), and that their secretory stage will depend on the stage of development of the plant material as well as on the essential oil seasonal variation.

Miguel *et al.* (2005) also found a high variability on the chemical composition and oil yield in *T. carnosus* oils, depending not just on the collection site and plant part used but also on the harvesting period. According to the authors, such variability may reflect not only genetic diversity and heterogeneity in environmental conditions but also a differential secretory capacity that depends on the developmental stage of the plant material. Omidbaigi *et al.* (2005) with studying effect of various harvest times on quality and quantity of thyme (*T. critriodorus* (pres.) Schreb) showed that the highest essence yield (2.21%) was obtained in beginning of blooming. Khorshidi *et al.* (2010) investigated four various stages in two region to assess the effect of climate and phenological stages on essence percentage of Denaian thyme (*T. daenensis* Celak.). Results showed higher essence percentage in full blooming stage in both regions (3.4 and 2.93% in Malaayer and Hamedan, respectively). The data show that the main components are linalool and nerolidol (Geréd-Csegedi, 1972), trans-β-caryophyllene (Pavel and Ristič, 2007) or thymol and carvacrol (Kisgyörgy *et al.*, 1983), which means that the species has a great variability depending on the genetic factors or soil and climate conditions.

The biosynthesis of secondary metabolites, although controlled genetically, is affected strongly by environmental influences. Agricultural factors have a critical effect on quantitative and qualitative characteristics of thyme, which finally result in plant growth and yield increment.

Therefore, this study was achieved to assess the quality/quantity of essential oils in thyme species (*T. kotschyanus*) in the flowering stage on the field condition.

MATERIAL AND METHODS

The experiment was carried out at Badiei Research Station in 2008 under filed conditions. The latitude and longitude of region is 34° and 45' north and 50° and 31' east and its height is 990 m above the sea level. Climate of the region is warm and semidry and the average rainfall and the area temperature according to 16 years statistics are 180-200 mm and 15-16°C, respectively. *Thymus kotschyanus* seeds (10 populations) were collected from various natural habitats of Iran and transferred into a new uniform environment. The experiment was carried out as a randomized complete block design with three replicates.

Cultural practices, control of weeds were given as needed during the growth season according to the local recommendations.

Isolation of the essential oils

After collection, the flowering aerial parts materials were shade dried at room temperature and placed in paper pockets. Samples transferred to Laboratory of Medicinal Plants of Qom Agriculture and Natural Resources Research Center. In order to estimate the rate of essential oils the distillation method was used (Sefidkon and Rahimi Bigdeli, 1999). Dry plant matter were milled to a powder in an electric blender. The essential oil of all air-dried samples (100 g) was isolated by hydrodistillation for 4 h, using a Clevenger-type apparatus according to the method recommended in British Pharmacopoeia (British Pharmacopoeia, 1988). The essential oil yield of samples were calculated based on dry weight, and then the oil was dried over anhydrous sodium sulfate.

Identification of essential oil compounds

Gas chromatography (GC)

GC analysis was performed using an Ultra Fast Chromatograph (Thermo-UFM), equipped with a Ph-5 column (10 m × 0.1 mm, film thickness 0.4 m). Helium used as a carrier gas (0.5 ml/min). Oven temperature was kept at 60˚C for 3 min and then programmed to 285˚C at rate of 80˚C/min and for 5.8 min kept at this temperature. Injector and detector (FID) temperature were 280˚C. The relative percentages of compounds were calculated by the Cromatopec software CR4-A, without considering correction factor.

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis was performed using varian 3400 gas chromatograph equipped with a FID and a DB-5 capillary column (30 m; 0.25 mm; 0.25 µm film thickness). The GC oven temperature was kept at 40 °C for 5 min and programmed to 250 °C at a rate of 4 °C/min. carrier gas was helium (with 99.999% purity) with a linear velocity of 31.5 cm/s, split ratio 1 s, ionization energy 70 eV and a mass range of 40-300.

Identification of compounds

The components of oils were identified by comparison of their mass spectra and retention indices with those published in the literature (Adams, 2007), comparison of their mass spectra of peaks with those obtained from GC-MS library, and by comparison of their relative retention times with those of authentic samples on a capillary column (Davies, 1990).

Statistical analysis

Statistical analysis was carried out through SAS software version 9.0 (SAS Institute Inc., Cary, NC, USA), while drawing graphs was done using Microsoft Office Excell 2007 software.

RESULTS

The overage oil yield of flowering aerial parts of thyme are given in *Fig. 1*. The highest oil yields were obtained from Mazandaran 2 (2.5%) and Rudbar (2.3%) populations and lowest oil yields were obtained from Avan (1.1%) and Alamut (1.09%) populations. The oil yields of three populations of thyme in region around the Damavand mountain 0.95 to 1.8% were reported by Jamshidi *et al.,* 2006, while in Mazandaran 1, Mazandaran 2, Rudbar and Semnan populations by 2.5, 2, 2.3 and 1.9% essential oil yields, respectively, had a higher yield.

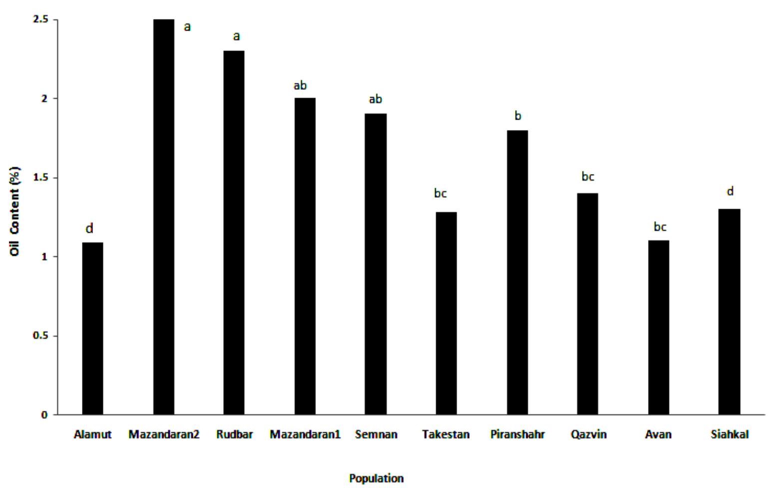

Figure 1 - Average oil yields of different populations

Table 1 - Main essential oil components (%) in different populations of *Thymus kotschyanus* from Iran

Compound	RRI	Avan	Mazandaran2	Takestan	Siahkal	Mazandaran1	Semnan	Alamut	Rudbar	Qazvin	Piranshahr
P-Cymene	1046	-	3.9	6.3	2.48	.38	7.74	.49	6.8	6.4	4.4
1,8-Cineole	1061	1.31	2.3	2.36	8.39	3.49	2.16	1.18	3.04	9.12	.53
Linalool	1104	39.05	6.5	3.35	8.98	7.2	-	4.08	.5	4.3	-
Borneol	1210	.68	3	3.05	3.16	1.11	1.32	2.97	2.86	3.8	1.48
α-Terpineol	1227	1.79	11.08	21.3	10.8	3.55	1.11	6.65	17.1	18.6	-
Thymol	1305	12.91	2.45	16.19	3.95	4.69	60.8	25.2	21.74	25.08	78.65
Carvacrol	1324	1.84	49.38	36.48	28.44	4.5	11.59	6.4	39.68	8.49	7.04
α- Terpinyl acetate	1363	.94	2.7	-	-		6.61	-	-	-	-
Geranylacetate	1368	6.24	1.6	-	4.4	61.69	-	.72	.4	-	-
E-caryophyllene	1482	4.94	.94	.95	2.3	1.04	1.9	1.35	1.01	3.7	2.26
Methylcarvacrol	1264	-	3.8	2.01	-	-	1.65	-	-	4.2	1.52
Neral	1243	.58	.61	-	-	-	-	.99	-	-	-
Cis-sabinene hydrate	1095	-	-	2.42	3.77	-	-	2.12	-	-	-
-Terpineneγ	1080	-	-	-	-	-	.9	-	-	-	-
Limonene	1056	2.57	-	-	-	4.7	-	-	-	-	-
Geraniol	1269	20.27	-	-	7.9	-	-	42.9	-	-	-
Geranial	1289	1.85	-	-	-	-	-	2.03	-	-	-

Considering that drought and heat stress can limit photosynthesis in *T. vulgaris* plants and alter nutrient uptake and carbon, sugar, amino acid and inorganic ion fluxes. In this situation, the plant received stress signal, and then plant by reducing the production of primary metabolites increased the production of secondary metabolites (essential oil) redirect the resistance to stress (Figueiredo *et al.*, 2008). As a results, climate condition (the average annual rainfall of 180-200 mm and mean annual temperature of 15-16 °C) is one of important factor in increasing essential oil production of studied population.

The dominant component of essential oil compounds in our study were somewhat different with most previous reports, that can resulted from genetic differences as well as geographic and climatic conditions of collection place (*Table 1*). In total, in essential oil of flowering aerial parts of ten studied populations, 17 compounds were identified. Different populations of *T. kotschyanus* showed considerable variation in term of type and percentage of essential oil compounds. In populations, compounds identified in essential oil, representing of total oil as follow (*Table 2*): Piranshahr (95.88), Qazvin (88.69), Rudbar (93.13), Alamut (97.08), Mazandaran 1 (92.35), Siahkal (84.57), Takestan (94.57), Mazandaran 2 (88.26) and Avan (95.42). Although these ten populations had almost same essential oil components, however, there were qualitative and quantitative differences among populations.

Table 2 - Compounds identified in essential oil of different populations of *Thymus kotschyanus*

No.	Population	Sesquiterpene hydrocarbons	Oxygenated monoterpene	Monoterpene hydrocarbons	Total identified	Number of identified compounds
1	Piranshahr	2.3	92	4.5	95.88	7
2	Qazvin	4.4	87.93	7.6	83.69	9
3	Rudbar	1.08	91.6	7.3	93.13	9
4	Alamut	.5	98.14	1.3	97.08	13
5	Semnan	1.9	89.1	9	95.78	10
6	Mazandaran1	1.1	94.4	5.5	92.35	10
7	Siahkal	2.9	93.9	3.2	84.57	10
8	Takestan	1	92.4	6.6	94.57	10
9	Mazandaran2	1	94.6	4.4	88.26	12
10	Avan	5.2	92.1	2.7	94.97	13

The highest level of thymol was obtained from Piranshahr (78.65%) and Semnan (60.80%) populations and lowest level of it obtained from Mazandaran 2 (2.45%), Siahkal (3.95%) populations. The highest level of carvacrol was exist in Mazandaran 2 (49.38%) and Rudbar (39.68%) populations. A α-terpinyl was not detected in Piranshahr population, and the it's highest and lowest level were determined in Takestan (21.3%) and Semnan (1.11%) populations, respectively. The variation in relative amount of carvacrol, thymol and α-terpinyl in different populations of *T. kotschyanus* are shown in *Fig. 2*.

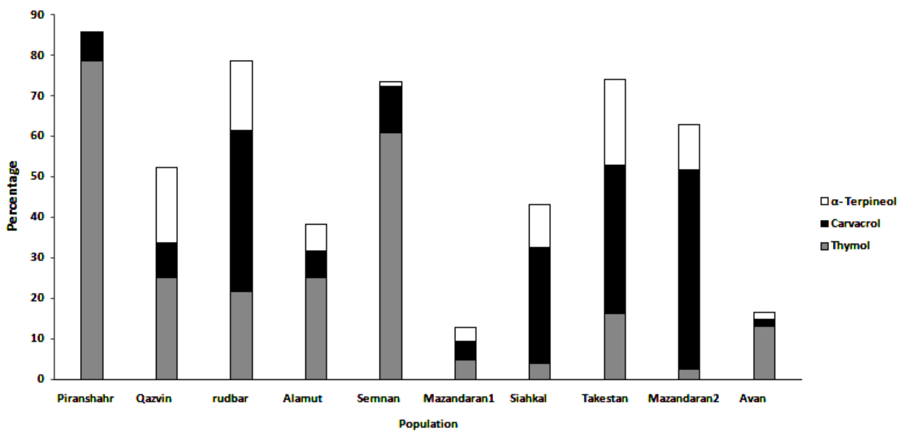

Figure 2 - Differences in the relative amounts of carvacrol, thymol and α-terpineol in essential oils of different populations

Given that an environmental conditions were equal to all studied populations of thyme, genetic variance of studied populations may be a major cause of the high variation in essential oil components. In general, cineol, borneol, thymol, carvacrol and (E)-caryophyllene were observed in all populations. Geraniol was only observed in Alamut (42.9%), Siahkal (7.9%) and Avan (20.27%) populations, while Avan (39.05%) and Rudbar (0.5%) populations had a highest level of linalool. The geranyl acetate in small amount was found in Alamut (0.72%), Rudbar (0.4%), Siahkal (4.4%), Mazandaran 2 (1.6%) and Avan (6.24%) populations, while Avan (39.05%) and Mazandaran 1 (61.69%) populations containing a large amount of geranyl acetate. A 1,8 - cineol was observed in all studied populations, and the its highest and lowest level were determined in Qazvin (9.13%) and Piranshahr (0.53%) populations, respectively. *T. kotschyanus* oil from Armenia was reported to contain thymol (35.48%), cymene (17.74%), carvacrol (11.65%), α-pinene (8.83%) and α-terpineol (6.50%) as main constituents (Kasumov, 1996).

While in our study first two compounds revealed as main constituents, but in this study, amount of para-cymene in all populations is very low and α-terpineol only in Semnan population and in very small amounts (0.9%) has been found. In general, α-terpineol in many previous studies on thymus have shown as main constituents, while in samples of essential oil of this study, this compound even not found. Also in this study, the linalool and α-terpineol were found as the main constituents of essential oil, which is in contrast with many previous reports for this species (Nickavar *et al.*,2005; Bagci *et al.*, 2004; Sefidkon and Rahimi Bigdeli, 1999). This difference is likely due to chemotype differences as a consequence of environmental and climatic conditions that prevail on studied plants.

Based on cluster analysis using of essential oil components of ten populations, the oil samples have been grouped in two chemical types (*Fig. 3*). (A) thymol/linalool type, which includes eight populations, was divided into five subgroups with thymol and linalool as the main compound; (B) thymol/carvacrol type that determined by high levels of thymol and carvacrol and lack of linalool. Type B consists of Piranshahr and Semnan populations, that high level of thymol (60.8-78.65%) and similar amounts of methyl carvacrol (1.9-2.26%) and lack of linalool and α-terpineol (minimum in the Semnan population) in these populations lead to this to populations separated from other populations and placed in a separate group. Rudbar and Semnan populations with the highest level of para-cymene (6.3-6.8%) and high levels of carvacrol (36.64- 39.68%) were placed in a first subgroup. Siahkal and Mazandaran 2 populations were placed in the second group that contain high amounts of linalool (6.5- 8.98%) and α-terpineol (10.8-11.08%), and also amount of para - cymene (2.48-3.9%) and borneol (3-3.16%).

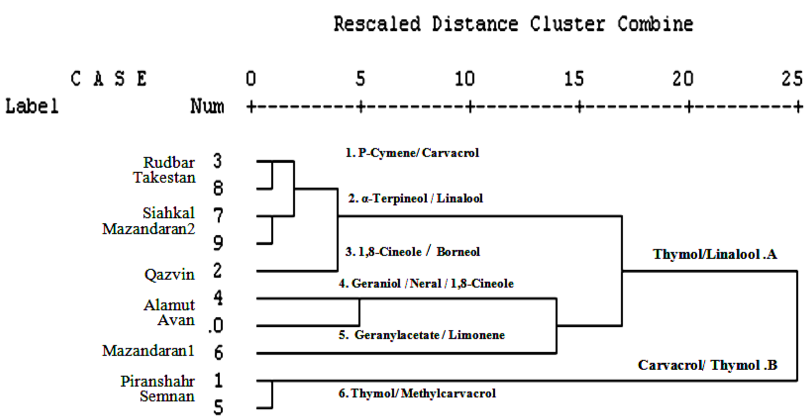

Figure 3 - Chemical grouping types of thyme essential oil components

Table 3 - Results of simple correlation among the components of essential oil in *Thymus kotschyanus*

	P-Cymene	Linalool	Borneol	Terpineol α	α-Terpinyl acetate	Geranyl acetate	Ecaryo phyllene	Neral	Terpinene γ	Limonene	Geraniol	Geranial	Oil content
P-Cymene	1												
Linalool	-.606	1											
Borneol	.35	-.55	1										
-Terpineolα	.26	-.22	.83**	1									
α-Terpinyl acetate	.38	-.139	-.31	-.36	1								
Geranyl acetate	-.29	.063	-.23	-.28	-.18	1							
E-caryophyllene	-.21	.761*	-.26	-.23	-.02	-.19	1						
Neral	-.58	.35	.01	-.22	-.006	-.17	.072	1					
-Terpineneγ	.26	.25	-.33	-.36	.91**	-.13	-.03	-.2	1				
Limonene	-.62	.26	-.62*	-.2	-.16	.9**	.12	-.01	-.15	1			
Geraniol	-.59	.31	-.02	-.25	-.16	-.12	.16	.85**	-.15	.03	1		
Geranial	-.65*	.59	-.22	-.32	-.12	-.12	.2	.82**	-.16	.16	.92**	1	
Oil content	.21	-.57	.06	-.05	.3	.17	-.62*	-.28	.16	-.07	-.57	-.66*	1

Qazvin population due to highest level of methyl - carvacrol (4.2%), borneol (3.8%) and 1,8 - cineol (9.12%) were placed in a separate group. Alamut and Avan populations were placed in fourth subgroup, because of presents of geraniol (20.27-42.9%) and neral (0.58-0.99%) and same amount of 1,8 - cineol (1.18-1.31%). Mazandaran 1 population with the highest level of geranyl acetate (61.29%) were placed in a fifth subgroup.

Previously, different types of chemical has been reported for this species of thyme (Torras *et al.*,2007; Sefidkon *et al.,* 2005; Lozine and Venskutonis, 2005; Blanquer *et al.,*1998). Results of the simple correlation between the essential oil components and yield are shown in *Table 3*. There was a significant positive and negative correlation between many essential oil components. The most important: positive correlation of borneol and terpineol (r = +0.83), terpinene and terpinene acetate (r =+0.91), geranyl acetate and limonene (r =+0.9), neral and geraniol (r =+0.85), geranyal and neral (r =+0.82) in the 1% significance level and linalool and caryophyllene (r =+0.76) in the 5% significance level. There were significant ($P < 0.05$) negative correlation between the borneol and limonene (r = -0.66) and p-cymene and geranyal (r = -0.65). Traits such as essential oil yield and amount of geranyal (r = -0.66) and (E) - caryophyllene (r = -0.64) showed a significant negative correlation, that increases the difficulty of making simultaneous improvement in these traits.

CONCLUSION

The biological effects of essential oils that derived from plant material strongly influenced by their constituents. The high diversity of thymus essential oil compounds that observed in this study and other studies. So, increased the possibility of selection of colonies of this plant with specific biological activity for use in the pharmaceutical industry, food, cosmetics and sanitary.

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