

## ESSENTIAL OIL COMPOSITION OF *THYMUS TRAUVETTERI* KLOKOV & DESJ. AT DIFFERENT GROWING ALTITUDES IN MAZANDARAN, IRAN

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**ABSTRACT.** *Thymus trauvetteri* Klokov & Desj. (*Lamiaceae*) is a permanent species that grows in some mountain rangeland of Iran including Mazandaran province. The aerial parts of *Thymus trauvetteri* were collected during flowering stage in June 2014, from mountain rangelands of Mazandaran province, in North of Iran. Around samples collected from four altitudes (2100 m, 2400 m, 2700 m and 3000 m) in mountain region of Mazandaran province. The goal of current research was to assess the effect of altitude on the chemical composition and function of essential oil in *Thymus trauvetteri*. The essential oil were obtained by hydrodistillation and analyzed by gas chromatography (GC) and gas spectrometry (GC-MS). Based on the results, the essential oil content is between 1.01-1.51% at different altitudes. The highest essential oil (1.51%) was extracted at an altitude of 2400 m, while it was opposite (1.01%) at an altitude of 3000 m. The main compounds essential oil of *Thymus trauvetteri* samples were

identified: thymol (5.93%-49.75%), carvacrol (1.78%-54.02%), and *p*-cymen (6.98%-19.07%). According to the results, altitude was significantly ( $p \leq 0.05$ ) effective on essential oil, thymol, carvacrol and *p*-cymen rates according to results of correlation analysis. The highest percentage of essential oil is at an altitude of 2400 m and the lowest is 3000 m above sea level. The highest percentage of thymol is in L3 (2700 m) and lowest is in L1 (2100 m). The highest percentage of carvacrol is in L3 (2700 m) and lowest is in L4 (3000 m). The highest percentage of *p*-cymen is in L1, L2, L3 (2100, 2400 and 2700 m, no significant difference) and lowest is in L4 (3000 m). Variations in essential oil rates and compositions may be due to on genetic, ecological or individual variability.

**Keywords:** thymol; carvacrol; mountain rangelands.

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## INTRODUCTION

The genus *Thymus* L. (*Lamiaceae*) consists of about 350 species of herbaceous perennials and subshrubs (Morales, 1986). The mediterranean region can be described as the center of the genus (Stahl-Biskup, 1991). A number of 18 *Thymus* species has been reported in Flora Iranica and six of them have been known endemic (Abousaber *et al.*, 2002; Mozaffarian, 1998). *Thymus* species are commonly used as tonic, carminative, digestive, antitussive, expectorant and for the treatment of cold in Iranian traditional medicine. Recent studies imply that these species have strong antibacterial activities (Vila, 2002). The Iranian popular name for the genus is "Avishan" (Rechinger, 1982). *Thymus trauvetteri* is distributed in Mazandaran, from sea-level up to altitudes of 2000 m, growing on loam and loam sandy soils (Ghelichnia, 2010). *Thymus* species are well known as medicinal plants because of their biological and pharmacological properties. In traditional medicine, the leaves and flowering parts of *Thymus* species are widely used as tonic and herbal tea, flavouring agents (condiment and spice), antiseptic, antitussive and carminative, as well as treating colds (Alimirzaee *et al.*, 2009; Zargari, 1990).

*Thymus* oils and extracts are widely used in pharmaceutical, cosmetic and perfume industry, as well as for the purpose of flavoring

and preservation of several food products (British Pharmacopoeia, 1988). Recent studies have showed that *Thymus* species have strong antibacterial, antifungal, antiviral, antiparasitic, spasmolytic and antioxidant activities (Sefidkon and Askari, 2002; Zargari, 1990). Many studies on composition of essential oils from different *Thymus* species have been carried out, one of which is *T. kotschyanus*. The published results reveal that major volatile constituents obtained from the aerial parts of the plant are thymol, carvacrol, *p*-cymene,  $\alpha$ -terpinene,  $\beta$ -caryophyllene, etc. (Guseinov *et al.*, 1987; Kasumov and Gadzhieva, 1980; Kulieva *et al.*, 1979; Sefidkon *et al.*, 1999).

The genus *Thymus* has made it one of the most popular plants throughout the entire world, due to its volatile constituents. Therefore, there is a considerable research interest in the compositional analysis of *Thymus* essential oils obtained from the aerial parts of the plant (Vila, 2002). The essential oil substances are thymol, carvacrol, *p*-cymene,  $\beta$ -pinene,  $\gamma$ -terpinene,  $\beta$ -caryophyllene, 1-borneol, 1,8-cineole etc. (Rustaiyan *et al.*, 2000; Sefidkon and Askari, 2002). It is believed that a part of these activities is due to its volatile constituents. Severity of environment associated with increasing altitude in mountain ecosystems can affect medicinal plants growth, as well as their chemical compositions. These variations might be due to the presence of different hemotypes, plants adaptation to the surrounding

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environment, and developmental stage.

It has been revealed that altitude has significant positive effect on the quality and quantity of essential oils of *Thymus fallax* in Lorestan natural habitats (Mohammadian *et al.*, 2015). Avci (2011) showed that altitude was significantly effective on essential oil, carvacrol and thymol rates of *Thymus praecox* ssp. *skorpilii* in Turkey.

According to a study conducted on *Thymus serpyllum*, altitude in most areas has a negative impact on the quantity of oil (Abu-Darwish *et al.*, 2009). In agreement, Habibi *et al.* (2007) also reported a negative correlation between the altitude and the quantity of essential oil in wild thyme oil (*Thymus kotschyanus*) grown in Taleghan. Takaloo *et al.* (2012) studied the composition of the oil from *Thymus migricus* and showed that the highest yields were obtained in the flowering stage and at the lowest altitude. However, evaluating the effect of environmental factors on essential oil of *Thymus kotschyanus* in Iran found the altitude with a positive effect on the amount of essential oil, while soil pH had a negative effect on the oil quantity of this species (Aminzadeh *et al.*, 2010).

Tabrizi *et al.* (2010) showed that the amount of oil in *Thymus transcaspicus* is correlated with the altitude and the quality of the oil depends on the region. The aim of this research paper is to determine the chemical combination of the essential oils collected from aerial parts of *T. trauvetteri* in North of Iran, during

the flowering period in five points altitude and effects of altitude on the amount and composition of essential oils.

## MATERIAL AND METHODS

### Plant material

The aerial parts of *Thymus trauvetteri* were collected during flowering stage, from mountain rangelands of Mazandaran province, in North of Iran, around. Samples collected from five altitudes (L) (2100 m, 2400 m, 2700 m and 3000 m). Voucher specimens were identified and deposited at the herbarium of Research Institute of Forests and Rangelands (RIFR), Tehran, Iran.

### Isolation of the essential oils

After collection, the flowering aerial parts materials were shade dried at room temperature (22-26°C) and placed in paper pockets. Samples transferred to Laboratory of Research Institute of Forests and Rangelands (RIFR), Tehran, Iran. In order to estimate the rate of essential oils, the distillation method was used (Sefidkon *et al.*, 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; Maisonneuve, 1983). The essential oil yield of samples were calculated based on dry weight, and then the oil was dried over anhydrous sodium sulfate.

### Identification of compounds

The constituents of the essential oils were identified by calculation of 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 comparison of their mass spectra with those of the internal reference mass spectra library or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature (Adams, 2001). For quantification purpose, relative area percentages obtained by FID were used, without the use of correction factors.

### Statistical analysis

The populations were compared for rate and components of essential oil by one-way analysis of variance (ANOVA) at SPSS 16 (statistical package program). Correlation analysis was also applied to determine the relations among the altitudes, rate and components.

## RESULTS AND DISCUSSION

Mountainous areas of Mazandaran province in Northern Iran are the most important habitats of *Thymus* species. Different species of *Thymus* growing from 1300 m to

3500 m above sea level. The species *T. trauvetteri* has the most habitat in Mazandaran. The essential oil content of the dried flowering aerial parts of *T. trauvetteri* obtained by hydro-distillation, were yellow color and a distinct sharp odor. The geographic characteristics and altitudes of sampling points and the percentage of essential oils content of *T. trauvetteri* are shown in *Table 1*. Based on the results, the essential oil content is between 1.01-1.51% at different altitudes. The highest essential oil (1.51%) was extracted at an altitude of 2700 m, while it was opposite (1.01%) at an altitude of 3000 m.

The main compounds from interpretation of spectra by GC and GC / Mass essential oil samples to identify, thymol (5.93%-49.75%), carvacrol (1.78%-54.02%), *p*-cymen (6.98%-19.07%), geraniol (0.30%-19.40%) and borneol (1.52%-5.84%) (*Table 2*). The *p*-cymen chemotype yield is higher at lower and middle altitudes (2100 m, 2400 m) and decreases at higher altitudes (3000 m).

**Table 1 - Geographical coordinates of sampling locations and the essential oil percentage of *Thymus trauvetteri* growing at different altitudes in Mazandaran**

Altitudes (m)	Latitude	Longitude	Essential oils (%)
L1 (2100 m)	36° 13 ' 37" N	52° 11 ' 23" E	1.14
L2 (2400 m)	36° 05 ' 56" N	52° 43 ' 57" E	1.45
L3 (2700 m)	36° 04 ' 52" N	52° 47 ' 23" E	1.51
L4 (3000 m)	36° 05 ' 9" N	51° 50 ' 26" E	1..01

L = location

The main components oil essential of *T. trauvetteri* in 2100 m altitude are carvacrol (28.01%), geraniol (19.40%), *p*-cymene

(19.07%), borneol (5.84%) and thymol (5.93%). The main components oil essential of *T. trauvetteri* in 2450 m altitude are carvacrol

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(54.02%), *p*-cymene (18.64%), thymol (9.29%) and borneol (3.51%). The main components oil essential of *T. trauvetteri*, in 2700 m altitude, are thymol (49.75%), *p*-cymene (16.55%), carvacrol (10.69%) and

borneol (5.89%). The main components oil essential of *T. trauvetteri*, in 3000 m altitude, are linalool (24.23%),  $\alpha$ -terepinol (27.84%), geraniol (11.37%), thymol (7.32%) and *p*-cymene (6.98%).

**Table 2 - Essential oil compositions and their percentage in *Thymus trauvetteri* growing at different altitudes in Mazandaran**

No.	Compounds	Altitudes			
		L1 (2100 m)	L2 (2400 m)	L3 (2700 m)	L5 (3000 m)
1	$\alpha$ -Thujene		1.6	1.24	
2	$\alpha$ -Pinene	2.34	1.89	2.95	1.51
3	Camphene	2.69	1.15	1.79	
4	$\beta$ -Pinene		0.90		
5	Myrcene	0.51	0.90	0.39	4.05
6	$\delta$ -Terpinene	2.30	2.97	0.99	4.67
7	<i>p</i> -cymene	19.07	18.64	16.55	6.98
8	1,8-Cineol	3.49		1.53	0.54
9	Linalool	0.17	0.09		24.23
10	Borneol	5.84	3.51	5.89	1.52
11	Terpinen-4-ol				
12	$\alpha$ -Terpineol	0.15	0.08		27.84
13	Methyl thymol	1.31		1.13	
14	Methyl carvacrol	0.51		1.89	
15	Geraniol	19.40	0.30		11.37
16	Thymol	5.93	9.29	49.75	7.32
17	Carvacrol	28.01	54.02	10.69	1.78
18	(E)-Caryophyllene	1.76	1.35	1.55	1.86
19	Geranyl acetate	1.75			0.52
20	$\alpha$ -Terepinene	2.30	1.57	0.46	0.59
21	Thymoquinone				
22	Total	97.53	98.26	96.8	94.8

L=Locations

The thymol chemotype yield is the highest in the 2700 m altitude and its lowest value is at in 2100 m altitude. The carvacrol chemotype yield is the highest value in the 2700 m altitude (68.39%) and its lowest value is at 3000 m altitude. The *p*-cymen chemotype yield is the highest in 2100 m, 2400 m and 2700 m altitudes and the lowest in 3000 m altitude. According to the

results, altitude was significantly ( $p \leq 0.05$ ) effective on essential oil, thymol, carvacrol and *p*-cymen rates and to results of correlation analysis (Tables 3 and 4). The highest percentage of essential oil is at an altitude of 2400 m and the lowest is 3000 m above sea level. The highest percentage of thymol is in L3 (2700 m) and lowest is in L1 (2100 m). The highest percentage of

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carvacrol is in L3 (2700 m) and lowest is in L4 (3000 m). The highest percentage of *p*-cymen is in L1, L2,

L3 (2100, 2400 and 2700 m, no significant difference) and lowest is in L4 (3000 m).

**Table 3 - The amount of essential oil, thymol, carvacrol and *p*-cymene rates of *Thymus trauvetteri* Klokov. J alas var. *laniger* (Borbás) J alas**

Locations	Essential oil (%)	Thymol (%)	Carvacrol (%)	<i>p</i> -cymene
L1	1.14 <sup>c*</sup>	5.93 <sup>d</sup>	28.01 <sup>b</sup>	19.07 <sup>a</sup>
L2	1.51 <sup>a</sup>	9.29 <sup>b</sup>	10.69 <sup>c</sup>	18.64 <sup>a</sup>
L3	1.45 <sup>b</sup>	49.75 <sup>a</sup>	54.02 <sup>a</sup>	16.55 <sup>a</sup>
L4	1.01 <sup>d</sup>	7.32 <sup>c</sup>	1.78 <sup>d</sup>	6.98 <sup>b</sup>

\*The columns with different letters mean statistically different according to LSD ( $\rho \leq 0.05$ ) test.

**Table 4 - Relations among the studied characters**

Characteristics	Essential oil	Thymol	Carvacrol	<i>p</i> -cymene
Altitude	-17.5	0.27	-0.68 <sup>*</sup>	-0.87 <sup>**</sup>
Essential oil	ns	0.68 <sup>*</sup>	0.44	0.62 <sup>*</sup>

The results of the present study focused on the effects of altitude factor of the quantity of essential oil in *T. trauvetteri*. These results indicate that altitude factors could have an effect on the quality of the oil, which corresponds with the results of previous work on this genus (Boira and Blanquer 1998; Omid Beigi, 1995). The percentage essential oil is high with increasing altitude and then decreasing in higher altitudes. also the percentage of essential oil is decreasing in low altitudes. This is in agreement with the results of some previous works on the essential oil of different species of the this genus (Mohammadian *et al.*, 2015, Yavari *et al.*, 2010) and contrary to the results of Habibi *et al.* (2007), Takaloo *et al.* (2012), Abu-Darwish (2009), Imani Dizajeyekan *et al.* (2016), which stated with increasing altitude,

essential oil production will be limited.

According to the results, altitude has a positive effect on the percentage of carvacrol and thymol. The lowest amount of carvacrol is in the highest altitude (3000 m) and the lowest thymol is in the lowest altitude (2100 m) (Table 2). This is in agreement with the results of some previous works on the essential oil of *Thymus kotschyanus* (Habibi *et al.*, 2007). This is in agreement with the result of essential oil of *Thymus carmanicus* (Ghasemi *et al.*, 2013). According to the results, altitude has a positive effect on the percentage of essential oils and essential oil increases with increasing altitude.

The study of on the essential oils of *T. trauvetteri* from another location of South of Iran shown that the percentage of thymol (24.43%), carvacrol (50.07%), *p*-cymene

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(10.09%),  $\delta$ -terpinene (7.78%) and  $\alpha$ -pinene (5.29%) were in flowering stage (Shahnazi *et al.*, 2007). In contrast with this, the study shows that the compounds of essential oil are similar, but the amount of percentage of essential oil is different. Although comparison between compounds obtained from this study and other reports, shows some similarities, but there are considerable quantitative and qualitative differences between these samples. These variations in the essential oil composition might have arisen from several differences (climatic, seasonal, geographical, and geological).

### CONCLUSIONS

The aerial parts of *Thymus trauvetteri* were collected during flowering stage, from mountain rangelands of Mazandaran province, in the north of Iran. Samples were collected from four altitudes (2100 m, 2400 m, 2700 m and 3000 m). In conclusion, our results demonstrate the essential oil content is between 1.01-1.51%, at different altitudes. The highest essential oil (1.51%) was extracted at an altitude of 2400 m, while it was opposite (1.01%) at an altitude of 3000 m. According to the results, altitude has a positive effect on the percentage of carvacrol and thymol. The lowest amount of carvacrol is in the highest altitude (3000 m) and the lowest thymol is in the lowest altitude (2100 m). Altitude has a positive effect on the percentage

of essential oils and essential oil increases with increasing altitude.

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