

ESSENTIAL OIL COMPOSITION OF *THYMUS FALLAX* FISCH. & C.A. MEY. AT DIFFERENT GROWING ALTITUDES IN MAZANDARAN, IRAN

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ABSTRACT. *Thymus fallax* Fisch. & C.A. Mey. (*Lamiaceae*) is a permanent plant that grows in some mountain rangelands of Mazandaran province in Iran. The aerial parts of *Thymus fallax* were collected during flowering stage from mountain rangelands of Mazandaran province, in North of Iran. Around samples were collected from three altitudes (2400 m, 2700 m and 3000 m a.s.l.), in mountain regions 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 fallax*. 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 1.12 - 1.61% at different altitudes. The result of study show that the highest concentrated essential oil (1.61%) was extracted in the lowest altitude (2400 m), while it was opposite, (0.1.12%) in the

highest altitude (3000 m). The main compounds of essential oil are: thymol (5.95% - 10.06%), carvacrol (13.63% - 69.04%), *p*-cymene (4.19% - 12.18%) and borneol (4.72% - 5.66%). According to the results, altitude has a negative effect on the percentage of essential oils and essential oil decreases with increasing altitude. The altitude has a negative effect on the percentage of thymol and the content of thymol decreased with increasing altitude. The altitude has a positive effect on the percentage of carvacrol and the content of carvacrol increased with increasing altitude.

Keywords: carvacrol; mountain rangelands.

INTRODUCTION

The genus *Thymus* L. (*Lamiaceae*) consists of about 350 species of herbaceous perennials and subshrubs (Morales, 1986).

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The mediterranean region can be described as the center of the genus (Stahl-Biskup, 1991). *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). 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 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 (Mirzaee *et al.*, 2012; 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). 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, β -pinene, γ -terpinene, β -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 environment, and developmental stage. 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, γ -terpinene, β -caryophyllene, etc. (Guseinov *et al.*, 1987; Kasumov and Gadzhieva, 1980; Kulieva *et al.*, 1979; Sefidkon *et al.*, 1999).

According to a study conducted on *Thymus serpyllum*, altitude in the 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 of *Thymus kotschyanus*

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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 aerial parts of *T. fedtschenkoi* from North of Iran, during the flowering period, in five points altitude and effects of altitude on the amount and composition of essential oils. *T. fallax* species is an endemic species in Iran and Turkey, but mostly distributed in Iran. It is element of Iran-Turan (Rechinger, 1982). In spite of existence many reports of the essential oil composition and biological activity of *Thymus* species, only a few reports have been published

on the essential oil of *T. fallax* (Barazandeh, 2004; Goze *et al.*, 2009). *Thymus fallax* is widely distributed in Mazandaran province of Iran, from 2400-3500 m altitudes, growing on sandy and siliceous soils (Ghelichnia, 2010). 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).

The goal of current research is to assess the effect of altitude on the chemical composition and function of essential oil in *Thymus fallax*.

MATERIALS AND METHODS

Plant material

The aerial parts of *T. fallax* were collected during flowering stage, from mountain rangelands of Mazandaran province, in North of Iran. Random plot sampling design was used for collecting from 2400, 2300 and 2000 m altitude with three replications and 50 m intervals in each altitude class. The size of used plots was 10×10 m². Voucher specimens were identified by Dr. Ziba Jamzad and deposited at the herbarium of Research Institute of Forests and Rangelands (RIFR), Tehran, Iran.

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

Altitudes (m)	Latitude	Longitude	Essential oils (%)
2400	35° 51' 45.12" N	52° 04' 36.05" E	1.61
2700	35° 54' 07.18" N	52° 03' 07.60" E	1.18
3000	35° 55' 26.96" N	52° 03' 41.05" E	1.12

Isolation of the essential oil

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 (Maisonneuve, 1983; 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 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* grow from 1300 m to 3500 m above sea level. The species *T. fallax* has the most habitat in Mazandaran. The essential oil content of the dried flowering aerial parts of *T. fallax* obtained by hydrodistillation, 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. fallax* are shown in *Table 1*. Based on the results, the essential oil content is between 1.12 and 1.61% at different altitudes (*Table 1*). The highest concentrated essential oil (1.61%) was extracted in the lowest altitude (2400 m), while it was opposite (1.12%) in the highest altitude (3000 m). There is a significant difference between altitude and the percentage of essential oil. The percentage of essential oil decreases with increasing of altitude ($p \leq 0.05$). The main components of the essential oils were thymol (5.95% – 10.06%), carvacrol (13.63% – 69.04%), *p*-cymene (4.19% – 12.18%) and borneol (4.72% – 5.66%) (*Table 2*). The thymol chemotype yield is higher at lower and middle altitudes (2400 m, 2700 m) and decreases at higher altitude

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(3000 m). There is no significant difference in the percentage of thymol between the heights of 2400 m and

2700 m, but a significant difference showed at an altitude of 3000 m ($p \leq 0.05$).

Table 2 - Amount of essential oil compositions of *Thymus fallax* growing at different altitudes in Mazandaran

No.	Compounds	Altitudes		
		2400 m	2700 m	3000 m
1	Camphene	1.5	2.65	-
2	α -Pinene	7.9	1.49	0.25
3	α -Phellandrene	5.1	2.19	1.14
4	Myrcene	2.89	1.47	1.51
5	Terpineolene	1.03	0.32	0.08
6	<i>p</i> -Cymene	7.73	12.18	4.19
7	γ -Terpinene	3.68	8.68	-
8	1,8-Cineole	2.02	1.26	2.72
9	γ -Terpineol	2.80	0.66	1.59
10	Fenchone	1.45	-	-
11	Linalool	1.30	0.18	0.77
12	Camphor	0.32	0.17	0.46
13	Pinol	-	0.25	-
14	Borneol	4.72	5.12	5.66
15	α -Terpineol	10.88	0.49	0.54
16	Bornyl acetate	1.45	0.15	0.07
17	Geraniol	9.27	4.35	0.30
18	Methyl thymol	2.47	-	2.97
19	Thymol	10.04	10.06	5.95
20	Carvacrol	13.63	41.84	69.04
21	Geranyle acetate	0.37	2.17	-
22	β -Selinene	0.19	0.12	0.07
23	E-Caryophyllene	1.38	1.37	0.55
24	Spathulene	0.11	0.37	0.09
25	B-Caryophyllene	-	0.35	0.86
26	Menthol	-	-	0.16
27	Caryophyllene oxid	0.39	0.12	-
Total		98.01	98.01	99.84

The carvacrol chemotype yield is the highest value in the 3000 m altitude (69.04%) and its lowest value is at 2400 m altitude (13.63%). The relationship between altitude and amount of carvacrol showed a significant difference ($p \leq 0.05$). The *p*-cymene chemotype yield is higher at middle altitudes (2700 m) and

decreases at higher altitude (3000 m). The relationship between altitude and amount of *p*-cymene showed a significant differences ($p \leq 0.05$). The amount of borneol in all altitudes is relatively the same. There is no significant difference between borneol levels at different altitudes ($p \leq 0.05$). The results of the present study

focused on the effects of altitude factor of the quantity of essential oil in *T. fallax*. 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). There is a significant negative correlation between altitude and the amount of essential oil and thymol (-0.94 and -0.84). There is a significant positive correlation between amount of carvacrol and altitude (0.99). There

is no significant relationship between amount of *p*-cymene and borneol (-0.42 and 0.54) (Table 3).

According to the results altitude has a positive effect on the percentage of carvacrol and the lowest amount of carvacrol is in the lowest altitude (2400 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).

Table 3 - Relations among the studied characters

Characteristics	Essential oil (%)	Thymol (%)	Carvacrol (%)	<i>p</i> -Cymene	Borneol (%)
Altitude	-0.94**	-0.84**	0.99**	-0.42 ^{ns}	0.54 ^{ns}

** = significant ($p \leq 0.01$); ns = no significant

The altitude has a negative effect on the percentage of thymol. The results of this study showed that the content of thymol decreased with increasing altitude and the highest amount of thymol is in the lowest and middle altitudes (2400 m and 2700 m) and the lowest is in the highest altitude (3000 m) (Table 4). This is agreement with the result of essential oil of *Thymus carmanicus* in Iran (Ghasemi *et al.*, 2013). According to the results, altitude has a negative effect on the percentage of essential oils and essential oil decreases with increasing altitude. This is in agreement with the results of some previous works on the essential oil of different species of the this genus (Habibi *et al.*, 2007; Takaloo *et al.*, 2012; Abu-Darwish *et al.*, 2009; Imani Dizajeyekan *et al.*, 2016), which states

with increasing altitude, essential oil production will be limited and contrary to the results of Mohammadian *et al.* (2015) and Yavari *et al.* (2010). The study of essential oils of *T. fallax* from Turkey has shown that the main components were carvacrol and thymol and *p*-cymene, respectively (Tumen *et al.*, 1999). Three chemotype of this study are similar to my study. The major constituents of the essential oil were carvacrol (30.2%), geraniol (15.4%) and *p*-cymene (7.7%) in north of Iran (Morteza-Semnani *et al.*, 2004). In contrast with this study, the results of my study show that the amount of carvacrol in the essential oil of *T. fallax* is high (69.04%), that this amount has not been observed in other studies in north of Iran. The study has shown that the main constituents of

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the essential oil were carvacrol (69.2%), *p*-cymene (15.4 %), thymol (5.3%) in the west of Iran (Rustaiee *et al.*, 2011). This is in agreement with the results of my study but the borneol is not a main component of this study. The study of essential oils of *T. fallax* has shown that the main compounds of *T. fallax* are thymol (43.43 – 61.14%), borneol

(3.89 – 7.01%), carvacrol (3.88 – 6.5%) and *p*-cymene (4.58 – 6.0%) in east Azerbaijan in Iran. Although the main of the compounds are similar to my study, the amount of carvacrol is much higher in compared to this study, and this is related to the sampling of *T. fallax* essential oil at higher altitudes (up to 2500 m), which has not been done in Iran.

Table 4 - The amount of essential oil, thymol, carvacrol and *p*-cymene rates of *Thymus fallax* at different altitudes

Location	Essential oil (%)	Thymol (%)	Carvacrol (%)	<i>p</i> -Cymene	Borneol
2400 m	1.61 ^{a*}	10.04 ^a	13.66 ^c	7.73 ^b	4.72 ^a
2700 m	1.18 ^b	10.06 ^a	41.84 ^b	12.18 ^a	5.12 ^a
3000 m	1.01 ^c	5.95 ^b	69.04 ^a	4.19 ^c	5.66 ^a

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

CONCLUSIONS

The aerial parts of *Thymus fallax* were collected during flowering stage, from mountain rangelands of Mazandaran province, in the North of Iran. Samples were collected from four altitudes (2400 m, 2700 m and 3000 m). In conclusion, our results demonstrate the essential oil content is between 1.12 – 1.61%, at different altitudes. The highest essential oil (1.61%) was extracted in the lowest altitude (2400 m), while it was opposite (1.12%) in the highest altitude (3000 m). There is a significant difference between altitude and the percentage of essential oil. The thymol chemotype yield is higher at lower and middle altitudes (2400 m, 2700 m) and decreases at higher altitude (3000 m). The carvacrol chemotype yield is the highest value

in the 3000 m altitude (69.04%) and its lowest value is at 2400 m altitude (13.63%).

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