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ESSENTIAL OIL COMPOSITION OF *THYMUS FEDTSCHENKOI* RONNIGER AT DIFFERENT GROWING ALTITUDES IN MAZANDARAN, IRAN

H. GHELICHNIA[1]*

*E-mail: ms_ghelichh@yahoo.com

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ABSTRACT. *Thymus fedtschenkoi* Ronniger (Lamiaceae) is a permanent, that grows in some mountain rangelands of Iran, including Mazandaran province. The aerial parts of *Thymus fedtschenkoi* were collected during flowering stage in June 2012, from mountain rangelands of Mazandaran province, in north of Iran. Samples were collected from five altitudes (1300 m, 1600 m, 2000 m, 2400 m and 3000 m) in mountain region of Mazandaran province. The goal of current research was to assessment the effect of altitude on the chemical composition and function of essential oil in *Thymus fedtschenkoi*. 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 0.92-1.31%, at different altitudes. The highest content of essential oil (1.31%%) was extracted in the highest altitude (3000 m), while it was opposite (0.92%) in the lowest altitude (1300 m). The main essential oil compounds of *Thymus fedtschenkoi* samples were thymol (8.62%-36.86%), carvacrol (6.787%-68.39%), γ-terpinene (1.473T-6.461%), p-cymen (5.764%-16.204%) and linalool (0.465%-6.457 6.8%). According to the results, altitude has a positive effect on the percentage of essential oils and essential oil increases 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: thymol; mountain rangelands.

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

[1] Forest and Rangeland Research Department, Mazandaran Agricultural and Natural Resources Research Center, AREEO, Sari, Iran

(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 fedtschenkoi* L. is widely distributed in Mazandaran, from sea level up to altitudes of 1400 m, growing on sandy and siliceous 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 (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). Recent studies have showed that *Thymus* species have strong antibacterial, antifungal, antiviral, antiparasitic, spasmolytic and antioxidant activities (Sefidkon & Asgari, 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, c-terpinene, b-caryophyllene, etc. (Guseinov *et al.,*1987; Kasumov & 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, β-pinene, γ-terpinene, β-caryophyllene, 1-borneol, 1,8-cineole, etc. (Rustaiyan *et al.,*2000; Sefidkon & Asgari, 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.

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). 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 has a positive effect on the amount of essential oil, while high soil pH had a negative effect on the oil quantity of this species and effective chemical substances of such species in salty and alkaline soils is less (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. 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.

MATERIALS AND METHODS

Plant material

The aerial parts of *Thymus fedtschenkoi* were collected during flowering stage, from mountain rangelands of Mazandaran province, in the north of Iran. Samples were collected from five altitudes (1300 m, 1600 m, 2000 m, 2400 m and 3000 m). Voucher specimens were identified by Dr. Ziba Jamzad 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.,* 2000). 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 (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.

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. fedtschenkoi* has the most habitat in Mazandaran.

The essential oil content of the dried flowering aerial parts of *T. fedtschenkoi*, 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. fedtschenkoi* are shown in *Table 1*. Based on the results, the essential oil content is between 0.92-1.31%, at different altitudes. The highest essential oil (1.31%) was extracted in the highest altitude (3000 m), while it was opposite (0.92%) in the lowest altitude (1300 m).

The main compounds from interpretation of spectra by GC and GC / Mass essential oil samples to identify, thymol (8.62%-36.86%), carvacrol (6.787%-68.39%), γ-terpinene (1.473%-6.461%), p-cymene (5.764%-16.204%), linalool (0.465% - 6.457%) are shown in *Table 2*.

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

Altitudes (m)	Latitude	Longitude	Essential oils (%)
1300	36° 11' 34.3" N	52° 12' 4.3" E	0.92
1600	36° 12' 37.9" N	52° 11' 19.8" E	1
2000	36° 13' 25.8" N	52° 11' 9.9" E	1.09
2400	36° 16' 2.17" N	52° 11' 48.8" E	1.10
3000	36° 66' 22.9" N	52° 22' 41.7" E	1.31

The linalool chemotype yield is higher at lower and middle altitudes (1300 m, 2400 m) and decreases at higher altitudes (3000 m). The main components oil essential of *T. fedtschenkoi* in 1300 m altitude are p-cymene (14.556%), *γ*-terpinene (6.461%), linalool (6.457%), geraniol (5.305%), *α*-terpineol (7.754%), thymol (30.046%) and carvacrol (6.787%). The main components oil essential of *T. fedtschenkoi* in 1600 m altitude are p-cymene (16.204%), borneol (3.465%), geraniol (21.011%), thymol (49.241%) and carvacrol (10.201%). The main components oil essential of *T. fedtschenkoi* in 2000 m altitude are p-cymene (13.915%), *γ*-terpinene (3.905%), thymol (36.860%) and carvacrol (23.159%). The main components oil essential of *T. fedtschenkoi* in 2400 m altitude are p-cymene (5.764%), linalool (6.941%),

geraniol (21.011%), α-terpineol (7.504%), thymol (9.698%) and carvacrol (29.754%). The main components oil essential of *T. fedtschenkoi* in 3000 m altitude are α-terpinene (4.30%), thymol (8.62%) and carvacrol (68.39%). The thymol chemotype yield is the highest in the 1300 m,1600 m and 2400 m altitudes and its lowest value is at 2400 m and 3000 m altitude.

Table 2 - Essential oil compositions and their percentage in *Thymus fedtschenkoi* growing at differentaltitudes in Mazandaran

No.	Compounds	*RIa*	Altitudes				
			1300 m	1600 m	2000 m	2400 m	3000 m
1	α-Thujone		1.003	1.288	1.076	0.404	-
2	α-Pinene		3.210	2.799	1.746	0.447	1.64
3	Camphene		1.072	2.085	1.337	0.786	
4	β-Pinene		-	0.534	0.367	-	-
5	Myrcene		1.698	0.500	0.475	0.735	0.84
6	α-Terpineol		-	1.539	1.681		0.11
7	p-Cymene		14.556	16.204	13.915	5.764	7.86
8	1,8-Cineol		1.555	2.911	1.323	2.444	0.58
9	γ-Terpinene		6.461	1.473	3.905	3.129	-
10	Linalool		6.457	0.465	0.616	6.941	0.90
11	Borneol		1.987	3.465	2.942	2.607	2.90
12	Terpinen-4-ol		-	-	0.752	-	-
13	α-Terpineol		7.794		0.656	7.504	
14	MethylThymol		0.559	1.644	1.473	-	-
15	Methyl carvacrol		0.377	-	0.512	-	-
16	Geraniol		5.305	-	1.158	21.011	-
17	Thymol		30.046	49.241	36.860	9.698	8.62
18	Carvacrol		6.787	10.201	23.159	29.754	68.39
19	(E)-Caryophyllene		2.585	1.635	2.148	1.221	1.17
20	Geranyl acetate		4.684	-	-	4.687	-
21	α-Terepinene		1.873	-	-	1.046	4.30
22	Thymoquinone		-	1.489	-	-	-
24	Total		98	97.47	96.10	98.17	97.31

The carvacrol chemotype yield is the highest value in the 3000 m altitude (68.39%) and its lowest value is at 1300 m altitude. The (E)-caryophyllene chemotype yield decreases directly with altitude and the highest in 1300 m altitude and the lowest in 3000 m altitude, whereas γ-terpinene has the highest yield even at a lower altitude. The geraniol chemotype yield increase directly with altitude, but there are no in essential oils at an altitude more than 2400 m and its maximum value in 2400 m altitude (21.011%). The study of essential oils of *T. fedtschenkoi* var. *handelii* from Turkey has shown that the main components were carvacrol

and thymol and linalool, respectively (Başer *et al.*, 2001).

The results of the present study focused on the effects of altitude factor of the quantity of essential oil in *T. fedtschenkoi.* 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 & Blanquer 1998; Omid Beigi, 1995). 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 (1300 m) (*Table 2*). This is in agreement with the results of some previous works on the essential oil of *T. kotschyanus* (Habibi *et al.,* 2007). The altitude has a negative effect on the percentage of thymol. The results of this study showed that the content ofthymol decreased with increasing altitude and the highest amount of thymolis in the lowest altitude (1300 m) and the lowest is in the highest altitude (3000 m) (*Table 2*). This is in agreement with the result of essential oil of *T. carmanicus* (Ghasemi Pirbalouti *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. 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) and Dizajeyekan *et al.* (2016), which stated with increasing altitude, essential oil production will be limited.

The study of on the essential oils of *T. fedtschenkoi*, from another location of Iran, has shown that the percentage of thymol (31.8%), carvacrol (24.3%), *p*-cymene (12.3%) and 1,8-cineol (5.8%) were in flowering stage (Abousaber *et al.,* 2002).

The study of essential oils of *T. fedtschenkoi* var. *handelii*, from Turkey, has shown that the main components were carvacrol and thymol and linalool, respectively (Başer *et al.*, 2001). The study has shown that linalool (17.29%), bornyl acetate (9.19%), borneol (10.4%), thymol (9.19%) and carvacrol (6%) were also determined in *T. fedtschenkoi* var. *handelii* from Ararat mountains in Turkey (Meriçli, 1986). In contrast with other studies, this study shows that the amount of carvacrol in the essential oil of *T. fedtschenkoi* is very high (68.39%), that this amount has not been observed in other studies.

CONCLUSION

The aerial parts of *Thymus fedtschenkoi* were collected during flowering stage, from mountain rangelands of Mazandaran province, in the north of Iran. Samples were collected from five altitudes (1300 m, 1600 m, 2000 m, 2400 m and 3000 m). In conclusion, our results demonstrate the essential oil content is between 0.92-1.31%, at different

altitudes. The highest essential oil (1.31%) was extracted in the highest altitude (3000 m), while it was opposite (0.92%) in the lowest altitude (1300 m). 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 (1300 m). The content of thymol decreased with increasing altitude and the highest amount of thymolis in the lowest altitude (1300 m) and the lowest is in the highest altitude (3000 m).

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