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Comparative Study of the Essential Oil Chemical Composition of *Thymus Kotschyanus* Boiss. & Hohen var. *kotschyanus* from Iran

Ali Mazooji*[1], Fahimeh Salimpour[2], Mahmoud Danaei[1], Samaneh Akhoondi Darzikolaei[3], Kianoosh Shirmohammadi[1]

[1]*Department of Biology, Roudehen Branch, Islamic Azad University, Roudehen, Iran*
[2]*Department of Biology, North Tehran Branch, Islamic Azad University, Tehran, Iran*
[3]*Young Researchers Club, Science and Research Branch, Islamic Azad University, Tehran, Iran*

ABSTRACT

The essential oil obtained from the aerial parts of Thymus Kotschyanus Boiss. & Hohen var. kotschyanus, growing wild in Iran, was analyzed by using GC and GC-MS. There were 10 compounds in the volatiles from T. Kotschyanus var. kotschyanus, comprising 97.38% of the total oil in 0.93 % (w/w) yield. The main constituents of the species were Thymol (89.08%) and γ-Terpinene (4.62%). Three common chemical compositions namely, α-Pinene, Borneol and Thymol, have been found among six populations. There is not Carvacrol in T. kotschyanus var. kotschyanus which is the major difference between this study and the previous ones. On the other hand, Gadouk Col population represents the highest amount of Thymol (89.08%) in its essence. The quantitative and qualitative differences in the volatile constituents can be considered as chemotaxonomic significance and it may be attributed to their different ecological and geographical origin factors.

Key words: Lamiaceae, Volatile oil, Thymol, Iran.

INTRODUCTION

The genus *Thymus* L., belonging to the Lamiaceae family and consists of 215 genera. The Mediterranean area is said to be the origin of this kind of genus [1-3]. This is a genus of small herbs and shrubs and is represented by 34 species growing in different parts of Iran, of which two are endemic [4]. *Thymus* species are well known as medicinal plants because of their biological and pharmacological properties. In traditional medicine, leaves and flowering parts of *Thymus* species are widely used as tonic and herbal tea, antiseptic, antitussive and carminative as

well as treating colds. *Thymus kotschyanus* is an aromatic and medicinal plant that grows wild in Iran (Azerbaijan, Gilan, Mazandaran, Qazvin and Tehran provinces) [5].

The volatile oil chemical constituents of *Thymus kotschyanus* Boiss. & Hohen var. *glabrescens* Boiss. have been analyzed in Turkey [6]. The essential oil chemical composition of *T. kotschyanus* was identified Thymol (38.0%), Carvacrol (14.2%) and 1, 8-Cineole (13.2%) as the main components of the oil of the species collected from Dizin in Iran [7]. The effect of distillation methods and stage of plant growth on the essential oil content and composition of this species was investigated [8]. The essential oils obtained from the aerial parts of *T. daenensis* Celak. and *T. kotschyanus* were analyzed by using GC and GC/MS. Thirty one components accounting for 98.7% of *T. kotschyanus* oil were identified [9]. The volatile oil chemical composition of *T. kotschyanus* var. *kotschyanus* from the eastern Anatolian region in Turkey was analyzed [10]. The essential oils of the aerial parts of *T. kotschyanus* Collected during the full flowering period from the suburb of Behshahr (North of Iran) were isolated by hydrodistillation and analyzed by GC and GC/MS. Twenty one constituents were identified in *T. kotschyanus* oil [11]. The effect of altitude on essential oil and components in wild thyme (*T. kotschyanus*) from Taleghan region was studied [12]. The effect of evaluation for quality and quantity of essential oil of *T. kotschyanus* (Damavand-Tar) has been studied**.** Generally, 37 different chemical compositions were analyzed by GC-MS. This study showed that essence of this species has high content of Thymol and Carvacrol which are two medically important [13]. The effect of distillation methods and plant growth stages on the essential oil content and composition of *T. vulgaris* L. was done [14]. The comparison of volatile organic compounds of this species using hydrodistillation and headspace solid phase microextraction gas chromatography mass spectrometry have been carried out [15]. Chemical characterization of volatile components of *T. serpyllium* L. using Microwave distillation (MD) ,a new method of essential oil extraction, have been compared with a conventional technique, hydor-distillation (HD). There was no obvious difference in the quality of essential oils obtained by the kinds of extraction methods [16]. Composition of essential oils of leaves stems and roots of *T. kotschyanus* var. *pseuderiophorus* Rech. F.P.P from wild populations in the north of Iran was analyzed by GC/MS [17]. The essential oils composition and antioxidant properties of *T. kotschyanus* were studied [18].

The aim of this study is the comparison of the chemical composition essential oil of *Thymus Kotschyanus* Boiss. & Hohen var. *kotschyanus*, growing wild in Iran, with the previous studied populations and species.

MATERIALS AND METHODS

Plant material: The aerial part of *Thymus kotschyanus* var. *kotschyanus* was collected from Firouzkouh Road, Gadouk Col, 2100 m (N 35˚50', E 52˚55'), in Sept. 2011 (IRAN-56840). The plant was identified by following Flora Iranica [5], and the voucher specimens are preserved in the herbarium of the Ministry of Jihad-e-Agriculture ("IRAN") at the Iranian Research Institute of Plant Protection (Tehran, Iran).

Isolation of the volatile oils: The air-dried aerial parts of the plant were subjected to hydrodistillation for four hour using a Clevenger-type apparatus. The oil was kept at 4°C in the sealed brown vials until required.

Gas chromatography-Mass spectroscopy: Analytical gas chromatography was carried out using a Thermoquest 2000 GC with capillary column DB-5 (30 m. 0.25 mm i.d., 0.25 μm film Thickness); carrier gas, He; split ratio, 1:25; and using a flame ionization detector. The column temperature was programmed at 50°C for 1 min. and then heated to 265°C at a rate of 2.5°C/min. and then kept constant at 265°C for 20 min. GC-MS was performed by Agilent Technology (U.S) on a Thermoquest 2000 with a quadruple detector, on capillary column DB-5 (GC); carrier gas, He; flow rate, 1.5 ml/min. the column was held at 50°C for 1 min. and programmed up to 265°C at rate of 2.5°C/min, then kept constant at 256°C for 20 min. The MS operated at 70 ev ionization energy. Retention indices were calculated by using retention times of *n*-alkanes that were injected after the oil at the same chromatographic conditions. Quantitative data were obtained from the electronic integration of the FID peak areas. The components of the oils were identified by comparison of their mass spectra and retention indicates with Wiley library and those published in the literature [19].

Table 1- Chemical composition (%) of the essential oil of *Thymus kotschyanus* var. *kotschyanus*

No.	Components	%	Rt	KI
1	α-Pinene	0.64	9.74	943
2	Camphene	0.60	10.24	956
3	β-Myrcene	0.27	12.18	997
4	α-Terpinene	0.33	13.22	1024
5	1,8-Cineole	0.70	13.83	1041
6	γ-Terpinene	4.62	15.43	1097
7	Borneol	0.36	19.99	1168
8	Thymol	89.08	26.58	1293
9	cis-Caryophyllene	0.56	31.57	1406
10	Isoaromadendrene	0.22	37.41	1498
	Total	**97.38**		

Rt = Retention time; KI= Kovats Index, %= Relative percentage obtained from peak area

Table 2- Comparison the essential oil composition (%) from various populations of *Thymus kotschyanus*

No.	Components	*T. kotschyanus* var. *kotschyanus* Gadouk Col (%)	*T. kotschyanus* var. *kotschyanus* Turkey [10]	*T. kotschyanus* Damavand (H1) [13]	*T. kotschyanus* Dizin [7] (%)	*T. kotschyanus* Lorestan [18] (%)	*T. kotschyanus* var. *glabrescens* Turkey [6] (%)
1	**α-Pinene**	**0.64**	**0.7**	**1.24**	**1.3**	**0.5**	**3.2%**
2	Camphene	0.60	0.7	0.55	0.3	-	4.1
3	β-Myrcene	0.27	-	-	-	0.7	-
4	Myrcene	-	0.9	0.93	-	-	-
5	α-Terpinene	0.33	0.8	0.74	-	1.2	2.1
6	1,8-Cineole	0.70	5.9	1.17	13.2	1.4	-
7	γ-Terpinene	4.62	3.3	3.11	0.9	11.4	-
8	**Borneol**	**0.36**	**7.7**	**1.38**	**0.7**	**4.4**	**0.5**
9	**Thymol**	**89.08**	**48.0**	**4.32**	**38.0**	**39.7**	**0.7**
10	*cis*-Caryophyllene	0.56	-	-	-	-	-
11	Isoaromadendrene	0.22	-	-	-	-	-
12	α-Thujene	-	0.2	0.89	2.2	0.5	-
13	β-Pinene	-	0.6	-	1.5	0.2	-
14	2-β-Pinene	-	-	0.2	-	-	-
15	ρ-Cymene	-	2.0	-	2.2	5.4	0.9
16	Linalool	-	0.1	0.17	4.4	0.1	4.5
17	Terpinen-4-ol	-	0.8	0.10	0.4	2.5	-
18	Carvacrol	-	2.3	75.75	14.2	7.6	44.2
19	β-Caryophyllene	-	2.9	0.40	0.9	-	1.1
20	*trans*-Caryophyllene	-	-	0.40	-	-	-
21	Germacrene-D	-	0.7	-	0.3	1.3	-
22	δ-Cadinene	-	0.3	-	0.2	-	-
23	3-Octanone	-	-	0.19	-	0.2	-
24	α-Phellandrene	-	0.1	0.18	-	0.1	-
25	β-Phellandrene	-	-	0.34	-	-	-
26	δ-3-Carene	-	-	0.07	-	-	-
27	Carvacrol methyl ether	-	-	0.16	-	-	-
28	*cis*-Sabinene Hydrate	-	0.9	0.78	-	2.9	-
29	Sabinene	-	0.1	-	-	0.1	-
30	α-Terpinolene	-	-	0.08	-	-	-
31	Terpinolene	-	0.2	-	-	0.2	0.9
32	*L*.Carvone	-	-	0.11	-	-	-
33	Aromadendrene	-	-	0.02	-	-	-

Table 1. (Continued)

No.	Components	*T. kotschyanus* var. *kotschyanus* Gadouk Col (%)	*T. kotschyanus* var. *kotschyanus* Turkey [10]	*T. kotschyanus* Damavand (H1) [13]	*T. kotschyanus* Dizin [7] (%)	*T. kotschyanus* Lorestan [18] (%)	*T. kotschyanus* var. *glabrescens* Turkey [6] (%)
34	Tricyclene	-	-	-	-	0.1	1.0
35	1-Octen-3-ol	-	-	-	-	0.1	-
36	3-Octanol	-	tr.	-	-	0.1	-
37	Limonene	-	0.4	-	-	0.2	-
38	(+)-Limonene	-	-	-	-	-	2.7
39	*cis*-Ocimene	-	-	-	-	0.1	-
40	*trans*-Ocimene	-	-	-	-	0.7	-
41	Camphor	-	-	-	-	0.1	-
42	β-Fenchyl alcohol	-	-	-	-	0.2	-
43	α-Terpineol	-	0.2	-	-	0.3	-
44	Citral	-	-	-	-	0.3	-
45	Geraniol	-	-	-	-	0.2	-
46	Bornyl acetate	-	-	-	-	0.2	-
47	Geranyl acetate	-	-	-	-	0.1	-
48	β-Bourbonene	-	-	-	-	0.1	-
49	β-Elemene	-	-	-	-	0.1	-
50	β-Gurjunene	-	-	-	-	1.7	-
51	γ-Gurjunene	-	-	-	-	2.2	-
52	Valencene	-	-	-	-	0.1	-
53	Ledene	-	-	-	-	0.1	-
54	Bicyclogermacrene	-	0.3	-	-	0.3	-
55	β-Bisabolene	-	0.6	-	-	0.1	-
56	γ- Bisabolene	-	-	-	-	1.2	-
57	Spathulenol	-	1.5	-	-	0.3	-
58	Caryophyllene oxide	-	tr.	-	-	0.6	-
59	*cis*-hexanol	-	-	-	-	-	1.1
60	5-methyl-3-heptanone	-	1.0	-	-	-	-
61	(*E*)-β -ocimene	-	2.8	-	-	-	-
62	γ -terpineol	-	0.7	-	-	-	-
63	Thymol methylether	-	4.1	-	-	-	-
64	(*Z*)-β -farnesene	-	0.3	-	-	-	-
65	γ- Cadinene	-	0.1	-	-	-	-
66	Cubenol	-	0.1	-	-	-	-

Table 1 (Continued)

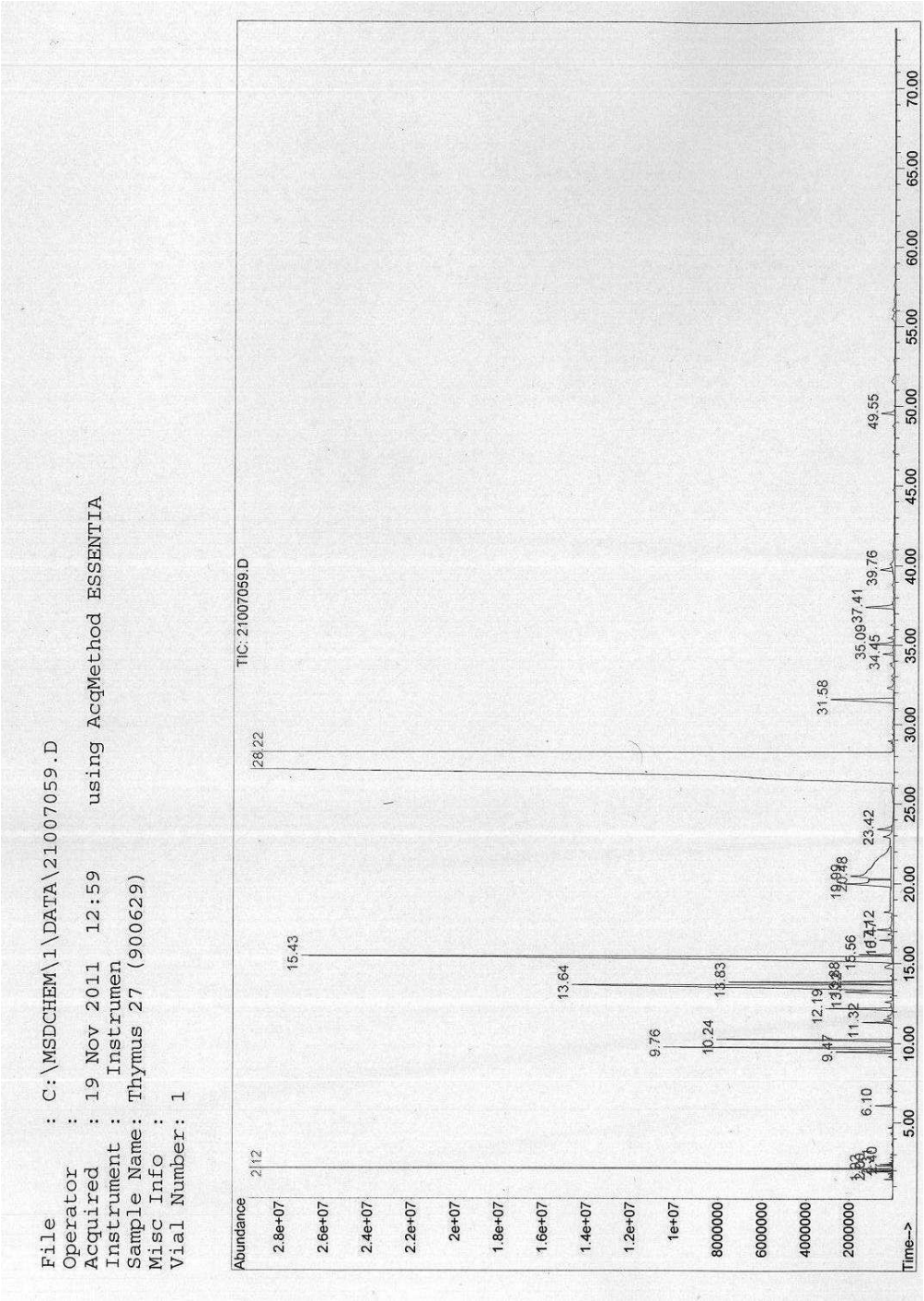
No.	Components	*T. kotschyanus* var. *kotschyanus* Gadouk Col (%)	*T. kotschyanus* var. *kotschyanus* Turkey [10]	*T. kotschyanus* Damavand (H1) [13]	*T. kotschyanus* Dizin [7](%)	*T. kotschyanus* Lorestan [18] (%)	*T. kotschyanus* var. *glabrescens* Turkey [6] (%)
67	*T*-cadinol	-	0.7	-	-	-	-
68	α-cadinol	-	0.1	-	-	-	-
69	Caryophyllenol-II	-	0.1	-	-	-	-
70	Phytol	-	0.1	-	-	-	-
	Total Identified	**97.38**	**92.3**	**97.19**	**80.7**	**90.3**	**67**

tr, trace (<0.1%).

Table 3- Percentage of Thymol and Carvacrol in various populations of *Thymus kotschyanus*

Speceis	Thymol (%)	Carvacrol (%)
***T. kotschyanus* var. *kotschyanus* (Gadouk Col)**	89.08	-
***T. kotschyanus* var. *kotschyanus* (Turkey) [10]**	48.0	2.3
***T. kotschyanus* (Damavand (H1)) [13]**	4.32	75.75
***T. kotschyanus* (Dizin) [7]**	38.0	14.2
***T. kotschyanus* (Lorestan) [18]**	39.7	7.6
***T. kotschyanus* var. *glabrescens* (Turkey) [6]**	0.7	44.2

Figure 1- Chromatogram of *Thymus kotschyanus* var. *kotschyanus*



RESULTS AND DISCUSSION

The hydrodistillation of the aerial parts of *Thymus kotschyanus* var. *kotschyanus* gave strong yellow oil with a distinct sharp odor in the yield of 0.93% (w/w), basis on dry weight. Ten

compounds representing 97.38 % of the oil were identified. The main ones were Thymol (89.08%) and γ-Terpinene (4.62%) (Table 1). Figure 1 shows the chromatogram of *T. kotschyanus* var. *kotschyanus*. In particular, oxygenated monoterpenes were the most abundant compound group of the oil (90.14%), but the amount of monoterpene hydrocarbons was notable (6.46%) and the sesquiterpene fraction of the oil was relatively low, representing 0.78 of the total oil. Table 2 indicates the comparison of the essential oil composition (%) from six populations of *Thymus kotschyanus.* There are three common chemical compositions namely, α-Pinene, Borneol and Thymol, among these populations. Table 3 indicates the percentage of Thymol and Carvacrol in various populations of *Thymus kotschyanus*. The absence of Carvacrol, as mentioned in this table, is the major difference between the present study and the previous studies. Thymol (89.08%) possesses the highest percentage in Gadouk Col population.

The qualitative and quantitative differences between the chemical compositions of the populations of the current studies and the previous ones may be attributed to their different ecological and geographical origin factors and it can be considered as a chemotaxonomic significance.

In conclusion, the oil of the investigated *Thymus* variety is rich in phenol content especially, thymol (89.08%), it can be considered as substitute for *Thymus vulgaris* L. (Common thyme) oil for medicinal purposes and other applications.

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