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Quantitative and Qualitative study of *Thymus fallax* essential oil in two habitats of East Azarbaijan province of Iran

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ABSTRACT

Essential oil analysis of *Thymus fallax* Fisch. &C.A.Mey was performed as a first attempt in East Azerbaijan province, Iran. . The results show that quantity of oil yields are varied between 0.53% to 0.91% with respect to dry samples weight. Thymol (43.43 - 61.14 %), borneol (3.89 - 7.01 %), carvacrol (3.88- 6.5 %) and p-cymen (4.58 - 6 %) are the main compounds of essential oil found in both sampling sites.

Keywords: *Thymus fallax, essential oil, thymol, borneol, p-cymen.*

1. INTRODUCTION

Thyme is a familiar name to all botanists due to its wider distribution and greater consumption by ordinary people in their daily life. Since a long time, Thymus L.has been one of the most important and known plant, considered as medicinal and edible herbs. East Azarbaijan province in Iran has nearly 7 species belong to the genus Thymus, with local name as "Kahlik Outi". *Thymus fallax* is one of the species belong to this genus [8]. T. fallax is perennial herbs standing on a wooden base with a height of 15 to 30 cm with sturdy and thick roots. Branched sturdy stems, wooden-base with floral branches to a height of 10 to 15 cm, more or less covered with hairs short and rectangular cut, oval leaves sharply at the base, whitish pink flowers integrated in a short capitol that clear at the middle spring [4,14]. Thyme oil with thymol and carvacrol compositions has been the most important export goods worldwide. Thymus can be used in various foods, including meat and meat products, spices, condiments, etc [11]. White thyme oil either liquid or powder as a disinfectant and aromatic seasoning are applied in many food products, including alcoholic and non alcoholic beverages, frozen dairy desserts, gelatin

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and desserts containing rice flour. [11]. Nowadays, many pharmaceutical products are made of *Thymus vulgaris* and has been widely used by patients. These are including Thyme Arta drops, tablets, syrup of thymecs and teamian which have been named as the main products for cough and mucus production. [7]. The effect of the Lorestan (western of Iran in the Zagros Mountains) natural habitats altitude and physico-chemical properties of the soil on *Thymus fallax* oil showed that a significant positive correlation between altitude and carvacrol, thymol compounds and thymol as major constituent [12]. Another work on the essence of this species composition has been conducted in another province of Iran, showing that carvacrol, thymol, p-cymene, γ - terpinene and geraniol are the main compounds of essential oil [13]. A study of antifungal and essential components of *Thymus fallax* in Turkey showed that thymol (41.8%), ortho - cymen (26.75%) and gamma-terpinene (15.84%) are considered as major manufacturer [15]. Carvacrol (68.19%) was the main compound of T. fallax essential oil detected in Turkey [18]. Research conducted in 2010 on the essences of T. fallax indicated that the carvacrol (69.2%), p-cymene (15.4%), thymol (5.3%) gama-terpinene (4.5%) are the main components [16]. Para-cymen, carvacrol, β- Ocimene and γ- terpinene are the main compounds of T. fallax which were reported from Turkey [10]. Study on antibacterial effects of some plants essence such as T. fallax in Turkey showed that thymol, carvacrol, p-cymene, thymol methyl ether and gamma-terpinene were the most important ingredients [9]. According to the research conducted in Turkey on the biological effect of T. fallax essential oil, carvacrol (46.15%) considered as a main part of oil was taken in Sivas (the eastern part of the Central Anatolia region of Turkey) [5]. According to the study carried out on the effect of environmental factors on the quantity of *Thymus serpyllum* L. oil, altitude in most regions had a negative impact on the quantity of oil [1]. In another study, Habibi et al. [15] on wild thyme oil *Thymus kotschyanus* Boiss. & Hohen. conducted on the quality and quantity oil samples collected from Taleghan. The results showed that altitude versus quantity of essential oil of this species have the negative correlation [6]. This means that with increasing altitude, the quantities of quantitative and qualitative essential oils were reduced.The main objective of this research, evaluation of quantitative and qualitative changes of *Thymus fallax* essential oil in two habitats of East Azarbaijan province of Iran.

2. MATERIALS AND METHODS

2.1. Plant Materials

In order to evaluate the *Thymus fallax* essential oil, an experiment was carried out in natural habitats of East-Azerbaijan, Iran. Samples were collected from two determined sites including Mianeh and Bozgoush) at the middle of flowering time (50%). Sampling sites characters are shown in table 1. Soil samples characteristic are shown in Table 2.

Table 1. Characteristics of natural habitats of sampling sites

No	area	longtitude	latitude	Altitude(m)	Slope direction	average rain(mm)	average temperature(°C)
1	Bozgoush	47° 47′ 49.5″	37° 46′ 19.83″	2728	south	450	6.3
2	Mianeh	47° 54′ 11.4″	37° 21′ 27.3″	1644	north	286.9	14

Table 2. The results of Soil factors of sampling sites

No	area	EC ms/cm	pH	T.N.V %	OC %	P(ava)ppm	N %	K (ava) ppm	Sand %	Silt %	Clay %
1	Bozgoush	0.75	7.9	13.6	2.06	3.13	0.21	387	62.3	17.33	20.33
2	Mianeh	0.51	7.87	15.28	0.94	15	0.1	70.9	71.7	14.3	14

2.2. Essence Extraction

The essence of 100 grams dried samples were extracted using hydrodistillation (Clevenger apparatus) method for 2.5 hours and then were dried over anhydrous sodium sulfate. Species essence percentage was calculated based on the percentage of dry matter. Qualitative analysis of oil samples was performed using GC and GC / MS method [2,3,17], and the combinations were identified.

2.3. GC and GC/MS

Thermo-UFM and chrom card A/D data analysis was used as GC apparatus. Column character: ph-5 made by thermo with 10m length, 0.1nm inner diameter with 0.4µm thickness dimethyl siloxane phenyl 5%. GC was programmed as follows: initial column temperature 60°C and raised till 285°C, 80°C increase in every minute finally stopped 3 minutes at terminate temperature. Injection chamber temperature: 280°C, FID detection unit temperature:290°C, He as carrier gas with 0.5 Kg/cm2 pressure. Varian 3400 coupled by Saturn II with the ion trap system 70eV ionization energy, DB-5 column (30m length,0.25 mm ID, 0.25µm cover thickness) was used for GC-Mass analysis. The apparatus was programmed as follows: 35 pound/inch2 gas pressure, 40-250°C column temperature by increase 3°C/min, injection chamber temperature 260°C and transfer line temperature 270°C. Constituents in essential oil were determined by RI (Retention Index), library refrences, GC/MS lab data and standard Mass spectrums [2,3,17].

3. RESULTS AND DISCUSSION

The highest and the lowest percentage of essential oils in Mianeh and Bozghoush sampling sites were 0.91% and 0.53%, respectively. We found that the main compounds in both sites were γ-terpinene (1.8-2.2%), borneol (3.9-7 %), thymol (43.4- 61.1%) and carvacrol (3.9-6.5%) (Table 3). With regard to eco-physiological factors in both sampling sites, the Mianeh with an altitude (1644 m), the average temperature (14 °C) and annual rainfall (286.9 mm) generated the highest yields (0.91 %). Bozghoush with an altitude (2728 m), the mean annual temperature (6.3 °C) and annual falls (450 mm) generated the lowest yields (0.53 %). With regard to soil factors, Mianeh toward to Bozghoush had lower E.C (Electro Conductivity:0.51-0.75 ms/cm), higher T.N.V (Total Neutralizing Value:15.28-13.6%), lower organic matter:0.94-2.06%, higher phosphorus:15-3.13 ppm, lower nitrogen:0.1-0.21%, lower potassium:70.9-387ppm and lighter texture, respectively. Comparing the results of this study showed that temperature, T.N.V, phosphorus and light texture are appropriate factors for increasing efficiency of essential oils. Altitude, rainfall, electrical conductivity, organic matter, nitrogen and potassium have an inverse functions related to oil yields. (Table 2). In Mianeh and Bozghoush, thymol is the main oil constituent (61.1%) and (43.3 %), respectively. (Table 3). The results of this research in terms of the essential oil main compounds have been agreed by Onaran [15], Mohammadian [12] and Kotan [9] studies. On the other hand, our findings were different with Tumen [18], Rustaiee [16], Goze [5], Morteza semnani [13] and Kucukbay [10] studies. Based on our results, it seems that the altitude factor has a negative effect on quantity of essential oil of *Thymus fallax* so that the Mianeh with lower altitude (1644 meters) has higher efficiency than the Bozghoush (2728 m). Our findings were in agreement with Abu Darvish et al. [1] studied on *Thymus serpyllum* and Habibi et al. [6] studied on the *Thymus kotschyanus*.

Table 3. *Thymus fallax* essential oil compounds

No	compounds	RI	Bozgoush (%)	Mianeh (%)
1	*ρ- Cymene*	1051	6	4.58
2	*1,8-Cineole*	1066	10.27	2.08
3	*γ- Terpinene*	1080	2.22	1.85
4	*Linalool*	1109	1.93	0.62
5	*Borneol*	1211	3.89	7.01
6	*α-Terpineol*	1223	7.23	−−
7	*Geraniol*	1267	−−	6.23
8	*Thymol*	1315	43.43	61.14
9	*Carvacrol*	1326	3.88	6.5
10	*-Terpinyl αacetate*	1367	3.12	−−
11	*E-Caryophyllen*	1493	0.84	2.33
Monoterpenes hydrocarbon			8.22	6.43
Oxygenated monoterpenes			73.75	83.58
Sesquiterpens hydrocarbons			0.84	2.23
Total			82.81	92.24
Essential Oil %			0.53	0.91

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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