

Original Article

Effect of Harvesting Time on Essential Oil Content and Composition of *Thymbra spicata*Asghar Mosleh Arani¹, Mostafa Naderi² and Sayyed Mostafa Goldansaz^{3*}¹Department of Natural Resource, Yazd University, Yazd, Iran.²Department of Natural Resource, Yazd University, Yazd, Iran.³Department of Natural Resource, Sari University of Agriculture Sciences and Natural Resources and Iranian Medicinal Plant Society, Iran

Article History: Received: 30 August 2014/Accepted in revised form: 15 February 2015
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Abstract

This study aims to investigate the essential oils content of *Thymbra spicata* L. in two times before flowering and full flowering in shirpenah area in Ilam Province. Both samples were dried out in shade and without humidity. Then 50 grams of each sample were powdered after obtaining the essential oils by hydro distillation using a Clevenger apparatus for 2 hours. Resulted oils were dried by anhydrous sodium sulfate and kept in tightly closed vials at 4 °C before chemical analyses. Finally both essential oil samples were analyzed by GC and GC/MS. Outcomes show that essential oils content in full flowering was (%4.04) more than before flowering (%3.64). The compositions of essential oils in before flowering were 7 compounds and major constituents were γ -terpinene (15.5%) and carvacrol (79.3%). The composition of essential oils in full flowering were 11 and the major composition also were carvacrol (70.5%) and γ -terpinene (14.9%). α -pinene, β -pinene, α -phellandrene and cis-sabinene hydrate were not found in compositions of before flowering. It is figured that flowering time is the best for obtaining the higher essential oils content and composition in *T. spicata*.

Key words: Ilam province, *Thymbra spicata*, Before flowering, Full flowering**Introduction**

Thymbra spicata L. is a perennial herb from Lamiaceae that is represented in Iran by one specie [1]. *Thymbra* L. has several names: common thyme, herba thyme, red thyme, in Arabic it is called Za'atar, in Kurdesh is called Jatra [2] and in Iran is named Gole Zufae. It is one of the 20 species listed in the Red Book of endangered species has been [3]. *T. spicata* is found in countries bordering the eastern Mediterranean and

Iran. The used parts of the spices are leaves, flowers, bulbs, fruits, stems, rhizomes and other plant parts [4]. In Turkey, its new leaves or dried flowers are consumed as a tea and used in preparing salads. From ancient times, in addition to spices and their derivatives being used for flavoring foods and beverages and for medication, essential oils have also been highly valued for their use as antimicrobials [5]. The plant is used in folk medicine to treat asthma, bronchitis, cough, rheumatism and many other diseases [6]. The leaves have recently gained much popularity as a remedy to combat hypercholesterolemia [7].

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Besides, the dried plant, softened in boiled water used to be applied to wounds as a drug [5]. Also it possesses analgesic and due to its anti-bacterial and fungal widely used in the treatment of rheumatism. It is used in the treatment of lung cancer [6]. Chemical constituents of the essential oils of the plant in different area were reported previously. Al-sheibany et al. 2005 [2] in Baghdad, Inan et al. 2011[8] in Adiyaman (Turkey), Unlu et al. 1994 [9], in Sivas (Turkey), Tumen et al 2009 [10] in different region in Turkey announced the oils of *T. spicata*. Carvacrol is the main ingredient of this plant like other Lamiaceae family such as thymus, satureja and Caridothymus [11]. Carvacrol with p-cymene and γ -terpinene comprise more than 70% active ingredient [12]. In addition, Thymbra in most countries has been reported to be used in the medicine industries as a raw material because of its valuable properties [13]. It seems to be interesting that the essential oil prevents germination of many weed and it can be employed to combat weeds [14]. Investigations on different times of harvest identify the elements and compounds in different plant varieties and it is an issue that has been focused in many studies. This implies that to obtain the highest essential oil percentage time should be carefully chosen. Due to its medicinal property and other activity, the present research has been done to determine the best time to harvest that obtains maximum performance.

Material and Methods

Soil and climate information

The climatic and soil characteristics according to data from the Bureau of Meteorology and Soil common tests were evaluated, respectively.

Plant material

The plant material of *T. spicata* were collected on March and May 2012 in two times of before flowering and full flowering in Shirpenah area, Ilam Province, Iran, and were dried in the shade at room temperature. Then 50 grams of each community was powdered after those essential oils were obtained by hydro distillation using a Clevenger apparatus for 2 hours. Resulted oils were dried anhydrous sodium sulfate and kept in tightly closed vials at 4 °C before chemical analyses. The quantitative and qualitative analyses of the oils were performed by GC and GC-MS, respectively.

Gas chromatography-flame ionization detector (GC-FID) and Gas chromatography-mass spectrometry (GC-MS) analysis

The GC analysis was performed using a Thermoquest gas chromatograph with a flame ionization detector (FID). The analysis was carried out on a fused silica capillary BPX-5 column (30 m \times 0.25 mm i.d.; film thickness 0.25 μ m). The injector and detector temperatures were kept at 250 °C and 300 °C, respectively. Nitrogen was used as the carrier gas at a flow rate of 1.1 ml/min; the oven temperature program was 60–250 °C at the rate of 4 °C /min and finally held isothermally for 10 min; split ratio was 1:50. GC-MS analysis was carried out by a Thermoquest-Finnigan gas chromatograph equipped with a fused silica capillary BPX-5 column (30 m \times 0.25 mm i.d.; film thickness 0.25 μ m) coupled with a TRACE mass (Manchester, UK). Helium was used as the carrier gas with ionization voltage of 70 eV. Ion source and interface temperatures were 200 °C and 250 °C, respectively. Mass range was from 35 to 456 amu. The oven temperature program was the same as given above for the GC.

Identification and quantification of the essential oil compounds

The constituents of essential oils were identified by calculation of their retention indices under temperature-programmed conditions for *n*-alkanes (C₆–C₂₄) and the oil on a BPX-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 (NIST, Adams and Wiley 7.0) or with authentic compounds (purchased from Sigma-Aldrich and Merck Co.) and confirmed by comparison of their retention indices with authentic compounds or with those reported in the literature [15]. For quantification purposes, relative area percentages obtained by FID were used without the use of correction factors.

Results

The results of climate and soil condition are shown in Table 1. Also the compositions of *T. spicata* are given in Table 2.

Discussion

T. spicata in the Lamiaceae family is a perennial plant that prefers dry sunny hillsides and high dry

meadows. They have considerable ornamental value and bloom from February to June. They are also popular with herbalists because the oil contains carvacrol and γ -terpinene, phenols with important range of important biological activities and pharmacological properties. The plant is used in the traditional medicinal system of Turks, Greeks, Egyptians and Romans to treat asthma and bronchitis besides, the use in food industry for flavor, aroma and preservation [16].

In this present study, the oil content of two stages of *T. spicata* in question varied from 3.64 - 4.04. In other words the essential oil content fluctuated considerably by different harvesting times, that it has a light yellow essential oil, of which content was affected by harvesting times. In this investigation minimum percentage of *T. spicata* essential oil was found about 3.64% at before flowering harvest time and it increased at full flowering nearly 4.04%. Muller-Riebau et al.1997, Tonçer and Kızıl 2005, also stated that the highest fresh herb yield was obtained at flowering time than that of other maturity stages [17,18]. In some studies, it was shown that the oils isolated from the leaves and from the flowers were markedly

different in showing that the essential composition depends on the developmental stage of the plants and on the amount of flowers present in the plant material used to isolate the essential oil [8,10].

The essential oil samples analyzed by GC/Mass. Results showed that eleven constituents were identified in *T. spicata* essential oil and monoterpene hydrocarbons were the major portion of all samples before the flowering and full flowering stage. The main components of the oils were carvacrol (70.5-79.3%), γ -terpinene (14.9-15.5%) and p-cymene (4.3-6.1%). The results were similar to the previous studies [17,18]. α -pinene, β -pinene, α -phellandrene and cis-sabinene hydrate were not found in compositions of before flowering. In addition to other components, which were of less abundance are showed in Table 2.

As a result, essential oil content and composition affected by many factors that have been reported by several authors [4,19]. Also the variation in the essential oil composition could be attributed to both interactions between genetic (biotic) and environmental (abiotic) factors. According to this investigation flowering period largely affected the amount of the major compounds.

Table 1 Climatic and soil conditions of the studied *Thymbra spicata* L. from Iran

Climatic condition		Soil condition			
			0-15 cm	15-30 cm	Total
Mean annual temp	18.5	Electrical conductivity [dS/m]	5.2	3.56	4.5
Maximal mean annual temp	25.7	pH	7.43	7.71	7.57
Minimal mean annual temp	9.8	OC [%]	0.7	0.47	0.58
Rainfall [mm/year]	644	OM [%]	1.21	0.81	1
Number of frost day	34	Caco ₃ [%]	76.4	73.9	75.2
Monthly sunshine hours	252	Sand	35	37.6	36.3
The mean relative humidity [%]	25	Clay	21	18.6	19.8
Wind speed [m/s]	16	Silt	44	43.6	43.8
Wind	South	Soil texture	Sandy loam	Sandy loam	Sandy loam

Table 2 Compare of essential oil composition of before flowering and full flowering

No	RI	Compounds	%	
			Before flowering	Full flowering
1	928	α -thujene	0.1	1.3
2	935	α -pinene	-	0.4
3	978	β -pinene	-	0.1
4	992	Myrcene	0.1	1.4
5	1006	α -phellandrene	-	0.1
6	1018	α -terpinene	0.3	2.7
7	1025	p-cymene	4.3	6.1
8	1060	γ -terpinene	15.5	14.9
9	1076	cis-sabinene hydrate	-	0.2
10	1310	Carvacrol	79.3	70.5
11	1420	(z)-caryophyllene	0.3	2.1
Total identified			99.9	99.8

RI, retention indices relative to C6–C24 n-alkanes on a BPX-5 column

These results could be explained that the date of harvest has a large effect on the components studied. Carvacrol was constant compounds in both samples. Comparison of the main components of the Iran *T. spicata* with other countries showed that the phenolic compound, carvacrol, was the major component, which is in concordance with the carvacrol chemotype previously reported growing in Turkey, Lebanese and Greece [20, 10]. Runner-up Unlu et al 2009 on his research indicated that the major compounds detected in the essential oil were carvacrol (60.39%), γ -terpinene (12.95%), and p-cymene (9.61%) [9]. Ozel et al 2003 [21] found that the essential oil composition under different temperature conditions at 100 °C minimum quantity of compounds about 12 and a maximum temperature at 175 °C nearly 19 were identified. The lowest percentage of oil with a temperature of 100 °C (2%) and the highest at 150 °C (4.3%), respectively.

Conclusion

It is figured that flowering time is the best for obtaining the higher essential oils content and composition in *T. spicata*. Also it is important to compare and explore the variants of essential oils from different provenances, since this will most probably affect their potential biological activities, jeopardizing their use either in food industries or for medical purposes.

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