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Essentiol oil composition of *Thymus kotschyanus* Bioss. & Hohen

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Abstract

Thymus Kotschyanus Boiss. & Hohen is one of the native medicinal plants of Iran. The chemical compositions of the essential oils were obtained from aerial part of 4 populations of *T. Kotschyanus* from Azarbayejangharbi province of Iran. The populations with three replications were arranged in a randomized completely block design (RCBD). The thyme populations that were collected during the full flowering phase and analyzed by gas chromatography (GC) and gas chromatography coupled to mass spectrometry (GC–MS).

The major essential oils were identified in populations of Azarbayejangharbi-I (0.85%) and Azarbayejangharbi-II (0.84 %). There were identified 13 chemical compounds in the essential oils, the majority including, Thymol, Borneol, Gamma terpinene, Cymne (ortho) and (z) Caryophyllene. Also, the results demonstrated that the most phytochemical variation due to genetical factors. Thus we can management of these types chemical protection on the domestication, conservation and creation of gene banks, propagation and breeding of chemical types can be programmed.

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Introduction

In floristic studies has been detected about 250 species of thyme in the world and about 18 species in Iran (Jamzad, 2006), that 14 species and subspecies has reported (Rechinger, 1982), previously. It is distributed in provincees of Zanjan, Azerbayejan, Kordestan, Hamedan, Lorestan, Isfahan, Kohkeluyh Boyer-Ahmad, Chahar Mahal Bakhtiari, Fars and Markazi (Rechinger, 1986). Also, it has been reported 37 species in Turkey, 136 species in USSR and 17 species in Flora Iranica (Mozaffarian, 1996; Jamzad, 1994).

The species of *Thymus kotschyanus* is one of the densest concentrations of natural habitats at an elevation of 1800 to 2800 meters at Southern side of the Alborz, Iran (Habibi *et al.*, 2004). *Thymus kotschyanus* is the woody-herbs, bush, almost straight, short stature, stem with many branches, bulging veins in the under the leaf surface and corolla is white or light pink, flowering time is late spring to the mid-summer (Jamzad, 2006). The chemical composition and yield of different EOs show wide variation, depending on the herbal source, chemo-type of the plants pieces, and the analytical methods used (Jardim *et al.*, 2008).Thyme contains the 0.8% to 2.6% essential oil, that also essential oil of thyme's aerial parts are contains, tannins, saponins and antiseptics (Leung and Foster, 1996).The essential oils of the aerial parts of *T. Kotschyanus* collected during the full bloom from the suburb of Behshahr (North of Iran), then were isolated by hydro-distillation and analyzed by GC and GC/MS. Twenty one constituents were identified in *T. kotschyanus* oil (Morteza-Semnani *et al.*, 2006). Phytochemical analysis of Thymus species have confirmed the occurrence of phenolic compounds such as thymol, carvacrol, thymonin, caffeic acid and rosmarinic acid, terpenoids, flavonoids and saponins in the plant (Blumenthal *et al.*, 2000). Which is used nowadays on a large scale in the food and cosmetic industries. In addition, it has been shown to exhibit a range of biological activities such as antibacterial, antifungal, insecticidal, analgesic and antioxidant properties (Thompson, 1996; Ultee and Smid, 2001). The aim of this study is the comparison of the chemical composition essential oil of *T. kotschyanus* populations grown under similar condition.

Materials and methods

Plant material

In this study, the essential oils amount of four *T. kotschyanus* populations were evaluated [Table 1]. The experimental samples were selected from the Collection of Medicinal Plants in Agricultural Research and Natural Resources Center of Markazi province (Arak) which is located in the 12 km from the city of Arak with altitude of location was 1760 m (N 49° 46', E 34° 04').

The three replications of four treatments (population's thyme) were arranged in a randomized completely block design (RCBD).The aerial parts of the plants were harvested at the flowering stage. The harvested materials were air-dried in a shaded place at a convenient temperature and in an air-flow during 5 days. The air-dried organs were ground to a homogeneous fine-grade powder. The samples were transferred to phytochemical analysis laboratory, for determine the percentage of essential oils according to the European pharmacopoeia method (Conseildel Europe, 1983).

Isolation of the volatile oil

50 g of sample was subjected to hydro-distillation by a Clevenger extraction apparatus for 4 hours. The volatile oil was collected in the graduated tube. The collected volatile oil was dried over anhydrous sodium sulphate and stored at 4 °C in sealed dark glass flasks prior to compositional analysis. The content of volatile oil obtained was v/w (British Pharmacopoeia, 1988).

GC/MS analysis

The analysis of the essential oils was performed using a gas chromatography method (GC/MS), interfaced with a mass selective detector equipped with a polar Agilent HP-5ms (5%-phenyl methyl poly siloxane) capillary column (30 m × 0.25 mm I. d. and 0.25 μm film thickness). Column thermal was regulated In this way program: The oven temperature was set at 50 °C and stopped at this temperature for 5 min thermal gradient temperature rise of three degrees Celsius per minute to 240 °C with a temperature increase rate of 15 °C per minute to 300 °C and hold for three minutes at this temperature, the injection room temperature was 290 °C and helium as the carrier gas flow rate (flow) 0.8 mm per minute was used. Mass spectrometry used 70 e V the ionization of voltage Aglet 5973 model method EI ionization and ionization source temperature 220 degrees Celsius. Spectrum of obtained from the standard compounds were identified by comparison of mass spectra and retention index computed using (RI) and were confirmed by injection of normal hydrocarbons. Percent of each of compounds according to the area under the curve in the spectrum of GC chromatogram were obtained using area normalization (Moradi, *et al.*, 2011).

Data obtained from based on statistical design of experiments, using SAS software underwent analyzed Duncan's test were used to compare the mean.

Results and discussion

Essential oil content

The content of the essential oils from the four populations were obtained from 1.4 to 8.5% based on dry weight. Essential oil content of the four populations was obtained from 0.15 to 0.85% based on dry weight (Table 2). More than 67.29% (13 compounds) of the essential oils composition were identified in each sample. The highest percentage of essential oil was belonged to population of Azarbayejangharbi-I (0.85%) and Azarbayejangharbi-IV (0.84%) and the lowest percentage were belonged to population of Azarbayejangharb-III to the level of 0.15% (Table 2). Variation in oil yield can be attributed to external and internal factors of plant. Previously studies indicated that ecological conditions, climate and harvest time (Cabo *et al.*, 1982; Putievsky and Basker, 1977), height (Gouyon *et al.*, 1988), chemotype structure of populations, year factor (Ozguven and Tansi , 1988) and agronomic factors (Naghdi badi *et al.*, 2004) could be affected on quality and quantity of thyme. According to a study (Bezic *et al.*, 2005) was reported that the cultivated *Satureja montana* and *S. cuenifolia* populations in the same weather had significant difference in the essential oil yield, and it was related to genetic variability. *T. kotschyanus* populations in this study were cultivated in similar environmental conditions. Therefore, the most of these differences could be due to genetical factors.

Table 1. Location characteristics of 4 populations.

Population no.	Altitude(m)	Latitude, N	Longitude, E
I	1389	45° 22' 45"	36° 55' 12"
II	1524	44° 57' 60"	37° 57' 34"
III	1478	45° 07' 14"	37° 17' 80"
IV	1600	45° 55' 56"	38° 56' 60"

Table 2. Chemical composition (%) of the essential oil of *Thymus kotschyanus*.

Populations	Essentialoil	Camphor	Carvacrol	Thymol	Borneol	Gamma-Terpinene	1.8-Cinole	Cymne (ortho)	Carvacrol methyl ether	(z) Caryophyllene
I	0.85a	2.70b	5.14b	20.99b	12.68a	2.82b	6.35a	8.87c	8.18a	2.54c
II	0.71b	-	9.62a	-	2.59c	-	2.40c	3.79d	3.87b	19.67a
III	0.15c	6.92a	5.00b	5.23c	4.31b	-	3.35c	16.35b	2.98b	4.11b
IV	0.84a	-	5.58b	23.28b	-	11.72a	4.94b	30.10a	-	-
RT	-	21.68	28.75	28.49	23.22	16.08	14.39	14.08	25.41	35.5

* Means in each column followed by the same letter are not significantly different (P < 0.05)

RT= Retention time.

Chemical composition

It was found important differences in the amounts of the major components, mainly of thymol (5.23-20.99%), borneol (2.59-12.68%), gamma- terpinene (2.8-11.72%), cymne- ortho (3.79-30.10%) and (z) caryophyllene (2.54-19.67%) (Table 2). In addition, findings of this study showed that some compounds (no tabled) such beta- bourbonene (2.86%) in the population of Azarbayejangharbi-I, bornyl acetate (4.93%) and thymol methyl ether (13.8%) in the population of Azarbayejangharbi-II, linalool (6.78%) and terpinen-4-ol (6.69%) in the population of Azarbayejangharbi- III were found. Previous research (Nikvar *et al.*, 2005) showed that the most composition of essential oil in *T. kotschyanus* was 38.6% thymol, 33.9% carvacrol, 7.3% α- cymene, and 5.2% gamma-terpenes. Another study by Sefidkon and Askari (2002) confirmed that the essential oil of *T. kotschyanus* were containing 20 compositions before flowering and 25 compounds in flowering stage, respectively, 93.5 and 99.3 percent of the oil formed. Also, in another study by Kasumov (1988) demonstrated the major compounds of essential oil of *T. kotschyanus* is included the 35.48% thymol, 11.65% carvacrol, 17.47% p-cymene and 6.50% gamma-terpene.

Table 3. Class composition of four populations.

Terpenes	% in sample			
	I	II	III	IV
Monoterpene hydrocarbons (MH	15.78	2.48	17.79	5.75
Oxyganated monoterpenes(MO)	54.68	52.55	42.43	48.33
Sesquiterpene hydrocarbons(SH)	5.46	21.40	4.47	9.70
Oxyganated sesquiterpens(SO)	-	-	-	-
Others	8.18	8.80	2.60	15.93
Total	84.10	85.23	67.29	5.75

Classification of chemical composition

The highest percentage of monoterpene hydrocarbons (MH), oxygenated monoterpenes (MO), sesquiterpene hydrocarbons (SH) and oxygenated sesquiterpenes (SO) were observed in population of Azarbayejangharbi-III (17.79%), Azarbayejangharbi-I (54.68%) and Azarbayejangharbi-II (21.40%), respectively. The lowest of these compounds were obtained in population of Azarbayejangharbi-II (2.48%), Azarbayejangharbi-III (42.43%) and Azarbayejangharbi-III (4.47%), respectively [Table 3]. In this study, oxygenated monoterpenes were the main group of constituents in all populations (42.43-54.48%). A studies on composition of essential oils from different Thymus species indicated that oxygenated monoterpenes were the main group of the constituents in all populations (67.2-75.4%) (LmHgia *et al.*, 2000).

Conclusions

T. kotschyanus is an important medicinal plant and has a special position in the world. In the essential oil of this plant, 13 components were identified that mainly include Thymol, Borneol, Gamma terpinene, Cymne (ortho) and (z) Caryophyllene. The highest amount of essential oil was related to the population of Azarbaijangharbi-I (0.85%) and Azarbaijangharbi-IV (0.84%). In the next step of the work, we are going to determine the influence of ecological factors in order to adaptation/domestication of this plant and also creating gene bank for using in breeding programs.

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References

Bezic N, Skocibusic M, Dunkic V. 2005. phytochemical composition and antimicrobial activity of *Satureja montana* L. and *satureja cuneifolia* var *ten*. essential oils. Acta Botanica Croatia **64,** 313-322.

Blumenthal M, Goldberg A, Brinckmann J. 2000. Herbal Medicine Expanded Commission E Monographs. Integrative Medicine Communications, Newton, 376-378 P.

British Pharmacopoeia. 1993. HMSO, London.

Cabo J, Crespo ME, Jimenez J, Navarro C, Risco S. 1982. Seasonal variation of essential oil yield and composition of *Thymus hyemalis*. Planta Medica, 380-382 P.

Conseildel Europe, Pharmacope Europe Henne. 1983. Maisonneuve S.A. Sainte RuSne **1.**

Gouyon PH, Vernett PH, Guillerm JL, Valdeyron G. 1988. Polymorphisms and environmental the adaptive value of the oils *Thyme vulgaris*. Heredity **57,** 59-66.

Habibi H, Fakhr-Tabatabaee M, Bigdeli M. 2006. Effect of Altitude on Essential Oil and components in Wild Thyme (*Thymus kotschyanus*) Taleghan Region. Pajouhesh and Sazandegi **73,** 2-10.

Jamzad Z. 1994. Thymus and Satureja of Iran. Research Institute of Forests and Rangelands Press, 1-15 P.

Jamzad Z. 2009. Thymus and Satureja of Iran. Research Institute of Forests and Rangelands press, 171 p.

Jardim CM, Jham GN, Dhingra OD, Freire MM. 2008. Composition and antifungal activity of the essential oil of the Brazilian *Chenopodium ambrosioides* L. Journal of Chemical Ecology **34,** 8-12.

Kasumov YOF. 1988. Chemical compositon of essential oils of Thymus specis in the flora of Armenia. Chemistry of Natural Products **24(1),** 121-122.

Leung AY, Foster S. 1996. Encyclopedia of common natural bingredients: used in food , drugs, and cosmetics. A wiley Interscience Publication - John wiley and sons, Ins, 649 P.

LmHgia R, Salgueiro Roser V, Xavier T, Salvador C, Igueral O, Paiva AH, Proenc D, Cunha TA. 2000. Chemotaxonomic study on *Thymus villosusfrom* Portugal. Biochemical Systematics and Ecology **28,** 471-482.

Moradi R, P, Rezvani Moghaddam M, Nasiri Mahallati A, Nezhadali. 2011. Effects of organic and biological fertilizers on fruit yield and essential oil of sweet fennel (*Foeniculum vulgare* var. dulce). Spanish Journal of Agricultural Research **9(2),** 546-553.

Morteza-Semnani K, Rostami B, Akbarzadeh M. 2006. Essential oil composition of *Thymus kotschyanus* and *T. pubescens* from Iran. Journal of Essential Oil Research **18,** 272-274.

Mozaffarian V. 1996. Culture of the plant name of Iran. Tehran Contemporary Culture press, 75 p.

Naghdi Badi H, Yazdani D, Sajad MA, Nazari F. 2004. Effective of spacing and harvesting time on herage yield and quality/quantity of oil in thyme, *Thymus vulgaris* L.. Industerial Crops and Products **19(3),** 231-236.

Nikvar B, Mojab F, Dolat- Abadi R. 2005. Analysis of the essential oils of Thymus species from Iran. Food Chemistry **90(4),** 609-611.

Ozguven M, Tansi S. 1998. Drug yield and essential oil and ontogenetical variation. Turkish Journal of Agriculture and Forestry **22,** 535-542.

Putievsky E, Basker D. 1977. Experimental cultivation of marjoram oregano and basil. Journal of Horticultural Science **52,** 181-188.

Rechinger KH. 1982. Labiatae in Flora Iranica, Vol 150.

Rechinger KH, Hedge IC. 1986. Umbelllifera in Flora Iranica, Graz, **162.**

Sefidkon F, Askari F. 2002. Essential oil composition of 5 Thymus species. Iranian Medicinal and Aromatic Plants Research **12**, 29 -51.

Thompson DP. 1996. Inhibition of growth of mycotoxigenic *Fusarium* species by butylated hydroxyanisole and/or carvacrol. Journal of Food Protection **59,** 412-415.

Ultee A, Smid EJ. 2001. Influence of carvacrol on growth and toxin production by *Bacillus cereus*. International Journal of Food Microbiology **64,** 373-378.