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Analysis of the essential oils of two *Thymus* species from Iran

Bahman Nickavar [a,b,*], Faraz Mojab [a,b], Reza Dolat-Abadi [c]

[a] *Pharmaceutical Sciences Research Center, Shaheed Beheshti University of Medical Sciences, P.O. Box 14155-6153, Tehran, Iran*
[b] *Pharmacognosy Department, School of Pharmacy, Shaheed Beheshti University of Medical Sciences, P.O. Box 14155-6153, Tehran, Iran*
[c] *Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*

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Abstract

The essential oils obtained from the aerial parts of *Thymus daenensis* subsp. *daenensis* and *Thymus kotschyanus* were analyzed by using GC and GC/MS. Twenty six compounds representing 99.7% of *T. daenensis* subsp. *daenensis* oil were identified. The main ones were thymol (74.7%), *p*-cymene (6.5%), β-caryophyllene (3.8%) and methyl carvacrol (3.6%). Thirty one components accounting for 98.7% of *T. kotschyanus* oil were identified. The major constituents were thymol (38.6%), carvacrol (33.9%), γ-terpinene (8.2%) and *p*-cymene (7.3%). Both oils were found to be rich in monoterpene phenols, especially thymol and carvacrol.

Keywords: *Thymus daenensis* subsp. *daenensis*; *Thymus kotschyanus*; Essential oil; Chemical composition; Labiatae

1. Introduction

The genus *Thymus* L. (Labiatae) consists of about 215 species of herbaceous perennials and subshrubs. The Mediterranean region can be described as the center of the genus (Stahl-Biskup & Saez, 2002). This genus is represented in Iranian flora by 14 species, four of which (*Thymus carmanicus*, *Thymus daenensis* subsp. *daenensis* and *T. daenensis* subsp. *lancifolius*, *Thymus persicus* and *Thymus trautvetteri*) are endemic (Rechinger, 1982). The Persian name of the genus is "Azorbeh" and/or "Avishan" (Mozaffarian, 1998).

Thymus species are commonly used as herbal tea, flavoring agents (condiment and spice) and medicinal plants (Stahl-Biskup & Saez, 2002). Among the species grown in Iran, *Thymus daenensis* Celak. and *Thymus kotschyanus* Boiss. and Hohen. are more widely used for these purposes. Infusion and decoction of aerial parts of *Thymus* species are used as tonic, carminative, digestive, antispasmodic, anti-inflammatory, antitussive, expectorant and for the treatment of colds in Iranian traditional medicine (Amin, 1991; Zargari, 1990). Recent studies have showed that *Thymus* species have strong antibacterial, antifungal, antiviral, antiparasitic, spasmolytic and antioxidant activities (Stahl-Biskup & Saez, 2002).

The aromatic and medicinal properties of the genus *Thymus* have made it one of the most popular plants throughout all of the world. It is believed that a part of these activities is due to the volatile constituents. Therefore, there is a considerable research interest towards the compositional analysis of *Thymus* essential oils (Stahl-Biskup & Saez, 2002). 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, γ-terpinene, β-caryophyllene, etc (Guseinov, Kagramanova, Kasumov, & Akhundov, 1987; Kasumov, 1988; Kasumov & Gadzhieva, 1980; Kulieva, Guseinov, Kasumov, & Akhundov, 1979; Rustaiyan et al., 2000; Sefidkon & Dabiri, 1999; Sefidkon, Jamzad, Yavari-Behrouz, Nouri-Sharg, & Dabiri, 1999). Contrary to *T. kotschyanus*, the study concerning the composition of *T. daenensis* oil is very limited. In a previous investigation on *T. daenensis* Celak. subsp. *lancifolius* (Celak.) Jalas oil, Sajjadi and Khatamsaz (2003) reported thymol (73.9%), carvacrol (6.7%), *p*-cymene (4.6%), β-bisabolene (1.5%), terpinen-4-ol (1.4%), borneol (1.1%) and spathulenol (1.0%) as

* Corresponding author. Tel.: +98-21-8773521; fax: +98-21-8795008.
E-mail address: bnickavar@yahoo.com (B. Nickavar).

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the main constituents. However, to the best of our knowledge, there is no previous report on the chemical composition of the essential oil of *T. daenensis* Celak subsp. *daenensis.*

The aim of the present work is to determine the essential oil composition of Iranian *T. daenensis* subsp. *daenensis*, and carry out a comparative evaluation between this oil and the oil of the similar species, *T. kotschyanus*. This study will be useful to identify and compare the bioactive compounds of both oils.

2. Materials and methods

2.1. Plant materials

The flowering aerial parts of *T. daenensis* subsp. *daenensis* and *T. kotschyanus* were collected from the west of Iran, Province Hamadan, in June 2002. The plants were identified and authenticated by Dr. Gh. Amin at the Faculty of Pharmacy, Tehran University of Medical Sciences. Voucher specimens were deposited in the Herbarium of Pharmacognosy Department, School of Pharmacy, Shaheed Beheshti University of Medical Sciences.

2.2. Isolation of the essential oils

The dried aerial parts of the plants (50 g) were separately hydrodistilled in a Clevenger-type apparatus for 3 h. The oils were dried over anhydrous sodium sulfate and kept at 4 °C in the sealed brown vials until required.

2.3. Analysis of the oils

GC analyses were carried out on a HP-6890 gas chromatograph equipped with a FID and a DB-5 capillary column (30 m × 0.25 mm; 0.25 μm film thickness). The oven temperature was held at 50 °C for 0.5 min then programmed at 2.5 °C/min to 265 °C. Other operating conditions were as follows: carrier gas, N_2 with a flow rate of 1.5 ml/min; injector temperature, 250; detector temperature, 300 °C; split ratio, 1:10.

GC/MS analyses were performed on a Thermoquest 2000 GC coupled with Thermofinnigan Mass system and a DB-1 capillary column (30 m × 0.25 mm; 0.25 μm film thickness). The operating conditions were the same conditions as described above but the carrier gas was He. Mass spectra were taken at 70 eV. Mass range was from *m/z* 35–375 amu.

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 indices with those published in the literature (Adams, 1995; Swigar & Silverstein, 1981) and presented in the MS computer library (WILEY275.L).

3. Results and discussion

The hydrodistillation of the aerial parts of *T. daenensis* subsp. *daenensis* and *T. kotschyanus* gave pale yellow oils with a yield of 2.4% ± 0.1 (v/w) and 1.2% ± 0.1 (v/w), on dry weight basis, respectively. The general chemical profiles of the tested oils, the percentage content of the individual components and retention indices are summarized in Table 1. The chemical class distribution of the oil components is also reported in Table 2.

In the oil of *T. daenensis* subsp. *daenensis*, twenty six components were identified, which represented about 99.7% of the total detected constituents. The major constituents of the oil were thymol (74.7%), *p*-cymene (6.5%), β-caryophyllene (3.8%) and methyl carvacrol

Table 1
Chemical composition of *T. daenensis* subsp. *daenensis* and *T. kotschyanus* essential oils

Compound[a]	RI[b]	Content (rel. %)	
		T. daenensis subsp. *daenensis*	*T. kotschyanus*
Tricyclene	925	0.9	0.4
α-Thujene	931	0.8	0.6
α-Pinene	946	0.2	0.2
β-Pinene	975	0.2	0.1
Myrcene	992	1.1	1.3
α-Phellandrene	1005	0.1	0.2
Δ-3-Carene	1010	0.1	0.1
α-Terpinene	1016	1.0	1.3
p-Cymene	1027	6.5	7.3
Limonene	1032	1.0	0.5
(*Z*)-β-Ocimene	1039	–	0.2
(*E*)-β-Ocimene	1049	–	2.6
γ-Terpinene	1062	2.6	8.2
(*E*)-Sabinene hydrate	1069	0.2	–
Linalool	1090	0.1	0.1
Borneol	1168	–	0.2
Terpin-4-ol	1180	–	0.1
Methyl thymol	1238	–	0.1
Methyl carvacrol	1246	3.6	0.1
Thymol	1297	74.7	38.6
Carvacrol	1305	1.3	33.9
β-Caryophyllene	1416	3.8	0.2
Aromadendrene	1436	0.1	0.1
α-Humulene	1449	0.1	–
Germacrene-D	1475	–	0.1
Bicyclogermacrene	1492	0.1	0.1
β-Bisabolene	1509	0.3	1.0
γ-Cadinene	1522	0.1	0.1
Elemol	1543	0.2	0.2
Spathulenol	1577	0.1	0.1
Caryophyllene oxide	1581	0.4	0.1
α-Cadinol	1640	–	0.5
n-Nonacosane	–	0.1	0.1
Total		99.7	98.7

[a] Compounds listed in order of elution.
[b] RI (retention index) measured relative to *n*-alkanes (C_9–C_{18}) on the non-polar DB-5 column under conditions listed in Section 2.

Table 2
Class composition of *T. daenensis* subsp. *daenensis* and *T. kotschyanus* essential oils

Class of compounds	*T. daenensis* subsp. *daenensis* (%)	*T. kotschyanus* (%)
Monoterpene hydrocarbons	14.5	23.0
Monoterpene alcohols	0.3	0.4
Monoterpene phenols	79.6	72.7
Sesquiterpene hydrocarbons	4.5	1.6
Sesquiterpene alcohols	0.3	0.8
Others	0.5	0.2
Total	99.7	98.7

(3.6%). Other components were present in amounts less than 3% (Table 1). In particular, monoterpene phenols were the most abundant compound group of the oil (79.6%). In the oil of the other species (*T. kotschyanus*), 31 compounds, constituting 98.7% of the oil, were identified. Thymol (38.6%) was the major component, followed by carvacrol (33.9%), γ-terpinene (8.2%), *p*-cymene (7.3%) (Table 1). Similar to *T. daenensis* subsp. *daenensis*, monoterpene phenols were also the most abundant compound group of this oil (72.7%). Therefore, both oils are rich in monoterpene phenols and poor in other terpenoids (Table 2). From Tables, it is evident that there are many qualitative similarities between the oils although the amounts of some corresponding compounds are different.

In regard to the previously reported contents of the essential oil of *T. kotschyanus*, it is interesting to point out that there are no important qualitative differences between the present work and those studies but there are some quantitative differences indicating that environmental factors strongly influence its chemical composition.

Comparison of the volatile compounds of *T. daenensis* subsp. *daenensis* oil with data that have been published on the oil composition of *T. daenensis* subsp. *lancifolius* shows that there are some qualitative and quantitative differences between the two oils. These chemical differences can be most probably explained by the variability of the plant subspecies and the existence of different chemotypes.

The oils of the two investigated species are rich in monoterpene phenols (especially, thymol and carvacrol) and due to this high phenol content, they can be considered as substitutes for *Thymus vulgaris* (common thyme) oil for medicinal purposes and other applications. In addition, the Iranian *T. daenensis* subsp. *daenensis* may be a potential thymol-rich source for commercial cultivation.

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