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Original Article

Essential oil composition of two *Thymus kotschyanus* Boiss. varietes from Elazığ (Turkey)

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Summary. In this study aerial parts essential oil of *T. kotschyanus* Boiss. & Hohen. var. *kotschyanus* and *T. kotschyanus* Boiss. & Hohen. var. *glabrescens* Boiss. were analyzed by HS-SPME/GC-MS. As a result forty one and thirty nine components were identified representing 90.4% and 89.3% of the oil, respectively. Thymol (31.2%), carvacrol (19.5%), *p*-cymene (11.2%) and γ-terpinene (8.4%) were detected the main compounds of *T. kotschyanus* var. *kotschyanus*; thymol (26.3%), carvacrol (24.3%) and *p*-cymene (17.6%) were detected the major constituents of *T. kotschyanus* var. *glabrescens*. With this study, chemotypes of studied taxa were detected carvacrol and thymol. In addition studied plant samples were found to be rich in respect to essential oils and the results discussed natural product, renewable resources and chemotaxonomy.

Keywords: *Thymus*, essential oil, HS-SPME/GC-MS.

Introduction

The genus *Thymus* L. is in the Lamiaceae family; this family occurs in more than 7200 species across about 240 genera which are classified in 7 subfamilies, which have a world-wide distribution (1). *Thymus* belonging to the Lamiaceae family includes about 350 species, existing mainly in Europe, Western Asia and the Mediterranean regions (2). *Thymus* is represented in Turkey by 39 species and 59 taxa, and the ratio of endemism in the genus is 53% (3). The oils of *Thymus* taxa are widely used as an antiseptic agent in many pharmaceutical preparations and as a flavouring agent for many kinds of food products. The genus *Thymus* has numerous species and varieties, and their essential oil compositions have been studied earlier (4).

Thymus kotschyanus Boiss. & Hohen. is suberect freely branching dwarf shrub lacking prostrate basal branches, corolla white or pale pinkish, this species in Turkey that grows on bare stony mountain slopes, between an altitude of 800-2250 m. *Thymus kotschyanus* has three varietes (var. *glabrescens*; var. *eriophorus*; var. *kotschyanus*) in Flora of Turkey (5). Many species of *Thymus* taxa have been widely used in folk medicine in the world for their carminative, antispasmodic, emmenagogic and tonic properties (6). The species of this genus are rich in essential oils and were characterized by a great variability of both morphology and chemotypes (7). Many studies on the antimicrobial and antioxidative activity (6) of these oils have been reported. On the other hand, several extracts of these plants were tested for their pharmacological (8) and other activity (9). Essential oil of this plant is a rich source of thymol and carvacrol which has been reported to possess the highest antioxidant activity (10). Medicinal and aromatic plants are valued for their biological activities which can be justified from the fact that about 80% of the local population still depend on these plants for primary health care. The formation and accumulation of essential oil in plants has been reviewed by many workers (11). The compounds from the plant based essential oil are useful as an alternative therapy, either directly or as models for new synthetic products (12). It is clear from these studies that these secondary plant metabolites have potential uses in medical procedures and applications in the cosmetic, pharmaceutical and food industries (13).

This paper reports the results of HS-SPME/GC-MS analyses essential oil from aerial parts of *T.kotschyanus* var. *kotschyanus* and *T. kotschyanus* var. *glabrescens*; to determined chemotypes and to potential usefulness of studied samples.

Materials and Methods

Plant materials

T. kotschyanus var. *kotschyanus* was collected at the flowering stage in July 2015 in vicinity of Pinarlar village, (Keban/Elazığ/Turkey). *T. kotschyanus* var. *glabrescens* was collected from vicinity of Aslankasi village (Keban/Elazığ/Turkey), in July 2015. The voucher specimens have been deposited in the department of Park and Garden Plants of Bingol University.

HS-SPME Procedure

"Five grams powder of aerial part of studied samples were carried out by a (HS-SPME) head space solid phase microextraction method using a divinyl benzene / carboxen / polydimethylsiloxane (DVB/CAR/PDMS) fiber, with 50/30 um film thickness; before the analysis the fiber was pre conditioned in the injection port of the gas chromatography (GC) as indicated by the manufacturer. For each sample, 5 g of plant samples, previously homogenized, were weighed in to a 40 ml vial; the vial was equipped with a "mininert" valve. The vial was kept at 35°C with continuous internal stirring and the sample was left to equilibratefor 30 min; then, the SPME fiber was exposed for 40 min to the headspace while maintaining the sample at 35°C. After sampling, the SPME fiber was introduced into the GC injector, and was left for 3 min to allow the analyzes thermal desorption. In order to optimize the technique, the effects of various parameters, such as sample volume, sample headspace volume, sample heating temperature and extraction time were studied on the extraction efficiency as previously reported by Verzera et al. (4).

GC-MS Analysis

"A Varian 3800 gas chromatograph directly inter faced with a Varian 2000 ion trap mass spectrometer (Varian Spa, Milan, Italy) was used with injector temperature, 260°C; injection mode, splitless; column, 60 m, CP-Wax 52 CB 0.25 mm i.d., 0.25 um film thickness. The oven temperature was programmed as follows: 45°C held for 5 min, then increased to 80°C at a rate of 10°C/min, and to 240°C at 2°C/min. The carrier gas was helium, used at a constant pressure of 10 psi; the transfer line temperature, 250°C; the ionisation mode, electron impact (EI); acquisit ion range, 40 to 200 m/z; scan rate, 1 us^{-1}. The compounds were identified using the NIST (National Institute of Standardsand Technology) library (NIST/WILEY/EPA/NIH), mass spectral library and verified by the retention indices which were calculated as described by Van Den Dool and Kratz (15). The relative amounts were calculated on the basis of peak-area ratios. The identified constituents of studied samples are listed in Table 1.

Results and Discussion

In this study the aerial parts essential oil of *T. kotschyanus* var. *kotschyanus* and *T. kotschyanus* var. *glabrescens* were analyzed and thymol (31.2%, 26.3%), carvacrol (19.5%, 24.3%) and *p*-cymene (11.2%, 17.6%) were detected the main compounds of *T. kotschyanus* var. *kotschyanus* and *T. kotschyanus* var. *glabrescens*, respectively. *T. kotschyanus* var. *kotschyanus* and *T. kotschyanus* var. *glabrescens* included high concentrations of thymol (31.2%, 26.3%) and carvacrol (19.5%, 24.3%), respectively (Table 1).

Karaman et al., (2001), reported that; the major components of *Thymus revolutus* Celak from Turkey were carvacrol (43.13%), γ-terpinene (20.86%), *p*-cymene (13,94%), β-caryophyllene (5.40%) and in low percentages thymol (4.62%). Several previous researches on another Turkish *Thymus* taxa showed that the main components of the oils were carvacrol and thymol in *T. cilicicus* (16); germacrene-D in *T. cariensis* and *T. haussknechtii* (13); thymol, *p*-cymene and carvacrol in *T. atticus* and *T. roegneri* (17); 1,8-cineole (21.5%) in *T. haussknechtii* and thymol (47.5%) in *T. kotschyanus* var. *kotschyanus* (4). In this study there are some similarities and differences in the essential oil contents from cited researches; the differences in the oil composition may be due to the collection time and geographic factors. In another study, thymol (47.5%) and carvacrol (53-70%) were the main cons-

Table 1. The identified constituents of studied samples (%).

Compounds	**RRI**	***T. kotschyanus* var. *kotschyanus* (%)**	***T. kotschyanus* var. *glabrescens* (%)**
α-Thujene	975	1.2	0.8
α-pinene	980	0.5	-
Camphene	982	0.3	0.2
Verbenene	990	0.2	0.1
Benzaldehide	995	-	0.1
Sabinene	1015	0.9	0.2
β-pinene	1020	0.3	0.4
1-octan-3-ol	1024	0.4	-
Mrycene	1035	1.5	0.1
α-phellandrene	1040	0.2	1.1
α-terpinene	1042	1.7	0.5
P-cymene	1050	11.2	17.6
Limonene	1055	0.4	-
1,8-cineole	1085	2.2	6.4
Terpinolene	1090	0.2	0.1
β-Ocimene	1100	0.2	0.1
γ-terpinene	1119	8.4	5.3
Sabinen-hydrate	1127	0.2	0.1
Linalool	1148	1.2	1.1
Camphor	1155	0.1	0.4
trans-pinocarveole	1166	0.1	-
Borneol	1200	0.3	0.5
Nerol	1232	0.1	0.2
Terpinen-4-ol	1245	0.6	0.2
Geraniol	1250	0.1	0.4
Thymol methı-ether	1290	1.1	1.6
Thymol	1297	31.2	26.3
Carvacrol	1302	19.5	24.3
Bornyl acetate	1337	-	0.1
Eugenol	1339	0.1	-
Neryl acetate	1342	0.2	0.1
α-copaene	1360	0.4	0.1
β-caryophyllene	1366	-	2.6
β-bourbene	1394	0.2	0.1
β-elemene	1400	-	0.1
Aromadendrene	1406	-	0.1
α-Humulene	1418	0.2	0.3
Germacrene-D	1465	0.1	0.1
Bicyclogermacrene	1470	0.1	0.2
γ-muurolene	1477	0.2	0.1
β-bisabolene	1482	0.2	0.3
δ-cadinene	1490	1.4	0.2
β-sesquiphellandrene	1492	0.1	-
Cis-α-bisabolene	1495	1.4	0.5
Spathulenol	1545	-	0.2
Caryophyllene oxide	1555	1.3	0.1
α-Cadinol	1615	0.2	-
Total		90.4	89.3

RRI*: Relative Retention Index

tituents of *T. kotschyanus* var. *kotschyanus* and *T. kotschyanus* var. *glabrescens*, respectively; the other main components were borneol (7.7%), 1,8-cineole (5.9%) and thymol methyl ether (4.1%) (18). Thymol and carvacrol were the main constituents in this study; the other major components in the oil of studied samples were *p*-cymene and 1,8-cineole (Table 1). Sefidkon et al., (19) determined the major constituents in *Thymus kotschyanus* from Iran as carvacrol (40%), thymol (10%) and γ-terpinene (8.55%).

Significant quantitative differences between the two oils were apparent only between the two isomeric phenols, carvacrol and thymol, and their biosynthetic precursors γ-terpinene and *p*-cymene; the concentration of other components varied greatly among the two oils (Table 1). In another study, floral budding, flowering and seed essential oil of *T. caramanicus* were analyzed by GC and GC-MS; as a result carvacrol was the major compound in all samples. The ranges of major constituents were as follow: carvacrol (58.9-68.9%), *p*-cymene (3.0-8.9%), γ-terpinene (4.3-8.0%), thymol (2.4-6.0%) and borneol (2.3-4.0%) (20, 21) reported that; in the oil of *T. daenensis* subsp. *daenensis*, twenty six constituents were detected, which represented about 99.7% of the total detected compounds and the major constituents of the oil were thymol (74.7%), *p*-cymene (6.5%), β-caryophyllene (3.8%) and methyl carvacrol (3.6%), other components were present in amounts less than 3%. In the present study studied *Thymus* samples were shown to contain mainly carvacrol, thymol, *p*-cymene; whereas other compounds detected only low percentages (Table 1); these differences probably depending on the different analytical method, environmental factors and different plant material investigated.

In the oil of *T. kotschyanus* var. *kotschyanus*, forty one components were identified, which represented about 90.4% of the total detected constituents. The major constituents of the oil were thymol (31.2%), carvacrol (19.5%), *p*-cymene (11.2%) and γ-terpinene (8.4%); other components were present in amounts less than 3% ; in particular, monoterpene phenols were the most abundant compound group of the oil (Table 1). In the oil of the other species (*T. kotschyanus* var. *glabrescens*), 39 compounds, constituting 89.3% of the oil, were identified; thymol (26.3%), carvacrol (24.3%), *p*-cymene (17.6%) and 1,8-cineole (6.4%) were reported the main compounds (Table 1). Similar to *T. kotschyanus* var. *kotschyanus*, monoterpene phenols were also the most abundant compound group of this oil. Therefore, both oils are rich in monoterpene phenols and poor in other terpenoids (Table 1). From table 1, it is evident that there are many qualitative similarities between the oils although the amounts of some compounds are different. In regard to the previously reported contents of the essential oil of *Thymus* taxa, it is interesting to point out that there are no important qualitative differences between the present work major compounds and those studies but there are some quantitative differences indicating that environmental and some other factors strongly influence its essential oil composition. Comparison of the volatile compounds of *T. kotschyanus*. var. *kotschyanus* and *T. kotschyanus* var. *glabrescens* oils (Table 1) with data that have been published on the oil composition of cited *Thymus* raxa in the literature shows that there are some qualitative and quantitative differences between the studied *Thymus* oils. These chemical differences can be most probably explained by the variability of the plant subspecies, varietes and the existence of different chemotypes.

In conclusion, the oils of the two investigated *Thymus kotscyanus* varietes are rich in monoterpene phenols (especially, thymol and carvacrol) and due to this high phenol content, they can be considered as substitutes for common Thyme (*Thymus vulgaris*) oil for medicinal purposes and other applications; so the Eastern region of Turkey (Elazığ) *T. kotschyanus*. var. *kotschyanus* and *T. kotschyanus* var. *glabrescens* may be a potential thymol-rich source for commercial cultivation. Furthermore, it is possible to say that, the essential oils of *T. kotschyanus*. var. *kotschyanus* and *T. kotschyanus* var. *glabrescens* have carvacrol/thymol chemotypes in Eastern region of Turkey. *T. kotschyanus*. var. *kotschyanus* and *T. kotschyanus* var. *glabrescens* evidenced a similarity, with reference to the presence of the main constituents; carvacrol, thymol, γ-terpinene and *p*-cymene were among the major compunds in both varietes. Also the percentages of 1,8-cineole, borneol and other compounds were comparable. In addition, the essential oil results have given some clues on the chemotaxonomy and potential usability of these *Thymus* varietes as natural product. According to these results, studied plants were found to be rich in respect to essential oils; so these plants may be used different purposes in industry, ethnobotany and can be cultivated to richened natural products. In addition, many plant taxa are threatened due to overharvesting for medicinal or other use, so there is great need to protect plant diversity; there is also a need to improve more sustainable ways of obtaining industrial products from renewable resources. Furthermore the existence of different valuable compounds in the selected *Thymus kotschyanus* varietes was revealed by the detailed oil composition characterisation performed in this study, thus demonstrating their applicability for medicinal and pharmaceutical purposes; and in the cosmetic and beverages industry.

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