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CHEMICAL COMPOSITION OF THE ESSENTIAL OILS FROM *Thymus transcaspicus* IN NATURAL HABITATS

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Thymus from Lamiaceae contains different species with variable chemical constituents [1]. Due to interspecific hybridization, the number of species from this genus is reported to be 350 [2], with 14 species from Iran [3]. The genus *Thymus* have been widely used in folk medicine in the world and also in medicinal and nonmedicinal aspects in the food, pharmaceutical, and cosmetic industries as a flavoring agent (condiment and spice), culinary herb, and herbal medicine in the treatment of a variety of illnesses [2, 4, 5].

These species are rich in monoterpenoid phenols, which contain important constituents such as thymol and carvacrol. The ecological role of secondary metabolites in *Thymus* can be associated with adaptation to the environment and interactive competition with other plants and also a chemical defense against herbivory and plant pathogens. There is evidence indicating the importance of the diversity of monoterpenes as an adaptation strategy to different environments [2].

There is evidence on the antifungal [6], antibacterial and antiparasitic [7, 8], antioxidant [9, 10], and antispasmodic [2, 11] activities from chemical extracts of *Thymus* genus. Thymol, carvacrol, *p*-cymene, and γ-terpinene are the main constituents of the *Thymus* genus [12–15].

Khorasan thyme (*Thymus transcaspicus* Klokov) [16] is native to Iran and Turkmenistan with a limited distribution in the Northeast of Iran from 1700 to 2800 m altitude [17, 18]. Although this species is native to Iran, there is not much information on the chemical composition, particularly its essential oil constituents. Miri et al. [19] reported 56.4% thymol, 7.7% γ-terpinene, 7.6% carvacrol, and 6.3% *p*-cymene from this species.

The purpose of the present investigation was to analyze the chemical constituent of plant material from *T. transcaspicus* in different habitats in Khorasan, Northeast of Iran.

Table 1 shows that the range of essential oils was from 1.2% to 2.3% in different habitats, with the highest in Tiwan (2.3%). In general, 51 constituents were identified in the essential oils (Table 1) with 43 (representing 100% of the total amount) in Reiin, 36 (representing 96.3% of the total amount) in Pakotal, 43 (representing 99.4% of the total amount) in Laeen Kohneh, and 40 (representing 99.0% of the total amount) in Tiwan. Thymol and carvacrol were the main constituents. This has also been reported elsewhere [20]. The thymol content for Pakotal, Laeen Kohneh, and Reiin were 54.3, 45.3, and 44.9%, respectively, and these values were 8.4, 13.1, and 13.3% for carvacrol. However, the trend was somehow different for the Reiin habitat where the content of carvacrol and thymol were 47.3 and 5.3%, respectively. The borneol content for this area was 7.1%.

Based on these findings, two distinct chemotypes were recognized, where the thymol chemotype was from Pakotal, Laeen Kohneh, and Tiwan, and the carvacrol chemotype was from Reiin. These differences can be associated with the probable hybridization of different species in the area. This has also been reported in the literature [21]. Pluhar et al. [22] studied the essential oil variability of *T. pannonicu*s and *T. praecox* growing wild in natural habitats and reported that the most common chemotype of *T. pannonicu*s was thymol/*p*-cymene, while for *T. praecox* a geraniol/germacrene D/β-caryophyllene chemotype was also recorded. The thymol concentration was positively correlated with humus and Na, K, Mg, and Cd contents of the soil.

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TABLE 1. The Principal Essential Oil Components of *T. transcaspicus* from Different Natural Habitats and Cultivated Conditions, Area, %

Compound	RI	A (1.6)	B (1.3)	C (2.0)	D (2.3)	E (1.2)
o-Xylene	801	0.4	–	–	–	–
α-Thujene	926	1.3	0.7	1.0	0.8	1.2
α-Pinene	935	2.5	0.9	1.3	1.2	1.9
Comphene	949	2.6	1.1	0.9	0.8	1.8
1-Octen-3-ol	961	0.5	0.7	0.3	0.2	–
3-Octanone	965	0.3	0.3	0.3	0.2	0.2
Sabinene	972	–	–	–	–	0.5
β-Pinene	975	0.5	0.2	0.3	0.3	–
3-Octanol	979	0.2	–	0.1	–	–
Myrcene	980	–	–	–	–	1.3
β-Myrcene	982	1.3	1.0	1.6	1.6	–
α-Phellandrene	1000	0.3	0.1	0.4	0.4	0.3
$^3\Delta$-Carene	1008	0.1	0.0	0.1	0.1	–
p-Cymene	1013	–	–	–	–	12.4
α-Terpinene	1016	6.8	5.2	10.1	9.8	–
1,8-Cineol	1025	1.9	1.4	1.4	1.9	2.6
Z-α-Ocimene	1035	–	–	0.1	–	–
γ-Terpinene	1050	5.8	4.2	8.6	7.7	10.6
trans-Sabinene hydrate	1057	1.4	2.0	1.7	1.1	–
α-Terpinolene	1082	0.3	0.1	0.3	0.5	0.3
Linalool	1084	0.2	0.3	0.1	–	0.1
cis-Sabinene hydrate	1087	0.4	0.5	0.5	0.3	0.4
Camphor	1127	0.7	1.0	1.2	0.3	2.0
Borneol	1156	7.1	6.6	2.2	0.5	3.8
4-Terpineol	1166	1.2	1.3	1.2	2.5	1.2
α-Terpineol	1176	0.2	0.5	–	1.3	0.3
Dihydrocarvone	1181	0.6	–	0.3	0.0	–
Pulegone	1206	1.2	–	–	–	–
Carvacrol methyl ether	1216	0.1	3.0	0.7	0.3	0.1
Carvone	1225	–	–	–	0.1	–
Thymol methyl ether	1226	0.3	–	–	3.1	2.7
Thymol	1268	5.3	54.3	45.4	45.0	43.1
Carvacrol	1288	47.4	8.4	13.2	13.3	8.7
Bornyl acetate	1289	0.6	–	–	–	–
Thymyl acetate	1328	–	0.4	0.6	0.3	0.2
Carvacryl acetate	1348	0.6	–	0.1	–	–
α-Copaene	1385	0.1	–	–	–	–
β-Bourbonene	1394	0.2	0.1	0.1	0.1	–
β-Caryophyllene	1424	–	–	–	–	0.5
trans-Caryophyllene	1429	1.1	0.2	1.8	1.1	–
Z-β-Farnesene	1445	–	–	–	–	0.2
Aromadendrene	1448	0.5	0.1	0.2	0.3	–
α-Humulene	1459	–	–	0.1	–	–
γ-Muurolene	1477	0.4	0.2	0.2	0.1	–
Germacrene D	1485	–	0.1	–	–	–
Valencene	1499	0.6	0.1	0.3	0.2	–
β-Bisabolene	1505	2.9	0.5	0.8	0.6	–
γ-Cadinene	1513	0.2	0.1	0.1	0.1	–
δ-Cadinene	1520	0.6	0.2	0.2	0.2	–
trans-β-Bisabolene	1533	–	–	–	–	1.9
cis-α-Bisabolene	1535	0.7	0.5	0.4	2.0	–
Spathulenol	1573	0.2	0.1	0.2	0.2	–
Caryophyllene oxide	1580	0.1	–	0.4	0.2	–
Nonadecane	1899	0.2	0.1	0.1	0.5	–

TABLE 1. (continued)

Compound	RI	A (1.6)	B (1.3)	C (2.0)	D (2.3)	E (1.2)
α-Cedrol	1922	0.3	–	0.4	0.1	–
trans-Phytol	2108	–	–	0.2	–	–
Nonadecanal	2113	–	–	0.1	0.1	–
Total identified compounds		100.0	96.3	99.4	98.9	98.2

The retention indices of compounds relative to C_6-C_{24} *n*- alkanes on DB-1 column were determined.
Studied sites: (Natural habitats of A: Reiin – Northern slopes of Aladagh mountain (longitude: 57° 02′E; latitude: 37° 24′N; altitude: 2048 m), B: Pakotal – Northern slopes of Aladagh mountain (longitude: 57° 22′E; latitude: 37° 16′N; altitude: 2032 m), C: Laeen Kohneh – Northern slopes of Hezar-masjed mountain (longitude: 59° 24′E; latitude: 37° 02′N; altitude: 1800 m), D: Tiwan – Northern slopes of Hezar-masjed mountain between Daregaz and Allah-o-akbar slopes (longitude: 58° 35′E; latitude: 37° 27′N; altitude: 2300 m)) and E: Cultivated condition.

Miri et al. [19] found 47constituents in Khorasan thyme, which were 99.5% of the total recognized constituents in the essential oils. Other studies [2] have shown that environmental conditions have a profound effect on the chemical constituents of plants. This can be due to differences in soil, climate, and geographical variability [21]. However, other studies [21] found no relationship between environmental conditions and the essential oil content of Spanish thyme (*Thymus zygis*). Hudaib and Aburjai [20] found that *T. vulgaris* from natural habitats contain high essential oils compared with similar cultivated plants.

In our investigation a comparison was made between essential oils from wild and cultivated plants (samples were taken from a separate experiment) [23], and it was found that the cultivated plants had 25 constituents, which was 98.2% of the total essential oil content (Table 1). Therefore, it appears that the essential oils from the wild samples contained more constituents compared with the cultivated samples.

In conclusion, the essential oils of *T. transcaspicus* from different habitats varied (1.3–2.3%), possibly due to different environmental conditions, and two distinct chemotypes were identified. Thymol and carvacrol were the main constituents, and plants from natural habitats contained higher amounts.

Study Sites. Ecological, edaphic, and topographic criteria of the natural habitats are presented in Table 1. Plant materials from these habitats were collected in 2006 during the flowering stage. Voucher specimens were deposited in the herbarium of the Ferdowsi University of Mashhad, Iran. For comparison purposes, samples from a field (longitude: 59° 28′E; latitude: 36° 15′N; altitude: 985 m) where this species was grown [23] were also analyzed.

Isolation of Essential Oil. The shade air-dried and finely powdered aerial parts (flowerheads and leaves) of the plant (30 g samples) were extracted by hydrodistillation for 3 h, using a Clevenger-type apparatus, giving a yellow oil which was dried with anhydrous sodium sulfate and stored in a sterilized vial at 4°C until analysis by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Yield percentage was calculated as volume (mL) of essential oil per 100 g of plant dry matter.

Gas Chromatography Analysis. GC-FID analysis of the oil was conducted using a Thermoquest-Finnigan instrument equipped with a DB-1 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 μm). Nitrogen was used as the carrier gas at a constant flow of 1.1 mL/min. The oven temperature was raised from 60°C to 250°C at a rate of 8°C/min and held for 20 min. The injector and detector (FID) temperatures were kept at 250°C and 280°C, respectively.

Gas Chromatography-Mass Spectrometry Analysis. GC/MS analysis was carried out on a Thermoquest-Finnigan Trace GC/MS instrument equipped with a DB-1 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was raised from 60°C to 250°C at a rate of 8°C/min and held for 20 min; transfer line temperature was 250°C. Helium was used as the carrier gas at a flow rate of 1.1 mL/min with a split ratio equal to 1/50. The quadrupole mass spectrometer was scanned over the 35–65 amu with an ionizing voltage of 70 eV and an ionization current of 150 μA.

Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those reported in the literature [24].

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