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Chemical Composition of Leaf and Flower Essential Oils of Two *Thymus* spp. from Western Himalaya

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ABSTRACT

Thymus species (Lamiaceae) are considered to be very beneficial whether used as food or as a medicament. Essential oils (EOs) derived from leaves and flowers of *Thymus serpyllum* and *Thymus linearis* grown in northern India were analyzed by GC and GC-MS. A total of 37 components forming 94.8-98.4% of EO composition were identified. The EOs of both species were rich in thymol, *p*-cymene and γ-terpinene. Thymol was higher in the EO of *T. linearis* (74.6-75.8%) compared to *T. serpyllum* (51.9-70.1%). The amount of thymol methyl ether, *p*-cymene, 1-octen-3-ol, camphor and borneol was relatively higher in *T. serpyllum* EO. Further, phenolic monoterpenes were higher in flower EOs of both species than in leaf EOs.

Keywords: *Thymus serpyllum*, *T. linearis*, Hydrodistillation, Essential oil, GC-MS, thymol
Abbreviations: GC, gas chromatography; **GC-MS**, gas chromatography-mass spectrometry

INTRODUCTION

The genus *Thymus* L. (Lamiaceae), commonly known as thyme in English consists of about 215 species of herbaceous perennial and sub-shrubs that have achieved great commercial importance. The Mediterranean region can be described as the centre of the genus (Stahl-Biskup 2002). In India the genus is represented by two species viz. *T. linearis* (native) and *T. serpyllum* (exotic) (Jalas 1973). Thyme is one of the most widely used culinary herbs. The dried leaves are used for food flavouring and the source of essential oil (EO) in pharmaceutical and cosmetic industries. A number of benefits in human and animal wellbeing have been associated with the use of thyme EO by the industry (Youdim *et al*. 2002). At this point, this plant can be considered as a potential impulse of new trends in food, pharmaceutical and cosmetic industries (Echeverrigaray *et al*. 2001). Recent studies have showed that thyme EOs have strong antibacterial, antifungal, antiviral, antiparasitic and antioxidant activities (Davidson and Naidu 2000; Stahl-Biskup 2002; Parajuli *et al*. 2005; Dababneh 2007; Al-Fatimi *et al*. 2010). The antiseptic, antioxidative, insecticidal, preservative and anesthetic properties of thyme EO are owed mainly to the presence of thymol, carvacrol, geraniol and other volatile components (Van-Den Broucke and Lemli 1981). The antioxidant potential of thyme EO has shown the uses of this product by the food industry and its effectiveness as a dietetic supplement (Youdim and Deans 1999).

The chemical polymorphism of thyme EO has been reviewed by Stahl-Biskup (1991). The most important components found in this genus are thymol and carvacrol followed by linalool, *p*-cymene, γ-terpinene, borneol, terpinen-4-ol and 1,8-cineole (Sfaei-Ghomi *et al*. 2009). In India, the EO composition of this genus has also been studied on a few occasions (Mathela *et al*. 1980; Verma *et al*. 2009a, 2010a). However, detailed research work has not been undertaken so far from this region.

The chemical composition of aromatic plants is significantly influenced by the plant part (Wang and Liu 2010), season and plant ontogeny (Hudaib *et al*. 2002; Jordan *et al*. 2006; Ebrahimi *et al*. 2008; Verma *et al*. 2009b), location of growing (Cabo *et al*. 1986), and drying (Venskutonis 1997; Verma *et al*. 2010b). A literature survey revealed that the EO of leaves and flowers of *T. serpyllum* and *T. linearis* have not been studied separately to date, therefore the present paper deals with the detailed analysis of the oils by GC and GC-MS.

MATERIALS AND METHODS

Plant material

The fresh flowering herbs of *T. linearis* and *T. serpyllum* were collected during summer (21st April and 25th June, respectively) from the experimental farm of the Central Institute of Medicinal and Aromatic Plants, Research Centre, Purara, Uttarakhand, India. The reproductive (flowers) and vegetative (leaves) parts of both species were separated and dried under shade. The site is located at an altitude of 1250 m in the Kattyur valley, western Himalayas. Climatologically, it is categorized as a temperate zone. The monsoon usually breaks in June and continues up to September.

Extraction of EOs

The EO of leaf and flower of both *Thymus* spp. was extracted separately by hydrodistillation for 3 hrs using a Clevenger-type apparatus (Clevenger 1928). The percentage EO content (v/w) was estimated on a dry weight basis. The oil samples obtained were dehydrated over anhydrous sodium sulphate and kept in a cool and dark place before analyses.

Gas chromatography (GC)

The GC analyses of the oil samples was carried out on a Perkin-Elmer Auto XL GC and Nucon gas chromatograph model 5765 equipped with a FID using two different stationary phases PE-5 (60 m × 0.32 mm; 0.25 μm film coating) and CP-Wax 52 CB (30 m × 0.32 mm × 0.25 μm film thickness) fused silica columns, respectively. Hydrogen was the carrier gas at 1.0 mL/min. Oven temperature programming was done from 70-250°C at 3°C/min for PE-5 and from 70-230°C at 4°C/min for CP-Wax 52 CB. The injector and detector temperatures were 210 and 230°C, respectively.

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Table 1 Essential oil composition of leaf and flower of *Thymus serpyllum* and *T. linearis*.

Compound*	*RI*[a]	*RI*[b]	Peak area (%)					
			Thymus serpyllum				*Thymus linearis*	
			A	B	C	D	B	D
α-Pinene	1026	941	1.1	0.4	0.8	1.1	0.3	0.3
Camphene	1065	954	1.1	0.4	0.1	0.2	t	t
β-Pinene	1105	982	t	T	t	t	t	t
β-Myrcene	1158	994	0.1	0.2	0.8	0.6	0.2	0.2
α-Terpinene	1177	1019	0.1	0.4	0.9	0.8	0.4	0.4
Limonene	1185	1034	-	t	t	t	t	t
1,8 Cineole	1196	1038	-	t	t	t	t	t
γ-Terpinene	1240	1065	6.9	6.2	9.2	6.8	9.5	8.2
p-Cymene	1271	1029	9.4	7.0	3.1	3.5	5.1	3.2
α-Terpinolene	1278	1089	t	t	t	t	t	t
3-Octanol	1394	1001	t	0.1	t	t	-	t
(*Z*)-Linalool oxide	1435	1070	-	t	-	-	t	-
1-Octen-3-ol	1448	986	2.6	3.1	1.3	1.3	t	-
(*E*)-Sabinene hydrate	1463	1069	0.6	0.6	0.4	0.6	0.4	0.5
α-Copaene	1481	1374	-	-	-	t	t	-
Camphor	1507	1147	3.5	2.5	0.2	0.4	-	-
Linalool	1536	1103	-	t	-	t	t	t
Bornyl acetate	1585	1285	0.1	0.1	-	t	t	t
β-Caryophyllene	1594	1418	1.1	1.5	1.2	1.5	0.6	1.0
Thymol methyl ether	1594	1220	10.4	5.8	2.3	2.6	t	-
Carvacrol methyl ether	1604	1230	0.1	0.1	0.1	0.1	t	t
Terpinen-4-ol	1606	1180	2.5	2.8	2.9	3.0	2.0	2.3
α-Humulene	1660	1457	-	t	-	t	-	-
α-Terpineol	1682	1192	0.1	0.2	0.5	0.5	t	t
Borneol	1695	1167	2.5	2.8	1.2	1.4	0.8	0.8
Geranial	1740	1277	0.1	t	t	-	-	-
Bicyclogermacrene	1747	1495	0.8	1.1	0.1	0.7	1.8	1.9
Geranyl acetate	1750	1373	0.9	1.5	1.2	1.6	-	-
p-Cymen-8-ol	1846	1185	0.1	t	0.3	0.2	0.9	2.5
Thymyl acetate	1867	1350	0.1	t	t	t	t	0.1
Carvacryl acetate	1880	1368	-	-	-	-	t	-
Caryophyllene oxide	2004	1584	-	t	t	-	t	t
Spathulenol	2143	1579	-	t	-	t	t	-
Eugenol	2161	1362	-	t	t	-	-	0.1
epi-α-Cadinol	2178	1643	-	-	t	-	0.1	0.1
Thymol	2196	1306	51.9	59.5	70.1	66.8	74.6	75.8
Carvacrol	2233	1320	t	0.6	0.8	1.1	1.3	1.0
Class composition								
Monoterpene hydrocarbons			18.7	14.6	14.9	13.0	15.5	12.3
Oxygenated monoterpenes			10.4	10.5	6.7	7.7	4.1	6.1
Phenolic monoterpenes			62.5	66.0	73.3	70.6	75.9	77.0
Sesquiterpene hydrocarbons			1.9	2.6	1.3	2.2	2.4	2.9
Oxygenated sesquiterpenes			-	t	t	t	0.1	0.1
Aliphatic			2.6	3.2	1.3	1.3	t	t
Total identified			96.1	96.9	97.5	94.8	98.0	98.4
Essential oil (%)[#]			0.80	0.94	0.39	1.30	2.50	3.33

[*] Mode of identification: Retention Index (RI), MS (GC-MS); [a] Retention indices on CP-WAX 52 CB column;
[b] Retention indices on PE-5 column; A: Shade dry leaves (vegetative stage); B: Shade dry leaves (flowering stage);
C: Fresh flower; D: Shade dry flower; [#] Dry weight basis except 'C' which is calculated on fresh weight basis; t: Trace (<0.10%)

The injection volume was 0.02 µL neat (syringe: Hamilton 1.0 µL capacity, Alltech USA) and the split ratio was 1: 30.

Gas chromatography-mass spectrometry (GC-MS)

GC-MS analysis of the EO samples was carried out on a Perkin Elmer AutoSystem XL GC interfaced with a Turbomass Quadrupole Mass Spectrometer fitted with an Equity-5 fused silica capillary column (60 m × 0.32 mm i.d., film thickness 0.25 µm) The oven temperature was programmed from 60-210°C at 3°C/min using helium as the carrier gas at 1.0 mL/min. The injector temperature was 210°C, injection volume 0.1 µL prepared in *n*-hexane, split ratio 1: 40. MS were taken at 70 eV with a mass scan range of 40-450 amu.

Identification of components

Constituents were identified on the basis of a Retention Index (RI, determined with reference to homologous series of *n*-alkanes, C_9-C_{24}, under identical experimental conditions, co-injection with standards or known EO constituents, MS Library search (NIST and WILEY), by comparing with the MS literature data (Davies 1990; Adams 1995). The relative amounts of individual components were calculated based on the GC peak area (FID response) without using a correction factor.

RESULTS AND DISCUSSION

The EOs were obtained by hydro-distillation from shade-dried leaves and flowers of *T. serpyllum* and *T. linearis* and subsequently analyzed by GC (RI) and GC-MS. The identified components with their relative percentages are reported in **Table 1**. The percentage EO content (v/w) in the shade-dried leaves of *T. serpyllum* varied from 0.8% (vegetative stage) to 0.94% (flowering stage), but was 0.39% in fresh and 1.3% in shade-dried flowers. However, the EO content was higher in the shade-dried leaves (2.5%) and flowers (3.33%) of *T. linearis* than of *T. serpyllum*. In total, 36 constituents representing 94.8-97.5% of *T. serpyllum* and 33 constituents forming 98.0-98.4% of *T. linearis* EO were

Table 2 Essential oil composition of *Thymus* spp.

Species	Country	Thymol (%)	Carvacrol (%)	*p*-Cymene (%)	γ-Terpinene (%)	Reference
Thymus serpyllum	India	60.0	2.0	-	-	Mathela *et al.* 1980
T. serpyllum	India	58.8	1.0	5.7	3.4	Verma *et al*. 2009a
T. serpyllum	Pakistan	53.3	10.4	8.8	-	Ahmad *et al.* 2006
T. serpyllum	Iran	18.7	0.4	20.7	22.7	Sefidkon *et al*. 2004
T. vulgaris	Brazil	44.7	2.4	18.6	16.5	Porte and Godoy 2008
T. vulgaris	Turkey	46.2	2.4	9.9	14.1	Ozcan and Chalchat 2004
T. vulgaris	Cuba	36.6	6.5	17.6	17.6	Pino *et al*. 1997
T. vulgaris	Cuba	37.4	4.4	26.1	0.9	Perez *et al*. 2007
T. vulgaris	Spain	36.3	2.0	27.8	13.1	Arraiza *et al.* 2009
T. linearis	India	52.3-66.6	1.0-5.3	1.8-21.6	1.9-12.5	Verma *et al.* 2010a
T. thracicus	Turkey	15.7	18.2	25.4	11.1	Akcin 2006
T. longicaulis	Turkey	16.7	1.9	15.0	1.8	Akcin 2006
T. pseudopulegioides	Turkey	22.1	2.3	13.7	1.3	Akcin 2006
T. kotschyanus	Iran	38.0	14.2	2.2	0.9	Rustaiyan *et al*. 1999
T. hyemalis	Spain	26.2	1.0	30.4	14.3	Jordan *et al*. 2006
T. munbyanus	Algeria	37.7	8.4	14.2	10.1	Hazzit *et al*. 2006
T. caramanicus	Iran	5.3	68.9	6.0	4.6	Ebrahimi *et al*. 2008
T. numidicus	Algeria	51.0	9.4	0.5	-	Saidj *et al.* 2008
T. fontanesii	North Africa	67.8	1.7	13.0	15.9	Ghannadi *et al*. 2004
T. pulegioides	Italy	26.3	4.7	19.9	-	Martino *et al*. 2009
T. alpestris	Slovakia	41.0	3.6	8.8	7.9	Martonfi 1992
T. alpestris	Slovakia	2.4	47.0	6.6	7.3	Martonfi 1992

identified and quantified. Although, the qualitative composition of both the EOs was almost the same there was considerable variation in the quantitative composition due to plant part and species.

The major components of the *T. serpyllum* EO were thymol (51.9-70.1%), γ-terpinene (6.2-9.2%), *p*-cymene (3.1-9.4%), thymol methyl ether (2.3-10.4%), camphor (0.2-3.5%), 1-octen-3-ol (1.3-3.1%), terpinen-4-ol (2.5-3.0%), borneol (1.2-2.8%), geranyl acetate (0.9-1.6%), β-caryophyllene (1.1-1.5%), α-pinene (0.4-1.1%), bicyclegermacrene (0.1-1.1%), camphene (0.1-1.1%) and carvacrol (<0.1-1.1%). However, the amount of thymol, γ-terpinene, α-terpinene, β-myrcene and *p*-cymen-8-ol were higher in fresh flowers of *T. serpyllum* than the dried leaves and flowers. Furthermore, the percentage of thymol methyl ether, *p*-cymene and camphor was higher in the leaves collected at the vegetative stage. On the other hand, 1-octen-3-ol, borneol and bicyclogermacrene were relatively higher in the leaves collected at the flowering stage of *T. serpyllum*. The major components of the EO from leaves and flowers of *T. linearis* were almost the same as from *T. serpyllum*. Nevertheless, the percentage of thymol was higher in the leaves (74.6%) and flowers (75.8%) of *T. linearis* than of *T. serpyllum*. Other major components of *T. linearis* leaves and flowers were γ-terpinene (9.5 and 8.2%), *p*-cymene (5.1 and 3.2%), terpinen-4-ol (2.0 and 2.3%), bicyclogermacrene (1.8 and 1.9%), *p*-cymen-8-ol (0.9 and 2.5%) and carvacrol (1.3 and 1.0%).

It was interesting to note that the phenols and alcohols (thymol, *p*-cymen-8-ol and terpinen-4-ol) accumulated more in the reproductive part, while thymol methyl ether and *p*-cymene accumulated more in the vegetative part of both *Thymus* species. Thyme EO with high thymol content strongly inhibited bacterial and fungal growth (Broucke 1983; Davidson and Naidu 2000; Dorman and Deans 2000; Iten *et al*. 2009). Thus, considering the compositional variation, it could be said that the flowers would be of more medicinal worth than leaves because the former possessed more phenolic monoterpenes than the latter.

The EO composition of genus *Thymus* has been evaluated from various countries and majority of its members were dominated by phenolic monoterpene viz. thymol or carvacrol (**Table 2**). The present study and earlier reports from India showed that the EO of *Thymus* spp contained thymol as principal component and percentage of this component varied from 51.9 to 75.8% with the maximum in *T. linearis*. Further, the **Table 2** clearly indicated that the percentage of thymol observed in Indian *Thymus* was quite higher than that reported from Iran, Brazil, Turkey, Cuba, Spain, Algeria, Italy and Slovakia. In addition to this, the chemical profile of our tested EOs was found in good agreement with the quality standards of European Pharmacopoeia (EP, 2002) for thyme oil. Thymol content (EP limits 36.0-55%) was found either well within the limit or higher than the limit of EP. Therefore, due to high thymol and EO content and hence; therapeutic potential, Indian thyme (*T. linearis*) can be considered as better option of common thyme oil (*T. vulgaris*).

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