Comparative chemical composition of the essential oil of *Thymus vulgaris* L. from different geographical sources

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Summary

Variations in the essential oil composition of *Thymus vulgaris* L. cultivated in Estonia and in other European countries were determined using capillary gas chromatographic analysis methods. Fifty-nine components were identified, representing over 95% of the total oil yield. The principal components in the oils of common thyme were thymol (0.9%–75.7%), carvacrol (1.5%–83.5%), p-cymene (4.3%–34.4%), γ-terpinene (0.9%–19.7%), linalool (0.4%–4.8%), (E)-β-caryophyllene (0.5%–9.3%) and terpinen-4-ol (tr.–3.8%). The sum of phenolic compounds (thymol and carvacrol) in the oils studied varied from 19.4% to 84.4%, and the sum of their precursors (p-cymene and γ-terpinene) ranged from 5.7% to 38.5%. Thymol content was predominant in the oils of Holland (65.5%) and of Estonia (75.7%) but carvacrol content predominated in the Greek thyme oil (83.5%). Armenian thyme oil contained only 17.0% of thymol, but it was rich in neral and citronellol (32.5%), borneol (4.3%), citronellal (4.0%), 1,8-cineol (4.0%) and methyl eugenol and thymol acetate (7.5%). In Estonia, the thymol, thymol–carvacrol and thymol–p-cymene-γ-terpinene chemotypes of the common thyme are distinguishable.

Key words: Thymus vulgaris L., Labiatae, common thyme, essential oil, different geographical sources, thymol, carvacrol, p-cymene, γ-terpinene

Within the genus *Thymus* there are many species and subspecies. Most of them, including *Thymus vulgaris* L., contain thymol and carvacrol as the main components, whereas the variations occur in the concentrations of 1,8-cineole, camphor, citral, carvone, monoterpene alcohols, as well as acetates and sesquiterpene alcohols [1-14]. These chemotypes, especially rich in phenolic terpenoids, showed strong antioxidant activities [15, 16]. Only two Thymus species are known in Estonia. Common thyme (*Thymus vulgaris* L.) is cultivated and wild thyme (*Thymus*

serpyllum L.) grows wild. A study of essential oil composition of wild thyme originating from various natural places of growth in Estonia showed the presence of at least three chemotypes [17]. Contrary to the literature data concerning other countries, thymol and carvacrol were not the main components of the Estonian wild thyme oil.

In the present work we determined the composition of the essential oil, using commercial common thyme samples from different European countries and samples cultivated in Estonia. The differences in the contents of the biologically active constituents were studied. Concentrations of the main thyme oil constituents from Estonia were compared to samples of other European countries.

MATERIALS AND METHODS

Plant materials (commercial *Thymi herba*) were obtained from retail pharmacies of various European countries in 2000 (France), 2001 (Hungary, Holland), 2002 (Russia, Greece, Estonia), and 2003 (Scotland, Moldavia, Armenia). The Estonian samples were gathered in summers of 2001, 2002 and 2003 from different places of growth in Estonia. Voucher specimens have been deposited at the Institute of Pharmacy, University of Tartu, Estonia.

Capillary gas chromatography

The essential oil was isolated from dried herb of common thyme by the distillation method described in the European Pharmacopoeia [18]. The oils were analysed using a Chrom-5 chromatograph with FID on two fused silica capillary columns (50 m × 0.20 mm i.d.) with nonpolar polydimethylsiloxane (NB-30) and polar polyethylene glycol 20M (NB-20M) stationary phases (Nordion, Finland). Film thickness of both stationary phases was 0.25 μm. Helium was used as a carrier gas, with split rate 1:150 and the flow rate 20–25 cm/sec. The temperature programme was from 50–250°C at 2°C/min, the injector temperature was 250°C. A 3390A Hewlett-Packard integrator was used for data processing.

Gas chromatography/mass spectrometry

The GC-MS data were obtained on a Hewlett-Packard 5988A instrument. The MS conditions were as follows: EI mode 70 eV, ion source temperature 200°C. GC conditions were 60–280°C at 5°C/min with an internal hold time of 2 minutes. Helium was used as a carrier gas at a flow rate of 20 cm/sec. A fused silica capillary column AT-5, poly(5%-phenyl-95%-dimethylsiloxane), was used (25 m × 0.25 mm i.d., film thickness 0.25 μm). The injector temperature was 280°C.

Identification and quantitative evaluation

Compounds were identified by comparing the retention indices (RI) of the GC peaks on NB-30 and NB-20M columns with the RI values of standard compounds, our RI data bank and the literature [19-21]. The results obtained were confirmed by GC-MS. The quantitative composition of the oils was calculated on the basis of the GC peak areas on the NB-30 column without FID response factor correction, using the normalisation method.

RESULTS AND DISCUSSION

The RI values of essential oil components of *Thymus vulgaris* L. on two columns of different polarity, the percentage composition of the thyme oils from Estonia and other European countries are presented in Table 1.

Table 1.

Composition of the essential oil from *Thymus vulgaris* L. of different origins, %.

compound	retention index		content, %
	NB-30	NB-20M	
tricyclene	920	1010	0-0.1
α-thujene	924	1021	0-1.7
α-pinene	931	1019	0-1.5
camphene	945	1063	0-1.9
sabinene	967	1118	0-0.7
1-octen-3-ol	968	1454	0-1.3
β-pinene	971	1115	tr-1.1
myrcene	984	1162	tr-5.1
α-phellandrene	998	1167	0-0.3
3-carene	1005	1148	0-0.2
α-terpinene	1011	1180	tr-1.4
p-cymene	1015	1270	4.3-34.4
1,8-cineole	1022	1205	0.1-4.0
limonene	1024	1195	tr-7.9
(Z)-β-ocimene	1028	1232	tr-0.2
(E)-β-ocimene	1040	1250	0-0.3
γ-terpinene	1050	1240	0.9-19.7
cis-linalool oxide*	1056	1420	0-1.0
trans-sabinene hydrate	1058	1466	0-0.6
trans-linalool oxide	1076	1455	0-0.2
terpinolene	1081	1276	0-0.4
linalool	1089	1551	0.4-4.8
camphor	1123	1513	0-3.8
citronellal	1143	1480	0-4.0
isoborneol*	1152		0-3.0
borneol	1154	1720	0-4.3
p-cymen-8-ol*	1162	1860	0-0.4
terpinen-4-ol	1166	1602	0-3.8

α-terpineol	1177	1713	0-1.5
(Z)-dihydrocarvone	1181		0-0.5
thymol methyl ether	1218	1580	tr-3.3
neral and citronellol	1220 1222	1677 1800	0-32.5
carvone	1224	1735	0-3.7
carvacrol methyl ether	1230	1584	0-2.2
geraniol	1243	1855	0-5.8
geranial	1264	1725	0-1.5
(E)-anethole and isobornyl acetate*	1264 1262	1837	0-1.1
bornyl acetate	1273	1574	0-2.4
thymol	1280	2197	0.9-75.7
carvacrol	1290	2210	1.5-83.5
methyl eugenol*	1332	1920	0-7.5
thymol acetate*	1334		
α-terpinyl acetate	1335	1700	0-0.4
carvacryl acetate*	1347		0-0.9
neryl acetate	1353	1724	0-0.3
α-copaene	1371	1485	0-0.6
β-bourbonene	1380	1510	0-0.3
(E)-β-caryophyllene	1418	1589	0.5-9.3
α-ionone	1426		0-0.5
bicyclosesquiphellandrene*	1436		0-0.3
α-humulene	1449	1658	0-0.8
alloaromadendrene	1457	1632	0-0.5
γ-muurolene	1472	1690	0-0.8
germacrene D	1478	1700	0-4.3
α-muurolene	1494	1720	0-0.6
bicyclogermacrene*	1490	1722	0-0.8
β-bisabolene	1500	1736	0-2.6
γ-cadinene	1505	1744	0-0.5
δ-cadinene	1517	1746	0-1.0
hedycaryol*	1530	2077	0-0.6
selina-3,7(11)-diene*	1540		0-2.4
germacrene-B*	1555		0-1.0
spathylenol	1570	2124	0-1.0
caryophyllene oxide	1575	1980	0.1-2.5
γ-eudesmol*	1612		0-0.2
T-cadinol	1630	2170	0-0.5
α-cadinol	1646	2217	0-0.4
farnesol*	1659		0-0.7
component groups:			
aliphatic compounds			tr-1.3
monoterpenes			8.3-42.1
(p-cymene + γ-terpinene)			5.7-38.5
oxygenated monoterpenes			40.4-86.8
(thymol + carvacrol)			19.4-84.4
sesquiterpenes			0.3-17.6
oxygenated sesquiterpenes			0.1-4.5
total, %			96.0-99.8

The components identified in the highest yields are printed in bold; tr – traces (<0.05%),
* – tentatively identified.

Fifty-nine components were identified in the samples studied, representing over 95% of the total oil. The main compound group in the oils was oxygenated monoterpenoids (40.4%–86.8%), including phenols (thymol and carvacrol): 19.4%–84.4%. Monoterpenes constituted 8.3%–42.1% of the oils, including phenolic precursors (p-cymene and γ-terpinene): 5.7%–38.5%. Sesquiterpenes made up 0.3%–17.6% of the thyme oils. The major sesquiterpenes in the oils were (E)-β-caryophyllene (0.5%–9.3%), germacrene D (0%–4.3%), β-bisabolene (0%–2.6%) and selina-3,7(11)-diene (0%–2.4%). The other sesquiterpenes made up less than 1% in all the samples. From the oxygenated sesquiterpenes identified in the thyme oils only caryophyllene oxide (0.1%–2.5%) was found to form over 1%.

A comparison of thyme oil composition from samples of different geographical sources showed some variability of the majority of biologically active constituents. In the oils of Greek origin, carvacrol amounted to 83.5%. In other samples studied, this value varied from 2.2% to 4.1%. In the case of two thyme samples from Estonia and Holland the oil contained more thymol (75.7%, 67.5% and 65.5%, respectively) than the other samples (0.9–49.0%). The sum of concentrations of precursors of phenols, p-cymene and γ-terpinene, varied from 5.7% to 38.5%, and these values were lowest in the oils from Armenia (5.7%) and Greece (7.8%). The total concentration of four major constituents (thymol, carvacrol, p-cymene and γ-terpinene) in the thyme oils studied ranged from 67.7% to 92.2%. The only exception was the oil from Armenia, where this value formed only 25.1%. The Armenian thyme oil was rich in neral and citronellol (32.5%), methyl eugenol and thymol acetate (7.5%), borneol (4.3%), citronellal (4.0%) and 1,8-cineol (4.0%).

As shown in Table 2, the thymol chemotype is clearly distinguishable in the Estonian samples 6 and 7 (content of thymol 75.7% and 67.5%, respectively). Samples 4, 8 and 10 were rich in thymol (22.5%–45.1%) and carvacrol (29.9%–34.6%), while samples 1, 2, 3 and 5 were rich in thymol (41.7%–49.0%) and p-cymene (14.6%–22.2%). Unlike the other oils studied, sample 9 contained relatively little thymol, carvacrol and p-cymene (total 45.6%), but it was rich in monoterpenes (myrcene – 5.1%) and sesquiterpenes (β-caryophyllene – 9.3%, germacrene D – 4.3%).

The results of this work have established noticeable quantitative differences in the case of biologically active compounds in common thyme oils from different geographical sources. Consequently the pharmacological effects of these medicinal plants, being of a basically antimicrobial and antibacterial nature, are also likely to differ.

The oil from Holland and two oils from Estonia belong to the thymol chemotype, while the oils from France, Hungary, Russia and Scotland belong to the thymol–p-cymene rich chemotype. Only in Estonia, the thymol–carvacrol and thymol–p-cymene–γ-terpinene chemotypes are distinguishable. The oil from Greece was found to be of a carvacrol-rich chemotype. Unlike the other oils, the oil from Armenia contained high quantities of neral and citronellol.

Table 2.

Concentration of the main essential oil components of *Thymus vulgaris* L. from different geographical sources.

tested samples	concentration, %						
	myrcene	p-cymene	γ-terpinene	linalool	terpinen-4-ol	thymol	carvacrol
France	0.8	28.1	4.5	2.4	tr.	45.7	3.8
Hungary	0.3	25.5	1.1	1.9	0.9	45.6	4.1
Holland	0.4	10.0	1.9	2.0	0.8	65.5	2.8
Russia	0.7	22.5	3.9	2.9	1.1	48.0	3.4
Greece	0.8	6.6	1.2	0.8	0.5	0.9	83.5
Scotland	0.5	34.4	4.1	4.8	2.3	31.5	3.5
Moldavia	tr.	16.4	0.9	1.8	3.8	47.8	2.6
Armenia	0.3	4.6	1.1	0.4	2.2	17.0	2.4
Estonia:							
sample 1	1.7	22.2	10.9	2.1	tr.	49.0	2.2
sample 2	1.7	20.2	9.1	2.3	0.2	49.0	2.9
sample 3	2.5	14.6	19.7	1.8	0.2	47.2	1.9
sample 4	0.4	6.5	3.4	2.0	0.9	45.1	29.9
sample 5	0.7	16.9	9.2	2.9	0.7	41.7	10.1
sample 6	0.4	4.3	3.8	2.1	0.8	75.7	4.1
sample 7	1.2	11.6	6.2	2.1	0.2	67.5	2.8
sample 8	0.7	16.4	4.9	1.7	0.3	28.5	34.6
sample 9	5.1	7.9	7.0	2.7	0.4	29.2	1.5
sample 10	1.4	17.7	9.6	2.2	1.1	22.5	32.1
sample 11	1.6	6.2	4.4	0.7	0.6	39.4	6.0

CONCLUSIONS

The principal components in the essential oils of common thyme from different geographical sources are thymol, carvacrol, p-cymene, γ-terpinene, linalool, (E)-β-caryophyllene and terpinen-4-ol.

In Estonia, the thymol, thymol–carvacrol and thymol–p-cymene–γ-terpinene chemotypes of the common thyme are distinguishable.

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PORÓWNANIE SKŁADU CHEMICZNEGO OLEJKU Z TYMIANKU POSPOLITEGO (*Thymus vulgaris* L.) Z RÓŻNYCH REJONÓW

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Streszczenie

Różnice składu chemicznego olejku uzyskanego z tymianku pospolitego (*Thymus vulgaris* L.) uprawianego w Estonii i innych krajach europejskich określono za pomocą metody kapilarnej chromatografii gazowej. Określono 59 składników, tworzących w sumie ponad 95% składu olejku. Głównymi składnikami olejków uzyskiwanych z tymianku pospolitego były tymol (0,9%–75,7%), karwakrol (1,5%–83,5%), p-cymen (4,3%–34,4%), γ-terpinen (0,9%–19,7%), linalol (0,4%–4,8%), (E)-β-kariofylen (0,5%–9,3%) oraz terpinen-4-ol (od ilości śladowych do 3,8%). Łączna ilość związków fenolowych (tymolu i karwakrolu) w badanych olejkach wynosiła od 19,4% do 84,4%, a łączna ilość ich prekursorów (p-cymenu i γ-terpinenu) – od 5,7% do 38,5%. Zawartość tymolu była najwyższa w olejkach uzyskiwanych z tymianku pochodzącego z Holandii (65,5%) i Estonii (75,7%), natomiast w olejku uzyskiwanym z roślin pochodzących z Grecji dominował karwakrol (83,5%). Olejek pozyskiwany z tymianku rosnącego w Armenii zawierał tylko 17,0% tymolu, charakteryzował się natomiast wysoką zawartością neralu i citronelolu (32,5%), borneolu (4,3%), citronelalu (4,0%), 1,8-cineolu (4,0%) oraz metyloeugenolu i octanu tymolu (7,5%). W wypadku tymianku pospolitego rosnącego w Estonii można wyróżnić chemotypy tymolu, tymolu-karwakrolu oraz tymolu-p-cymenu-γ-terpinenu.

Słowa kluczowe: Thymus vulgaris L., Labiatae, tymianek pospolity, olejek, różne źródła geograficzne, tymol, karwakrol, p-cymen, γ-terpinen