Content and composition of the essential oil of *Thymus serpyllum L.* growing wild in Estonia

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Key words: *Thymus serpyllum L., essential oil composition, chemotypes, Estonia.*

Summary. *Objective. The aim of this work was to analyze the essential oil content and its composition in the drug (Serpylli herba) of wild thyme (Thymus serpyllum L.) originating from 20 different natural places of growth in Estonia.*

Material and methods. The quantitative content of essential oil was determined according to the method of European Pharmacopoeia. Gas chromatographic analysis of essential oils was carried out using a gas chromatography with flame ionization detector on two fused silica capillary columns with bonded stationary phases NB-30 and SW-10. The identification of the oil components was accomplished by comparing their retention indices on two columns with the retention indices values of standard compounds, with our retention indices data bank and with literature data. The results obtained were confirmed by gas chromatography / mass spectrometry.

Results. The content of essential oil is between 0.6 and 4 ml/kg and usually is not in conformity with European Pharmacopoeia standard (3 ml/kg). There were 55 components identified in the essential oil of wild thyme of Estonian origin. Differently from the data in the literature of foreign countries, thymol and carvacrol are not the main components of the essential oil of wild thyme growing in Estonia. The main components here are (E)-nerolidol, caryophyllene oxide, myrcene, (E)-β-caryophyllene and germacrene D.

Conclusion. In Estonia, the (E)-nerolidol–caryophyllene oxide, (E)-nerolidol–myrcene and myrcene chemotypes of wild thyme drug are distinguishable.

Introduction

Wild thyme (*Thymus serpyllum* L.) is regarded as a source of essential oil containing aromatic terpenes. Since 2003, the drug of wild thyme (*Serpylli herba*) belongs to the European Pharmacopoeia (EP) (1). The content of the essential oil in wild thyme drug varies in great extent depending on the origin of the plants, but it is between 0.1 and 0.6% (2), or the difference is larger to some extent – between 0.1 and 1% (3–4). The EP standard to the essential oil content is 3 ml/kg (~0.3%) (1). The components of the essential oil of wild Thyme were determined in the 1960s geraniol, terpineol, citronellol, borneol, linalol, nerolidol, citral, cineol (eucalypol), carvacrol, thymol, bornylacetat, geranylacetat, nerylacetat, linalylacetat, terpinylacetat, citronellal, camphen, α-pinen, and limonen (5). Nowadays, the main components are considered to be thymol, carvacrol, p-cymol, linalol, a-pinene and other terpenes (2).

The chemotypes of common thyme (*Thymus vulgaris* L.*)*, which is with the essential oil content quite close to wild thyme, are widely known. Depending on the source, six or even seven chemotypes are distinguished (6–7). The chemical content of the essential oil of large thyme (*Thymus pulegioides* L.) has been studied in Lithuania, and the occurrence of citral–geraniol chemotype and carvacrol chemotype was noticed (8). In the sense of chemotypes, wild thyme is not much studied.

As the main components of the essential oil of wild thyme, thymol (up to 30% in essential oil) and carvacrol (up to 20%) are named (2–3, 6). However, according to I. Rasooli and S. A. Mirmostafa (2002), there is more γ-terpinene (>22%) and p-cymene (>20%) in the essential oil of wild thyme. The authors thirdly mention thymol (>18%). By contrast, the content of carvacrol in the essential oil is insignificant (~1%) (9). While studying the populations of wild thyme in Alta region, Russia, it became clear that the content of the essential oil depends greatly on the altitude of the place of growth. The main components of a drug, gathered from 150 meters above the sea level, were *trans*-nerolidol (29.8%), 1,8-cineol (14%) and *cis*-β-terpineol (8.2%) and it contained less camphor,

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β-myrcene and p-cymol. However, in case of a drug, gathered from 500–700 meters above the sea level, carvacrol (29.6%), γ-terpinene (17.2%) and p-cymol (14.5%) dominated, and there was less 1,8-cineol (5.6%). It is interesting that in both cases, content of thymol was not higher than 2% (10).

As in many species in the thymus family, the essential oil content varies greatly; there is a reason to think that wild thyme could also have different chemotypes within one growing area.

In the present work, the possible occurrence of chemotypes of wild thyme growing wild in Estonia, were studied. Previously, the essential oil content in the drug and its accordance to the EP standards were studied.

Materials and methods

As the research material, the whole dried aerial part of wild thyme *(Serpylli herba)* was used. The samples were gathered in July and August of 2002 and 2003 by the pharmacy students from 20 different places of growth in Estonia. The plants were identified on the basis of macroscopic characteristics; the drug was dried in the temperature of 30–40°C.

The essential oil was isolated from dried plant material by the distillation method of the EP (1) using 50 g of cut drug, a 1000 ml round-bottomed flask, and 500 ml of water as the distillation liquid. The distillation time was 2 h at a rate of 2–3 ml/min. 0.2 ml of hexane was added to take up the essential oil.

The essential oils were analyzed using a Chrom-5 chromatograph with flame ionisation detector (FID) on two fused silica capillary columns (50 m × 0.20 mm i. d.) with stationary phases NB-30 and SW-10 (film thickness 0.25 μm). Carrier gas helium with the split ratio 1:150 and the flow rate 17–25 cm/sec was applied. The temperature program was from 50–250ţC (NB-30) and 70–230ţC (SW-10) at 2ţC/min, the injector temperature was 200ţC. A Hewlett-Packard Model 3390A integrator was used for data processing. The identification of the oil components was accomplished by comparing their retention indices (RI) on two columns with the RI values of standard compounds, with our RI data bank and with literature data. The results obtained were confirmed by gas chromatography/mass-spectrometry (GC/MS) (11, 12). Entire process of chromatographic analysis took from six to eight hours for one oil sample.

The quantitative composition of the oils was calculated in peak areas without using correction factors. The relative standard deviation (calculated by SPSS 10.0 for Windows) of percentages of oil components of repeated GC analyses of single oil sample did not exceed 5%.

The mass spectra of the oil components were recorded at 70 eV on Hewlett Packard GC/MS 5988A instrument, the mass range 30–350 amu, ionization energy 10–250 eV. The fused silica capillary column (10 m × 0.20 mm) with HP-17 as stationary phase was used. The oven temperature was from 50ţC to 250ţC at 8ţC/min. The injector temperature was 280ţC.

Results and discussion

In the samples studied, the essential oil content varied between 0.6 and 4 ml/kg. The average essential oil contents did not statistically differ in the drug samples gathered from the North-Estonian and West-Estonian coasts and from the Estonian inland. To some extent, the essential oil content is higher in the drugs gathered in August. Only one drug with the content of 4.0 ml/kg was in conformity with EP standard (at least 3 ml/kg). In 12 drugs, additionally studied by pharmacy student R. Reiman (13), the essential oil content reached only to one fourth or half compared to the EP standards (0.6–1.2 ml/kg). It is hard to say, whether wild thyme herb growing in Estonia should be considered of low quality, or the EP standard should be revised.

There were 55 components identified in the essential oils of wild thyme growing in Estonia (Table 1). In addition to those, there are *cis*-linalol oxide, *trans*-sabinene hydrate, *cis*- and *trans*-verbenon, borneol, hedycaryol, selina-3.7(11)-diene, ledol and α-farnesol tentatively identified. Concentration of separate components in the oil varies greatly depending on the places of growth of the plants. In many cases, the studied drugs contain one or other component only in traces, but in certain samples their content in essential oil ranges in tens of percents: for example, geranyl acetate (up to 46.4%), linalyl acetate (up to 31.4%), geraniol (up to 30.3%), and myrcene (up to 20.2%). The content of (E)-nerolidol (0.3–70.1%) varies the most; it ranges even by hundreds of times. If only the average content of the main components in the essential oil, which is the largest in case of (E)-nerolidol (24.87% on average) and caryophyllene oxide (11.29%) is considered, we could bring out two chemotypes. If in addition to percentual content also the values of variation coefficients (Table 1) are considered, then the changes in the content of myrcene, linalol, linalyl acetate, geraniol, thymol, carvacrol, geranyl acetate, (E)-β-caryophyllene, germacrene D, (E)-nerolidol and caryophyllene oxide should deserve more attention.

Table 1. **Composition of the essential oil of wild thyme (*Thymus serpyllum* L.) growing in Estonia**

Compound	RI NB-30	RI SW-10	Range, %	Average, % n=20	Variation coefficient
A-Pinene	929	1027	tr.–2.4	0.59	1.39
Camphene	943	1072	**tr.–7.3**	**1.70**	**1.34**
Sabinene	965	1123	0–0.6	0.20	1.05
1-Octen-3-ol	968	1436	tr.–3.0	1.02	1.00
and β-Pinene	970	1115			
Myrcene	984	1162	**tr.–20.2**	**5.65**	**1.20**
and 3-Octanol	986	1375			
α-Terpinene	1010	1178	0 - 0.3	0.05	1.60
p-Cymene	1013	1273	0–0.6	0.15	1.27
1,8-Cineole	1022	1211	**0.2–5.4**	**1.62**	**1.14**
and **Limonene**	1024	1204			
(Z)-β-Ocimene	1028	1221	0–0.3	0.07	1.43
(E)-β-Ocimene	1039	1240	tr.–3.7	1.23	1.04
γ-Terpinene	1050	1246	0–0.6	0.16	1.19
cis-Linalol oxide*	1055	1420	tr.–3.4	0.17	4.47
trans-Sabinene hydrate*	1058	1460	0–0.3	0.12	1.00
Terpinolene	1080	1281	0–0.2	0.06	1.17
Linalool	1089	1546	**0.4–22.8**	**3.38**	**1.53**
and α-Thujone	1090	1424			
β-Thujone	1098	1409	tr.–2.4	0.12	4.50
Camphor	1123	1500	**tr.–10.7**	**3.13**	**0.81**
cis-Verbenol*	1132		0–0.7	0.19	1.37
trans-Verbenol*	1150		0–0.3	0.07	1.43
Borneol	1156	1690	**tr.–6.4**	**1.53**	**1.30**
Terpinen-4-ol	1165	1592	tr.–1.5	0.52	0.58
α-Terpineol	1177	1692	**0.4–5.4**	**2.13**	**0.90**
Nerol	1217	1790	tr.–2.2	0.28	1.96
Geraniol	1242	1824	tr.–1.4	0.11	3.00
Linalyl acetate	1244	1543	**tr.–31.4**	**1.93**	**3.62**
Bornyl acetate	1274	1556	tr.–2.3	0.94	0.71
Thymol	1282	2153	tr.–2.9	1.07	0.70
Carvacrol	1292	2157	0.1–3.5	0.89	0.91
Neryl acetate	1345	1724	tr.–0.7	0.09	2.11
Geranyl acetate	1362	1732	**tr.–46.4**	**4.67**	**2.35**
α-Copaene	1373	1485	tr.–0.9	0.18	1.50
β-Bourbonene	1383	1510	tr.–2.0	0.48	1.13
β-Elemene	1385		0–0.6	0.12	1.33
(E)-β-Caryophyllene	1418	1589	**1.4–13.3**	**7.06**	**0.56**
(E)-β-Farnesene	1448	1662	0–0.5	0.09	1.67
α-Humulene	1450	1658	tr.–1.7	0.34	1.35
Alloaromadendrene	1456	1624	tr.–1.6	0.31	1.16
Germacrene D	1478	1673	**0.1–12.4**	**6.52**	**0.51**
Bicyclogermacrene	1489	1676	tr.–1.8	0.49	1.04
and α-Muurolene	1490	1712			
α-Farnesene	1497	1722	tr.–1.9	0.38	1.47
β-Bisabolene	1500	1736	tr.–2.5	0.89	0.76
γ-Cadinene	1502	1746	**tr.–5.2**	**0.48**	**2.35**
δ-Cadinene	1518	1748	0.1–2.8	0.83	0.82
Hedycaryol*	1535	2077	0–0.5	0.12	1.17
Selina-3,7(11)-diene*	1540		tr.–2.4	0.65	1.05
Germacrene B	1550	1800	0–0.8	0.22	1.14
(E)-Nerolidol	1554	2035	**0.3–70.1**	**24.87**	**0.75**
Spathylenol	1572	2104	**tr.–5.1**	**1.60**	**0.89**
and Germacren-4-ol	1576	2015			
Caryophyllene oxide	1578	1972	**0.3–45.0**	**11.29**	**0.97**
Viridiflorol	1587	2041	tr.–1.4	0.18	1.83
Ledol*	1598	2100	tr.–1.8	0.44	0.95
T-Cadinol	1632	2156	tr.–3.9	0.84	1.35
α-Cadinol	1644	2218	tr.–2.2	0.93	0.85
α-Bisabolol	1677	2206	**tr.–6.4**	**1.16**	**1.70**
α-Farnesol*	1700	2200	tr.–3.2	0.45	2.13
Yield of oil			0.6–4.0	1.34	0.60

tr. – trace (<0.05%); 0 – not determined; * tentatively identified.

Based on the literature, thymol and carvacrol can be hardly considered as the main components due to their low content. In the essential oil in wild thyme growing in Estonia: 0–2.9% and 0.1–3.5% correspondingly, and in 10 samples there was less than 1% of thymol, and in 14 samples there was less than 1% of carvacrol (Table 2). By contrast, we consider (E)-nerolidol (up to 70.1%), caryophyllene oxide (up to 45.0%), myrcene (up to 20.2%), (E)-β-caryophyllene (up to 13.3%), and germacrene D (up to 12.4%) as the main components of the essential oil of wild thyme growing in Estonia. In a few samples, there is relatively high content of geranyl acetate (only in one case 46.4%), linalyl acetate (in one case 31.4%), linalol (higher contents 4.4, 10.7 and 22.8%), etc.

Among the essential oils of the drugs analyzed, the (E)-nerolidol chemotype is clearly distinguishable; its content dominates in 12 cases out of 20. It is possible to distinguish 2 types of oils rich in (E)-nerolidol: in some of them, besides (E)-nerolidol, caryophyllene oxide dominates (in 7 out of 12 oils); in the rest of the oils, it was myrcene to a significant extent. So as chemotypes we could more exactly point out (E)-nerolidol – caryophyllene oxide and (E)-nerolidol – myrcene. There are also samples, which show relatively high content of myrcene and very low content of (E)-nerolidol, but at the same time, it contains relatively high concentration of (E)-β-caryophyllene. That gives us reason to consider the occurrence of the mycrene chemotype to be possible. In addition to the ones mentioned above, our analysis reveals some single drugs with untypical essential oil content. For example, in a few drugs, caryophyllene oxide (45.0 and 17.7%) dominates. From the aspect of content, also α-bisabolol and germacrene D deserve to be mentioned, but there is very little content of (E)-nerolidol. Wild thyme herb, which contains relatively high amount of linalyl acetate (31.4%) and linalol (22.8%), is also exceptional. Thirdly, a drug rich in (E)-nerolidol and with high content of geranyl acetate could be mentioned. The essential oil content of wild thyme herb growing in Estonia seems to differ from the essential oil contents of the drugs analyzed elsewhere and mentioned in the literature sources. This, and also the

Table 2. **Content of the main components, thymole and carvacrole in the essential oil of wild thyme (*Thymus serpyllum* L.) growing in Estonia**

No	Essential oil content, ml/kg	Myrcene, %	Thymol, %	Carvacrol, %	(E)-β-Caryophyllene, %	Germacrene D, %	(E)-Nerolidol, %	Caryophyllene oxide, %
1	0.6	1.4	0	0.1	8.6	**11.2**	1.7	1.6
2	0.8	0.1	0.2	0.1	**7.0**	6.6	**30.1**	**24.0**
3	2.5	0.8	0.5	0.3	3.8	3.3	**52.0**	**4.2**
4	0.9	0	1.9	0.9	4.6	**5.9**	**4.8**	**45.0**
5	1.1	0	0.8	0.5	3.4	4.3	**32.9**	**25.0**
6	1.5	0.2	2.9	1.7	3.1	3.8	**49.5**	**11.2**
7	0.9	0.6	1.0	0.5	3.5	1.7	**27.6**	**20.8**
8	1.0	0	1.0	0.3	1.8	**2.8**	**70.1**	**4.5**
9	4.0	0.3	0.5	0.3	5.7	5.3	**20.5**	**6.4**
10	1.2	0	0.9	0.3	**13.3**	6.8	**28.4**	**16.4**
11	0.6	**11.2**	0.9	0.6	**11.0**	3.3	4.2	**10.8**
12	1.2	0.4	2.9	3.5	**13.2**	**12.4**	2.7	**17.7**
13	1.8	**8.6**	1.4	2.0	4.6	**7.9**	**24.3**	2.5
14	0.7	**20.2**	0.8	0.7	9.4	**10.2**	**15.2**	2.2
15	0.8	**6.4**	1.0	0.8	5.4	**7.2**	**34.5**	5.7
16	1.5	**18.6**	1.3	1.3	**13.0**	**11.4**	2.2	8.2
17	2.1	**15.3**	1.0	1.1	5.4	**6.2**	**33.1**	1.5
18	1.5	**10.7**	0.5	0.5	2.2	3.1	**30.0**	1.4
19	1.4	6.9	0.8	0.8	**11.7**	**10.5**	**27.6**	7.4
20	0.6	**10.5**	1.1	1.5	**10.5**	**11.2**	6.0	9.2

0 – not determined.

large variations in the essential oil content depending on the place of growth, can be considered to be in connection with Estonia's geographical situation towards the sea and with the varying relief of Estonian inland.

Conclusions

The herb of wild thyme growing in Estonia usually is not in conformity with European Pharmacopoeia standards in the aspect of the essential oil content. In the essential oils of wild thyme from different natural places of growth in Estonia, 55 components were identified. The contents of those components varies tens or even hundreds times in different drugs.

Differently from the data of foreign countries, thymol and carvacrol are not the main components of wild thyme growing in Estonia. The main components here in Estonia are (E)-nerolidol, caryophyllene oxide, myrcene, (E)-β-caryophyllene and germacrene D.

In Estonia, it is possible to distinguish between different chemotypes of wild thyme herb – the chemotypes of (E)-nerolidol–caryophyllene oxide, (E)-nerolidol–myrcene and myrcene.

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Thymus serpyllum L., augančio Estijoje, eterinio aliejaus tyrimas

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Raktažodžiai: *Thymus serpyllum* L., eterinio aliejaus sudėtis, chemotipai.

Santrauka. *Darbo tikslas*. Ištirti eterinio aliejaus sudėtį paprastojo čiobrelio (*Thymus serpyllum* L.) augalinėje žaliavoje (*Serpylli herba*), surinktoje iš skirtingų natūralių augaviečių Estijoje.

Medžiagos ir metodai. Eterinio aliejaus kiekio nustatymas atliktas pagal Europos Farmakopėjos metodą. Eterinio aliejaus dujų chromatografinė analizė atlikta naudojant dujų chromatografijos jonizacinės liepsnos detektorių, dvi sujungtas silicio kapiliarų kolonėles su skirtingomis stacionariomis fazėmis NB-30 ir SW-10. Aliejaus komponentų identifikacija atlikta lyginant jų sulaikymo trukmės rodiklius dviem kolonėlėms su šių rodiklių standartinių junginių reikšmėmis, su mūsų nustatytų sulaikymo trukmės duomenų banku bei literatūros duomenimis.

Rezultatai. Eterinio aliejaus kiekis žaliavoje svyruoja nuo 0,6 iki 4 ml/kg ir kai kurių bandinių neatitinka Europos Farmakopėjos standarto (3 ml/kg). Estijoje surinkto paprastojo čiobrelio eteriniame aliejuje identifikuoti 55 komponentai. Skirtingai nuo užsienio šalių literatūros duomenų timolis ir karvakrolis nėra pagrindiniai paprastojo čiobrelio, augančio Estijoje, eterinio aliejaus komponentai. Pagrindiniai eterinio aliejaus komponentai: (E)-nerolidolis, kariofileno oksidas, mircenas, (E)-β-kariofilenas ir germakranas D.

Išvados. Iš Estijoje surinkto paprastojo čiobrelio žaliavos išskirti chemotipai: (E)-nerolidolio – kariofileno oksido, (E)-nerolidolio – mirceno ir mirceno.

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