Chemical diversity of essential oil of *Thymus pulegioides* L. and *Thymus serpyllum* L. growing in Lithuania

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Twenty five samples of *Thymus pulegioides* L. and 13 samples of *Thymus serpyllum* L. were collected from 18 localities in Lithuania and the composition of their essential oil was analysed by capillary gas chromatography and mass spectrometry. Five chemotypes of *T. pulegioides* have been defined: (1) thymol; (2) carvacrol; (3) thymol/carvacrol; (4) citral/geraniol; (5) linalool. Six chemotypes of *T. serpyllum* have been defined: (1) 1,8-cineole; (2) E-carvyl acetate; (3) E-*β*-ocimene; (4) caryophyllene oxide; (5) *β*-caryophyllene; (6) *cis*-*p*-ment-2-en-1-ol.

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

INTRODUCTION

Two *Thymus* species, *T. pulegioides* and *T. serpyllum,* grow in the wild in Lithuania [1]. *T. pulegioides* can accumulate up to 1.21% of essential oil [2], while *T. serpyllum* produces considerably lower amount of oil (up to 0.58%) [3].

In southern countries *T. serpyllum* accumulates high amounts of thymol: 42.63% (in Pakistan) [4], 64.6% (in India) [5], 81.5% (in Armenia) [6]. However, in colder climatic areas such as Scandinavian countries, Siberia, *T. serpyllum* synthesises only negligible amounts of thymol [7] or does not at all [8]. The absence of thymol was reported in three different phenotypes of *T. serpyllum* collected in Western Lithuania (Kuršių Nerija, Juodkrantė) [3].

Chemical polymorphism is characteristic of *T. pulegioides* as well as of many other *Thymus* species. For instance, 5 chemotypes were described in Slovakia, (fenchone, linalool, citral/geraniol, thymol and carvacrol [9]). Thymol (to 37.24%) and carvacrol (to 35.24%) chemotypes were described as exceptional in Norway [10]. Four chemotypes of *T. pulegioides* were described up to now in Lithuania (Vilnius region): α-terpinyl acetate [11], citral/geraniol [12, 13], carvacrol [12,13], and thymol [12].

The present study presents the results of essential oil variability and chemical polymorphism of *T. pulegioides* and *T. serpyllum* collected in Lithuania.

MATERIALS AND METHODS

Aerial parts of 25 samples of *T. pulegioides* (from 11 different localities of Lithuania) and 13 samples of *T. serpyllum* (from 7 different localities) were collected at the flowering phase in July of 1995–1998.

Voucher specimens have been deposited in the Herbarium of the Institute of Botany, Vilnius, Lithuania. The samples were dried at room temperature and used for analysis. The essential oils were isolated by hydrodistillation in a European Pharmacopoeia apparatus during two hours.

The essential oils were diluted in diethyl ether (20 µl in 1 ml) and analysed with a Fisons 8261 gas chromatograph with a flame ionisation detector (FID) on a fused silica capillary column DB-5, 25 m, i.d. 0.32 mm, film thickness 0.5 µm. Helium was used as a carrier gas with a flow rate of 1.6 ml/min; detector temperature was 260 °C, oven temperature was programmed from 40 °C to 250 °C at the rate of 4 °C/min. The split injector was heated at 250 °C, split ratio was 15:1. The data were processed on a DP 800 integrator.

For identification, essential oils were also analysed on a HP 5890 (II) instrument equipped with a 5971 series mass selective detector in the electron impact ionisation mode at 70 eV and the following GC parameters: split inlet 1:10; helium as carrier gas at a flow rate of 2 ml/min; fused silica HP5 MS column (Hewlett Packard, crosslinked 5% phenyl methyl silicone) 30 m long, 0.25 mm id, 0.25 µm film thickness, temperature program from 40 to 250 °C increasing at 4 °C/min.

RESULTS AND DISCUSSION

Totally 86 compounds have been identified in essential oil of *T. pulegioides* by capillary GC and coupled GC/MS. Five main groups of the samples were observed according to the major constituents in the essential oil. The following chemotypes of *T. pulegioides* have been defined: (1) thymol – 3 samples (T); (2) carvacrol – 8 samples (C); (3) thymol/carvacrol – 3 samples (T/C); (4) citral/geraniol – 10 samples (C/G); (5) linalool – 1 sample (L). The range of the content of the quantitatively major compounds in the chemotypes is presented in Table 1. The content of thymol was to 30.91% (in plants of thymol chemotype), carvacrol to 32.76% (in samples of carvacrol chemotype). The T/C chemotype can

Table 1. **Variation intervals in the content of the main constituents in various *Thymus pulegioides* L. chemotypes (% in the essential oil)**

Compound	Thymol	Carvacrol	Thymol/Carvacrol	Citral/Geraniol	Linalool
Myrcene	1.14–1.50	0.1–1.57	1.11–1.27	0.00–1.07	0.23
p-Cymene	10.21–11.36	7.52–27.43	13.40–16.03	0.00–0.12	0.07
γ-Terpinene	8.70–9.89	11.06–30.55	8.02–20.70	0.00–1.44	0.04
Linalool	0.00–0.05	0.00–0.65	0.00–0.58	0.11–0.72	**80.29**
cis-p-Menth-2-en-1-ol	0.00–2.80	0.00–3.87	0.00–5.13	0.00–10.76	–
Thymol methyl ether	4.94–11.84	0.00–1.49	2.53–3.04	–	–
Carvacrol methyl ether	2.65–4.29	3.74–8.61	3.03–4.27	–	0.13
Nerol	–	–	–	**0.84–18.89**	–
Neral	–	0.00–1.95	–	**0.00–17.37**	–
Geraniol	0.28–1.39	0.00–1.23	0.00–2.75	**2.52–43.78**	–
Geranial	–	–	–	**11.39–29.36**	–
Thymol	**26.05–30.91**	0.03–9.82	**11.77–14.20**	0.00–0.23	–
Carvacrol	0.73–1.51	**5.85–32.76**	**12.40–22.61**	0.00–1.90	–
β-Caryophyllene	10.13–10.27	5.21–11.95	5.05–7.99	2.23–15.76	5.68
Germacrene D	0.76–2.24	0.31–3.13	0.63–1.23	0.41–5.63	2.67
β-Bisabolene	3.31–4.69	2.57–6.40	4.73–8.99	3.53–12.8	1.37
Caryophyllene oxide	1.08–1.15	0.59–1.59	0.25–1.20	1.03–4.86	0.52

Table 2. **Variation intervals in the content of the main constituents in various *Thymus serpyllum* L. chemotypes (% in the essential oil)**

Compound	1,8-Cineole	E-Carvyl acetate	E-β-Oci-mene	Caryophyllene oxide	β-Caryophyllene	*Cis-p*-Ment-2-en-1-ol
α-Pinene	2.34–3.44	2.59	–	–	0.95–2.00	2.88
Camphene	2.78–5.52	3.18	3.13	0.00–0.23	3.6	9.50
Sabinene	1.32–1.53	1.63	–	–	0.09–0.18	–
β-Pinene	2.31–2.83	2.42	–	–	0.42–0.74	0.62
Myrcene	5.57–9.68	12.15	5.66	0.00–0.42	9.10–15.85	7.30
1,8-Cineole	**29.50–30.32**	13.87	–	0.93–1.63	0.00–0.74	–
E-β-Ocimene	0.36–3.29	0.80	**34.78**	–	1.10–2.87	0,74
Linalool	0.12–2.02	1.26	–	0.90–3.03	0.00–1.47	1.85
Cis-p-Ment-2-en-1-ol	0.00–0.09	–	1.47	0.00–0.14	0.00–6.52	**24.06**
Camphor	1.22–5.6	0.90	–	9.46–12.08	2.35–6.23	4.93
Borneol	0.34–3.17	0.83	4.10	4.67–27.05	0.71–4.28	6.02
E-Carvyl acetate	0.00–0.15	**21.58**	–	–	–	–
α-Copaene	0.08–0.16	–	8.68	0.13–0.54	0.12–0.13	0.13
β-Bourbonene	0.56–1.21	0.47	-	1.22–2.02	0.68–0.72	2.00
β-Caryophyllene	4.52–16.09	5.43	2.35	0.17–1.05	**23.32–27.18**	3.41
Germacrene D	3.17–13.75	3.82	–	0.00–0.38	13.5–20.38	3.01
β-Bisabolene	0.43–0.72	0.47	–	–	6.66–8.46	3.76
Caryophyllene oxide	2.31–6.06	2.29	–	**25.43–27.15**	0.11–4.31	13.83
epi-α-Cadinol	0.18–1.96	4.66	1.63	2.24–14.13	0.09–0.67	–

be considered as an intermediate type, because the content of thymol and carvacrol is similar (Table 1). The samples of C/G chemotype had four components (nerol, neral, geraniol and geranial) characteristic of this chemotype. The essential oil composition of L chemotype of *T. pulegioides* was the simplest one in terms that it contained an extremely high content of linalool (>80%), while the percentage of this compound in the other samples did not reach 1%. It is worth mentioning that a very high content of linalool (54.8%) was determined in L chemotype of *T. pulegioides* samples from Slovakia [9].

Totally, 53 compounds have been identified in the essential oil of *T. serpylum* by capillary GC and coupled GC/MS. Thymol, carvacrol, neral, nerol, geranial and geraniol were not detected in any of the samples of *T. serpyllum* analysed. Six main groups of the samples were observed according to the major constituents in the essential oil. The following chemotypes of *T. serpyllum* have been defined: (1) 1,8-cineole – 2 samples; (2) E-carvyl acetate – 1 sample; (3) E-β-ocimene - 1 sample; (4) caryophyllene oxide – 2 samples; (5) β-caryophyllene – 2; (6) *cis-p*-ment-2-en-1-ol – 1 sample. The range of the content of the major quantitatively compounds in the defined chemotypes is presented in Table 2.

Under Lithuanian climatic conditions *T. pulegioides* accumulates more essential oil with more components in it than in *T. serpyllum*. Some chemotypes of *T. pulegioides* accumulate pharmacologically important components such as thymol and carvacrol. Meanwhile, these components were not found in *T. serpyllum* growing under the local climatic conditions.

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***THYMUS PULEGIOIDES* L. IR *THYMUS SERPYLLUM* ETERINIŲ ALIEJŲ CHEMINĖ ĮVAIROVĖ LIETUVOJE**

S a n t r a u k a

Ištirta 25 *Thymus pulegioides* ir 13 *T. serpyllum* pavyzdžių, surinktų įvairiose Lietuvos vietose, eterinių aliejų cheminė sudėtis. *T. pulegioides* rūšyje buvo išskirti 5 chemotipai: timolio (šio komponento eteriniame aliejuje gali susikaupti iki 30,91%), karvakrolio (iki 32,76%), timolio-karvakrolio, citralio-geraniolio ir linalolo (iki 80,29%). *T. serpyllum* rūšyje buvo išskirti 6 chemotipai: 1,8-cineolio (iki 30,32%), E-carvilo acetato (gali susikaupti iki 21,58%), E-β-ocimeno (iki 34,78%), kariofileno oksido (iki 27,15%), β-kariofileno (iki 27,18%) ir *cis-p*-ment-2-en-1-olio (iki 24,06%).

Mūsų klimatinėmis sąlygomis augantys *T. pulegioides* sukaupia daugiau eterinio aliejaus, pasižyminčio įvairesne sudėtimi, nei *T. serpyllum*. Kai kurie *T. pulegioides* chemotipai kaupia timolį ir karvakrolį – farmakologiškai svarbius komponentus. Tuo tarpu mūsų klimatinėse sąlygose augančiuose *T. serpyllm* augaluose šių komponentų neaptinkama.