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PHARMACY

Comparative analysis of content and composition of essential oils of *Thymus vulgaris* L. from different regions of Europe

Ain Raal[a*], Tetiana Gontova[b], Maie Palmeos[a], Anne Orav[c], Galiya Sayakova[d] and Oleh Koshovyi[a,b]

[a] Institute of Pharmacy, Faculty of Medicine, University of Tartu, Nooruse 1, 50411 Tartu, Estonia
[b] National University of Pharmacy, Pushkinska 53, 61002 Kharkiv, Ukraine
[c] Institute of Chemistry, Tallinn University of Technology, Akadeemia tee 15, 12618 Tallinn, Estonia
[d] Asfendiyarov Kazakh National Medical University, Tole Bi 94, 050000 Almaty, Kazakhstan

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Abstract. Common thyme (*Thymus vulgaris* L., Lamiaceae) is one of the most well-known plants in the world, widely used in medicine, culinary arts, and cosmetics. The aim of this study was to analyse the content and composition of essential oils (EO) obtained from the *T. vulgaris* commercial samples grown in Estonia and in various European countries. Gas chromatography was used to characterize the quality and diversity of chemotypes of the studied plant raw material of different origins. The samples of the *T. vulgaris* herb contained 3–28 mL/kg of EO, and in five samples it was below the European Pharmacopoeia (Ph. Eur.) requirement (minimum 12 mL/kg). In total, 44 EO compounds were identified in the essential oils of the studied *T. vulgaris* samples. Among them, five principal compounds were carvacrol (2.3–87.5%), thymol (0.9–71.2%), p-cymene (0.3–26.0%), γ-terpinene (0.1–16.1%), and (E)-β-caryophyllene (0.6–9.2%). It was concluded that the main compound of *T. vulgaris* EO (n = 37) was thymol with an average content of 41.1%. Monoterpenoids (90.5%) and sesquiterpenoids (5.7%) were the most dominating groups of terpenoids in the studied EO. Regarding the minimum and maximum content of key compounds in EO, none of the studied samples (n = 22) fully met the requirements of Ph. Eur. 11. The content of the four most important terpenoids (thymol, carvacrol, p-cymene, and γ-terpinene) in the EO of *T. vulgaris* does not change much from the beginning to the end of the flowering period. Correlations between the content of the most important (>2%) components (n = 14) of the studied EO (n = 37) showed a level >0.9 in several cases. Among seven studied chemotypes of *T. vulgaris* EO, five contained thymol as one of the main components.

Keywords: common thyme, gas chromatography, chemotype, thymol, carvacrol, p-cymene, γ-terpinene.

INTRODUCTION

Thymus vulgaris L. from the Lamiaceae family is widely used in various fields of medicine, nutrition, cosmetology, and food and other industries. It is an evergreen semi-shrub wildly distributed in the Mediterranean, south of France, northwest of Italy, the Iberian Peninsula, and the Balearic Islands [1] and cultivated in several countries as a medicinal herb and an essential oil (EO) and spice plant [2]. Phenolic complexes containing flavonoids, phenolic acids (ursolic, caffeic), and EO are obtained from the herb *T. vulgaris* on an industrial scale [3,4]. According to the requirements of the European Pharmacopoeia (Ph. Eur.) 11, the minimum content of EO in *T. vulgaris* should be 12 mL/kg [5].

T. vulgaris is widely used in medicine due to its expectorant, antitussive, antispasmodic, anthelmintic, carminative, and diuretic properties [6–9]. The EO of *T. vulgaris* shows antispasmodic, anti-inflammatory, antifungal [10], antiviral, cytotoxic, antioxidant [6,11–13], and antimicrobial properties and is used as a natural antiseptic in the pharmaceutical and food industries [10,14,15].

[*] Corresponding author, ain.raal@ut.ee

The monoterpenes thymol and carvacrol are effective against food pathogens, such as *Salmonella* spp. and *Staphylococcus aureus* [16,17]. Therefore, *T. vulgaris* EO can protect packaged food products [12]. Thymol is a species-specific monoterpene of species of the genus *Thymus* and may be effective alone [4,18]. It has a more significant potential for use in combination with conventional antibiotics, reducing the minimum therapeutic amount of the latter [4]. Carvacrol is a flavouring agent in baked goods, sweets, beverages, and chewing gum. The compounds accumulate in cell membranes and cause intracellular leakage of adenosine triphosphoric acid and potassium ions, which ultimately leads to the death of the microorganism. Thymol and carvacrol can be used as pesticides because natural monoterpenes break down relatively quickly and are almost completely eliminated from living organisms after 24 hours. The half-life of thymol is 16 days in water and 5 days in soil [19,20]. Thus, it is important to study the level of thymol and carvacrol in *T. vulgaris* samples from different origins with a focus on the chemotypes of the oil.

The purpose of the work was to compare the content and composition of EO samples from the herb *T. vulgaris* harvested in Estonia and other European countries, to investigate the variation in the content of biologically active components from the phase of plant development, to establish the qualities of the EO compared to the requirements of Ph. Eur. 11, and to determine the chemotypes of *T. vulgaris*.

MATERIALS AND METHODS

Plant material

The plant material (samples 1–22) of *T. vulgaris* was obtained from local Estonian sources and commercial samples from various origins (Table 1). The raw material had been dried indoors at room temperature. Samples 23–37 were obtained from community pharmacies or health shops from different countries: 23, 29, 35 – Estonia; 24 – Lithuania; 25 – Latvia; 26 – Spain; 27 – Italy; 28, 30 – Germany; 31 – Czechia; 32 – England; 33 – Ukraine; 34 – Austria; 36 – Turkey; 37 – Georgia. Voucher specimens of Estonian samples (#Lam/Thy16–17) with commercial *T. vulgaris* samples have been identified and deposited at the Institute of Pharmacy, University of Tartu, Estonia.

Isolation of essential oil

EO was isolated from the dried raw material of *T. vulgaris* by the distillation method described in Ph. Eur. 11 using 30 g of crushed raw material, a 1000 mL round-bottomed flask, and 400 mL of water as the distillation liquid. The distillation time was 2 h at a rate of 3–4 mL/min.

Capillary gas chromatography

Gas chromatography (GS) analysis used a Chrom 5 chromatograph with FID on two fused silica capillary columns with bonded stationary phases SPB-5 (30 m × 0.25 mm, Supelco) and SW-10 (30 m × 0.25 mm, Supelco). The film thickness of both stationary phases was 0.25 µm. Carrier gas was He with a split ratio of 1:150, and the flow rate of 30–35 cm/s was applied. The temperature programme was 50–250 °C at 2 °C/min, and the injector temperature was 200 °C. A Hewlett-Packard Model 3390A integrator was used for data processing.

The oil components were identified by comparing their retention indices (RI) on two columns with the RI values of reference standards, our RI data bank, and the literature data [21–23]. Gas chromatography/mass spectrometry (GC/MS) confirmed the results obtained. The percentage composition of the EO was calculated in peak areas (nonpolar column) using the normalization method without correction factors. The relative standard deviation of percentages of oil components of three repeated GC analyses of a single oil sample did not exceed 5%.

Statistics

GC results were analysed using IBM SPSS Statistics to obtain correlation coefficients. The correlation coefficient, or Pearson's coefficient, measures the linear relationship between two variables, giving a value between +1 and –1. At the same time, 1 is an indicator of full positive correlation, 0 means no correlation, and –1 is a negative correlation. This means that, for example, for a correlation coefficient of 1, all data points lie on a straight line with a positive slope [5,24,25].

RESULTS AND DISCUSSION

Content of essential oil

Samples 2, 4, and 21 had the highest EO content (26–28 mL/kg; Table 1). Among the three analysed samples of Greek origin, two accumulated EO in significant quantities; out of 19 samples from Estonia, 14 met the requirements of Ph. Eur. 11. Both samples (15,16) of *T. vulgaris* cultivated under unnatural conditions in an apartment contained EO below the Ph. Eur. 11 standard norm (minimum 12 mL/kg).

Table 1. Content of essential oil in the samples of *T. vulgaris* and compliance with the requirements of Ph. Eur. 11

Sample	Origin	Content of essential oil per 1 kg of the drug (mL/kg)	Compliance with the requirements of Ph. Eur. 11 (minimum 12 mL/kg)
1	Kubja farm commercial herb	14	satisfactory
2	Loodusravi farm commercial herb	26	satisfactory
3	Energia farm fresh plants (beginning of flowering)	19	satisfactory
4	Energia farm fresh plants (full bloom)	27	satisfactory
5	Energia farm fresh plants (end of flowering)	24	satisfactory
6	Vadi farm commercial herb	20	satisfactory
7	Santa Maria commercial spice	23	satisfactory
8	Meira commercial spice	13	satisfactory
9	Greek commercial herb (Peloponnesus)	3	unsatisfactory
10	Greek commercial herb (Metoxi)	18	satisfactory
11	Rimi market commercial herb	13	satisfactory
12	Tartu open food market I fresh plants	13	satisfactory
13	Tartu open food market II fresh plants	9	unsatisfactory
14	Karepa farm	7	unsatisfactory
15	City apartment fresh plants (seeds from Suvipiha)	4	unsatisfactory
16	City apartment fresh plants (Puutarhurin)	9	unsatisfactory
17	Greek commercial herb (Nikn)	19	satisfactory
18	Hansa Herbs fresh plants	12	satisfactory
19	Grüne Fee fresh plants	13	satisfactory
20	Polish commercial herb (Kotanyi)	20	satisfactory
21	Energia farm commercial herb I	28	satisfactory
22	Energia farm commercial herb II	22	satisfactory

Composition of essential oil

A total of 44 substances were identified in the *T. vulgaris* EO samples 1–22 (Table 2). The five principal compounds of oils were carvacrol (2.3–87.5%), thymol (0.9–71.2%), p-cymene (0.3–26.0%), γ-terpinene (0.1–16.1%), and (E)-β-caryophyllene (0.6–9.2%). It is interesting to note for comparison that the EO of *T. Serpyllum*, another species of the same plant family, collected from 33 different growing places in Estonia, contained both thymol and carvacrol only up to 4% [26]. Conversely, the concentration of thymole in the *T. serpyllum* EO from Ukraine was 53% [27].

Among the identified substances in most samples, thymol, p-cymene, α-terpinene, γ-terpinene, linalool, borneol, carvacrol, β-myrcene, and (E)-β-caryophyllene were present, which impart a specific aroma to EO and exhibit various pharmacological effects. Considerable interest in thymol-based pharmaceuticals, cosmetics, and nutraceuticals is due to studies evaluating the potential therapeutic use of this compound in treating disorders affecting the respiratory, cardiovascular, and nervous systems, diabetes, and human neurodegenerative diseases. In addition, thymol exhibits antioxidant, immunomodulating, anticarcinogenic, anti-inflammatory, and antispasmodic activity [4,11,28–30]. Results from in vitro and in vivo studies show that carvacrol possesses a variety of biological and pharmacological properties, including antioxidant, antibacterial, antifungal, anticancer, anti-inflammatory, hepatoprotective, spasmolytic, and vasorelaxant [31]. Carvacrol and/or thymol protect kidneys against γ-irradiation-induced acute radiotherapy nephropathy, which might be attributed to their antioxidative, anti-inflammatory, and antiapoptotic activities [32]. A monocyclic monoterpene p-cymene is an alkyl-substituted aromatic compound that occurs naturally in the EO of aromatic plants, including the genus *Thymus* and *Origanum* [33].

Numerous studies have demonstrated the pharmacological properties of p-cymene, including antioxidant [34], anti-inflammatory [35], antiparasitic, anticancer [33], antidiabetic [36], antiviral, antitumour, antimicrobial [34], antibacterial, and antifungal activities. p-Cymene has also been reported to act as an analgesic, antiulcer [37], antinociceptive [33], immunomodulatory [37], vasorelaxant,

Table 2. Content of essential oil compounds in *T. vulgaris* samples 1–22

Compound	RI		Content (%)														
	SPB-5	SW-10	1*	2	3–5, 21–22	6	7	8	9	10	11	12–13	14	15–16	17	18	19	20
α-Thujene	922	1024	0.1	0.4	0.5–0.9	0.2	0.3	tr	0.1	0.3	0.3	0.4	tr	–	tr	0.1	0.4	0.3
α-Pinene	927	1021	0.3	0.6	0.4–1.1	0.6	0.9	0.1	0.5	0.4	0.3	0.4	tr	–	0.2	0.1	0.2	0.7
Camphene	940	1065	0.2	0.3	0.2–0.5	0.3	0.6	tr	0.3	tr	0.2	0.3–0.4	tr	–	0.1	tr	0.2	0.4
β-Pinene	968	1105	0.1	0.2	0.1–0.3	0.1	0.2	tr	tr	tr	0.1	0.1–0.2	tr	–	tr	0.2	0.1	0.1
1-Ocene-3-ol	976	1452	0.6	1.1	0.5–0.7	0.2	0.9	0.8	0.2	0.4	0.8	0.7–0.8	1.0	0.3–0.5	0.2	1.5	1.1	1.3
β-Myrcene	988	1160	0.5	1.2	1.3–2.3	1.0	0.9	0.1	0.9	1.6	1.3	1.3–1.4	0.5	–	0.2	0.9	1.4	0.6
α-Phellandrene	1002	1160	0.1	0.1	0.2–0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	–	tr	0.1	0.2	tr
Δ-3-Karene	1005	1140	tr	0.1	0.1	0.1	0.1	tr	0.1	0.1	0.1	0.1	tr	–	tr	0.1	0.1	0.1
α-Terpinene	1012	1173	0.5	1.0	1.5–2.1	1.3	0.9	0.1	0.7	1.4	1.4	1.4–1.6	0.6	tr	0.1	1.2	1.6	0.6
p-Cymene	1019	1265	10.6	21.1	4.5–7.5	10.2	26.0	5.0	6.0	11.4	9.3	14.3–14.4	4.8	0.3–0.4	5.1	6.3	3.9	24.7
Limonene	1022	1200	0.2	0.5	0.3–0.4	0.4	0.6	0.1	0.3	0.3	0.4	0.5	0.2	tr	0.1	0.2	0.3	0.3
1,8-Cineol	1024	1208	0.4	0.5	0.2–0.3	0.4	0.6	0.3	tr	0.2	0.2	0.2–0.4	0.1	0.2	tr	0.1	0.1	0.6
(Z)-β-Ozimene	1036	1235	–	tr	tr–0.1	–	tr	–	–	–	tr	tr	–	–	–	tr	tr	tr
(E)-β-Ozimene	1044	1250	tr	tr	0.1	tr	tr	–	tr	tr	0.1	0.1	tr	–	–	0.1	0.1	tr
γ-Terpinene	1053	1245	3.3	7.9	9.9–15.9	7.9	4.9	0.2	1.9	4.6	14.2	15.7	5.5	0.1–0.2	0.1	11.0	13.9	2.2
(Z)-Sabinene hydrate	1062	1463	0.4	0.7	0.5–0.8	0.3	0.4	0.5	0.1	0.1	1.1	1.1	1.2	1.1–1.5	–	1.8	1.6	0.4
Terpinolene	1082	1280	0.1	0.1	0.1–0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	–	tr	0.1	0.1	tr
(E)-Sabinene hydrate	1095	1544	tr	0.1	tr–0.1	tr	0.1	0.1	–	tr	0.1	0.1–0.2	tr	0.2–0.3	–	0.1	0.1	tr
Linalool	1100	1548	1.7	2.5	1.7–2.2	1.8	2.7	2.9	0.9	0.8	2.0	2.4–2.8	2.1	2.0–2.6	0.4	2.2	1.9	2.4
Borneol	1160	1695	0.4	0.6	tr–0.5	0.6	1.1	1.6	0.9	0.1	0.8	1.0–1.4	0.6	2.4–2.6	0.5	1.1	1.2	0.9
Terpinen-4-ol	1172	1595	0.6	0.5	0.1–0.8	0.7	0.6	1.2	0.7	0.7	0.5	0.5–0.6	0.5	0.2	tr	0.3	0.1	0.4
α-Terpineol	1192	1702	0.4	0.3	tr–0.4	tr	0.3	0.2	tr	0.1	0.1	0.1	tr	tr–0.2	0.1	0.1	0.1	0.2
Verbenone	1200	1710	0.2	0.1	tr–0.1	0.1	0.2	0.3	0.1	tr	0.1	0.1	0.1	–	–	–	–	–
Thymol methyl ether	1231	1584	0.5	0.4	0.1–0.3	0.5	0.8	0.9	tr	tr	0.2	0.4–0.8	tr	0.7–0.8	0.1	0.3	0.2	0.7
Carvacrol methyl ether	1239	1590	0.4	0.4	0.2–0.5	0.5	0.6	0.6	tr	0.1	0.2	0.5–0.7	tr	1.5–1.7	–	0.8	0.7	0.6
Thymol	1295	2172	66.2	48.2	53.9–63.0	61.8	45.8	70.9	5.2	0.9	51.9	45.7–46.7	71.2	44.0–48.2	1.4	63.0	62.1	54.7
Carvacrol	1300	2200	4.6	3.5	2.3–8.2	4.6	4.6	5.7	76.7	69.7	6.3	3.5–3.6	4.6	2.5–3.1	87.5	2.7	2.4	3.7
(E)-β-Caryophyllene	1410	1576	1.6	1.5	1.2–2.8	2.6	1.1	1.1	1.5	2.4	1.8	1.7–2.2	3.2	8.6–9.2	0.6	2.8	2.2	0.8
α-Humulene	1441	1645	0.1	0.1	tr–0.1	0.1	0.1	0.2	0.2	tr	0.3	0.1	0.1	0.4	tr	0.1	0.1	tr
γ-Muurolene	1467	1692	0.1	0.1	0.1–0.2	0.1	0.1	0.1	–	0.1	0.2	0.1–0.2	0.1	0.7–1.0	–	0.3	0.3	0.2
Germacrene D	1474	1695	0.1	0.1	tr–0.2	0.1	0.2	0.1	–	0.1	0.4	0.2	0.2	0.6–0.7	–	0.1	0.1	tr
β-Ionone	1484	1924	tr	0.1	tr–0.1	–	tr	0.1	–	–	0.2	0.1–0.2	tr	0.2	–	–	tr	tr

tr – the concentration of the component in the sample is less than 0.05%, * the samples are described in Table 1, – the component was not detected in the sample

Continued on the next page

Table 2. *Continued*

Compound	RI		Content (%)															
	SPB-5	SW-10	1*	2	3–5, 21–22	6	7	8	9	10	11	12–13	14	15–16	17	18	19	20
α-Muurolene	1497	1736	0.3	0.1	tr–0.2	tr	tr	–	tr	0.2	–	–	–	0.1–0.2	tr	tr	–	–
Bicyclogermacrene	1500	1721	0.1	0.2	tr–0.1	0.2	tr	0.3	0.2	0.3	0.4	0.3	tr	0.2	tr	tr	–	–
γ-Cadinene	1502	1740	0.3	0.3	tr–0.2	–	0.2	–	–	–	–	–	0.1	1.0–1.4	tr	0.3	0.2	0.1
δ-Cadinene	1514	1738	tr	0.2	tr–0.2	0.2	0.1	0.2	0.1	–	0.4	0.3	0.1	0.9–1.2	tr	0.3	0.2	0.1
Spatulenol	1567	2110	0.3	0.3	tr–0.1	0.4	0.9	1.6	0.1	–	0.1	tr	0.1	–	0.1	–	–	–
Caryophyllene oxide	1570	1957	0.6	0.3	0.1–0.2	–	–	–	0.6	0.5	0.3	0.3–0.5	0.6	1.0–1.4	0.8	0.2	tr	0.9
Caryophyllenol	1596	2400	0.1	0.3	0.2–1.0	0.1	0.5	0.3	0.2	0.2	tr	tr–0.1	tr	tr	tr	tr	tr	tr
Viridiflorol	1604	2078	tr	0.2	0.1–0.3	0.2	tr	0.1	–	–	tr	0.2	0.3	0.1–0.2	tr	–	tr	tr
Epiglobulol	1612	2200	0.4	0.3	–	0.3	0.3	–	–	0.2	–	–	–	0.2	tr	tr	tr	tr
τ-Cadinol	1632	2160	–	–	0.1–0.2	–	–	0.4	–	0.2	0.3	0.3–0.5	–	0.8–1.4	tr	0.2	0.2	0.2
α-Cadinol	1660	2218	0.2	0.1	tr	–	0.1	0.2	0.1	tr	tr	–	tr	tr–0.1	–	tr	tr	tr

tr – the concentration of the component in the sample is less than 0.05%, * the samples are described in Table 1, – the component was not detected in the sample

and neuroprotective agent. It exhibits an antitumour effect associated with the mechanism of apoptosis inhibition and cell cycle arrest [38].

Nadi et al. [39] found that the *T. vulgaris* extract, rich in thymol and carvacrol, has properties that may mitigate the severity of COVID-19. Thyme's ability to suppress pro-inflammatory cytokines, such as TNF-alpha and IL-6, while enhancing anti-inflammatory cytokines, such as TGF-beta and IL-10, has been determined. This suggests its potential to modulate the immune response to viral infections. In addition, thyme extract demonstrated inhibitory effects on the cytokines IL-1-beta and IL-8 at both the mRNA and protein levels, indicating complex effects on inflammatory pathways.

In total, a bicyclic sesquiterpene hydrocarbon (E)-β-caryophyllene was found in 295 species from 51 plant families [40]. It alleviates chronic pathologies characterized by inflammation and oxidative stress, including metabolic and neurological diseases [41]. Also, it shows beneficial effects on obesity, non-alcoholic fatty liver disease/non-alcoholic steatohepatitis, liver disease, diabetes, and cardiovascular disease. Borneol has anti-inflammatory, antioxidant, anti-apoptotic, and anticoagulant activity that improves energy metabolism. It also promotes the penetration of drugs into tissues through physiological barriers, such as the blood-brain barrier, mucous membranes, and skin [42]. The main biological properties of β-myrcene are anxiolytic, antioxidant, rejuvenating, anti-inflammatory, and analgesic properties [43].

The terpenes α-terpineol, β-caryophyllene, and γ-terpinene induce analgesia in a murine model of neuropathic pain [44]. The degree of activity of terpenes was α-terpineol > β-caryophyllene > γ-terpinene. Linalool is often an essential ingredient in perfumes and household detergents. It is used in food flavours and industry; linalool exhibits analgesic, anxiolytic, sedative, anti-inflammatory, antitumour and antibacterial effects [45].

Comparison of the composition of essential oil with the requirements of Ph. Eur. 11

The monograph *Thymi aetheroleum* of Ph. Eur. 11 [5] puts forward requirements for determining the content of seven components in *T. vulgaris* EO: β-myrcene (1.0–3.0%), γ-terpinene (5.0–10.0%), p-cymene (15.0–28.0%), linalool (4.0–6.5%), terpinen-4-ol (0.2–2.5%), thymol (36.0–55.0%), and carvacrol (1.0–4.0%). According to the monograph *Thymi herba*, the minimum content of thymol and carvacrol combined must be 40% in EO [5].

Table 3 shows that none of the studied samples fully met the requirements of Ph. Eur. 11 [5]. EO sample 2, which did not meet the requirements only for the content of linalool, differed the least from the requirements of the pharmacopoeia, and samples 1, 8, 9 and 17, in which the

Table 3. Compliance of the *T. vulgaris* essential oil quality of the samples with the requirements of Ph. Eur. 11

Sample	Content of the component (%)						
	β-Myrcene	γ-Terpinene	p-Cymene	Linalool	Terpinen-4-ol	Thymol	Carvacrol
1	0.5*	3.3*	10.6*	1.7*	0.6	66.2*	4.6*
2	1.2	7.9	21.1	2.5*	0.5	48.2	3.5
3	1.4	13.9*	4.5*	1.7*	0.5	62.0*	3.8
4	1.4	14.2*	4.8*	1.8*	0.8	60.8*	4.1*
5	1.3	9.9	6.7*	1.8*	0.5	57.5*	8.2*
6	1.0	7.9	10.2*	1.8*	0.7	61.8*	4.6*
7	0.9*	4.9*	26.0	2.7*	0.6	45.8	4.6*
8	0.1*	0.2*	5.0*	2.9*	1.2	70.9*	5.7*
9	0.9*	1.9*	6.0*	0.9*	0.7	5.2*	76.7*
10	1.6	4.6*	11.4*	0.8*	0.7	0.9*	69.7*
11	1.3	14.2*	9.3*	2.0*	0.5	51.9	6.3*
12	1.4	16.1*	14.3*	2.8*	0.5	46.7	3.5
13	1.3	15.7*	14.4*	2.4*	0.6	45.7	3.6
14	0.5*	5.5	4.8*	2.1*	0.5	71.0*	4.6*
15	–*	0.2*	0.3*	2.0*	0.2	48.2	3.1
16	–*	0.1*	0.4*	2.6*	0.2	44.0	2.5
17	0.2*	0.1*	5.1*	0.4*	0.2	1.4*	87.5*
18	0.9*	11.0*	6.3*	2.2*	0.05*	63.0*	2.7
19	1.4	13.9*	62.1*	1.9*	0.1*	62.1*	2.4
20	0.6*	2.2*	54.7*	2.4*	0.4	54.7	3.7
21	1.7	15.9*	58.6*	1.8*	0.3	58.6*	2.6
22	2.3	15.3*	53.9*	2.2*	0.1*	53.9	2.3

* does not meet the requirements of Ph. Eur. 11, – the component was not detected in the sample

content of six components did not meet the requirements, differed the most. None of the samples contained the required amount of linalool, 20 samples did not meet the requirements for p-cymene and 18 samples for γ-terpinene. The EO samples mostly met the requirements for the content of terpinen-4-ol. Most of the samples exceeded the established Ph. Eur. 11 standards for the content of carvacrol (11 samples), thymol (10) and γ-terpinene (9). The content of the compounds linalool (22 samples), p-cymene (16 samples) and β-myrcene (10 samples) were the lowest of the required limits. Regarding the carvacrol content, none of the samples contained concentrations below the required limits. All samples contained the sum of thymol and carvacrol exceeding the Ph. Eur. 11 limit of 40%.

Thus, *T. vulgaris* samples from different origins have highly variable EO composition. The chemical composition of plants differs depending on several factors, such as the region of growth, humidity, average daily temperature, amount of precipitation, type of soil, conditions and methods of cultivation, and harvesting and storage of raw materials [46–56]. The genetic variability and phenotypic plasticity of plants also influence the composition of EO [50,51].

Groups of essential oil compounds

Monoterpenoids were represented by 25 compounds, the content of which was 90.5%, of which 72.7% were aromatic substances (Table 4). Of the 16 sesquiterpenoids (5.7%), most were bicyclic (4.3%). The lowest content of compounds from the group of other substances was represented by ketone β-ionone, diterpenoid Δ-3-carene, and aliphatic compound 1-oken-3-ol.

Minimum and maximum levels of essential oil compounds

The substances with the highest content (>2%) were (E)-β-caryophyllene, γ-terpinene, p-cymene, carvacrol and thymol (Table 5). On average, the samples contained 49.6% thymol and 14.5% carvacrol, which (64.1%)

Table 4. Average content of groups of essential oil components in samples 1–22 of *T. vulgaris*

Group of components (Number of components in a group)	Average content in essential oils (%)
Monoterpenoids (C10)	
Aliphatic (4)	3.2
Aromatic (3)	72.7
Monocyclic (7)	10.4
Bicyclic (9)	3.3
Aromatic ether (2)	0.9
Total content (25 components)	90.5
Sesquiterpenoids (C15)	
Aliphatic (1)	0.1
Monocyclic (1)	0.1
Bicyclic (10)	4.3
Tricyclic (4)	1.2
Total content (16 components)	5.7
Other substances	
Aliphatic (1)	0.7
Monocyclic (1)	0.1
Diterpenoids (1)	0.1
Total content (3 components)	0.9

exceeded the requirement of Ph. Eur. 11 (40%). For the rest of the substances, the percentage points (PP) between the minimum and maximum concentration of compounds were from 1.7 to 0.1 PP. The differences between the concentrations of the main components thymol and carvacrol were especially significant (85.2 and 70.3 PP, respectively). This large difference is due to the samples where the component with the highest concentration was carvacrol rather than thymol (samples 9, 10 and 17 of Greek origin).

Since thyme is a plant of highly variable composition, the EO composition of which largely depends on growing conditions and genetic predisposition, only those components whose concentration in at least one sample exceeded 2% were investigated as the next step (14 of the 44 compounds) in samples 1–22 and 23–37 (Table 2; Table 6). The total content of 14 components comprised the most EO (86.4% on average). Of the analysed substances, the largest amount contained compounds belonging to one biosynthesis pathway, namely thymol (44.1%), p-cymol (12.3%), carvacrol (12.3%), and γ-terpinene (7.2%) (Fig. 1).

Correlations between the compounds of essential oil

The correlation coefficients between the contents of 14 components presented in Fig. 1 were determined. This study considered coefficients whose value exceeded 0.7

Table 5. Limits and average content of principal components in *T. vulgaris* samples 1–22

Component	Minimum content (%)	Maximum content (%)	Gap (percentage point – PP)	Average content (%)
Carvacrol	2.3	87.5	85.2	14.1
Thymol	0.9	71.2	70.3	49.1
p-Cymene	0.3	26.0	25.7	9.5
γ-Terpinene	0.1	16.1	16.0	8.1
(E)-β-caryophyllene	0.6	9.2	8.6	2.4
Borneol	0.1	2.6	2.5	0.9
Linalool	0.4	2.9	2.5	2.0
β-Myrcene	0.1	2.3	2.2	1.1
α-Terpinene	0.1	2.1	2.1	1.1

Table 6. Content of essential oil components in samples 23–37 from different countries

Origin	Concentration of components (%)																
	α-Pinene	Camphene	Myrcene + 3-Octanol	α-Terpinene	p-Cymene	Limonene + β-phellandrene	1,8-Cineole	γ-Terpinene	Linalool	Borneol	Terpinen-4-ol	α-Terpineol	Neral + thymol methyl ether	Carvacrol methyl ether	Thymol	Carvacrol	(E)-β-Caryophyllene
23. Estonia	0.4	0.2	1.1	1.1	6.1	0.3	0.2	7.7	1.8	0.2	0.6	0.1	–	0.1	70.2	3.0	2.1
24. Lithuania	0.8	0.5	0.9	0.8	24.6	0.5	0.7	4.2	2.6	0.7	1.1	0.2	0.9	0.1	45.6	3.6	1.5
25. Latvia	0.2	0.1	0.6	0.7	17.5	0.4	0.6	4.6	2.9	0.8	–	0.2	0.7	–	53.1	4.1	2.1
26. Spain	2.1	1.5	0.4	0.5	29.0	1.4	2.2	2.7	5.5	6.2	1.7	2.4	0.2	0.3	12.6	10.1	0.6
27. Italy	4.8	9.3	0.3	0.4	2.8	0.6	tr	1.4	3.2	32.5	2.2	7.7	–	3.0	10.4	6.7	3.2
28. Germany	1.1	0.9	1.0	0.7	31.4	0.7	0.5	2.8	2.5	0.8	0.8	0.1	2.2	0.7	40.7	4.2	1.4
29. Estonia	0.8	0.8	2.0	1.7	15.4	0.7	0.7	13.7	2.6	1.3	0.5	tr	0.4	0.5	48.5	2.4	0.9
30. Germany	1.2	1.2	1.8	1.4	36.7	0.7	1.2	8.1	4.2	1.7	0.9	0.2	1.2	0.1	27.7	1.8	0.7
31. Czechia	0.1	0.1	0.6	0.4	14.7	0.3	0.8	3.2	2.4	1.4	0.7	0.2	0.9	tr	58.8	4.5	0.7
32. England	3.3	3.3	0.7	0.6	3.2	0.6	tr	2.9	3.6	23.1	1.2	–	–	tr	22.3	10.9	4.2
33. Ukraine	0.4	0.4	1.4	0.4	8.1	0.8	1.0	3.8	1.1	0.8	1.1	–	2.6	3.7	2.9	18.3	3.7
34. Austria	0.9	0.6	0.9	0.9	21.8	0.5	0.6	5.7	2.2	0.7	0.5	0.1	–	0.6	51.8	3.5	1.1
35. Estonia	0.7	0.2	1.6	1.9	8.1	0.7	0.3	13.1	1.6	–	0.1	–	–	–	60.3	3.3	1.2
36. Turkey	0.7	0.1	1.4	1.7	11.2	0.5	tr	12.0	0.2	–	0.6	–	–	–	2.5	62.0	1.3
37. Georgia	0.4	0.4	0.4	1.1	14.5	1.0	1.0	2.6	0.3	1.5	0.8	0.3	0.3	–	43.1	4.5	2.8

tr – the concentration of the component in the sample is less than 0.05%, – the component was not detected in the sample

or was less than –0.7. For all data (samples 1–37), the strongest correlation was 0.941 ($p = 0.01$) between borneol and camphene. It was followed by α-pinene/camphene (0.929), camphene/α-terpineol (0.915) and α-pinene/borneol (0.913).

When analysing samples with thymol as the main component, the highest correlation was α-pinene/camphene (0.871) ($p = 0.01$). The following correlations were below 0.8: p-cymol/camphene (0.769), β-caryophyllene/carvacrol methyl ether (0.719), and borneol/carvacrol methyl ether (0.716). Looking at previously excluded samples separately (samples 9, 10, 17, 26, 27, 30, 32, 33, and 36), the strongest correlations were borneol/α-pinene (0.972), borneol/camphene (0.943), camphene/α-pinene (0.941), and camphene/α-terpineol (0.916). Some negative correlations were also found: linalool/carvacrol (–0.823), carvacrol/terpinen-4-ol (–0.774), and thymol/carvacrol (–0.737).

Dynamics of the essential oil main compounds within the flowering period of *T. vulgaris*

Data analysis on the dynamics of the accumulation of thymol, carvacrol, p-cymene, and γ-terpinene (in samples 3–5) showed no particularly large changes in the substance concentration between the beginning and full flowering of *T. vulgaris* (Fig. 2). At the end of flowering, the levels of compounds had changed more; the carvacrol levels had almost doubled. The correlation coefficient between p-cymene and γ-terpinene for samples 3–5 was –0.982 ($p = 0.05$). The correlation between thymol and p-cymene was –0.991. Differences were observed for γ-terpinene and thymol, where the coefficient had a positive value: 0.948 and 1.00, respectively. The correlation between thymol and carvacrol was –0.980 in samples from different growing seasons. The difference between the multipliers is relatively small, which gives reason to assume a negative relationship between the biosynthesis of the mentioned substances.

Chemotypes of *T. vulgaris* essential oil

In the literature [6,52], the main six chemotypes of *T. vulgaris* have been described, which are determined by one or, less often, by several components of the essential oil close in chemical nature, namely geraniol, linalool, α-terpineol, carvacrol, thymol, and trans-thujan-4-ol/terpinen-4-ol. The thymol chemotype is more com-

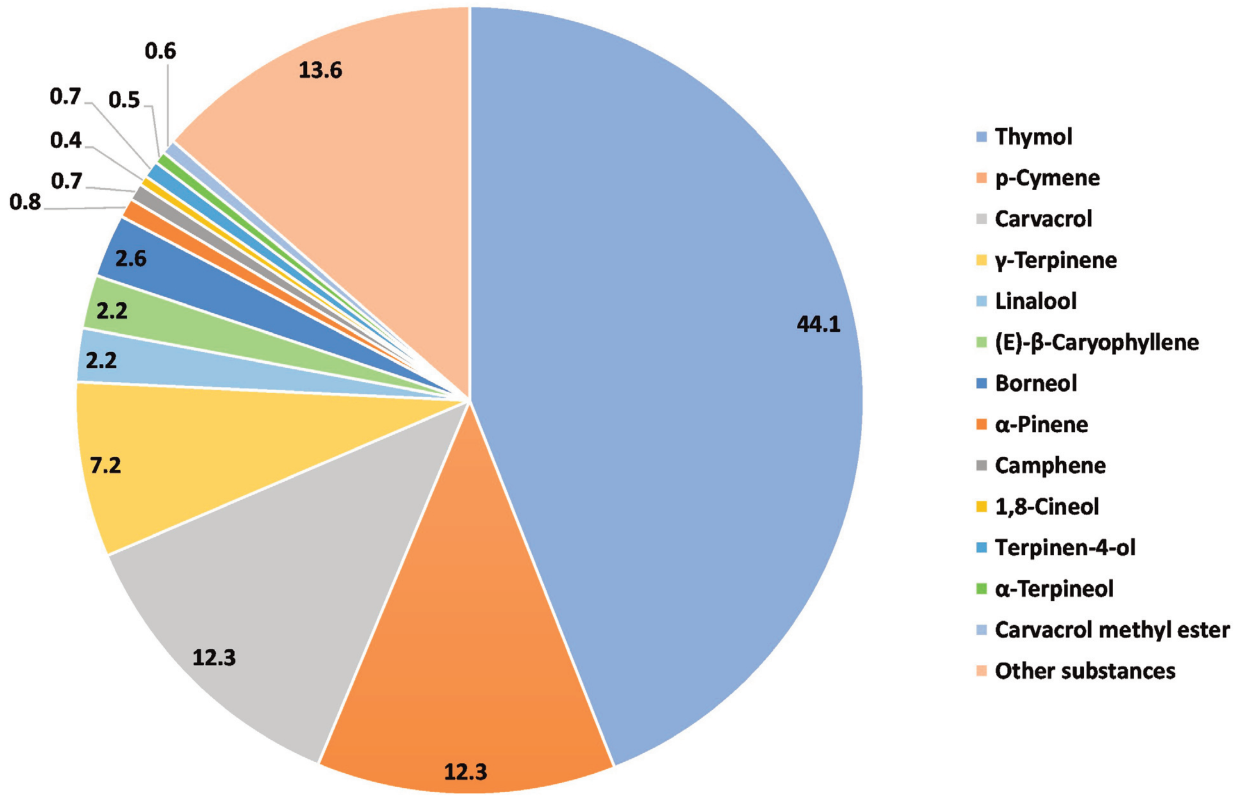

Fig. 1. Average content of components with a concentration of more than 2% in samples 1–37.

mon when the raw material is dominated by thymol (up to 50%), with γ-terpinene, p-cymol, and linalool also present. This chemotype is widely cultivated and used as a spice in the food industry, as well as in the formulation of medical drugs and cosmetics [54]. Less common is the linalool chemotype with the dominance of linalool (up to 85%) and the presence of α-terpineol, 1,8-cineole, and carvacrol. Satyal et al. [54] investigated the EO compositions, including monoterpenoid enantiomeric compositions, of four different chemotypes of *T. vulgaris* EO from Europe and performed a hierarchical cluster analysis to elucidate/distinguish different chemotypes of *T. vulgaris*.

In the present study, for the first time, an analysis of *T. vulgaris* chemotypes was carried out that characterized the enantiomeric distribution of monoterpenoids, which can have a profound effect on the bioactivity, taste, and aroma of the raw material. Most of the studied samples accumulated thymol in larger quantities but, considering the content of other components, seven chemotypes were distinguished (Table 7). The most common chemotype in the analysis was the p-cymol/thymol type, represented by 12 samples. The content of thymol in the samples was twice that of p-cymene. Seven samples of Estonian origin belonged to the thymol/γ-terpinene chemotype, in which thymol was also predominant (Table 7). Also, the thymol chemotype was determined among five samples from Estonia, in which thymol content ranged from 44 to 70.9%. The other six Estonian samples also accumulated a significant amount of thymol but, at the same time, also contained significant concentrations of γ-terpinene and p-cymene, so they were assigned to the thymol/γ-terpinene/p-cymene chemotype. Two samples (9 and 17) contained up to 82% of carvacrol and were assigned to the carvacrol chemotype. Such a chemotype has been described earlier [56]. In samples 27 and 32, the presence of borneol and a significant content of thymol and carvacrol were noted among the identified components. Therefore, they were assigned to the borneol/thymol/carvacrol chemotype. Chemotypes 1–4 were characterized by significant thymol content, while chemotypes 5 and 6 were characterized by carvacrol, both of which are aromatic compounds. Therefore, aromatization in these chemotypes is more intensive [56]. In chemotype 7, which has not been previously described in the literature, the content of borneol exceeded the thymol content by 1.7 times and carvacrol by 3 times.

In this study, it was established that most samples of *T. vulgaris* cultivated in different regions of Estonia and other European countries contained thymol and/or

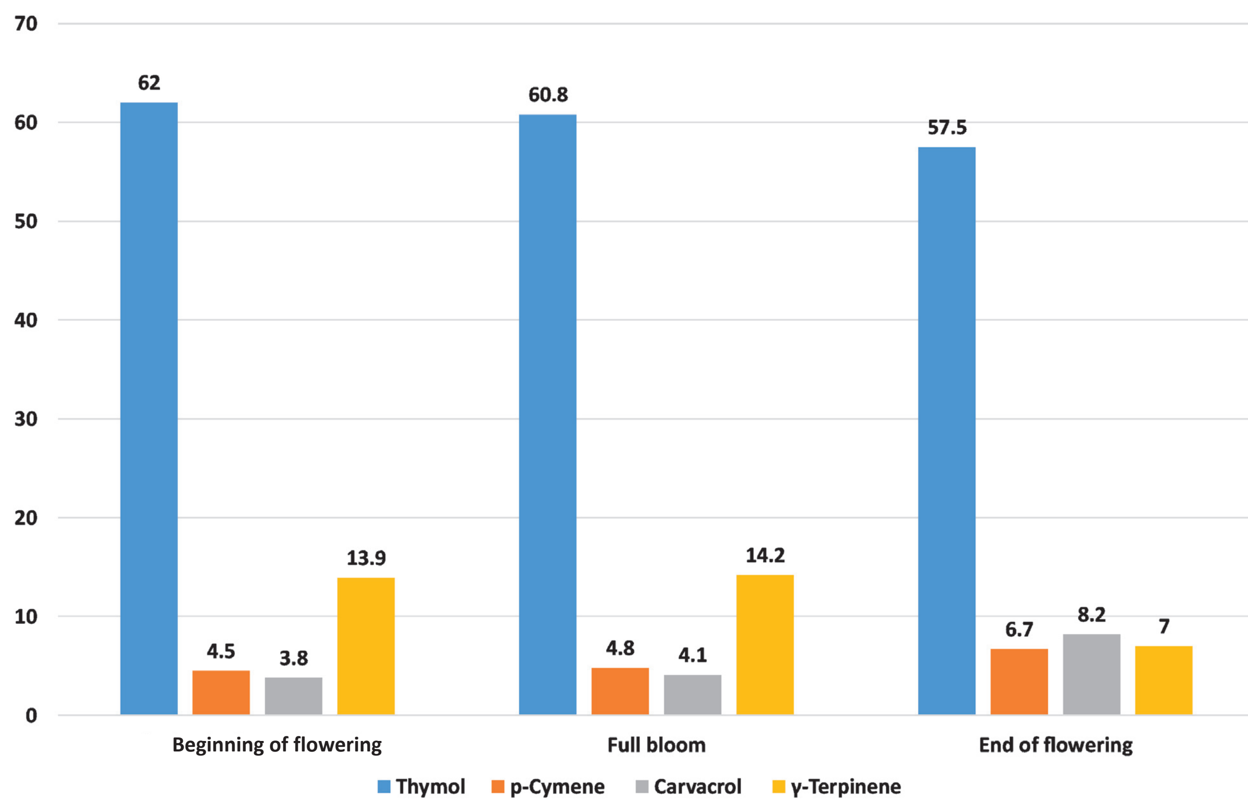

Fig. 2. Average content of components with a concentration of more than 2% in samples 1–37.

Table 7. *T. vulgaris* chemotypes of the studied samples 1–37

Chemotype No.	Components	Average substance content (%)	Samples	Number of chemotype samples
1	Thymol p-Cymene	45.7 21.2	1, 2, 7, 20, 24, 25, 26, 28, 30, 31, 34, 37	12
2	Thymol γ-Terpinene	59.4 13.3	3, 4, 5, 11, 18, 19, 21	7
3	Thymol	60.9	8, 14, 15, 16, 23	5
4	Thymol γ-Terpinene p-Cymene	52.8 13.6 11.7	6, 12, 13, 22, 29, 35	6
5	Carvacrol	82	9, 17	3
6	Carvacrol p-Cymene γ-Terpinene	50 10.2 6.8	10, 33, 36	2
7	Borneol Thymol Carvacrol	27.8 16.4 8.8	27, 32	2

carvacrol as the principal compounds. As a result of a relatively narrow shift in time, plants can acquire quite different chemical compositions. *T. vulgaris* is grown in Estonia as an annual crop, which undoubtedly affects the chemical composition and type of chemotypes. It can be assumed that the differences in the component composition of the studied samples of EO and chemotypes of *T. vulgaris* from those previously described in scientific articles are related to the different geographical locations of the plants, their growing conditions, stage of vegetation during harvesting, method of harvesting and drying, etc.

CONCLUSIONS

The quality of 22 samples of *T. vulgaris* EO was analysed based on the requirements of Eur. Ph. 11. The components of EO from 37 herb samples collected in Estonia and other European countries were identified and quantified. The dynamics of the accumulation of the main components of EO depending on the phase of plant development were analysed. The five principal compounds were carvacrol, thymol, p-cymene, γ-terpinene, and (E)-β-caryophyllene. Regarding the content of compounds in EO, none of the studied samples (n = 22) fully corresponded to the standards of Ph. Eur. 11. Correlations between the content of the most important (>2%) components (n = 14) were studied and found. Seven chemotypes of *T. vulgaris* EO were established; among them, five were rich in thymol content.

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Erinevatest Euroopa piirkondadest pärineva *Thymus vulgaris*'e eeterliku õli koostis

Ain Raal, Tetiana Gontova, Maie Palmeos, Anne Orav, Galiya Sayakova ja Oleh Koshovyi

Aed-liivatee (*Thymus vulgaris* L., Lamiaceae) on üks maailma tuntumaid taimi, mida kasutatakse ulatuslikult meditsiinis, kulinaarias ja kosmeetikas. Käesoleva töö eesmärk oli analüüsida gaasikromatograafiliselt Eestis kasvatatud ja muudest Euroopa riikidest pärineva *T. vulgaris*'e kaubanduslike droogide eeterliku õli sisaldust ja koostist ning iseloomustada erinevat päritolu droogide kvaliteeti ja kemotüüpe. Uuritud *T. vulgaris*'e ürdinäidised (n = 22) sisaldasid 3–28 mL/kg eeterlikku õli, mis viies näidises oli alla Euroopa farmakopöa miinimumnormi (12 mL/kg). Identifitseeriti 44 eeterliku õli komponenti, millest viis peamist olid karvakrool (2,3–87,5%), tümool (0,9–71,2%), p-tsümeen (0,3–26,0%), γ-terpineen (0,1–16,1%) ja (E)-β-kariofülleen (0,6–9,2%). *T. vulgaris*'e eeterliku õli (n = 37) põhikomponent, keskmise sisaldusega 41,1%, oli tümool. Uuritud eeterlikus õlis domineerivad monoterpenoidid (90,5%) ja seskviterpenoidid (5,7%). Tähtsamate koostisainete minimaalse ja maksimaalse sisalduse poolest ei vasta ükski uuritud eeterlik õli (n = 22) täielikult Euroopa farmakopöa standardnõuetele. Nelja olulisema terpenoidi (tümool, karvakrool, p-tsümeen, γ-terpineen) sisaldus *T. vulgaris*'e eeterlikus õlis ei muutunud kuigivõrd taimede õitsemisperioodi algusest lõpuni. Korrelatsioonid uuritud õlide (n = 37) tähtsamate (sisaldus >2%) komponentide (n = 14) kontsentratsioonide vahel näitasid mitmel juhul taset >0.9. Tehti kindlaks seitse *T. vulgaris*'e eeterliku õli kemotüüpi, millest viies domineerib või on üheks põhikomponendiks tümool.