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Comparative chemical composition of the essential oil of *Mentha* × *piperita* L. from various geographical sources

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Abstract. Variations in the essential oil composition of *Mentha* × *piperita* L., cultivated in different European countries, were determined. The oils were obtained in yields of 0.8–3.3% from dried samples. A total of 46 components were identified, representing over 90% of the total yield of oil. The principal components in the oils of peppermint were menthone (11.2–45.6%), menthol (1.5–39.5%), isomenthone (1.3–15.5%), menthyl acetate (0.3–9.2%), piperitone (0.8–5.9%), pulegone (0.1–13.0), 1,8-cineole (0.4–6.0%), limonene (1.0–5.9%), and *trans*-sabinene hydrate (traces – 6.2%). The ratio between menthol and menthone varied from 0.04 to 2.8 and between 1,8-cineole and limonene from 0.3 to 5.0. Menthol was predominant (37–40%) in the oils from Greece and Hungary. Russian peppermint oil contained only 1.5% menthol, but was rich in menthone (38.2%), isomenthone (15.5%), and pulegone (13.0%). Menthone (37.9–39.5%) and menthol (31.6 – 35.8%) were found in high concentrations in Estonian peppermint oil.

Key words: *Mentha* × *piperita* L., Labiatae, peppermint, essential oil, menthone, menthol, menthyl acetate.

INTRODUCTION

Peppermint (*Mentha* × *piperita* L.) is one of the most widely distributed aromatic and medicinal plants in the world, including Estonia. Peppermint contains usually 0.5–4% essential oil. The yield of oil depends on the age of the plant and on the cut [1], also on the vegetative period, as well on the cultivars. Peppermint leaves are harvested several times a year. The first cut in the second year gives the best yield, which decreases thereafter. The maximum essential oil content occurs shortly before the flowering season [1, 2].

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The preparation of peppermint oil and its composition have been widely studied [1–18]. The chief components of oil include menthol (30(35)–55%), menthone (14–20(32)%), 1,8-cineole (6–8(14)%), isomenthone (2–3(10)%), menthyl acetate (3–5%), neomenthol (2.5–3.5%), menthofurane (1(2)–7(9)%), limonene (1–5%), etc. The ratio of the 1,8-cineole to limonene contents exceeds usually 2 [4]. The total ester content is 4–9%. Other constituents of the oil are phellandrene, α- and β-pinene, jasmone, pulegone, *trans*-sabinene hydrate, acetaldehyde, acetic acid, valeric acid, etc. Jasmone is present in the oil in a rather low concentration (<0.1%), but it gives the essential oil of peppermint a sweet and pleasant taste [5]. There are also flavonoids (apigenin, diosmetin and luteolin glycosides, xanthomicrol, gardenin D), tannins (6–12%), triterpenes, bitter substances, rosmarinic acid, etc. in the leaves of peppermint [1, 2].

Uses of peppermint leaves described in pharmacopeias and in traditional systems of medicine are symptomatic treatment of dyspepsia, flatulence, and intestinal colic [3]. Internal application of peppermint oil is indicated in the case of cramps of the upper gastrointestinal tract and also bile ducts, catarrhs of the respiratory tract, inflammation of the oral and pharyngeal mucosa. External application is indicated to treat myalgia and neuralgia. The main component of the oil, menthol, has an antimicrobial, choleretic, insecticidal, carminative, spasmolytic effect on the smooth muscle of the intestine and a cooling effect on the skin [2]. Peppermint tea increases the production of bile; the effect is due to the essential oil and the flavonoids also play a part [1]. Limited scientific information is available about a sedative effect of drug and oil. In pharmacy, leaves and essential oil of peppermint are also used for taste improvement of pharmaceuticals. Large quantities of the oil of peppermint are consumed in making confectionery, liquors, cosmetics, toothpastes, and many other products [5].

According to European Pharmacopoeia [19] peppermint leaf (*Menthae piperitae folium*) as whole drug should contain not less than 12 mL/kg (1.2%) and as cut drug not less than 9 mL/kg (0.9%) of essential oil. The peppermint oil (*Menthae piperitae aetheroleum*) used in the pharmaceutical industry of Europe is essential oil obtained by steam distillation from the fresh aerial parts of the flowering plant of peppermint. The essential oil is a colourless, pale yellow or pale greenish-yellow liquid, which has a characteristic odour and taste followed by a sensation of cold. The limits of terpenes in oil should be within the following ranges: limonene 1.0–5.0%, cineole 3.5–14.0%, menthone 14.0–32.0%, isomenthone 1.5–10.0%, menthofuran 1.0–9.0%, menthyl acetate 2.8–10.0%, isopulegol – maximum 0.2%, menthol 30.0–55.0%, pulegone – maximum 4.0%, and carvone – maximum 1.0%.

In the present work we determined the composition of the essential oil using commercial peppermint samples from Estonia and from other European countries. The variation in the content of the biologically active constituents was studied. The quality of Estonian peppermint oil compared to the oils from other countries and to the European Pharmacopoeia requirements was determined.

EXPERIMENTAL

Materials

Plant materials (commercial *Menthae piperitae folium*) were obtained from retail pharmacies from different European countries in 2000 (Estonia, France), 2001 (Hungary, Belgium), and 2002 (Estonia, Russia, Greece, Ukraine). Voucher specimens have been deposited at the Department of Pharmacy, University of Tartu, Estonia.

Isolation of essential oil

The essential oil was isolated from dried leaves of peppermint by the distillation method described in the European Pharmacopoeia [19] using 20 g of crushed drug, a 500 mL round-bottomed flask, and 200 mL water as the distillation liquid. Xylene (0.5 mL in a graduated tube) was added to take up the essential oil. The distillation time was 2 h at a rate of 2–4 mL/min.

Capillary gas chromatography

GC analysis was carried out using a Chrom 5 chromatograph with FID on two fused silica capillary columns (50 m × 0.2 mm) with bonded stationary phases NB-30 (polydimethylsiloxane) and NB-20M (polyethylene glycol) from Nordion (Finland). Film thickness of both stationary phases was 0.25 μm. Helium with a split ratio 1 : 150 and flow rate 17–25 cm/sec was applied as the carrier gas. The temperature program was from 50 to 250 °C at 2 °C/min; the injector temperature was 200 °C. 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 reference standards, our RI data bank, and with literature data. The percentage composition of the oils was calculated in peak areas 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%.

RESULTS AND DISCUSSION

A total of 46 compounds, representing more than 90% of the total oil, were identified and quantitatively evaluated in the peppermint oils isolated from *Mentha* × *piperita* of different origins (Table 1, Fig. 1). The principal group of compounds in peppermint oils was oxygenated monoterpenes (66.6–88.4%). Among them menthone (11.2–45.6%), menthol (1.5–39.5%), isomenthone (1.3–15.5%), menthyl acetate (0.3–9.2%), 1.8-cineole (0.9–5.9%), and *trans*-sabinene hydrate (traces – 6.2%) occurred in largest amounts. The content of monoterpenes in the oils amounted to 15.6% (limonene 0.9–5.9%) and of sesquiterpenes to 8.8%

(caryophyllene 0.7–4.3%, germacrene D 1.1–3.1%). Oxygenated sesquiterpenes found in oils made up only 0.4–2.4%. All constituents of peppermint oil identified in this work were earlier mentioned in the literature [1–18].

Table 1. Composition of the essential oil from *Mentha* × *piperita* L. The components identified in the highest yields are in bold

Compound	RI* NB-30	RI NB-20M	Concentration, %							
			Est**		Fr	Hun	Bel	Rus	Ukr	Gr
			2000	2002	2000	2001	2001	2002	2002	2002
α-Thujene	922	1019	0.1	–	tr.	0.2	0.1	0.1	tr.	tr.
α-Pinene	929	1019	0.6	tr.	0.6	1.1	0.7	0.7	0.6	0.7
Camphene	942	1063	tr.	–	tr.	0.1	tr.	0.1	tr.	tr.
Sabinene	964	1118	0.7	0.1	0.7	0.7	0.7	0.5	0.3	0.4
β-Pinene	968	1106	1.0	0.2	0.8	1.4	0.8	0.7	0.7	1.0
Myrcene	982	1160	0.3	0.1	**4.4**	0.8	1.2	0.4	0.2	0.2
3-Octanol	984	1392	0.3	0.2	0.2	–	–	–	0.2	tr.
α-Terpinene	1008	1178	0.2	–	0.7	0.3	0.2	0.3	tr.	0.2
p-Cymene	1012	1270	0.1	0.2	0.1	0.2	0.1	0.2	–	0.1
1,8-Cineole	1020	1205	**6.0**	**4.3**	**5.7**	**5.8**	**5.3**	**0.4**	**1.9**	**4.3**
Limonene	1022	1195	**1.2**	**1.0**	**5.9**	**1.5**	**1.4**	**1.3**	**2.0**	**0.9**
(Z)-ß-Ocimene	1027	1228	0.4	0.1	1.8	0.5	0.3	0.1	0.2	0.2
(E)-ß-Ocimene	1038	1244	0.1	tr.	0.3	0.4	0.1	0.1	0.1	tr.
γ-Terpinene	1049	1238	0.3	tr.	0.2	0.5	0.4	0.5	tr.	0.3
***trans*-Sabinene hydrate**	1056	1466	**3.0**	**3.6**	**1.1**	**2.8**	**4.4**	**6.2**	tr.	**1.7**
Terpinolene	1081	1276	0.1	tr.	0.1	0.1	0.1	0.1	tr.	0.1
cis-Sabinene hydrate	1087	1549	0.1	0.2	0.2	–	0.1	0.1	0.2	0.3
Linalool	1089	1551	0.3	0.4	0.3	0.2	0.2	0.4	0.1	tr.
Menthone	**1133**	**1460**	**37.9**	**39.5**	**11.2**	**13.8**	**45.6**	**38.2**	**38.8**	**24.8**
Isomenthone	1145	1487	**3.6**	**3.7**	**1.3**	**4.5**	**5.5**	**15.5**	**6.4**	**3.1**
Menthofuran	1150	1477	0.9	0.5	0.6	0.1	0.7	0.3	0.9	0.8
Neomenthol	1157	1592	**1.9**	**2.4**	**2.2**	**2.7**	**2.3**	**0.8**	**2.2**	**3.4**
Menthol	1165	1637	**31.6**	**35.8**	**31.3**	**37.2**	**17.6**	**1.5**	**24.4**	**39.5**
Isomenthol	1172	1663	0.7	0.4	0.2	1.0	0.2	tr.	0.3	0.6
α-Terpineol	1177	1713	0.2	0.2	0.2	0.8	0.4	0.5	tr.	0.2
Pulegone	1218	1640	0.1	0.2	0.2	1.0	1.7	**13.0**	1.2	0.5
Carvone	1220	1735	–	–	–	–	–	**3.1**	0.6	0.3
Piperitone	1232	1730	0.8	0.8	**5.9**	0.8	0.9	**3.5**	0.8	0.7
Neomenthyl acetate	1264		0.1	tr.	0.3	1.5	tr.	0.3	0.1	0.5
Menthyl acetate	1281	1556	**1.2**	**1.1**	**5.3**	**7.2**	**3.2**	0.3	**9.2**	**6.3**
Isomenthyl acetate	1287		–	–	–	–	–	0.1	0.2	1.0
α-Copaene	1373	1484	–	0.1	–	–	–	tr.	tr.	0.1
β-Bourbonene	1381	1508	0.1	0.3	0.3	0.5	0.1	0.3	0.1	0.2
β-Elemene	1388		–	tr.	–	0.5	0.1	0.5	0.1	0.1
β-Caryophyllene	1418	1587	**1.5**	**0.7**	**4.3**	**2.4**	**1.4**	**2.6**	**1.8**	**2.0**
α-Humulene	1450	1657	0.1	0.3	0.2	0.6	0.2	0.1	0.2	0.4
(E)-β-Farnesene	1453	1632	0.2	–	0.2	0.2	tr.	0.1	0.1	tr.
Germacrene D	1478	1700	**2.7**	**1.1**	**3.1**	**1.7**	**1.7**	**1.3**	**2.2**	**2.0**
α-Muurolene	1493		0.3	0.1	0.4	0.9	0.2	0.8	0.1	0.3
γ-Cadinene	1502	1744	–	–	0.2	0.8	tr.	tr.	tr.	–

Table 1 continued

Compound	RI* NB-30	RI NB-20M	Concentration, %							
			Est**		Fr	Hun	Bel	Rus	Ukr	Gr
			2000	2002	2000	2001	2001	2002	2002	2002
δ-Cadinene	1518	1746	0.1	–	0.1	0.1	0.1	0.1	tr.	0.1
Spathylenol	1570	2124	–	0.3	0.2	0.8	0.1	1.2	0.1	0.2
Caryophyllene oxide	1575	1980	tr.	0.2	0.3	0.5	0.1	0.5	0.3	0.3
Viridiflorol	1585	2083	0.5	1.0	0.8	0.8	0.1	–	0.3	0.9
α-Cadinol	1644	2215	0.1	0.1	0.2	0.3	0.1	0.1	0.1	–
COMPONENT GROUPS:										
Aliphatic compounds			0.3	0.2	0.2	–	–	–	0.2	–
Monoterpenes			5.1	1.7	15.6	7.8	6.1	5.1	4.1	4.1
Oxygenated monoterpenes			88.4	93.1	66.6	79.4	88.1	84.2	87.3	88.0
Among them menthyl isomers			77.9	83.4	52.4	68.4	75.1	57.0	82.5	80.0
Sesquiterpenes			5.0	2.6	8.8	7.7	3.8	5.8	4.7	5.2
Oxygenated sesquiterpenes			0.6	1.6	1.5	2.4	0.4	1.8	0.8	1.4
Total			99.4	99.2	92.1	97.3	98.4	96.9	97.1	98.7
Menthol/menthone			0.83	0.91	2.79	2.70	0.39	0.04	0.63	1.59
1,8-Cineole/limonene			5.0	4.3	1.0	3.9	3.8	0.3	1.0	4.8
Oil yield, %			3.2	2.2	0.9	1.0	3.3	1.4	2.5	0.8

* RI – retention index; NB-30 and NB-20M – polydimethylsiloxane and polyethylene glycol stationary phases.

** Est – Estonia, Fr – France, Hun – Hungary, Bel – Belgium, Rus – Russia, Ukr – Ukraine, Gr – Greece.

– Not found.

tr. – traces (<0.05%).

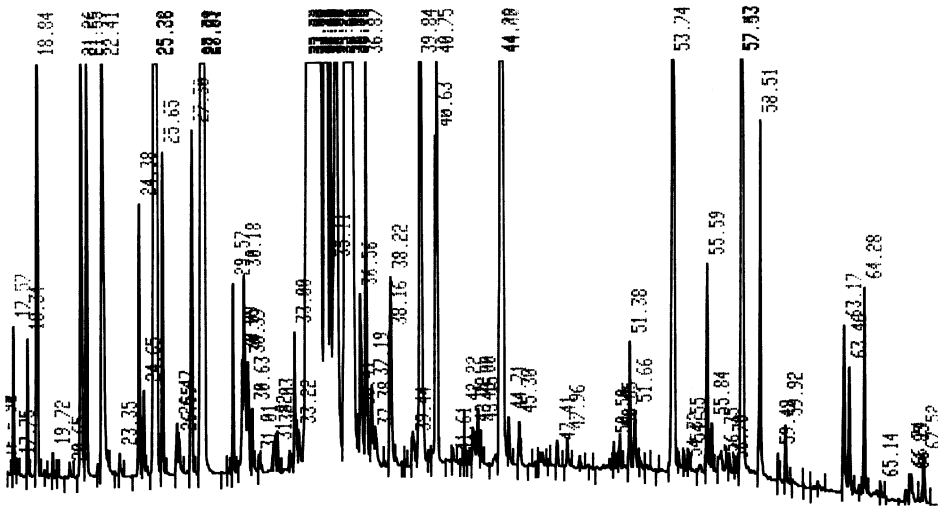

Fig. 1. Chromatogram of the essential oil from *Mentha* × *piperita* L. on polydimethylsiloxane column (NB-30).

The yield of essential oil from different peppermint samples varied in the range 0.8–3.3%. The higher yields (2.2–3.3%) were obtained from peppermint of Belgian, Estonian, and Ukrainian origins. A lower oil content (0.8–1.0%) was found in Grecian, French, and Hungarian peppermint. Only one cut drug (Grecian) with the content of 8 mL/kg of essential oil did not meet the European Pharmacopoeia standard (at least 9 mL/kg) [19]. The two commercial samples of Estonian peppermint (from the years 2000 and 2002) were quite similar. Only the content of menthol and menthone was a little higher and the content of monoterpenes was lower in 2002 than in 2000.

Comparison of the peppermint oil composition from different samples showed a high variability of concentrations of the majority of biologically active constituents (Fig. 2). The highest amount of menthol was found in Grecian peppermint oil (39.5%), and the smallest amount in Russian oil (only 1.5%). Estonian, French, and Hungarian oils contained 31.6–35.8% of menthol, Belgian and Ukrainian oils 17.6% and 24.4%, respectively. Belgian peppermint oil was rich in menthone (45.6%). In the samples from Estonia, Russia, Ukraine, and Greek this value varied from 24.8% to 39.5%. The smallest content of menthone was found in the samples of France and Hungary (11.2% and 13.8%, respectively). The oil from Ukraine contained more menthyl acetate (9.2%) than the other samples (0.3–6.3%). The content of menthyl acetate was very small (0.3%) in the oil of Russian peppermint. The ratio between the concentrations of menthol and menthone varied from 0.04 to 2.8 (Table 1). These values are the main quality characteristics of peppermint oil.

Variation of some other peppermint oil constituents was also observed. The concentration of isomenthone varied from traces to 15.5% (Russian origin) and the concentration of *trans*-sabinene hydrate varied from traces to 6.2% (Russian origin). Differently from the other samples the oil from France contained 4.4% myrcene and the oils from Russia and France 3.5% and 5.9% piperitone (in the

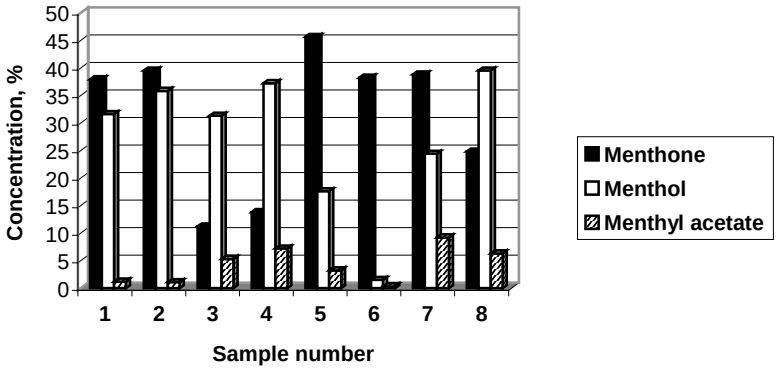

Fig. 2. Variation in the concentrations of the main components of peppermint oils isolated from samples of different European countries. Samples: 1 – Estonia 2000, 2 – Estonia 2002, 3 – France, 4 – Hungary, 5 – Belgium, 6 – Russia, 7 – Ukraine, 8 – Greece.

other samples these values were below 1.2% and 0.9%, respectively). The highest content of carvone (13.0%) was found in the oil of Russian origin. In the oils from other countries the carvone content was much lower (0–0.6%). The ratio between the concentration of 1,8-cineole (0.4–6.0%) and limonene (1.0–5.9%) varied in the interval 1–5.0 (as an exception, in the oil from Russia this ratio was 0.3). Sesquiterpenes in the peppermint oils were mainly represented by (E)-β-caryophyllene (0.7–4.3%) and germacrene D (1.1–3.1%).

CONCLUSION

The results of this work established noticeable quantitative differences in the quantity of biologically active compounds in peppermint oil from different origins. Consequently the organoleptic quality and pharmacological effects of these plants are also likely to differ. Estonian peppermint oil contained menthone and menthol in high quantities and menthyl acetate in low quantities. These values and a high yield of oil (up to 3.2%) showed a good quality of Estonian peppermint compared with other samples studied.

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Erinevatest geograafilistest paikadest pärit piparmündi (*Mentha* × *piperita* L.) eeterliku õli keemilise koostise võrdlus

Anne Orav, Ain Raal ja Elmar Arak

Erinevatest Euroopa riikidest pärit piparmündi lehtedest eraldati eeterlik õli veega destilleerimise teel (Euroopa farmakopöa meetod) ja saadud õlide koostis määrati kapillaargaasikromatograafilisel meetodil. Õli saagised kuiva taimmaterjali kohta jäid vahemikku 0,8–3,3%. Erinevate proovide põhikomponentide sisaldused varieerusid suures ulatuses: mentool 1,5–39,5%, mentoon 11,2–45,6%, isomentoon 1,3–15,5%, mentüülatsetaat 0,3–9,2%, piperitoon 0,8–5,9% ja pulegoon 0,1–13,0%. Mentooli ja mentooni sisalduse suhe oli väikseim Vene õlis (0,04) ja suurim Ungari ja Prantsuse õlis (2,7–2,8). Eesti piparmündiõli oli rikas mentooni (37,9–39,5%) ja mentooli (31,6–35,8%) poolest, sisaldades vaid vähesel hulgal mentüülatsetaati (1,1–1,2%).