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CHEMOTAXONOMY

Chemosystematic markers for the essential oils in leaves of *Mentha* species cultivated or growing naturally in Estonia

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Abstract. The content and composition of essential oils in leaves of *Mentha* × *piperita* L., *M. spicata* L., *M. arvensis* L., and *M. longifolia* (L.) Huds. from Estonia were investigated using hydrodistillation and GC–MS analyses. Some chemosystematic markers for the identification of *Mentha* species are presented. The essential oil yield of the studied species was 2.4–3.0%, 0.9–2.3%, 0.9–1.4%, and 0.7–2.5%, respectively. From the isolated essential oils, 75, 89, 67, and 69 compounds were identified and quantitatively evaluated, representing 92.2–99.5% of the total oil. The main constituents of *M.* × *piperita* oils were menthol (26.4–47.7%) and menthone (13.6–31.9%); the ratio of their content was usually between 1 and 3. Four chemotypes of *M. spicata* were determined: rich in carvone (62.1–67.4%), rich in piperitenone oxide (61.9%), rich in α-terpinyl acetate + *trans*-β-caryophyllene (61.9% and 11.4%, respectively), and rich in acetic acid,1-methyl-1-(4-methyl-5-oxo-cyclohex-3-enyl) ethyl ether (6.5%). The last two chemotypes were determined for the first time. Only the essential oils of *M. arvensis* were found to contain *trans*-1-octen-3-ol (3.8-7.7%) and neryl propionate (2.4% in both samples). Good chemosystematic markers for *M. arvensis* were also *trans*-(16.0–19.1%) and *cis*-β-ocimene (16.0–20.5%). The ocimene-rich chemotype was found by us for the first time. The chemosystematics of *M. longifolia* was found to be the most complicated, as many chemotypes have been determined by us and several other authors. In this study, a high concentration of carvone (51.4–57.6%) was characteristic of all analysed *M. longifolia* samples.

Key words: *Mentha* × *piperita*, *Mentha spicata*, *Mentha arvensis*, *Mentha longifolia*, essential oil, leaves, GC–MS.

INTRODUCTION

Several species of the genus *Mentha* are cultivated worldwide for the pharmaceutical, perfume, cosmetic, and food industries. Mints are characterized by great morphological variation, reflected in the high number of different taxonomic rank names. Recent studies conducted on the basis of morphological, cytological, and chemical markers have revealed that the genus *Mentha* consists of 18 species and includes 11 named hybrids (Krzyzanowska et al., 2011). The findings of our earlier study showed that the qualitative and quantitative content of different groups of terpenoids could be used in the chemosystematics of plant species (Orav et al., 2011a). Commercially, the most important mint species are *Mentha* × *piperita* L. (peppermint), *Mentha spicata* L. (spearmint), and *Mentha arvensis* L. var. *canadensis* (cornmint) (Hajlaoui et al., 2009).

For centuries, mints have been used as a folk remedy or in alternative medicine. They have been used to treat nausea, flatulence, vomiting in pregnancy, ulcerative colitis, jaundice, bronchitis, anorexia, dental caries, rheumatism, hysteria, infantile troubles, dysmenorrhea, amenorrhea, skin diseases, and fever. In addition, they have been consumed as a vermifuge and antidote (Jagetia and Baliga, 2002; Gursoy et al., 2009; Hajlaoui et al., 2009).

According to the European Pharmacopoeia (EP), *M.* × *piperita* leaf as a whole drug should contain not less than 12 mL/kg (1.2%) and as a cut drug, not less than 9 mL/kg (0.9%) of essential oil (European Pharmacopoeia, 2010). The EP postulates that terpenes in *M.* × *piperita* essential oil used in the pharmaceutical industry in Europe should be within the following ranges:

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limonene 1.0–5.0%, cineole 3.5–14.0%, menthone 14.0–32.0%, menthofuran 1.0–9.0%, isomenthone 1.5–10.0%, menthyl acetate 2.8–10.0%, isopulegol – maximum 0.2%, menthol 30.0–55.0%, pulegone – maximum 4.0%, and carvone – maximum 1.0%.

The content of *M.* × *piperita* oil and its composition have been widely studied also during the last three years (Schmidt et al., 2009; Sokovic et al., 2009; Bassole et al., 2010; Dolzhenko et al., 2010; Hussain et al., 2010a; Kizil et al., 2010; Zheljazkov et al., 2010b). The chief components of oil are menthone (11–46%), menthol (2–40%), isomenthone (1–16%), menthyl acetate (0.3–9%), pulegone (0.1–13%), piperidone (1–6%), limonene (1–6%), 1,8-cineole (0.4–6%), etc. (Orav et al., 2004).

Also the composition of *M. spicata* essential oil has been studied well by several authors, resulting in identification of different chemotypes (Edris et al., 2003; Chauhan et al., 2009; Mkaddem et al., 2009; Sokovic et al., 2009; Hussain et al., 2010a, 2010b; Kizil et al., 2010; Koliopoulos et al., 2010; Telci et al., 2010; Zheljazkov et al., 2010a, 2010b; Şarer et al., 2011). The major components identified in the oil of *Mentha arvensis* are menthol, *p*-menthone, isomenthone (Rao, 1999; Rao et al., 1999, 2000; Pandey et al., 2003; Hussain et al., 2010a, 2010b; Prasad et al., 2010). Menthone, menthol, pulegone, menthofuran, and other terpenes have been found to be principal constituents of *Mentha longifolia* (L.) Huds. (horse mint) from different countries and their chemo-geographical variation has been described by several authors (Viljoen et al., 2006; Asekun et al., 2007; Hajlaoui et al., 2008, 2009; Singh et al., 2008; Gursoy et al., 2009; Kakhky et al., 2009; Mkaddem et al., 2009; Hussain et al., 2010a; Koliopoulos et al., 2010).

Most *Mentha* species are characterized by great morphological variation, reflected in the high number of different taxonomic rank names. In spite of the rather stable qualitative oil composition of cultivated mints, in most wild-growing *Mentha* species a great chemical diversity is observed (Mimica-Dukic and Bozin, 2008). The findings of our earlier study showed that the qualitative and quantitative content of different groups of terpenoids could be used in the chemosystematics of plant species (Orav et al., 2011a).

The aim of the present study was to find chemosystematic markers for the identification of *Mentha* species.

EXPERIMENTAL

Plant material

The content and composition of essential oils hydrodistilled from leaves of *Mentha* × *piperita*, *Mentha spicata*, *Mentha arvensis*, and *Mentha longifolia* cultivated or growing naturally in Estonia was studied. All leaves of cultivated or wild *Mentha* species studied were collected from July to August 2011 from different locations in Estonia (Table 1). A herbarium of the collected plant species was made. The plant material

Table 1. *Mentha* spp. samples studied from Estonia

No.	*Mentha* spp.	Origin	Comments
1	*Mentha spicata* L.	Lääne-Viru county, Vihula rural municipality, Karepa village	Cultivated in Karepa herb farm
2	*Mentha spicata* L.	Tartu county, Võnnu rural municipality, Võnnu village	Cultivated in home garden
3	*Mentha spicata* L.	Jõgeva county, Puurmani rural municipality, Puurmani village	Cultivated in home garden
4	*Mentha* × *piperita* L.	Tartu county, Kambja rural municipality, Pangodi village	Cultivated in home garden
5	*Mentha longifolia* (L.) Huds.	Harju county, Kuusalu rural municipality, Pudisoo village	Cultivated in home garden
6	*Mentha* × *piperita* L.	Tartu county, Võnnu rural municipality, Võnnu village	Cultivated in home garden
7	*Mentha arvensis* L.	Tartu county, Võnnu rural municipality, Võnnu village	Growing wild
8	*Mentha arvensis* L.	Tartu county, Võnnu rural municipality, Võnnu village	Growing wild
9	*Mentha spicata* L.	Tartu county, Võnnu rural municipality, Võnnu village	Cultivated in home garden
10	*Mentha longifolia* (L.) Huds.	Botanical Garden of the University of Tartu	Cultivated in herb garden
11	*Mentha longifolia* (L.) Huds.	Botanical Garden of the University of Tartu	Cultivated in herb garden
12	*Mentha longifolia* (L.) Huds.	Botanical Garden of the University of Tartu	Cultivated in herb garden, different than No. 11
13	*Mentha* × *piperita* L.	Saare county, Saaremaa island	Cultivated by order of Vadi herb company, dried leaves obtained from pharmacy in Tartu, package labelled as 'Peppermint leaves'
14	*Mentha spicata* L.	Tartu county, Alatskivi rural municipality, Ronisoo village	Cultivated in home garden

was dried in a dark room at room temperature (20±2 °C) for ten days. Each sample was labelled, packed in a paper bag, and stored in the dark at room temperature until assayed in September 2011. Mint species were identified using the herbarium by botanist Dr Ülle Reier from the Department of Botany, University of Tartu. Samples Nos 13 and 14 were obtained from retail pharmacies in Tartu, Estonia; their packages were labelled as *Menthae piperitae folium*. The voucher specimens (No. Lamiaceae/Men1-13) are deposited in the Institute of Pharmacy, University of Tartu, Estonia.

Hydrodistillation of essential oil

The essential oil was isolated from dried plant material using the distillation method described in the European Pharmacopoeia (2010). To take up the essential oil 20 g of the whole drug and 0.50 mL of xylene were used. Distillation time was 2 h at a rate of 3–4 mL/min.

Analysis of essential oils

Capillary gas chromatography (GC–FID)

The essential oils were analysed using a Chrom-5 chromatograph (Laboratorni Pristroje Praha, Czech Republic) with a flame ionization detector (FID) on two fused silica capillary columns with two stationary phases: poly(5%diphenyl-95%dimethylsiloxane) (SPB^{TM}-5, 30 m × 0.25 mm, Supelco, Switzerland) and polar polyethylene glycol (SW-10, 30 m × 0.25 mm, Supelco, Switzerland). The film thickness of both stationary phases was 0.25 µm. Carrier gas helium (purity >99.999%), with a split ratio of 1 : 150 and flow rate of 30–35 cm/s, was applied. The temperature program was from 50 to 250 °C at 2 °C/min, the injector temperature was 250 °C. A Clarity Lite chromatography station (DataApex Ltd, Czech Republic) was used for data processing.

The components of the oils were identified by comparing their retention indices (RI) on two columns to the RI values of reference standards (from Sigma), to our RI data, and to literature data (Davies, 1990; Zenkevich, 1996, 1997, 1999). The results obtained were confirmed by GC–MS.

The percentage composition of the oils was calculated using the normalization method without correction factors. The relative standard deviation of the percentages of oil components in three repeated GC analyses of the single oil sample did not exceed 5%.

Gas chromatography/mass spectrometry (GC–MS)

GC–MS analysis of samples was carried out using a GCMS-QP2010 (Shimadzu, Japan) on a fused silica capillary column (30 m × 0.32 mm) with a bonded stationary phase: poly(5%-diphenyl-95%-dimethyl) siloxane (ZB-5, Zebron). For further details, see Raal et al. (2010).

RESULTS AND DISCUSSION

Content of essential oils

The yield of essential oil from different *M.* × *piperita* samples varied in the range of 0.1–3.0% (Table 2) and corresponded to the EP standard. As a comparison, in our earlier study, peppermint cut drugs from several countries contained 0.8–3.3% essential oil (Orav et al., 2004). The content of essential oil in *M. spicata* was 0.9–2.3%, in *M. arvensis* 0.9–1.4%, and in *M. longifolia* 0.7–2.5%. The oil yield from the leaves of other mints can be similar or even higher than that of *M.* × *piperita*, for example 2.9–3.2% in *M. spicata* (Edris et al., 2003; Şarer et al., 2011). According to Hussain and co-authors (2010a), *M.* × *piperita* contains 1.1–1.2%, *M. spicata* 1.0–1.2%, *M. arvensis* 0.9–1.7%, and *M. longifolia* 0.7–1.1% of essential oil.

Previously we found much more significant differences between the minimum and maximum yields of essential oils: 17-fold in *Coriandrum sativum* (Orav et al., 2011b), 16-fold in *Levisticum officinale* (Raal et al., 2008), 14-fold in *Thymus serpyllum* (Paaver et al., 2008), and 10-fold in *Chamomilla recutita* (Orav et al., 2010), collected or obtained as commercial samples from different retail pharmacies in various countries. Over years, the variance of oil yield in *Juniperus communis* branches was 14-fold (Raal et al., 2010). Therefore, instead of the content of essential oil, the yield of terpenoids should be the basis of the chemosystematics of the genus *Mentha*. Moreover, the content of oil can be affected by several factors such as climatic and vegetation variations.

Compositon of essential oils

Up to 75, 89, 67, and 69 compounds were identified and quantified in the oils isolated from *M.* × *piperita*, *M. spicata*, *M. arvensis*, and *M. longifolia*, representing 92.2–99.5% of the total oil (Table 2).

The *M.* × *piperita* oil samples studied contained 26.4–47.7% menthol. Menthol is mentioned as the main constituent of peppermint oil also by many other authors (Stojanova et al., 2000; Rohloff et al., 2001; Pino et al., 2002; Schmidt et al., 2009; Sokovic et al., 2009; Bassole et al., 2010; Dolzhenko et al., 2010; Hussain et al., 2010a; Kizil et al., 2010; Zheljazkov et al., 2010b). In our previous study, the essential oil composition of peppermint samples sold in pharmacies was determined. Samples from Estonia ($n = 2$) contained 31.6–35.8% menthol. Herbs from France, Hungary, Belgium,

Table 2. Content and composition (%) of essential oils of *Mentha* spp. Boldface highlights the main components (not less than 2%); tr – traces (<0.05%)

No.	Compound	RI		*M. spicata*			*M. × piperita*	*M. longifolia*	*M. × piperita*	*M. arvensis*		*M. spicata*	*M. longifolia*			*M. × piperita*	*M. spicata*
		SPB-5	SW-10	Sample number													
				1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	α-Thujene	924	1024	0.1	tr	0.1	0.1	–	–	0.1	0.2	0.1	0.1	0.1	tr	0.1	tr
2	**α-Pinene**	927	1020	0.7	0.7	0.7	0.8	0.5	0.8	**2.8**	**3.1**	0.3	0.7	0.3	0.6	0.7	0.7
3	Camphene	940	1069	tr	–	0.1	tr	tr	–	1.0	0.8	tr	tr	–	tr	tr	tr
4	**Sabinene**	968	1120	0.5	0.6	0.6	0.6	0.4	0.7	1.3		1.7	0.5	0.2	0.4	0.4	0.6
5	**β-Pinene**	970	1107	1.0	1.0	1.2	1.2	0.7	1.3	**9.1**	**13.0**	0.5	0.8	0.3	0.7	1.1	0.9
6	**1-Octen-3-ol**	980	1450	tr	–	0.1	0.1	–	–	**2.8**	**4.7**	tr	–	–	–	tr	–
7	**Myrcene**	990	1165	**2.3**	**2.4**	**5.5**	0.4	0.6	0.3	**2.2**	1.8	**5.8**	0.9	0.6	0.6	0.3	**3.3**
8	3-Octanol	995	1398	0.9	0.4	0.3	0.2	0.5	0.4	1.4	1.3	–	0.6	0.5	1.1	0.2	0.5
9	α-Phellandrene	1000	1068	0.1	0.1	–	–	0.1	–	tr	–	0.1	0.1	–	0.1	–	0.2
10	Isobutyl isovalerate	1005	1195	–	–	0.1	–	–	–	–	–	0.1	–	–	–	–	–
11	α-Terpinene	1012	1177	tr	–	0.1	0.2	0.1	0.2	0.1	0.1	0.1	tr	0.1	–	0.2	–
12	*p*-Cymene	1020	1264	–	–	–	0.1	–	0.1	–	–	tr	–	–	–	0.1	–
13	*trans*-2-Heptenyl acetate	1020	1405	–	–	–	–	–	–	0.1	0.3	–	–	–	–	–	–
14	**Limonene**	1025	1200	**10.4**	**11.8**	1.3	**8.9**	**10.3**	**9.8**	0.7	0.5	0.6	**8.2**	**2.6**	**18.8**	**2.8**	**13.9**
15	**1,8-Cineole**	1028	1209			**5.8**				**7.6**	**7.9**	**4.1**	–	1.5		**4.8**	–
16	***cis*-β-Ocimene**	1035	1237	0.5	0.7	1.6	0.7	0.2	0.6	**16.2**	**20.5**	1.3	0.4	0.3	0.2	0.4	0.8
17	*n*-Butyl-2-methyl butanoate	1037	1240	0.1	0.1	0.1	0.1	0.1	–	–	–	0.2	0.1	–	0.1	tr	–
18	***trans*-β-Ocimene**	1044	1250	0.2	0.3	0.2	0.2	0.1	0.2	**16.0**	**19.1**	0.5	0.1	0.1	0.1	0.1	0.3
19	γ-Terpinene	1052	1243	0.1	0.1	0.1	0.3	0.1	0.4	0.3	0.2	0.2	tr	–	tr	0.3	–
20	2-Methyl benzaldehyde	1058	1613	–	–	0.5	–	–	–	–	–	0.7	–	–	–	–	–
21	*trans*-2-Octen-1-ol	1058	1588	0.2	0.2	–	0.1	0.3	0.1	0.5	0.3	–	1.0	**2.7**	0.2	0.1	0.4
22	***cis*-Sabinene hydrate**	1063	1466	1.3	0.1	1.1	**2.6**	0.1	**2.7**	–	–	0.2	0.1	–	0.1	**3.2**	tr
23	Unidentified 1	1063	1558	–	–	–	–	–	–	0.2	1.3	–	–	–	–	–	–
24	Terpinolene	1083	1274	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.2	0.3	0.1	–	0.1	0.1	0.1
25	*trans*-Sabinene hydrate	1098	1540	–	–	–	0.3	–	0.5	–	–	–	–	–	–	0.7	–
26	Linalool	1100	1554	0.1	tr	0.4	–	tr	–	0.5	0.5	0.4	tr	–	tr	–	tr
27	Isoamyl isovalerate	1102	1300	tr	tr	tr	–	0.1	–	–	tr	0.3	0.1	0.1	0.2	–	0.1
28	Benzyl alcohol	1105		–	–	tr	–	–	–	tr	0.1	–	–	–	–	–	–
29	Amyl isovalerate	1106	1300	–	–	0.1	0.4	–	0.2	–	–	0.4	0.1	0.2	–	0.1	0.1
30	Octen-1-ol acetate	1113	1373	–	–	–	–	–	–	–	–	**2.7**	–	–	–	0.1	–

Table 2. *Continued*

No.	Compound	RI		*M. spicata*			*M. × piperita*	*M. longifolia*	*M. × piperita*	*M. arvensis*		*M. spicata*	*M. longifolia*			*M. × piperita*	*M. spicata*
		SPB-5	SW-10	Sample number													
				1	2	3	4	5	6	7	8	9	10	11	12	13	14
31	*trans*-*p*-Mentha-2,8-dienol	1118	1558	tr	tr	–	–	–	–	–	–	0.4	–	–	tr	–	0.1
32	3-Octanol acetate	1124	1333	0.1	0.3	0.3	tr	0.5	0.1	–	–	1.3	0.1	–	0.1	0.1	0.1
33	2(10)-Pinen-3-one	1124	1360	–	–	–	–	–	–	0.1	0.3	–	–	–	–	–	–
34	*cis*-*p*-Mentha-2,8-dienol	1129	1563	tr	–	–	0.1	–	–	0.2	0.2	0.3	–	–	tr	–	0.1
35	3,9-Epoxy-1-*p*-menthene	1132	1533	0.1	0.1	–	–	0.1	–	0.1	0.2	–	–	0.1	0.1	–	0.1
36	Camphor	1142	1500	–	–	–	–	–	–	–	0.1	–	–	–	–	tr	–
37	**Menthone**	1147	1454	–	0.1	0.1	**31.9**	tr	**13.6**	0.3	0.1	–	0.2	–	–	**30.1**	tr
38	**β-Citronellal**	1151	1468	–	–	–	–	–	–	–	–	0.3	–	–	–	–	–
39	**Menthofuran**	1159	1477	–	–	0.1	**3.3**	–	**3.9**	–	–	–	–	0.3	–	**4.5**	tr
40	Borneol	1160	1700	–	–	–	–	–	–	**1.9**	**1.5**	–	–	–	–	–	–
41	**Isomenthone**	1162	1468	0.1	0.3	0.3	**7.0**	tr	**2.8**	–	–	0.2	0.1	0.1	0.1	**2.4**	0.2
42	Isopinocamphone	1162		–	–	–	–	–	–	0.3	0.3	–	–	–	–	–	–
43	**Neomenthol**	1168	1598	–	–	–	**2.0**	–	**2.7**	–	–	0.1	–	–	–	**2.1**	–
44	**Menthol**	1171	1643	0.2	tr	–	**26.4**	tr	**47.7**	–	–	–	tr	0.1	0.1	**31.3**	0.1
45	3-Decanone	1171	1480	–	–	–	–	–	–	0.4	0.6	–	–	–	–	–	–
46	Terpinen-4-ol	1177	1602	0.1	–	0.3	tr	0.1	tr	0.1	0.2	0.3	–	–	–	0.1	–
47	Isomenthol	1186	1669	–	–	–	0.7	–	0.3	–	–	–	–	–	–	0.2	0.1
48	*p*-Cymen-8-ol	1186	1850	–	–	0.2	–	–	–	–	–	–	–	–	–	–	–
49	**α-Terpineol**	1190	1706	tr	–	0.2	tr	–	tr	**8.2**	**3.5**	**2.4**	–	–	–	0.1	–
50	Myrtenal	1190	1620	–	–	0.4	–	–	–	–	–	–	–	–	–	–	–
51	***cis*-Dihydrocarvone**	1193	1596	1.0	**4.8**	–	0.5	**3.7**	0.8	–	–	–	0.5	0.5	**2.3**	0.6	0.6
52	Dihydrocarveol	1193	1754	tr	1.5	–	–	1.3	–	–	–	–	–	–	–	–	tr
53	*cis*-2,6-Dimethyl-3,5,7-octatriene-2-ol	1193	1872	–	–	–	–	–	–	0.6	0.1	–	–	–	–	–	–
54	*trans*-2,6-Dimethyl-3,5,7-octatriene-2-ol	1202	1886	–	–	tr	0.1	–	0.1	0.3	0.4	–	–	–	–	0.1	–
55	*trans*-Dihydrocarvone	1206	1624	tr	–	–	–	–	–	–	–	tr	–	–	0.1	–	0.1
56	*n*-Octyl acetate	1216	1485	–	–	–	–	–	–	0.2	0.3	0.4	–	–	–	–	–
57	*trans*-Carveol	1216	1836	–	tr	–	tr	–	0.1	–	–	–	0.2	0.2	–	0.1	0.1
58	*cis*-3-Hexenyl isovalerate	1216	1506	–	–	0.4	–	–	–	tr	0.4	–	–	–	–	–	–

Continued overleaf

Table 2. *Continued*

No.	Compound	RI		*M. spicata*			*M. × piperita*	*M. longifolia*	*M. × piperita*	*M. arvensis*		*M. spicata*	*M. longifolia*			*M. × piperita*	*M. spicata*
		SPB-5	SW-10	Sample number													
				1	2	3	4	5	6	7	8	9	10	11	12	13	14
59	*cis*-Carveol	1227	1844	–	–	–	–	–	–	–	–	–	0.2	0.3	–	tr	0.1
60	*cis*-3-Hexenyl valerate	1235	1512	–	–	0.6	–	–	–	0.9	0.7	0.5	–	–	–	–	–
61	**Pulegone**	1236	1635	–	–	–	**2.2**	–	0.2	–	–	–	–	–	–	0.5	–
62	**Carvone**	1238	1730	**67.4**	**62.1**	–	–	**57.6**	–	tr	0.1	**2.3**	**56.8**	**51.4**	**56.4**	0.2	**66.5**
63	Hexyl isovalerate	1246	1575	–	–	tr	–	–	–	0.1	0.1	0.3	–	–	–	–	–
64	Piperitone	1250	1715	0.4	0.4	0.7	0.7	1.2	0.7	0.1	0.2	–	**2.7**	**2.4**	1.4	0.8	0.3
65	Methyl citronellate	1260	1588	–	–	–	–	–		–	0.2	–	–	–	–	–	–
66	*p*-Mentha-(7),8(10)-dien-9-ol	1266		0.1	0.2	–	–	0.2	–	–	–	0.3	0.1	–	0.1	–	0.1
67	Piperitone oxide	1273	1716	0.1	0.1	0.2	–	0.2	–	–	–	–	1.2	0.8	0.1	0.1	0.1
68	**1-Decanol**	1273	1770	–	–	–	–	–	–	**2.2**	1.5	–	–	–	–	–	–
69	Anethole	1280	1827	–	–	–	–	0.1	–	–	–	–	–	–	–	–	0.1
70	Dihydroedulan I	1280	1506	–	–	0.3	0.1	–	–	–	–	0.9	–	–	–	0.2	–
71	Neomenthyl acetate	1282	1629	0.1	0.1	–	0.1	0.1	0.2	–	–	tr	–	–	0.1	0.1	–
72	Bornyl acetate	1282	1575	–	–	tr	–	–	–	–	tr	–	–	–	–	–	–
73	4-Terpinenyl acetate	1284	1625	–	–	–	–	–	–	tr	0.4	–	–	–	–	–	–
74	Dihydroedulan II	1284	1540	–	–	0.5	–	–	–	–	–	–	–	–	–	–	–
75	Perillyl alcohol	1287	1890	0.1	0.1	–	tr	0.2	0.2	–	–	0.4	0.4	0.4	0.2	–	tr
76	**Menthyl acetate**	1290	1558	tr	tr	–	0.6	0.1	0.9	–	–	–	–	–	tr	0.6	0.1
77	Isomenthyl acetate	1296	1566	–	–	–	0.1	–	0.1	–	–	–	–	–	–	0.2	–
78	Dihydrocarvyl acetate	1304	1563	–	0.2	–	–	0.1	–	–	–	0.2	0.2	–	tr	0.1	–
79	*n*-Nonyl acetate	1311	1580	–	–	–	–	–	–	0.1	tr	–	–	–	–	–	–
80	β-Terpinyl acetate	1311	1648	–	–	–	–	–	–	–	–	0.5	–	–	–	–	–
81	Myrcenyl acetate	1315	1690	–	–	0.1	–	–	–	–	–	0.7	–	–	–	–	–
82	Isodihydrocarvyl acetate	1326	1642	tr	1.4	–	–	0.9	–	–	–	0.4	0.1	–	0.3	tr	0.1
83	*trans*-Carvyl acetate	1335	1730	0.1	0.2	–	–	0.1	–	–	–	–	–	–	0.3	–	0.1
84	δ-Elemene	1335	1477	–	–	0.1	–	–	–	–	–	–	–	–	–	–	–
85	**α-Terpinyl acetate**	1345	1695	0.1	–	–	–	–	–	–	–	**32.3**	–	–	–	–	–
86	Eugenol	1354	2165	–	–	–	–	–	–	–	–	0.4	–	–	–	–	–
87	α-Cubebene	1354	1449	–	–	–	–	–	–	0.1	tr	–	–	–	–	–	0.1
88	**Piperitenone oxide**	1358	1957	–	–	**61.9**	–	–	–	–	–	–	–	–	–	–	–
89	*cis*-Carvyl acetate	1360	1748	tr	0.3	–	0.1	0.3	–	–	–	0.7	–	–	0.1	tr	0.1
90	α-Copaene	1367	1480	0.1	tr	–	–	0.1	–	0.1	0.1	0.3	0.1	0.1	0.1	–	0.1
91	Geranyl acetate	1370	1758	–	–	–	0.1	–	tr	–	–	–	–	–	–	0.1	–

Table 2. *Continued*

No.	Compound	RI		*M. spicata*			*M. × piperita*	*M. longifolia*	*M. × piperita*	*M. arvensis*		*M. spicata*	*M. longifolia*			*M. × piperita*	*M. spicata*
		SPB-5	SW-10	Sample number													
				1	2	3	4	5	6	7	8	9	10	11	12	13	14
92	β-Cubebene	1375	1512	–	0.4	–	–	–	–	0.1	0.1	–	0.2	–	–	–	–
93	α-Bourbonene	1379	1508	1.3	0.7	tr	0.1	1.4	0.1	–	–	**2.6**	0.4	0.9	1.3	0.4	0.8
94	*cis*-3-Hexenyl hexanoate	1380	1650	–	–	–	–	–	–	0.4	0.2	–	–	–	–	–	–
95	*trans*-3-Hexenyl hexanoate	1385	1672	–	–	–	–	–	–	1.5	1.0	–	–	–	–	–	–
96	β-Elemene	1385	1587	0.4	0.7	0.3	0.1	0.3	0.1	–	–	0.7	0.4	0.3	0.3	0.2	0.9
97	3-Dodecanone	1393	1674	–	–	–	–	–	–	0.1	tr	–	–	–	–	–	–
98	*cis*-Jasmone	1393	1937	0.1	0.2	1.1	0.1	0.5	0.1	–	–	0.1	tr	–	0.1	0.1	0.1
99	***trans*-β-Caryophyllene**	1409	1584	**2.7**	**1.9**	**2.3**	1.2	**4.3**	1.2	**3.8**	**2.8**	**11.4**	**7.7**	**9.7**	**2.8**	**2.4**	1.9
100	***n*-Decyl acetate**	1412	1700	–	–	–	–	–	–	**5.0**	**2.4**	–	–	–	–	–	–
101	Unidentified 2, MW204	1420		0.2	0.1	0.1	tr	0.2	0.1	–	–	0.4	0.1	0.2	0.2	0.1	0.1
102	*cis*-Muurola-3,5-diene	1434	1580	0.1	0.1	0.4	–	0.6	–	–	–	0.2	tr	0.1	0.1	0.1	0.1
103	*cis*-Muurola-4(14),5-diene	1443	1613	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.1	0.5	0.3	0.5	tr	0.1	0.1
104	α-Humulene	1450	1664	0.2	–	0.3	tr	0.1	tr	0.2	0.2	0.3	0.5	0.4	0.1	0.1	–
105	*trans*-β-Farnesene	1456	1676	0.1	0.2	0.6	0.3	1.1	0.2	–	–	1.0	0.6	1.1	0.6	0.4	0.1
106	Alloaromadendrene	1459	1634	–	–	–	–	0.3		–	–	tr	0.1	0.3	0.2	–	–
107	**γ-Muurolene**	1473	1695	**2.7**	1.5	–	–	**2.6**	0.5	–	–	**3.2**	**9.4**	**12.0**	**4.2**	**1.6**	1.6
108	**Germacrene D**	1477	1700	–	–	**3.3**	**3.3**	–	1.8	**3.4**	1.8	–	–	–	–	1.2	–
109	γ-Elemene (Elixene)	1487	1678	0.5	0.2	0.7	0.4	0.3	0.3	0.4	0.1	0.9	0.7	1.0	0.3	0.6	0.2
110	*trans*-Muurola-4(14),5-diene	1494	1710	0.4	0.6	0.2	0.1	0.1	0.1	0.1	0.1	0.4	0.2	0.5	0.2	0.1	0.8
111	*n*-Pentadecane	1500	1500	–	–	–	–	0.1	–	–	–	–	–	–	–	–	–
112	γ-Cadinene	1503	1750	0.1	tr	0.2	0.1	0.2	tr	–	–	0.1	0.1	–	0.2	0.1	0.1
113	Cadina-3,9-diene	1512	1769	0.1	–	–	–	0.4	–	–	–	–	–	–	0.2	–	–
114	*cis*-Calamenene	1512	1815	–	–	0.1	–	–	–	–	–	–	–	–	–	–	–
115	δ-Cadinene	1516	1748	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.1	0.3	0.3	0.3	0.1	0.1	0.1
116	Cadina-1,4-diene	1528	1770	0.1	–	0.1	–	0.2	–	–	–	–	–	–	0.1	–	–
117	Caryophyllene epoxide	1540	1960	–	–	–	–	–	–	–	–	–	–	–	tr	–	–
118	Acetic acid,1-methyl-1-(4-methyl-5-oxo-cyclohex-3-enyl) ethyl ether	1561	2259	–	–	–	–	–	–	0.1	0.1	**6.5**	–	–	–	–	–
119	Spathulenol	1567	2120	0.1	0.1	0.1	–	0.1	–	0.3	0.1	0.2	0.1	0.1	0.1	0.1	0.1

Continued overleaf

Table 2. *Continued*

No.	Compound	RI		*M. spicata*			*M. × piperita*	*M. longifolia*	*M. × piperita*	*M. arvensis*		*M. spicata*	*M. longifolia*			*M. × piperita*	*M. spicata*
		SPB-5	SW-10	Sample number													
				1	2	3	4	5	6	7	8	9	10	11	12	13	14
120	Caryophyllene oxide	1570	1962	0.1	0.1	tr	0.1	0.2	0.1	0.2	0.2	0.4	0.6	0.7	0.2	0.2	0.1
121	Ethyl dodecanoate	1582	1830	–	–	–	–	–	–	0.3	0.2	–	–	–	–	–	–
122	Epiglobulol	1584	2080	0.2	–	–	0.7	0.7	0.6	–	–	–	–	–	0.9	1.1	0.1
123	Germacrene D-4-ol	1595	2052	0.1	–	tr	–	0.5	–	–	–	–	–	–	0.2	–	–
124	Humulene epoxide I	1604	2015	–	–	–	–	–	–	0.8	0.3	–	–	–	–	–	–
125	Cubenol	1606	2000	–	–	0.2	–	–	–	–	–	–	–	–	–	–	–
126	*cis*-3-Hexenyl phenyl acetate	1628	2221	–	–	–	–	–	–	0.8	0.6	–	–	–	–	–	–
127	Bicyclo[3.1.1]hept-2-en-4-ol, 2,6,6-trimethyl hexanoate	1630	2241	–	–	–	–	–	–	–	–	0.5	–	–	–	–	–
128	δ-Cadinol	1634	2161	tr	–	0.1	0.1	0.3	tr	–	0.1	–	0.2	tr	0.2	0.1	tr
129	α-Cadinol	1643	2218	0.2	tr	0.2	0.1	0.4	tr	–	–	0.5	0.3	0.4	0.2	0.1	tr
130	Isovaleryl decanoate	1645	2181	–	–	–	–	–	–	0.2	0.1	–	–	–	–	–	–
131	Selina-6-en-8-ol	1677	2267	0.1	0.1	–	–	0.2	–	–	–	0.3	0.2	0.4	0.1	–	0.1
132	Unidentified 3, MW 254	1760	2344	–	–	–	–	–	–	0.4	0.1	–	–	–	–	–	–
133	Hexahydrofarnesyl acetone	1840	2087	–	–	–	–	0.1	–	0.1	–	–	–	–	–	–	–
134	Palmitic acid	1972	2920	–	–	–	–	–	–	–	–	–	–	–	–	–	0.1
Total, %				98.7	97.9	97.4	99.5	96.8	98.2	98.0	98.1	97.0	99.2	95.2	98.1	99.4	98.7
Content of essential oil, %				2.1	2.1	0.9	3.0	1.4	2.4	0.9	1.4	1.1	2.2	0.7	2.5	3.0	2.3

See Table 1 for number and origin of samples.

Mass spectral data of unidentified compounds:

(1) – m/z: 150(100), 135(85), 91(52), 79(45), 107(32), 41(30), 96(27), 82(22)

(2) – m/z: 161(100),105(50),91(42), 81(25), 79(23), 55(20),41(20), 93(18), 120(16),133(15)

(3) – m/z: 114(100), 57(70), 43(60), 41(43), 66(40), 67(35), 71(30), 85(25)

– Not found.

Ukraine, Greece, and Russia contained menthol respectively 31.3%, 37.2%, 17.6%, 24.4%, 39.5%, and 1.5% (Orav et al., 2004). Thus, it may be concluded that other mint species are sometimes sold as *M.* × *piperita*.

Menthone, the second main constituent of *M.* × *piperita* oil, can also be used in the chemosystematics of peppermint. The oil of the *M.* × *piperita* sample from Pangodi village contained menthone even above the EP limit (32%). Menthone and menthol were the typical components of peppermint oils and can be used as chemical markers. The ratio between their content according to our current study was 0.05–3.4, but usually 1–3; the same ratio was found in our previous (Orav et al., 2004) and other studies (Pino et al., 2002; Schmidt et al., 2009; Sokovic et al., 2009). According to Rohloff and co-authors (2001), changes in menthone–menthol metabolism are related to reduced menthone content from the vegetative stage to full bloom.

Four chemotypes of *M. spicata* were found: rich in carvone (samples 1, 2, and 14; see Table 2), rich in piperitenone oxide (sample 3), rich in α-terpinyl acetate and *trans*-β-caryophyllene (both in sample 9). Acetic acid,1-methyl-1-(4-methyl-5-oxo-cyclohex-3-enyl) ethyl ether was determined only in sample 9. The studied oils of *M.* × *piperita* contained only low concentrations of carvone, piperitone oxide, and germacrene D, while α-terpinyl acetate was not present in these oils. Carvone was found to be one of the main constituents of *M. spicata* oils also in other studies: carvone alone or carvone and limonene by Edris et al. (2003), Chauhan et al. (2010), Hussain et al. (2010a, 2010b), Zheljazkov et al. (2010a); carvone and 1,8-cineole by Mkaddem et al. (2009) and Şarer et al. (2011). In addition, piperitone oxide and limonene (Koliopoulos et al., 2010) or pulegone and piperitone (Telci et al., 2010) were established as their main constituents. Only traces of menthone and menthol were present to a maximum of 0.1% in the spearmint oils analysed by us. However, *M. spicata* oil may sometimes contain remarkably high amounts of menthone or menthol but not both simultaneously. Hence, we found two new chemotypes of spearmint: rich in α-terpinyl acetate + *trans*-β-caryophyllene and rich in acetic acid,1-methyl-1-(4-methyl-5-oxo-cyclohex-3-enyl) ethyl ether. In addition, the *M. spicata* oils were found to contain more myrcene than other mint oils studied by us.

The presence of *trans*-1-octen-3-ol and neryl propionate was specific only to essential oils from *M. arvensis* (Table 2). Good chemosystematic markers for *M. arvensis* were also *trans*- and *cis*-β-ocimene, both being present in the concentration of 16% and more. This mint species contained also significantly more β-pinene and α-pinene, as well as 1-octen-3-ol than other mint species studied. In other studies (Rao, 1999; Rao et al., 1999; Pandey et al., 2003; Hussain et al., 2010a; Pandey et al., 2010; Prasad et al., 2010) menthol was determined as a principal compound of *M. arvensis* oil but *cis*-β-ocimene was not found. The ocimenes-rich *M. arvensis* chemotype was determined by us for the first time.

The present study showed that carvone should not be considered as a specific chemosystematic marker for *M. spicata* carvone-chemotype because carvone was found also in the oils of *M. longifolia*, whose chemosystematics is most complicated. In two samples out of four (Nos 10 and 11, collected from the same place) rather high concentrations of germacrene D with α-muurolene, limonene with 1,8-cineole (samples 5, 10, and 12), and *trans*-β-caryophyllene (samples 10 and 11) and less *trans*-dihydrocarvone (samples 5 and 12) and piperitone (samples 10 and 11) were found. Other authors identified the mentofuran-rich (Viljoen et al., 2006), piperitone oxide-rich (Hussain et al., 2010a), *cis*-piperitone oxide and piperitenone oxide-rich (Viljoen et al., 2006; Singh et al., 2008; Kakhky et al., 2009), menthone-rich (Asekun et al., 2007), menthone, menthol, and pulegone-rich (Hajlaoui et al., 2008), pulegone-rich (Mkaddem et al., 2009), carvone and limonene-rich, piperitenone oxide and 1,8-cineole-rich (Koliopoulos et al., 2010) chemotypes of *M. longifolia*. The oils of *M. spicata* contained much more myrcene (2.3–5.5%) than *M. longifolia* (0.6–0.9%). Quite large amounts of menthofuran were found in *M.* × *piperita* oils and a very low concentration in one *M. longifolia* sample (No. 11) from four. Hence, the chemosystematics of *M. longifolia* based on the terpenes composition seems to be rather complicated. Phenolic compounds may probably be better chemical markers for *M. longifolia* and *M.* × *piperita*. Krzyzanowska and co-authors (2011) showed that all their samples of *M.* × *piperita* had higher concentrations of ester of caffeic acid and 3,4-dihydroxyphenyllactic acid than *M. longifolia* samples.

The main results of our study are presented in Table 3.

Table 3. Some chemosystematic markers of *Mentha* species

Mentha species	Chemosystematic markers
Mentha × *piperita*	High concentration of both menthol and menthone, usually their ratio between 1–3. No α-terpinyl acetate
Mentha spicata	High chemical diversity of terpenes. Sometimes contain remarkably high amounts of menthone or menthol but not simultaneously both. 2–6% myrcene
Mentha arvensis	Presence of *trans*-1-octen-3-ol and neryl propionate. Rather high concentration of *trans*- and *cis*-β-ocimene. Significant amounts of β-pinene, α-pinene, and 1-octen-3-ol
Mentha longifolia	High chemical diversity of terpenes. 1–3% of piperitone. No germacrene D, 1-caffeic acid, and 3,4-dihydroxyphenyllactic acid

CONCLUSION

The content and composition of essential oils hydrodistilled from four *Mentha* species cultivated or growing naturally in Estonia were studied. Some specific chemosystematic markers for their identification are presented. The markers found in the oils studied by us should also be evaluated in a larger number of mint samples and in other mint species.

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Eestis kultiveeritavate ja looduslikult kasvavate *Mentha* liikide lehtede eeterlike õlide kemosüstemaatilised markerid

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On uuritud Eestist pärineva piparmündi (*Mentha* × *piperita*), rohemündi (*M. spicata*), põldmündi (*M. arvensis*) ja pikalehise mündi (*M. longifolia*) vesidestillatsioonil eraldatud eeterliku õli sisaldust ning GC-MS-meetodil selle koostist. On esitatud mõningad kemosüstemaatilised markerid erinevate mündiliikide identifitseerimiseks. Eeterliku õli sisaldus uuritud mündiliikides oli vastavalt 2,4–3,0%, 0,9–2,3%, 0,9–1,4% ja 0,7–2,5%. Nimetatud liikide eeterlikes õlides identifitseeriti ja määrati kvantitatiivne sisaldus vastavalt 75, 89, 67 ning 69 ühendil, mis moodustasid 92,2–99,5% õlide koguhulgast. *Mentha* × *piperita* eeterliku õli põhikomponendid olid mentool (26,4–47,7%) ja mentoon (13,6–31,9%); nende sisalduse suhe oli tavaliselt vahemikus 1–3. Tehti kindlaks neli *M. spicata* kemotüüpi: karvooni (62,1–67,4%), piperitenoonoksiidi (61,9%), α-terpinüülatsetaadi + *trans*-β-kariofülleeni (vastavalt 61,9% ja 11,4%) ning äädihkappe,1-metüül-1-(4-metüül-5-okso-tsükloheksa-3-enüül)etüüleetri (6,5%) kemotüüp, kaks nendest esmakordselt. *Trans*-1-okteen-3-ooli (3,8–7,7%) ja nerüülpropionaati (2,4% mõlemas proovis) leiti ainult *M. arvensis*'e eeterlikust õlist. Liik *M. arvensis*'e jaoks on headeks kemosüstemaatilisteks markeriteks ka *trans*- (16,0–19,1%) ja *cis*-β-otsimeen (16,0–20,5%), kusjuures otsimeeni kemotüüp on leitud esmakordselt meie poolt. Kõige komplitseeritum on *M. longifolia* kemosüstemaatika, nii meil kui ka kirjanduses on andmeid paljudest selle taimeliigi kemotüüpidest. Kõikidele *M. longifolia* uuritud proovidele oli iseloomulik suur karvoonisisaldus (51,4–57,6%).