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Chemical diversity in essential oil composition of *Mentha longifolia* (L.) Hudson subsp. *typhoides* (Briq.) Harley var. *typhoides* from Turkey

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The essential oil obtained by hydro-distillation of leaf and flower in *Mentha longifolia* (L.) Hudson subsp. *typhoides* (Briq.) Harley var. *typhoides* (Lamiaceae) collected on the flora of Tokat (Black Sea region of Turkey) has been investigated by gas chromatography (GC) and GC–mass spectrometry (GC–MS). As a result of cluster analysis of oil components, four chemotypes – piperitone oxide (PO), piperitone oxide/piperitenone oxide (PO/PNO), *p*-menthone/piperitone oxide (MN/PO) and *trans*-dihydrocarvone (DC) – were characterized in both leaf and flower oils. Piperitenone oxide/piperitone oxide (PNO/PO), piperitone oxide/*p*-menthone/pulegone (PO/MN/PL) and linalool (LI) are distinguished in flower oil, while piperitone oxide/carvacrol/thymol (PO/CR/TH), linalool/isomenthone (LI/IMN) and linalool/piperitone oxide (LI/PO) are distinguished in leaf oil. Samples of the chemotypes have been conserved in botanical garden of Agricultural Research and Application Center in Gaziosmanpasa University for biological activity studies in future.

Keywords: *Mentha longifolia* subsp. *typhoides*; essential oil composition; chemotype

Introduction

The genus *Mentha*, belonging to Lamiaceae family, includes eighteen species and eleven hybrids (1), among which several species, *M. spicata, M. canadensis, M.* × *piperita* etc., have economic importance due to their high-valued oil and good taste (2, 3). The species are used in perfumery, confectionary and pharmaceutical preparations. They are also used in different traditional medicinal treatments as herbal remedies and in the food industry as food additives and taste enhancers because of their good odors (4, 5).

Genus *Mentha* contain fifteen taxa belonging to eight species in the flora of Turkey (6, 7). *Mentha longifolia* is one of wild mint species in Turkey and includes three subspecies of two varieties. *Mentha longifolia* subsp. *typhoides* var. *typhoides* is a more intensive taxon than other taxa in the middle Black Sea region of Turkey. As a result of previous records, there is large chemotypic diversity in *Mentha* species. *Mentha longifolia* has linalool, carvone and piperitone oxide chemotypes as explained by Kokkini (8). Fleisher and Fleisher (9) reported additionally chemotypes with cineol/piperitone oxide/piperitone and pulegone from Egypt and Israel. Lawrence (10, 11) also reviewed oil composition of *Mentha* commercial and non commercial species, subspecies and varieties. According to the records, *M. longifolia* subsp. *typhoides* var. *typhoides* contained a rich chemical diversity and piperitone oxide, piperitenone oxide, pulegone and carvone were the major components. Similarly, Baser et al. (12) explained six different chemotypes with piperitone oxide, linalool, carvone, *trans*-sabinene hydrate, menthone and β-caryophyllene in *M. longifolia* subsp. *typhoides* var. *typhoides* from the Black Sea region of Turkey.

Oil composition depends upon both biotic (genetic, ontogeny, morphogenesis) and abiotic (climate, soil, temperature etc.) factors affecting plant growth. Morphogenetic variability is the variation of oil compositions according to different parts of the plant such as flower, leaf, root etc. Essential oil compositions were generally studied in the leaves of *Mentha* species, and there are very few records on comparison of essential oils from different parts of *M. longifolia*. We observed that flower yield is higher in the *Mentha longifolia* subs. *typhoides* var. *typhoides* than other wild species and subspecies. So, we aimed to evaluate the chemical variation of both flower and leaves, and to cluster according to the diversity in *M. longifolia* subs. *typhoides* var. *typhoides* from the middle Black Sea region of Turkey (Tokat).

Experimental

Plant material

Aerial parts of *Mentha longifolia* subsp. *typhoides* var. *typhoides* were collected during August of 2008 in

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Tokat flora (C-5 and C-6) located in the Middle Black Sea of Turkey. Twenty samples (100–150 g) were collected from different localities and dried at room conditions. The leaves and flowers were separated before essential oil distillation (Table 1). Plant herbariums of all samples were prepared and named botanically. Voucher specimens of the collected plants are kept in Herbarium of Uludag University, Bursa (BULU).

Isolation of essential oils

The essential oils were isolated from both flowers and leaves of each sample, separately, by hydro-distillation with a Clevenger apparatus. Air-dried plant material (flower and leaf) of 20-g samples were diluted with 300 mL distillated water (1:15 w/v). Distillations were performed for 2 hours. Essential oil were dried over anhydrous sodium sulfate and kept at 4°C until gas chromatography (GC) and GC–mass spectrometry (GC–MS) analysis (13).

GC and GC–MS analysis

GC analyses were performed using a Perkin–Elmer Clarus 500 Series GC system, in split mode, 50:1, equipped with a flame ionization detector (FID) and a mass spectrometer-equipped BPX-5 apolar capillary column (30 m × 0.25 mm, 0.25 m i.d.). Helium (1.0 mL/minute) was used as carrier gas. The injector temperature was set at 250°C and the FID was operated at 250°C. An initial column oven temperature of 50°C was elevated to 220°C at a rate of 8°C/minute and held for 5 minutes. The mass spectrometer conditions were as follows: transfer line temperature at 250°C, ion source at 250°C and the ionization energy at 70 eV. The standard components were available for the majority of the essential oil constituents and Kovats retention indices (RIs) were determined for all the sample components using the Van den Dool and Kratz (14) equation according to homolog *n*-alkane series retention times. The relative peak area percentages of compounds were calculated based on the FID data.

Identification of components

Identification of oil components was accomplished: (a) by comparison of the mass spectra with those of the Willey and NIST 08 computer mass libraries; (b) by comparison of RIs with those reported in the literature with BPX-5 column (15–19) and Adams (20).

Data analysis

Mean, standard deviation, minimum and maximum data of essential oil content (mL/100 g) were calculated and the relationship between oil content and altitude were analyzed for both flower and leaf parts. Cluster analysis was used to classify and to group all the chemotypes according to their main components. Cluster analyses based on selected components were calculated using the Euclidean distance measure. For the grouping of the chemotypes, the agglomerative and hierarchical methods were applied. The computations were performed using SPSS package software (Version 16).

Results and discussions

Essential oil yield

Essential oil contents varied between 1.00 and 3.06 mL/100 g in flowers, and between 0.86 and 2.46 mL/100 g in leaves. Mean data were 1.66 and 1.79 mL/100 g in flower and leaf oils, respectively. Although the minimum and maximum data of flower oil is higher than that of leaves, the mean data for leaves is higher as a result of causing lower values of standard deviation (±0.38). So, plasticity in flower oil was higher than that of the leaves. There are diverse correlations ($r = -0.40$) between oil content and altitude in flower oil, contrary to leaf oil, which was insignificant correlated with altitude. Oil contents of flowers in low altitudes generally are higher than that of high altitudes.

Essential oil composition

Compositions of both flower and leaf oils were identified by GC–MS, and the major components were subjected to cluster analysis. As a result of clustering, eight chemotypes were identified in leaf and flower oil (Figure 1). All chemotypes were summarized in Table 2 and, the mean values of essential oil composition in the chemotypes of flower and leaf oil are listed in Tables 3 and 4, respectively. Piperitone oxide and piperitenone oxide are major components in the species, and three chemotypes were characterized in the components from both leaf and flower essential oils. They are: piperitone oxide (PO), piperitone oxide/piperitenone oxide (PO/PNO) and piperitenone oxide/piperitone oxide (PNO/PO).

PO/PNO are major chemotypes in both leaf and flower oils and comprise nine and seven samples, respectively. The mean data of piperitone oxide and piperitenone oxide were 61.6% and 20.4% in leaves and, 52.4% and 32.3% in flowers, respectively. PO is another chemotype obtained in three and four samples in leaf and flower oils, respectively. The mean piperitone oxide contents of leaf and flower oil in the chemotype are 87.5% and 73.6%. Piperitone oxide contents in both chemotypes were higher in leaves than that of flowers.

PNO/PO is an additional chemotype characterized in flower oil with 43.1% and 28.2%, respectively, although there were no samples containing piperitenone oxide as maximum contents in leaf oil. So, the chemotype PNO/PO was characterized in two samples of flower oil.

Table 1. Locations and oil contents of plant samples.

No	Sample no.	Locations	Oil contents (mL/100 g)		Area			Herbarium no. BULU
			Flower	Leaves	Latitude	Longitude	Altitude	
1	K4	Çamlıca 3 km	2.26	2.06	40°24 235	36°12 527	647	33630
2	R10	Başçitftlik	1.50	2.26	40°33 502	36°10 048	1447	33649
3	R4	North of Niksar	1.91	1.66	40°34 000	36°57 609	361	33643
4	R7	Hanyeri village	1.70	1.80	40°30 794	36°02 176	608	33646
5	Y1	Dodurga	1.26	1.66	40°02 275	36°22 483	1125	33654
6	Y10	Artova	2.40	1.93	40°06 909	36°18 413	1168	33663
7	Y11	Aktepe	1.40	1.46	40°04 975	36°30 051	1124	33664
8	Y13	Bedirkale village	1.11	1.33	40°03 954	36°27 006	1122	33666
9	Y14	Yeşilyurt çıkışı	1.33	2.33	40°00 251	36°08 673	1068	33667
10	Y15	Artova kunduz	1.33	1.53	40°03 958	36°17 980	1086	33668
11	Y2	Artova yol ayrımı	1.26	1.80	40°11 867	36°29 180	1154	33655
12	Y3	Artova 10 km kala	1.00	1.53	40°08 325	36°21 425	1245	33656
13	Y7	Başören yol ayrımı	1.53	1.80	40°14 823	36°32 755	758	33660
14	Z1	Pazar/Tatar	1.57	1.80	40°16 206	36°14 370	546	33673
15	Z10	Pazar yol ayrımı	2.30	2.20	40°18 144	36°22 640	574	33681
16	Z11	Söngüt	1.80	0.86	40°19 272	36°23 372	558	33682
17	Z13	Köy hizmetleri	1.53	1.33	40°20 109	36°24 616	563	33684
18	Z2	Hacıpınar	3.06	2.46	40°16 092	36°10 094	538	33674
19	Z4	Kazgölü	1.13	1.86	40°16 202	36°09 441	536	33676
20	Z7	Zilegirişi	1.53	1.80	40°16 228	36°59 858	615	33678

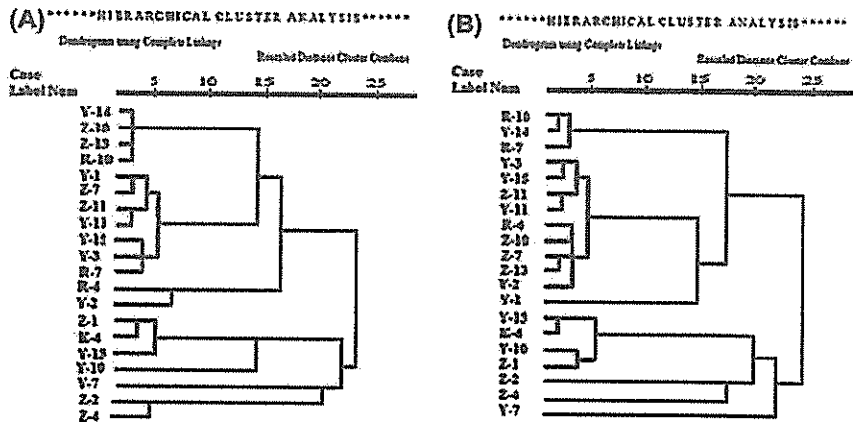

Figure 1. Clustering of flower (A) and leaf (B) oil compositions in *Mentha longifolia* subsp. *typhoides* var. *typhoides*.

Table 2. Chemotypes of flower and leaf oils in *Mentha longifolia*.

Chemotypes	Flower	Leaf
Piperitone oxide (PO)	R-10, Y-14, Z-10, Z-13,	R-7, R-10, Y-14
Piperitone oxide/piperitenone oxide (PO/PNO)	R-7, Y-1, Y-3, Y-11, Y-15, Z-7, Z-11	R-4, Y-2, Y-3, Y-11, Y-15, Z-7, Z-10, Z-11, Z-13,
Piperitenone oxide/piperitone oxide (PNO/PO)	R-4, Y-2	
p-Menthone/piperitone oxide (NM/PO)	Z-1, K-4, Y-13	K-4, Y-10, Y-13, Z-1
Piperitone oxide/carvacrol/thymol (PO/CR/TH)		Y1
Piperitonoxide/*p*-menthone/pulegone (PO/MN/PL)	Y-10	
trans-Dihydrocarvone (DC)	Y-7	Y-7
Linalool (LI)	Z-2, Z-4	
Linalool/isomenthone (LI/MN)		Z-2
Linalool/piperitone oxide (LI/PO)		Z-4

Piperitone oxide is also first or second major component in other three chemotypes of leaves and flower oils. The chemotypes are: *p*-menthone/piperitone oxide (MN/PO), piperitone oxide/carvacrol/thymol (PO/CR/TH) and piperitonoxide/p-menthone/pulegone (PO/MN/PL). MN/PO is characterized by four samples of leaf oil and three of flower oil. The contents of *p*-menthone and piperitone oxide were 49.5% and 18.2% in flower oil and, 47.3% and 16.2% in leaf oil, respectively. PO/CR/TH in leaf oil and PO/MN/PL in flower oil was represented by one sample.

Trans-dihydrocarvone (DC) is a chemotype found only in one sample of leaves and flowers. It was 69.6% in flower oil and 76.4% in leaf oil.

In our investigations, three chemotypes with linalool as major component were found. They are linalool (LI), linalool/isomenthone (LI/IMN) and linalool/piperitone oxide (LI/PO). While chemotype LI has two samples of flower oil, the other two chemotypes, LI/IMN and LI/PO, were characterized by one sample in leaf oils. Linalool contents are 61.3% in flower oil, while it is 46.8% and 54.9% in leaf oils of LI/IMN and LI/PO chemotypes, respectively. Isomenthone and piperitone oxide are second major components in the respective chemotypes of leaf oils with 34.8% and 32.7%, respectively.

Mentha is the most important genus in the *Lamiaceae* family because of high economic value, genetic and chemical diversity. Although there are nearly thirty species in the genus containing eighteen species and eleven hybrids according to Tucker and Naczi (1), some of them, *M. piperita*, *M. canadensis*, *M. spicata*, *M. citrata* etc. have economic value and are cultivated commercially (3–11). However, most species in the genus grow naturally worldwide and there are many studies on their essential oil compositions. *Mentha longifolia* has a worldwide distribution and is characterized by twenty-two subspecies (1). Scanning of the wild mint species of the Tokat region of Turkey, showed that the most common species were similar to reports of Harley (6). Quantitative differences of major components caused different groups of oils.

Table 3. Essential oil composition (%) in flower oil of *Mentha longifolia* subsp. *typhoides* var. *typhoides*.

				Chemotypes												
				PO		PO/PNO		PNO/PO		MN/PO		PO/MN/PL	LI		DC	
Components[a]	RI[b]	RI[c]	RI[d]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	1 sample	Mean	SD	1 sample	IM[e]
3-Octanol	1005	995	988	0.1	*0.0*	0.2	*0.1*	0.4	*0.2*	0.3	*0.2*	0.4	0.4	*0.0*	0.2	a,b
p-Cymene	1036	1046	1020	0.2	*0.0*	0.3	*0.2*	0.3	*0.2*	0.6	*0.2*	0.3	0.3	*0.0*	0.9	a,b
Limonene	1041	1050	1024	0.1	*0.0*	0.1	*0.0*	0.2	*0.0*	0.2	*0.0*	0.2	0.2	*0.0*	0.3	a,b,c
Eucalyptol	1045	1038	1026	0.0	*0.0*	0.2	*0.1*	0.2	*0.2*	0.4	*0.0*	0.2	0.3	*0.0*	0.4	a,b,c
γ-Terpinene	1071	1068	1054	0.0	*0.0*	0.0	*0.0*	0.0	*0.0*	0.0	*0.0*	0.0	0.1	*0.0*	0.0	a,b
cis-Sabinene hydrate	1079	1067	1065	0.1	*0.0*	0.4	*0.3*	0.0	*0.0*	0.0	*0.0*	1.0	0.0	*0.0*	0.0	a,b
Linalool	1109	1110	1095	0.3	*0.1*	1.0	*1.4*	4.9	*6.6*	0.2	*0.1*	0.0	**61.3**	*12.3*	0.2	a,b
3-Octanol acetate	1135	1125	1120	0.0	*0.0*	0.0	*0.0*	0.3	*0.2*	0.0	*0.0*	0.0	0.1	*0.0*	0.0	a,b
p-Menthone	1167	1156	1148	0.6	*0.1*	0.9	*0.3*	0.7	*0.3*	**49.5**	*10.2*	**26.6**	0.4	*0.0*	0.5	a,b
Isomenthone	1176	1167	1158	0.2	*0.0*	0.3	*0.1*	0.2	*0.1*	3.3	*0.2*	10.2	12.4	*0.0*	0.0	a,b
Menthol	1184	1173	1167	0.0	*0.0*	0.1	*0.0*	0.0	*0.0*	0.0	*0.0*	0.0	0.0	*0.0*	0.0	a,b,c
Isopulegone	1186	–		0.0	*0.0*	0.1	*0.0*	0.0	*0.0*	0.2	*0.1*	0.0	0.0	*0.0*	0.0	a,b
4-Terpineol	1188	1189	1174	0.0	*0.0*	0.1	*0.0*	0.0	*0.0*	0.0	*0.0*	0.0	0.0	*0.0*	0.0	a,b
α-Terpineol	1200	1198	1186	0.1	*0.0*	0.0	*0.0*	0.0	*0.0*		*0.0*	0.0	0.0	*0.0*	0.0	a,b
cis-Dihydrocarvone	1206	1193	1191	0.3	*0.1*	0.4	*0.2*	0.3	*0.1*	0.3	*0.1*	0.4	0.1	*0.0*	9.7	a,b
Dihydrocarveol	1207	1226	1192	0.0	*0.0*	0.0	*0.0*	0.1	*0.0*	0.6	*0.1*	0.2	0.0	*0.0*	0.7	a,b
trans-Dihydrocarvone	1215	1217	1200	0.1	*0.1*	0.2	*0.0*	0.5	*0.0*	0.0	*0.0*	0.0	0.0	*0.0*	**69.6**	a,b
Verbenone	1229	–	1204	0.0	*0.0*	0.2	*0.1*	16.1	*0.3*	0.3	*0.0*	0.2	0.0	*0.0*	0.0	a,b
Pulegone	1234	1250	1233	0.0	*0.0*	0.5	*0.5*	0.1	*0.0*	6.1	*0.5*	**14.7**	10.7	*0.0*	0.0	a,b,c
Carvone	1248	1259	1239	0.0	*0.0*	0.0	*0.0*	0.0	*0.0*	0.0	*0.0*	0.0	0.0	*0.0*	1.4	a,b,c
Geraniol	1252	1254	1249	0.2	*0.0*	0.0	*0.0*	0.0	*0.0*	0.0	*0.0*	0.0	0.1	*0.0*	0.0	a,b,c
Piperitonoxide	1271	–		**73.6**	*4.0*	**52.4**	*5.2*	**28.0**	*7.7*	**18.2**	*3.5*	**28.2**	8.1	*6.5*	6.2	a,b
Isopiperitenone	1286	–		0.1	*0.1*	0.2	*0.1*	1.5	*0.0*	0.0	*0.0*	0.0	0.0	*0.0*	0.0	a,b
Thymol	1304	1303	1289	0.2	*0.0*	3.3	*4.4*	0.2	*0.0*	5.6	*8.1*	0.4	0.0	*0.0*	0.0	a,b,c
Carvacrol	1314	1307	1298	0.0	*0.0*	2.6	*5.1*	3.1	*0.0*	0.2	*0.1*	0.2	0.0	*0.0*	0.7	a,b,c
p-Menthan-4-ol	1331	–		0.1	*0.0*	0.5	*0.3*	0.8	*0.0*	0.2	*0.0*	0.5	0.0	*0.0*	0.0	a,b
α-Terpinyl acetate	1367	–	1346	0.0	*0.0*	0.0	*0.0*	0.0	*0.0*	0.0	*0.0*	0.0	0.0	*0.0*	0.2	a,b
Piperitenone oxide	1383	–	1366	**16.2**	*4.8*	**32.3**	*9.9*	**43.1**	*18.9*	3.1	*1.3*	13.8	0.0	*0.0*	3.4	a,b
Caryophyllene	1442	1432	1417	1.2	*1.1*	0.7	*1.1*	1.0	*0.7*	0.3	*0.2*	0.2	0.6	*0.0*	0.0	a,b
Germacrene D	1502	1496	1484	0.0	*0.0*	0.4	*0.0*	0.0	*0.0*	0.0	*0.0*	0.0	0.1	*0.0*	0.0	a,b,c
Spathulenol	1604	1585	1577	0.0	*0.0*	0.2	*0.0*	0.1	*0.0*	0.0	*0.0*	0.0	0.0	*0.0*	0.0	a,b
Caryophyllene oxide	1612	1593	1582	1.0	*0.6*	1.3	*1.5*	0.8	*0.0*	1.9	*1.4*	0.9	0.0	*0.0*	3.1	a,b
				97.9	*0.6*	94.5	*2.8*	96.6	*2.1*	92.3	*3.2*	98.5	99.5	*0.0*	97.4	

Notes: [a]Components are listed in order of their elution from a BPX-5 column. [b]Linear retention index on BPX-5 capillary column. [c]Relative linear retention index from literature. [d]Retention indices of Adams' library. [e]Identification methods: MS (a) by comparison of the mass spectrum with those of the computer mass libraries Willey and NIST 08; RI (b) by comparison of RI with those reported in the literature; (c) by comparison of the retention time and MS spectrum of available authentic standards. Chemotypes: PO, piperitone oxide; PO/PNO, piperitone oxide/piperitenone oxide; PNO/PO, piperitenone oxide/piperitone oxide; MN/PO, *p*-menthone/piperitone oxide; PO/MN/PL, piperitone oxide/*p*-menthone/pulegone; LI, linalool; DC, *trans*-dihydrocarvone.

Table 4. Essential oil composition (%) in leaf oil of *Mentha longifolia* subsp. *typoides* var. *typoides*.

				Chemotypes										
				PO		PO/PNO		PO/CR/TH	MN/PO		DC	LI/IMN	LI/PO	
Components[a]	RI[b]	RI[c]	RI[d]	Mean	SD	Mean	SD	1 sample	Mean	SD	1 sample	1 sample	1 sample	IM[e]
3-Octanol				0.3	*0.3*	0.2	*0.0*	0.4	0.3	*0.1*	0.1	0.4	0.4	a,b
p-Cymene	1036	1046	1020	0.3	*0.1*	0.3	*0.1*	0.0	0.6	*0.3*	0.3	0.5	0.0	a,b
Limonene	1041	1050	1024	0.2	*0.0*	0.2	*0.1*	0.2	0.2	*0.1*	0.2	0.2	0.2	a,b,c
Eucalyptol	1045	1038	1026	0.2	*0.0*	0.2	*0.1*	0.2	0.2	*0.1*	0.1	0.3	0.3	a,b,c
γ-Terpinene	1071	1068	1054	0.0	*0.0*	0.0	*0.0*	0.0	0.0	*0.0*	0.0	0.1	0.1	a,b
cis-Sabinene hydrate	1079	1067	1065	0.2	*0.0*	0.3	*0.0*	0.2	1.1	*1.0*	0.1	0.0	0.0	a,b
Linalool	1109	1110	1095	0.2	*0.0*	1.9	*3.4*	3.3	0.5	*0.3*	0.4	**46.8**	**54.9**	a,b
3-Octanol acetate	1135	1125	1120	0.0	*0.0*	0.2	*0.1*	0.2	0.1	*0.1*	0.0	0.1	0.2	a,b
p-Menthone	1167	1156	1148	0.5	*0.3*	0.6	*0.3*	1.2	**47.3**	*15.3*	1.5	0.0	0.4	a,b
Isomenthone	1176	1167	1158	0.2	*0.0*	0.2	*0.1*	0.3	9.2	*5.9*	0.4	**34.8**	0.1	a,b
Menthol	1184	1173	1167	0.0	*0.0*	0.0	*0.0*	0.0	1.6	*0.5*	0.0	0.0	0.0	a,b,c
Isopulegone	1186	–		0.0	*0.0*	0.0	*0.0*	0.0	0.1	*0.0*	0.0	0.1	0.0	a,b
4-Terpineol	1188	1189	1174	0.0	*0.0*	0.0	*0.0*	0.0	0.5	*0.5*	0.0	0.0	0.0	a,b
γ-Terpineol	1200	1198	1186	0.0	*0.0*	0.0	*0.0*	0.0	0.6	*0.0*	0.0	0.3	0.1	a,b
cis-Dihydrocarvone	1206	1193	1191	0.2	*0.1*	3.8	*7.1*	1.1	0.4	*0.2*	5.5	0.2	0.1	a,b
Dihydrocarveol	1207	1226	1192	0.0	*0.0*	0.1	*0.0*	0.0	0.0	*0.0*	0.9	0.0	0.0	a,b
trans-Dihydrocarvone	1215	1217	1200	0.1	*0.0*	2.5	*3.5*	0.4	0.2	*0.1*	**76.4**	8.7	0.0	a,b
Verbenone	1229	–	1204	0.0	*0.0*	12.3	*0.0*	0.0	0.1	*0.0*	0.0	0.0	0.0	a,b
Pulegone	1234	1250	1233	0.0	*0.0*	0.1	*0.0*	0.1	2.9	*0.8*	0.1	0.0	0.0	a,b,c
Carvone	1248	1259	1239	0.0	*0.0*	0.0	*0.0*	0.0	0.0	*0.0*	0.3	0.0	0.0	a,b,c
Geraniol	1252	1254	1249	0.2	*0.0*	0.1	*0.0*	0.2	0.2	*0.0*	0.1	0.1	0.0	a,b,c
Piperitonoxide	1271	–		**87.5**	*3.1*	**61.6**	*10.1*	**55.3**	**16.2**	*6.6*	6.6	4.3	**32.7**	a,b
Isopiperitenone	1286	–		0.5	*0.0*	0.4	*0.3*	**0.0**	0.1	*0.0*	0.0	0.0	0.0	a,b
Thymol	1304	1303	1289	0.4	*0.0*	0.2	*0.1*	**10.4**	13.7	*19.3*	0.1	0.1	0.1	a,b,c
Carvacrol	1314	1307	1298	0.2	*0.0*	0.1	*0.0*	**17.1**	1.1	*0.0*	0.1	0.0	0.0	a,b,c
p-Menthan-4-ol	1331	–		0.1	*0.0*	0.2	*0.0*	0.3	0.1	*0.0*	0.0	0.0	0.0	a,b
α-Terpinyl acetate	1367	–	1346	0.0	*0.0*	0.0	*0.0*	0.0	0.0	*0.0*	0.0	0.0	0.0	a,b
Piperitenone oxide	1383	–	1366	6.5	*1.7*	**20.4**	*7.8*	2.0	4.3	*4.1*	3.6	0.0	6.8	a,b
Caryophyllene	1442	1432	1417	1.5	*0.8*	0.6	*0.7*	3.3	1.9	*2.0*	0.0	1.1	2.4	a,b
Germacrene D	1502	1496	1484	0.2	*0.0*	0.1	*0.0*	0.5	1.7	*0.0*	0.0	0.3	0.0	a,b,c
Spathulenol	1604	1585	1577	0.1	*0.0*	0.2	*0.1*	0.6	0.3	*0.1*	0.0	0.0	0.0	a,b
Caryophyllene oxide	1612	1593	1582	0.4	*0.1*	1.2	*0.7*	0.2	1.2	*0.6*	1.6	0.1	0.0	a,b
				98.8	*0.6*	98.2	*0.8*	97.4	96.9	*2.0*	98.4	98.4	98.7	

Notes: [a]Components are listed in order of their elution from a BPX-5 column. [b]Linear retention index on BPX-5 capillary column. [c]Relative linear retention index from literature. [d]Retention indices of Adams' library. [e]Identification methods: MS (a) by comparison of the mass spectrum with those of the computer mass libraries Wiley and NIST 08; RI (b) by comparison of RI with those reported in the literature; (c) by comparison of the retention time and MS spectrum of available authentic standards. Chemotypes: PO, piperitone oxide; PO/PNO, piperitone oxide/piperitenone oxide; PO/CR/TH, piperitone oxide/carvacrol/thymol; MN/PO, *p*-menthone/piperitone oxide; DC, *trans*-dihydrocarvone; LI/IMN, linalool/isomenthone; LI/PO, linalool/piperitone oxide.

As a result of clustering according to essential oil components, piperitone oxide and piperitenone oxides are two major components in two chemotypes, PO and PO/PNO, of leaf oil, and three chemotypes, PO, PO/PNO and PNO/PO, of flower oil. The components are most common components in subspecies and varieties of *M. longifolia* (10), but they are in fairly low amounts in cultivated *Mentha* species. Oumzil et al. (21) mentioned PO and PNO chemotypes from *M. suaveolens* collected in Morocco. The chemotypes were also characterized from *M. longifolia* and other mint species (22–24).

Our results showed that linalool, menthone, dihydrocarvone, pulegone were major components. Similarly, previous studies recorded different chemotypes with linalool, carvone, menthone, *trans*-sabinene hydrate, piperitone in *M. longifolia* and other *Mentha* species (10–12, 25).

Because of the rare components in the *Mentha* genus (26), thymol was confirmed with an NMR result. Although thymol and carvacrol were major components in *Origanum* and *Thymus* species, they were rare components and recorded as minor or trace amounts in *Mentha* species (27, 28). Contrary to previous studies, thymol and carvacrol were major components in one chemotype, PO/CR/TH. There is no record of thymol as a major component of *Mentha* genus in the literature, but Gulluce et al. (29) recorded 6.0% of thymol in *M. longifolia* subsp. *longifolia*, similar to our results.

As a result of this research, chemical diversity in essential oil of *M. longifolia* subsp. *typhoides* from the Tokat region of Turkey was identified and the chemotypes were transferred to the botanical garden for future bioactivity studies such as insecticidal, herbicidal, antifungal etc.

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Record 1 of 10

Title: Phylogentic relationship of cold tolerant Mentha arvensis variety 'CIM Kranti' with some released varieties as assessed through physiological and molecular analysis

Author(s): Dhawan, SS (Dhawan, Sunita S.); Mishra, A (Mishra, Anand); Gupta, P (Gupta, Pankhuri); Bahl, JR (Bahl, J. R.); Bansal, RP (Bansal, R. P.)

Source: JOURNAL OF APPLIED RESEARCH ON MEDICINAL AND AROMATIC PLANTS Volume: 10 Pages: 67-74 DOI: 10.1016/j.jarmap.2018.06.004 Published: SEP 2018

Abstract: An elite genotype of Mentha arvensis was developed through half sib progeny selection with cold tolerance and higher yields of essential oil during winters. Molecular variations were assessed among nine released commercial varieties and the new variety 'CIM-Kranti' by RAPD and ISSR. In total, 20 RAPD primers and 16 microsatellite primers were used to detect the polymorphism among ten varieties of Mentha arvensis including new genotype. Phylogenetic analysis revealed a close relationship of the variety 'CIM-Kranti' to 'Gomti' and 'Shivalik' genotype of Mentha arvensis. RAPD and ISSR analysis resulted in two different phylogenetic relationships of the 10 genotypes. Comparative physiological variations were assessed by analyzing antioxidants, glutathione reductase, lipid peroxidation assay and relative water content of the new variety CIM Kranti, which clearly demonstrated tolerance towards cold therefore having potential of giving higher additional essential oil yields during winters.

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eISSN: 2214-7861

Record 2 of 10

Title: A Comprehensive Study on Phytochemical Contents, Isolation and Antioxidant Capacities in wild mind, Mentha longifolia subsp. typhoides var. typhoides

Author(s): Ozen, T (Ozen, Tevfik); Telci, I (Telci, Isa); Gul, F (Gul, Fatih); Demirtas, I (Demirtas, Ibrahim)

Source: MOROCCAN JOURNAL OF CHEMISTRY Volume: 6 Issue: 4 Pages: 601-614 Published: 2018

Abstract: The current study is to determine variation in vitro antioxidant activities and chemical contents of different extraction solvents in aerial parts of wild M. longifolia subsp. typhoides var. typhoides taxons from Tokat, Turkey. The chemical and volatile compounds of M. longifolia were investigated with HPLC-TOF/MS and GC/MS. The highest total phenolic and flavonoid contents were found as 28.27 and 25.42 mg from R6W, respectively. Hesperidin, neohesperidin, taxifolin, fumaric acid, chlorogenic acid, naringin, 4-hydroxybenzoic acid, caffeic acid, protocatechuic acid and syringic acid were determined in all extracts. The linalool, menthone, isomenthone, piperitone oxide, pulegone, thymol, caryophyllene and caryophyllene oxide were major components in volatile compounds. The highest activities were observed significantly ($p < 0.01$) from R6M, R6W, R10EA and R5M in total antioxidant, superoxide-scavenging, metal-chelating and inhibition of lipid peroxidation activities, respectively. The isolated menthone (96.00%) was exhibited the higher inhibition of lipid peroxidation than standards. The results validate that M. longifolia possesses as a source of antioxidant potential for medicinal and foods.

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ISSN: 2351-812X

Record 3 of 10

Title: ANTI-BIOFILM, ANTIMICROBIAL AND CYTOTOXIC ACTIVITY OF ACHILLEA MILLEFOLIUM L. ESSENTIAL OIL

Author(s): Tutar, U (Tutar, Ugur)

Source: FRESENIUS ENVIRONMENTAL BULLETIN Volume: 27 Issue: 5A Pages: 3713-3720 Published: 2018

Abstract: This research was conducted to investigate the anti-biofilm and antimicrobial activities of essential oil from Achillea millefolium L. (EOAM) on clinical isolates of Staphylococcus aureus, as well as its chemical composition, and cytotoxic activity. The chemical composition of EOAM was analyzed by gas chromatography. Antimicrobial and antibiofilm activities were determined by broth micro-dilution method. Colorimetric assay was used to assess its cytotoxicity on human osteosarcoma (MG63), human breast cancer (MCF-7), and mouse fibroblast (L929) cell lines. Viability in the biofilm was studied using 2,3-bis (2- methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) assay. 3-Cyclopenten-l-one, 2-hydroxy-3-(3-methyl-2-butenyl) (28.94 %), piperitone oxide (25.74 %), carvacrol (15.41 %), eucalyptol (5.16 %) and limonene (3.23 %) were the major components of the essential oil. ICso value was found to be 28.07 mu g/mL, 19.02 mu g/mL, 41.35 mu g/mL in the MCF-7, MG-63, and L929 cell line, respectively. Minimum inhibitory concentration (MIC) values were found in the range of 12.5-25 mu l/mL, whereas bactericidal activities reached higher concentrations values in the order of 25 and 50 mu l/mL. Minimal biofilm inhibition concentration (MBIC) value was found to be 1.56-12.5 (mu l/mL) while minimal biofilm eradication concentration (MBEC) value was found to be 6.25-50 (mu l/mL). EOAM damaged viability in the biofilm at MIC value by 35.3-94.3 %. These findings suggest that EOAM contains components that may be useful for the development of potential phytotherapeutic agents against S. aureus infections.

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Record 4 of 10

Title: Pharmacological activity of Mentha longifolia and its phytoconstituents

Author(s): Farzaei, MH (Farzaei, Mohammad Hosein); Bahramsoltani, R (Bahramsoltani, Roodabeh); Ghobadi, A (Ghobadi, Ali); Farzaei, F (Farzaei, Fatemeh); Najafi, F (Najafi, Fariba)

Source: JOURNAL OF TRADITIONAL CHINESE MEDICINE Volume: 37 Issue: 5 Pages: 710-720 Published: OCT 2017

Abstract: Current paper reviews the pharmacological activities, therapeutic indications and phytochemicals of M. longifolia. This herb has been consumed traditionally for the treatment of various diseases, including gastrointestinal disorders, respiratory disorders, infectious diseases, inflammatory diseases, as well as menstrual disorders. In the modern era, various pharmacological activities have been confirmed for M. longifolia, such as anti-parasitic, antimicrobial, anti-insect, antimutagenic, antinociceptive, anti-inflammatory, antioxidant, keratoprotective, hepatoprotective, anti-diarrhea, and spasmolytic effects. The plant showed therapeutic benefits in irritable bowel syndrome, amenorrhea and oligomenorrhea, and oxidative stress-associated diseases as well. A vast variety of natural components such as flavonoids, phenolic acids, cinnamates, ceramides, sesquiterpenes, terpenes, and terpenoids have been suggested to be responsible for the pharmacological action of M. longifolia. These natural products can be considered as novel medicinal sources for developing new drugs. Further investigations to explore therapeutic efficacy, tolerability, and pharmaceutical properties of M. longifolia phytochemical agents are recommended. (C) 2017 JTCM. All rights reserved.

Accession Number: WOS:000413285000018

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Record 5 of 10

Title: Enantioselective GC Analysis of C-3-Oxygenated p-Menthane type Indian Mentha spicata var. viridis 'Ganga' Essential Oil

Author(s): Shanmugam, PV (Shanmugam, Pragadheesh V.); Saroj, A (Saroj, Arvind); Maurya, R (Maurya, Ranjana); Yadav, A (Yadav, Anju); Gupta, N (Gupta, Namita); Samad, A (Samad, Abdul); Chanotiya, CS (Chanotiya, Chandan S.)

Source: NATURAL PRODUCT COMMUNICATIONS **Volume:** 12 **Issue:** 3 **Pages:** 427-430 **Published:** MAR 2017

Abstract: Essential oil of Mentha spicata L. var. viridis 'Ganga', an indigenously developed variety, was chemically profiled using various gas chromatographic techniques. Piperitenone oxide was characterized as the most exclusive constituent (69.7%) along with a new C-3-oxygenated p-menthane alcohol, diosphenolene (1.6%). Enantiomeric discrimination revealed (4S)-(-)-limonene, (R)-(-)-linalool and (1S,2S)-(+)-piperitenone oxide as predominant enantiomers. The oil contained mainly C-3-oxygenated p-menthane monoterpenoids, which are distinctive of peppermint, instead of the characteristic C-6-oxygenated class of spearmint. The present findings will aid in understanding the pathway and cause of C-3-oxygenation in a spearmint taxon. The essential oil and pure piperitenone oxide showed growth inhibiting properties and thus, may be utilized in antifungal preparations for disease management of medicinal and aromatic plants.

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PubMed ID: 30549902
ISSN: 1934-578X
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Record 6 of 10

Title: Genetic elaborations of glandular and non-glandular trichomes in Mentha arvensis genotypes: assessing genotypic and phenotypic correlations along with gene expressions

Author(s): Mishra, A (Mishra, Anand); Lal, RK (Lal, R. K.); Chanotiya, CS (Chanotiya, C. S.); Dhawan, SS (Dhawan, Sunita Singh)

Source: PROTOPLASMA **Volume:** 254 **Issue:** 2 **Pages:** 1045-1061 **DOI:** 10.1007/s00709-016-1011-x **Published:** MAR 2017

Abstract: Mentha arvensis (corn mint) is well known for the production of menthol, a widely used commodity in pharma and flavoring industries and provides natural fragrances and products. Glandular trichomes are specialized hairs found on the aerial surface of vascular plants species producing specific secondary metabolite chemistry. Correlations were established among trichomes, oil yield, and major secondary metabolites. Nine improved, elite cultivars representing different M. arvensis genotypes were used for analysis. Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were estimated; results indicated the presence of considerable amount of genetic variability, thereby emphasizing wide scope of selection. Positive and significant associations were found among glandular trichomes, oil yield, essential oil constituents, and leaf morphology itself, whereas morphological parameters of leaf show positive and negative correlations to average number of trichome and essential oil constituents. Average number of glandular, non-glandular trichomes, their ratios, menthol content, and trichome number showed a good heritability. Trichomes were studied microscopically in leaf parts in all varieties for analyzing their distribution pattern. The trichome number variations showed significant correlation throughout the genotypes with essential oil yield and monoterpenoid constituents. Differential changes were analyzed for Glutathione S-transferases, Glutathione reductase, Malondialdehyde, phenolics, and chlorophyll content. Gene expressions were analyzed for biosynthesis genes and selected transcription factors TRANSPARENT TESTA GLABRA 1 (TTG1), ENOLASE 1, GLABRA 3, GTL 1, NUCLEAR TRANSCRIPTION FACTOR Y SUBUNIT B-6, WRKY transcription factor 22, putative WRKY 33, WRKY 17, WRKY 1, and WRKY 65-like for harnessing their relation with trichome development in M. arvensis genotypes.

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PubMed ID: 27515313
ISSN: 0033-183X
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Record 7 of 10

Title: Genetic, phenotypic, and phytochemical polymorphism in Eastern European populations of Mentha arvensis L.

Author(s): Shelepova, OV (Shelepova, O. V.); Semenova, MV (Semenova, M. V.); Enina, OL (Enina, O. L.); Schanzer, IA (Schanzer, I. A.)

Source: RUSSIAN JOURNAL OF GENETICS **Volume:** 53 **Issue:** 1 **Pages:** 59-66 **DOI:** 10.1134/S1022795416120139 **Published:** JAN 2017

Abstract: Variability of M. arvensis from five geographically distanced populations was examined using morphological traits and phytochemical composition of essential oil and with the help of DNA fingerprinting using ISSR markers. The population differentiation based on morphological traits was weak. Analysis of the essential oil composition provided the subdivision of the sample into three groups and, on the basis of the composition of ISSR amplicons, into four groups of specimens. A high degree of genetic polymorphism of M. arvensis and substantial, though incomplete, population differentiation were identified. It was demonstrated that the population of M. arvensis from the Komi Republic was the most genetically isolated, while the populations from Moscow and Penza provinces were weakly differentiated from each other. The population from the Republic of Belarus (near Grodno) was genetically and phytochemically considerably different from the other studied populations, although morphologically indistinguishable from them. We argue that the differentiation was caused not only by the isolation by distance but also owing to the formation of three different ecotypes adapted to different climatic conditions.

Accession Number: WOS:000394159700006
ISSN: 1022-7954
eISSN: 1608-3369

Record 8 of 10

Title: Anti-biofilm and antimicrobial activity of Mentha pulegium L essential oil against multidrug-resistant Acinetobacter baumannii

Author(s): Tutar, U (Tutar, Ugur); Celik, C (Celik, Cem); Karaman, I (Karaman, Isa); Atas, M (Atas, Mehmet); Hepokur, C (Hepokur, Ceylan)

Source: TROPICAL JOURNAL OF PHARMACEUTICAL RESEARCH **Volume:** 15 **Issue:** 5 **Pages:** 1039-U211 **DOI:** 10.4314/tjpr.v15i5.20 **Published:** MAY 2016

Abstract: Purpose: To investigate the antimicrobial and anti-biofilm activities of essential oil from Mentha pulegieum L. (EOMP) on multi-drug resistant (MDR) isolates of A. baumannii, as well as its phytochemical composition, antioxidant properties and cytotoxic activity.
Methods: The phytochemical composition of EOMP was analyzed by gas chromatography, while its antimicrobial activities were determined by disc diffusion and broth micro-dilution methods. Minimal biofilm inhibition concentration (MBIC) and minimal biofilm eradication concentration (MBEC) tests were used for assessment of its anti-biofilm properties. Viability in the biofilm was studied using 2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) assay, while colorimetric assay was used to assess its cytotoxicity on L929 cells
Results: D-isomenthone, pulegone, isopulegone, menthol and piperitenone were the major components of the plant extract. EOMP produced > 22 mm inhibition zone for the isolates, with minimum inhibitory concentration (MIC) and MBIC of 0.6 -2.5 and 0.6 -1.25 mu L/mL, respectively, while MBEC was >= 10 mu L/msL. EOMP damaged biofilm structures formed by A. baumannii strains at MIC by 26 - 91 %.
Conclusion: These results suggest that EOMP contains agents that may be useful in the development of new drugs against A. baumannii infections.

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Title: Evaluation of the Antibiofilm and Antimicrobial Properties of Ziziphora tenuior L. Essential Oil Against Multidrug-resistant Acinetobacter baumannii

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Source: INTERNATIONAL JOURNAL OF PHARMACOLOGY **Volume:** 12 **Issue:** 1 **Pages:** 28-35 **DOI:** 10.3923/ijp.2016.28.35 **Published:** 2016

Abstract: Acinetobacter baumannii is one of the most important gram-negative microorganisms which lead to opportunistic hospital-acquired infections. A great part of the infections it causes is produced by strain resistant to all the antibiotics used. In our study, the Essential Oil (EO) of the Ziziphora tenuior L. antimicrobial and antibiofilm effects on multidrug resistant (MDR) A. baumannii isolates were researched. In addition, antioxidant, cytotoxic activity and chemical composition of EO were investigated. As a result of the gas chromatography-mass spectrometry (GC-MS) analysis of the Z. tenuior EO pulegone was found as the major component by 74.37%. It was observed when the antimicrobial activity of the EO was examined that it had >30 mm disc diffusion values. Minimal Inhibition Concentration (MIC) values were found between 0.6-1.25 mu L mL(-1) and Minimal Bactericidal Concentration (MBC) values were found between 2.5-5 mu L mL(-1). Minimal biofilm inhibition concentration (MBIC) values of the EO were found as 0.3-1.25 mu L m L-1 and Minimal Biofilm Eradication Concentration (MBEC) value as 5-10 mu L mL(-1). It was seen that the MIC value damaged the biofilm formations constituted by the A. baumannii strains by 51-84%. At 25, 12.5 and 6.25% EO concentrations, no cytotoxic appeared for the fibroblast cells in terms of the cytotoxic activities (p>0.05). Findings that were obtained in our study seem promising for the development of phytotherapeutic agents that could be used in the treatment of the MDR A. baumannii infections.

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Record 10 of 10

Title: Essential Oil Composition of Mentha longifolia (L.) L. Collected from Garhwal Region of Western-Himalaya

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Source: JOURNAL OF ESSENTIAL OIL BEARING PLANTS **Volume:** 18 **Issue:** 4 **Pages:** 957-966 **DOI:** 10.1080/0972060X.2014.897594 **Published:** JUL 4 2015

Abstract: Mentha longifolia (L.) L. (family: Lamiaceae), commonly known as wild mint, is an extremely variable perennial and strong aromatic herb. The hydrodistilled essential oil of M. longifolia population growing wildly in high altitudes of Western-Himalayan region was analyzed using gas chromatography/flame ionization detector (GC-FID) and GC-mass spectrometry (GC-MS). A total of fifty-five constituents, forming 97.5 % of the total oil composition were identified. The oil was characterised by high amount of oxygenated monoterpenes (74.0 %) and sesquiterpene hydrocarbons (18.0 %). The characteristic constituents of the oil were trans- piperitone epoxide (48.7 %), piperitenone oxide (21.2 %), germacrene D (9.8 %), (E)-caryophyllene (2.3 %), 2-hydroxy piperitone (1.6 %), alpha-humulene (1.5 %), thymol (1.4 %), and alpha-longipinene (1.0 %). The essential oil composition of the presently studied M. longifolia population differed considerably, especially in trans-piperitone epoxide content with earlier reports from India.

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