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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

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## 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.

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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 |

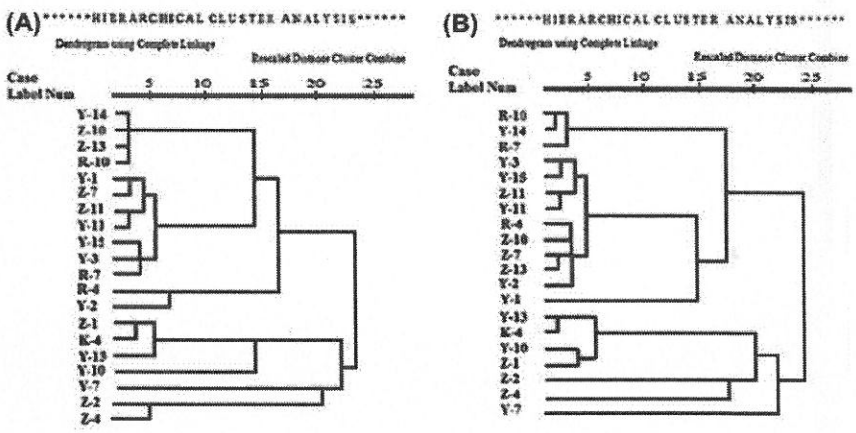



Figure 1. Clustering of flower (A) and leaf (B) oil compositions in *Mentha longifolia* subsp. *typhoides* var. *typhoides*.

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.

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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.

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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.

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

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