International Journal of Chemistry and Technology

http://dergipark.gov.tr/ijct

Research Article

Chemical analyzes and antioxidant activities of essential oils of four wild *Mentha* species growing in the Tokat and its districts

Tevfik OZEN[1,*], Isa TELCI[2], Fatih GUL[3], Ibrahim DEMIRTAS[3]

[1]*Department of Chemistry, Faculty of Arts and Sciences, Ondokuz Mayıs University, Samsun, 55139, Türkiye*

[2]*Department of Field Crops, Faculty of Agriculture, Süleyman Demirel University, Isparta, 32260, Türkiye*

[3]*Department of Chemistry, Faculty of Science, Çankırı Karatekin University, Çankırı, 18100, Türkiye*

Received: 13 October 2017, Revised: 19 November 2017; Accepted: 22 November 2017

[*]Corresponding author's e-mail address: tevfikoz@omu.edu.tr (T. Ozen)

ABSTRACT

The current work evaluated the chemical compositions of the essential oils (EOs) extracted from *Mentha longifolia* subsp. *typhoides* (Briq.) Harley var. *typhoides* PH. Davis, *Mentha spicate* L. subsp.*spicata*, *Mentha longifolia* (L.) Hudson subsp.*longifolia* and *Mentha villoso nervata* Opiz from gowning in the different locations of Tokat. The antioxidant activities of EOs were tested using total antioxidant activity, reducing power, inhibition of lipid peroxidation, metal chelating, H_2O_2, DPPH$^{\bullet}$ and $O_2^{\bullet-}$ scavenging activities. The chemical compositions of EOs were determined by GC and GC-MS. Significant differences were recorded between the percentages of many constituents depending on the geographical. The most important components were identified consecutively: linalool (nd-62.80%), menthone (0.31-60.81%), pulegone (nd-21.52%), isomenthone (nd-30.15%) and piperitoneoxide (nd-68.92%). The inhibition of lipid peroxidation, $O_2^{\bullet-}$ and H_2O_2 scavenging activities of the EOs were very effective but their reduction power values were low. The results obtained validate that EOs of four *Mentha* species possess a source of antioxidant potential for medicinal and foods.

Keywords: *Mentha* species, essential oils, chemical contents, antioxidant activity

Tokat ve ilçelerinde yetişen dört yabani nane (*Mentha*) türünün uçucu yağlarının kimyasal analizleri ve antioksidan aktiviteleri

ÖZ

Bu çalışma Tokat' ın farklı yerlerinde yetişen *Mentha longifolia* subsp. *typhoides* (Briq.) Harley var. *typhoides* PH. Davis, *Mentha spicata* L. subsp. *spicata*, *Mentha longifolia* (L.) Hudson subsp. *longifolia* ve *Mentha villoso nervata* Opiz*'* den ekstrakte edilen uçucu yağların kimyasal bileşimleri belirledi. Uçucu yağların antioksidan aktiviteleri toplam antioksidan aktivite, indirgeyici güç, lipid peroksidasyonunun inhibisyonu, metal şelat, H_2O_2, DPPH$^{\bullet}$ ve $O_2^{\bullet-}$ giderme aktiviteleri uygulanarak test edildi. Herbir uçucu yağın kimyasal bileşimleri GC ve GC-MS ile belirlendi. Coğrafyaya bağlı olarak birçok bileşenin yüzdesi arasında önemli farklılıklar kaydedildi. En önemli bileşenleri sırayla linalool (nd-% 62.80), menthone (% 0.31-60.81), pulegone (nd-% 21.52), isomenthone (nd-% 30.15) ve piperitoneoxide (nd-% 68.92) tespit edildi. Uçucu yağların lipid peroksidasyon inhibisyonu, $O_2^{\bullet-}$ ve H_2O_2 giderme aktiviteleri çok etkiliydi, fakat indirgeme gücü değerleri düşüktü. Elde edilen sonuçlar, dört nane (*Mentha*) türünün uçucu yağlarının tıbbi ve gıdalarda bir antioksidan potansiyel kaynak olduğunu doğrulamaktadır.

Anahtar Kelimeler: Nane **(***Mentha***)** türleri, uçucu yağlar, kimyasal bileşenler, antioksidan aktivite

1. INTRODUCTION

Mentha species are members of the Lamiaceae, which almost show a cosmopolitan distribution of moderate to the tropical regions.[1] They have been used for different purposes throughout the history with aim of usages of their various features. For example, *Mentha* oil is the world's oldest known herbal remedy.[2] Essential oils (EOs) are a mixture of complex, naturally occurring volatile compounds synthesized by plants as secondary metabolites in *Mentha* species.[3] EOs are abundant in flowers, leaves and seeds in plants. It is usually isolated by hydro-distillation methods.[4] Nowadays, about 3000 EOs are known, among which particularly 300 are commercial of vital importance for food, pharmaceutical, cosmetic, agronomic, sanitary and perfume industries. For example, D-carvone, D-limonene and geranyl acetate are used as perfumes, creams, soaps, as flavor agents for food product and as industrial solvents in household cleaners.[5] EOs in combination with vegetable oils are

also used in massages. Some of EOs appear to have medical features and are reported to have treated one or more diseases, and also are used in paramedical applications. Terpenoids and aromatic in the EOs components can also be used to enhance the efficacy of existing treatments. Hundreds of studies performed *in vitro* and *in vivo* in animals demonstrate their efficacy when used alone, without conventional chemotherapy or radiotherapy treatments.[6-7] Numerous experimental data indicate that toxicity focuses primarily on cancer cells without affecting healthy cells and it is important to investigate new effective natural products.[8]

In the present study, the EOs of four widely consumed wild Turkish mints, namely *M. longifolia* subsp. *typhoides* (Briq.) Harley var. *typhoides* PH. Davis, *M. longifolia* (L.) Hudson subsp. *Longifolia*, *M. spicate* L. subsp. *spicata* and *M. villoso nervata* Opiz collected from different locations of Tokat, Turkey were investigated in September 2014 for their chemical composition and their *in vitro* antioxidant properties. The antioxidant activities of EOs were evaluated by total antioxidant activity, reducing power, inhibition of lipid peroxidation, metal chelating, H_2O_2, DPPH· and $O_2^{\bullet-}$ scavenging assays for the first time for this region. And, there are no records of correlations between chemical compositions of the EOs and their antioxidant activities of the taxon from Tokat, Turkey. In addition to being used as spices, these *Mentha* samples are also used as foodstuffs and beverages. Moreover, it is known that they are also used as traditional medicines in the treatment of different diseases or conditions.[9] The main purpose of this study is to further explore the potential benefits of these *Mentha* species as a naturally occurring source of bioactive substances.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Chemicals

Folin–Ciocalteu reagent (FCR), nicotine adenine dinucleotide (NADH), tricholoroacetic acid (TCA), buthylated hydroxyanisole (BHA), α-tocopherol, buthylated hydroxytoluene (BHT), pyrocatechol, quercetin, phenazine methosulphate (PMS), 1,1-diphenyl-2-picryl-hydrazl (DPPH$^{\bullet}$), ferrous chloride, ammonium molybdate, $K_3[Fe(CN)_6]$, trolox, sodium acetate (CH_3COONa), sodium carbonate, thiobarbutiric acid (TBA), nitrobluetetrazolium (NBT) and ascorbic acid were provided from Sigma. The organic solvents used in analyses were of HPLC grade and purchased from Merck. All the other chemicals were supplied from other commercial sources.

2.1.2. Plant material

The fresh plant materials of *M. longifolia* subsp. *typhoides* (Briq.) Harley var. *typhoides* PH. Davis (K4; 40° 24 235, Y1; 40° 02 275, Y3; 40° 08 325, Y10; 40° 06 909, Z2; 40° 16 092, Z7; 40° 16 228, K2; 40° 23 744, R2; 40° 27 156, R7; 40° 30 794, Y12; 40° 03 187, Y13; 40° 03 954, Y15; 40° 03 958, Z4; 40° 16 202, R1; 40° 25 894, R4; 40° 34 000, Y4; 40° 03 187, Y7; 40° 14 823, Y11; 40° 04 975, Y14; 40° 00 251, Z1; 40° 16 206, Z10; 40° 18 144, Z11; 40° 19 272), *M. longifolia* (L.) Hudson subsp. *longifolia* (R5; 40º 31 737 616, R6 40º 30 739 596), *M. spicata* subsp. *spicata* (Z5; 40º 16 024 540, Y9; 39º 59 151 1030) and *M. villoso nervata* Opiz (K3; 40º 24 235 647, R9; 40º 32 068 1434) were collected in the different locations of Tokat city of Turkey in September 2014 (Figure 1). The samples were verified by Prof. Dr. Isa Telci and prepared in the Herbarium of Agriculture Faculty, Gaziosmanpasa University, Tokat-Turkey. The collected samples were air-dried and kept for analysis.

2.2. Methods

2.2.1. Analysis of EOs

2.2.1.1. GC-MS Analysis

The EOs were determined using Agilent Technologies GC 7890A with a built-in 5975 Triple Axis Detector MS system, equipped ionization system, HP5-ms (30 m x 250 mm x 0.25 mm) column and ionization energy for GC-MS detection (70 eV). The helium was used as carrier gas at a flow rate (1 mL min^{-1}). The same column temperate was achieved with GC analysis given above in our laboratory, by comparison of their mass spectral fragmentation patterns (WILLEY and NIST database/Chem Station data system), and then their retention indices were determined with reference to homologue series of *n*-alkanes (C6-C26).[10]

2.2.1.2. Separations of EO components

A sample of the EO was separated using a method described by Amzazi[11] using silica-gel column chromatography (*n*-pentane and *n*-pentane: diethyl ether (5:95, v/v)). The 600 fractions were eluted with a gradient of increasing solvent polarity (n-pentane: diethyl ether 100:0-90:10). All fractions were submitted to GC-MS analysis. The combined fractions were evaporated under air flow and determined by GC-MS.[11]

2.2.2. Antioxidant activity

2.2.2.1. Assay of total antioxidant activity by phosphomolybdate method

The total antioxidant activities of the EOs were evaluated to the method described in literature.[12] The assay was based on the reduction of Mo (VI) to Mo (V) by the EOs. The subsequent formation of green phosp-

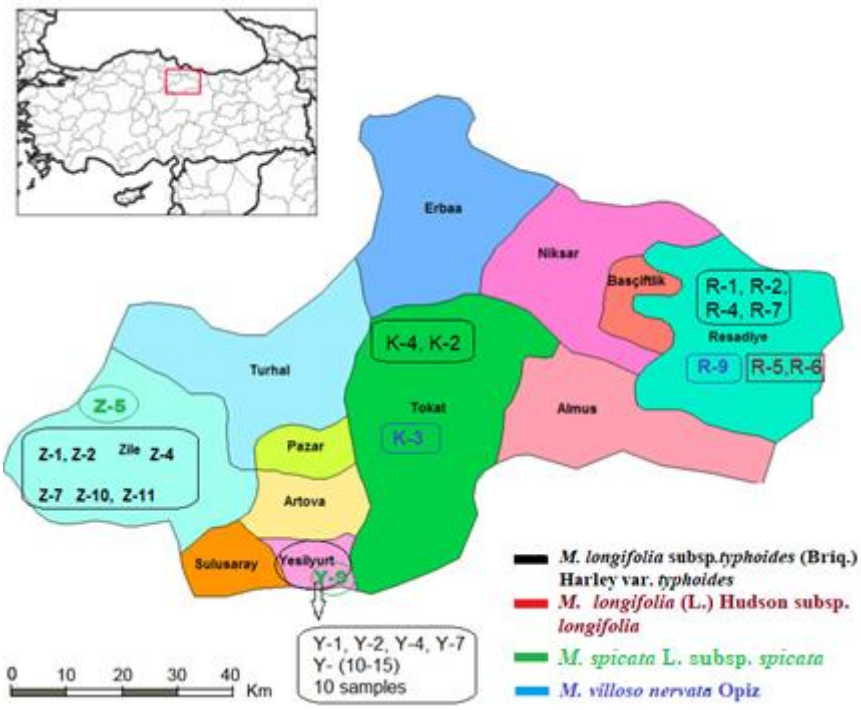

Figure 1. Distribution map of four *Mentha* species (*Mentha longifolia* subsp. *typhoides* (Briq.) Harley var. *typhoides* PH. Davis, *Mentha longifolia* (L.) Hudson subsp. *Longifolia*, *Mentha spicata* L. subsp. *spicata* and *Mentha villoso nervata* Opiz in Tokat and its Districts.

hate/Mo(V) compounds monitored at acidic pH. A 0.3 ml EO was added to 4 mM ammonium molybdate, 0.6 M sulfuric acid and 28 mM sodium phosphate, respectively. The mixture of the reaction was incubated at 95°C and measured absorbance at 695 nm. The total antioxidant activities of the EOs were calculated as an equivalent of α-tocopherol according to the extinction coefficient of $4x10^3$ M^{-1} cm^{-1} and expressed as µmol α-tocopherol/g sample.

2.2.2.2. Assay of reducing power

The reducing powers of the EOs were determined using Oyaizu Method with slight modification.[13] The EO samples (100 µg ml^{-1}) were added to phosphate buffer (0.2 M, pH 6.6) and $K_3Fe(CN)_6$ (10 g l^{-1}), respectively. After incubation for 25 min at 50 ^oC, TCA (2.5 ml; 10%) was added to the mixture and centrifuged at 3.000 x g for 20 min. Finally, 2.5 ml of supernatant was combined with water and $FeCl_3$ solution (0.5 ml; 0.1%). The absorbance of the mixture was read at 700 nm. It is indicated that the high absorbance of the sample has effective reducing power in the reaction condition that the reducing capacity had increased.

2.2.2.3. Assay of DPPH˙ scavenging activity

DPPH˙ (free radical) scavenging activities of the EOs were assayed according to the reference.[14] The DPPH˙ solution (2.7 ml; 1 mM) was mixed with the sample (100 µg ml^{-1}) in the test tubes. After incubating at room temperature in the dark for 30 min, the absorbance of the mixture was monitored at 517 nm. The reduction of sample absorbance indicated higher DPPH˙ scavenging activity. The activities were calculated as a percentage of DPPH˙ discoloration, using the following equation. For the free radical scavenging activity, the equation is

$$\% = [(A_{517\ (control)} - A_{517\ (sample)}) / A_{517\ (control)}] \times 100$$

where $A_{532\ (control)}$ is the absorbance of control and $A_{532\ (sample)}$ is the absorbance of the sample.

2.2.2.4. Assay of H_2O_2 scavenging activity

H_2O_2 scavenging activity of the sample was evaluated according to the reference.[15] A 1 ml extract solution was added in a flask to 0.1 mM H_2O_2 in phosphate buffer (pH 7.4), ammonium molybdate, 10 ml of 2 M H_2SO_4 and 7 ml of 1.8 M KI. The mixture was titrated with 5 mM $Na_2S_2O_3$ until the disappearance of yellow color. The activity was calculated as the percentage of H_2O_2 scavenging, using the following equation. For the H_2O_2 scavenging activity, the equation is

$$\% = [(V_{control} - V_{sample}) / V_{control}] \times 100$$

where $V_{(control)}$ is the volume of control and $V_{(sample)}$ is the volume of the sample.

2.2.2.5. Assay of superoxide scavenging activity

Superoxide scavenging activities of the EOs (100 µg ml^{-1}) were estimated according to a minor modification.[16] Superoxide anion radicals were generated in a PMS-NADH system by oxidation of NADH and assayed by reduction of NBT. Briefly, an EO sample was mixed with NBT (156 µM: 1 ml) and NADH (468 µM: 1 ml), respectively. Then, the reaction was started by adding PMS (60 µM: 0.1 ml). After incubating at room temperature, the absorbance of the mixture was monitored at 532 nm. A decrease in absorbance of the mixture indicates an increase in superoxide anion-scavenging activity.[17] The activities were calculated as inhibition of superoxide anion generation by the following equation. For the superoxide anion scavenging activity, the equation is

$$\% = [(A_{532(control)} - A_{532(sample)}) / A_{532(control)}] \times 100$$

where $A_{532\ (control)}$ is the absorbance of control and $A_{532\ (sample)}$ is the absorbance of the sample.

2.2.2.6. Assay of metal chelating (Fe^{2+}) activity

The metal chelating activities of the EOs (100 µg ml^{-1}) were measured according to the method in the literature with modifications.[18] In this method, ferrozine can form complex with Fe^{2+} (ferrozine-Fe^{2+}), effectively. In the presence of other chelating compounds, this complex is disrupted with chelator compounds. Briefly, EOs was mixed with of $FeCl_2$ (2 mM, 0.05 ml) solution. After 30 min incubation at 25°C, the reaction was initiated with the addition of 5 mM ferrozine. Each mixture was shaken vigorously and then left to stand at 25°C. The absorbance of the mixture was read at 562 nm. The activities were calculated as a percentage of inhibition of Fe^{2+}-ferrozine complex formation according to the following equation. For, the metal chelating activity, the equation is

$$\% = [(A_{562\ (control)} - Abs_{562\ (sample)}) / Abs_{562\ (control)}] \times 100$$

where $A_{562(control)}$ is the absorbance of control and $A_{562(sample)}$ is the absorbance of the sample.

2.2.2.7. Inhibition of lipid peroxidation

The inhibition of lipid peroxidation assay for EOs (100 µg ml^{-1}) was carried out using TBA method based on inhibition of linoleic acid peroxidation according to the literature.[19] Briefly, the EO sample was mixed with 500 µl of 20 mM linoleic acid, 50 µl of 100 µM phosphate buffer (pH 7.4), 150 µl of 20 µM ascorbic acid. The reaction of peroxidation was initiated by adding of 10 µM $FeSO_4$ (0.1 ml) and incubated at room temperature for 60 min. The mixture was added to 10% TCA solution and kept in a water bath (95 °C). After cooling at room temperature, butanol was added to the mixture and centrifuged at 4000 × g. After removing the supernatant, the formation of TBA reactive substance was monitored at 532 nm. The inhibition ration was calculated by the following formula. For the inhibition of lipid peroxidation, the equation is

$$\% = [(A_{532(control)} - A_{532\ (sample)}) / A_{532\ (control)}] \times 100$$

where $A_{532\ (control)}$ is the absorbance of control and $A_{532\ (sample)}$ is the absorbance of the sample.

2.2.3. Statistical Analysis

The data were presented as the mean ± standard deviation (S.D.). The results were analyzed by one-way ANOVA analysis of variance followed by Duncan's test and considered to be significant with $p < 0.05$ and $p < 0.01$ confidence level, statistically.

3. RESULTS AND DISCUSSION

3.1. Quantitative analysis

The retention indexes (RI) and retention time (RT) of EOs of leaves and flowers of wild *Mentha* species were determined as quantitative compositions. The major variations were determined among the parts of *Mentha* species and extraction applications. The chemical composition of essential oils of *Mentha* species was shown in Table 1-4. 30 compounds were identified in the EOs and the percentage of identified compounds ranged from nd to 68.92%. Compounds that were the most abundant in the EOs of *M. longifolia* subsp. *typhoides* (Briq.) Harley var. *typhoides* PH. Davis were linalool [Z-2L (45.68%), Z-2F (62.88%), Z-4L 52.32)], menthone [K-4L (60.81%), K-4F (50.15%), Y-10L (34.89%), Y-10F (27.54%), Y-13L (26.70%), Z-1F (35.25%)], isomenthone [Y-10L (16.85%), Y-10F (13.28%), Z-2L (30.15%), Z-2F (14.09%)], piperitoneoxide [K-4L (15.46%), K4-F (18.13), Y-1L (35.69%), Y-2L (28.62%), Y-2F (23.74%), Y-3L (63.15%), Y-3F (25.99%), Y-10L (24.00%), Y-10F (3106%), Z-7L (69.43%), K-2L (47.57%), R-2L (20.74%), Y-15L (63.81%), R-4F (25.34%), Y-4F (35.39%), Y-11F (46.78%), Y-14F (41.83%), Z-1F (13.93%), Z-10F (26.90%), Z-11F (49.22%)], thymol [Y-1L (14.07%), Y-12L (57.13%)], 1-acetoxy-p-menth-3-one (Y-1F 22.09%) and anhydroserricornin [K-2L (16.51%), Y-3F (14.82%), Z-10F (20.62%), R-2L (24.04%)] (Table 1-4). The menthone [K-3L (19.41%)] in *M. villoso nervata* Opiz and menthone [Y-9L (10.60%)], pulegone [Z-5L (21.52%)] in *M. spicate* L. subsp. *spicata* were the highest content because of growing at high altitude. The piperitone oxide is major component in *M. villoso nervata* Opiz [K-3L (56.19%); R-9F (68.92%)], *M.*

spicate [Y-9L (46.82%)] and *M. longifolia* (L.) **[**R-5F (47.60%)] (Table 1-4). The EOs components of *M. longifolia* subsp., *M. longifolia* (L.), *M. spicata* and *M. villoso nervata* Opiz show different compositions. When the EOs of *M. longifolia* subsp., *M. longifolia* (L.), *M. spicata* and *M. villoso nervata* Opiz are compared with the same species grown in different counties, the found results are the same major constituents with slightly different levels.[20,21] Also, many compounds in the EOs such as sabinene hydrate, linalool oxide, 3-octyl acetate, methylvaleric acid, 2-propylmalonic acid, 5-caranol, 4-terpineol, levomenthol, citronellol, carvone, linalyl acetate, dihydroedulan, isopulegol acetate, thymol acetate, dihydrocarvyl acetate, ascaridole, ethyl hydroden pimelate, formic acid, cubedol, α-humulene, jasmine lactone, hexanoic acid, cinerolone, dodecanoic acid and aromadendrene oxide were determined as trace amounts.

Table 1. Identified chemical components in EOs of wild *M. longifolia* subsp. *Typhoides* (Brig) Harley var. *typhoides* PH. Davis samples from Reşadiye (R) and center of Tokat (K)

Components	**RT (min)**	**RI**	**K-2**	**K-4**		**R-1**	**R-2**	**R-4**	**R-7**
			L	**L**	**F**	**F**	**L**	**F**	**L**
3-octanol	13.531	965	nd	0.43	nd	nd	nd	0.33	0.45
o-cymene	14.699	999	0.36	0.65	0.45	nd	nd	0.13	0.23
Eucalyptol	14.991	1009	0.26	0.24	0.23	nd	nd	0.10	0.13
Linalool oxide	17.105	1071	nd	0.30	nd	0.48	nd	0.14	0.23
Linalool	17.127	1072	nd	nd	nd	nd	nd	nd	nd
β-linalool	19.233	1132	0.48	60.81	50.15	1.26	0.69	0.65	0.38
Menthone	20.053	1156	nd	nd	nd	nd	nd	nd	0.07
α-terpineol	19.634	1144	0.48	5.85	5.35	0.50	0.48	0.42	0.31
Isomenthone	19.833	1150	nd	3.14	nd	nd	nd	nd	nd
Menthol	20.734	1175	0.43	0.22	0.36	68.30	0.30	0.33	0.18
Dihydrocarvone	22.212	1217	nd	3.34	5.68	nd	2.74	0.72	0.75
Pulegone	22.416	1224	nd	nd	nd	nd	nd	nd	nd
Linalyl acetate	22.797	1235	47.57	15.46	18.13	2.56	20.74	25.34	0.36
Piperitone oxide	23.700	1262	nd	nd	nd	nd	nd	1.10	1.13
Thymol	24.234	1277	0.77	nd	nd	nd	1.34	nd	0.56
Diosphenol	24.802	1293	nd	nd	0.46	nd	0.97	nd	nd
3-methylidenedecan-4-ol	26.431	1344	nd	nd	nd	nd	8.05	21.95	7.37
Humulene epoxide 2	26.865	1357	7.05	0.44	nd	nd	8.48	12.96	1.70
1-[tert-butyl(dimethyl)silyl]-4-(2,2-dimethyl-6-methylidenecyclohexyl)butan-1-ol	27.125	1365	nd	0.28	nd	0.26	nd	nd	nd
β-bourbonene	27.145	1366	0.79	nd	nd	nd	nd	nd	nd
7-(2-acetylcyclopropyl)-6,6-dimethylheptane-2,5-dione	27.826	1391	1.19	nd	nd	nd	2.42	nd	0.77
5-(tetrahydro-2-furanylmethyl)-2-heptanol	28.272	1399	nd	0.27	nd	1.31	nd	nd	1.45
□-caryophyllene	28.973	1422	2.08	0.22	nd	nd	0.48	nd	nd
1-acetoxy-p-menth-3-one	29.341	1434	7.44	nd	nd	nd	12.47	2.56	1.41
Anhydroserricornin	29.798	1449	16.51	0,41	0.56	0.12	24,04	8,70	8,02
2-tridecyn-1-yl hexanoate	31.306	1497	1.01	nd	nd	nd	1.15	1.43	0.20
3-(hydroxymethyl)-5-methoxyphenol	32.002	1521	nd	nd	nd	nd	0.35	nd	nd
Cinerolone	33.069	1558	0.51	nd	nd	0.21	0.22	nd	0.38
Spathulenol	33.296	1565	1.99	2.56	4.22	1.63	0.58	1.33	1.73
Caryophyllene oxide	40.067	1810	nd	0.61	nd	nd	0.52	nd	0.44

nd: not detected

Table 2. Identified chemical components in EOs of wild *M. longifolia* subsp. *typhoides* (Briq.) Harley var. *typhoides* PH. Davis samples from Yeşilyurt (Y)

Components	RT (min)	RI	Y-1		Y-2		Y-3		Y-4	Y-7	Y-10		Y-11	Y-12	Y-13	Y-14	Y-15
			L	F	L	F	L	F	F	F	L	F	F	L	L	F	L
3-octanol	13.531	965	nd	nd	0.20	nd	nd	nd	nd	0.15	nd	nd	nd	0.46	nd	nd	0.27
o-cymene	14.699	999	nd	nd	0.24	nd	nd	nd	nd	0.36	0.59	nd	nd	0.61	nd	nd	0.52
Eucalyptol	14.991	1009	nd	nd	nd	nd	nd	nd	nd	0.20	nd	nd	nd	7.13	nd	nd	0.35
Linalool oxide	17.105	1071	2.90	2.80	6.01	9.07	0.64	0.29	0.33	0.17	nd	nd	0.27	0.72	0.78	0.32	0.36
Linalool	17.127	1072	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
β-linalool	19.233	1132	1.36	1.02	0.84	0.95	0.68	1.17	1.03	0.80	34.89	27.54	1.26	2.84	26.70	0.60	0.89
Menthone	20.053	1156	0.14	nd	nd	0.15	nd	nd	nd	nd	nd	nd	nd	1.21	0.87	nd	nd
α-terpineol	19.634	1144	0.79	0.63	0.54	0.48	0.87	0.64	0.60	0.36	16.85	13.28	0.54	2.22	9.23	0.26	0.48
Isomenthone	19.833	1150	nd	nd	nd	nd	nd	nd	nd	nd	4.49	nd	nd	nd	1.82	nd	nd
Menthol	20.734	1175	1.12	0.55	0.73	0.53	nd	0.58	0.64	24.23	0.49	nd	0.45	0.72	0.69	0.41	nd
Dihydrocarvone	22.212	1217	0.29	1.00	0.94	nd	nd	2.35	nd	0.31	2.11	5.63	nd	0.75	2.37	nd	nd
Pulegone	22.416	1224	0.68	0.71	0.98	0.87	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Linalyl acetate	22.797	1235	35.69	2.51	28.62	23.74	63.15	25.99	35.39	5.13	24.0	31.06	46.78	2.30	nd	41.83	63.81
Piperitone oxide	23.700	1262	14.07	8.97	0.48	0.46	1.57	1.33	0.95	nd	nd	nd	nd	57.13	35.61	nd	0.58
Thymol	24.234	1277	nd	nd	0.52	nd	nd	nd	0.72	24.23	nd	nd	nd	nd	nd	1.68	nd
Diosphenol	24.802	1293	0.70	0.72	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
3-methylidenedecan-4-ol	26.431	1344	nd	nd	5.55	nd	nd	11.03	nd	nd	nd	nd	nd	nd	nd	nd	nd
Humulene epoxide	26.865	1357	0.27	4.01	8.58	0.75	nd	11.98	8.72	0.34	1.46	2.20	5.23	nd	nd	4.10	2.91
1-[tert-butyl(dimethyl)silyl]-4-(2,2-dimethyl-6-methylidenecyclohexyl)butan-1-ol	27.125	1365	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.64	2.30	nd
β-bourbonene	27.145	1366	0.79	1.20	0.62	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.42
7-(2-acetylcyclopropyl)-6,6-dimethylheptane-2,5-dione	27.826	1391	nd	nd	0.37	nd	nd	1.10	nd	nd	nd	nd	nd	nd	nd	nd	nd
5-(tetrahydro-2-furanylmethyl)-2-heptanol	28.272	1399	3.80	2.63	1.05	1.03	nd	nd	nd	nd	nd	nd	nd	2.66	3.36	nd	nd
□-caryophyllene	28.973	1422	nd	nd	0.59	nd	0.86	nd	0.63	nd	nd	nd	nd	nd	nd	nd	0.99
1-acetoxy-p-menth-3-one	29.341	1434	7.74	22.09	12.59	0.38	nd	3.56	nd	3.29	nd	nd	0.76	nd	nd	25.34	3.37
Anhydroserricornin	29.798	1449	0.94	4.22	7.94	0.37	nd	14.82	6.04	0.95	1.70	1.89	8.63	nd	nd	7.98	3.50
2-tridecyn-1-yl hexanoate	31.306	1497	nd	0.59	1.10	nd	nd	1.32	0.87	nd	nd	nd	0.69	nd	nd	0.50	0.34
3-(hydroxymethyl)-5-methoxyphenol	32.002	1521	nd	0.85	nd	nd	nd	2.70	1.34	nd	0.30	0.46	0.85	nd	nd	1.03	0.48
Cinerolone	33.069	1558	1.20	0.78	0.49	0.30	nd	nd	0.36	3.01	0.68	nd	nd	1.26	0.85	nd	0.99
Spathulenol	33.296	1565	0.85	0.53	1.46	2.04	3.22	0.76	1.39	nd	2.30	1.59	2.72	1.82	1.17	1.27	3.05
Caryophyllene oxide	40.067	1810	nd	nd	0.60	0.27	nd	nd	nd	0.63	0.57	nd	nd	nd	nd	nd	0.30

nd: not detected

3.2.1. Antioxidant activities

The antioxidant compounds affect different mechanisms such as retention of transition metal ions, inhibition of hydrogen abstraction, the breakdown of peroxides and radical clearing. One of the most important factors affecting antioxidant capacity is the ability of antioxidants to donate electrons. Because of the harmful effects of BHA and BHT on synthetic antioxidants, the antioxidant capacities of plant-derived natural polyphenols and EOs continue to be investigated and studied extensively.[22,23] Many methods have been developed to evaluate the antioxidant activities of naturally occurring compounds, crude extracts and EOs. Among these methods, the most commonly used assays are total antioxidant activity, inhibition of lipid peroxidation, reducing power, metal chelating, $DPPH^{\cdot}$ and $O_2^{\cdot -}$ scavenging activities.[24] The evaluation of the antioxidant activity of the EOs of the *Mentha* species is shown in Table 5. Statistical analysis of EOs activities showed a similarity between each other ($p < 0.01$). Since leaf and flower parts of *Mentha* species have different chemical contents of EOs, the effects of applied antioxidant activities have been affected.

3.2.1.1. Total antioxidant activity by phosphomolybdenum method

The phosphomolybdenum method has been widely used to evaluate the antioxidant activity of secondary metabolites, food and medicinal bioactive molecules.[25]

The antioxidants are able to prevent oxidation of a substrate exposed to the oxidation process and scavenge free radicals via deoxygenating processes.[26] The antioxidant capacities of EOs were tested for the formation of a green phosphomolybdenum (phosphate/Mo(V)) and compared with standard antioxidants and exhibited in Table 5. The experimental data indicated that EOs were likely to have the potential for reducting Mo(V) into Mo(IV) and to show statistically significant differences, ($p < 0.01$). The total antioxidant activities of EOs were lower than those of standards in terms of phosphomolybdenum reduction potential. The activities of K-4L, K-4F, K-1L, R-2L, Z-1F, Z-10F, Z-11F, R-9F and R-5F had the higher activity than among EOs due to containing a high amount of β-linalool (60.81%)-linalyl acetate (15.46%), β-linalool (50.15%)-linalyl acetate (18.13%), linalyl acetate (47.57%), linalyl acetate (20.74%), linalyl acetate (13.93%), linalyl acetate (26.90%), linalyl acetate (49.22%), piperitone oxide, (68.92%) and piperitone oxide (47.60%), respectively (Table 1-4).

3.2.1.2. Reducing power ($Fe^{3+} \rightarrow Fe^{2+}$ reduction)

The reduction power of EOs of *Mentha* species was determined by measuring the reduction of $Fe^{3+} \rightarrow Fe^{2+}$. The reducing power of the EOs detects the complex compound in purine blue color by the addition of $FeCl_3$ at 700 nm.[27] Another different defense mechanism to protect the cell against the hazardous effects of free radicals is the reduction ability of these molecules by the natural antioxidants. The EOs of *Mentha* species demonstrated reducing ability at 100 μg ml^{-1} (Table 5). As can be seen from Table 2 and 3, linalyl acetate is the major component of the Y-1F, Y-2L, Y-3F, Z-10F, Y-12L and Y-11F EOs studied here.

Table 3. Identified chemical components in EOs of wild *M. longifolia* subsp. *typhoides* (Briq.) Harley var. *typhoides* PH. Davis leaf (L) and flower (F) samples from Zile (Z)

Components	RT (min)	RI	Z-2		Z-7		Z-4	Z-1	Z-10	Z-11
			L	F	L	F	L	F	F	F
3-octanol	13.531	965	0.43	0.42	0.48	nd	0.32	nd	nd	nd
o-cymene	14.699	999	0.31	0.40	0.38	nd	0.30	0.32	nd	0.46
Eucalyptol	14.991	1009	0.19	0.30	0.83	nd	0.28	0.27	nd	nd
Linalool oxide	17.105	1071	45.68	62.88	0.41	0.33	52.32	nd	0.39	nd
Linalool	17.127	1072	nd	nd	nd	nd	nd	nd	nd	nd
β-linalool	19.233	1132	1.27	0.55	0.50	1.34	0.64	35.25	0.74	0.52
Menthone	20.053	1156	0.61	0.37	0.11	nd	0.14	nd	nd	nd
α-terpineol	19.634	1144	30.15	14.09	nd	0.85	0.42	6.03	0.45	0.34
Isomenthone	19.833	1150	nd	nd	nd	nd	nd	nd	nd	nd
Menthol	20.734	1175	0.24	0.10	0.21	0.77	0.20	0.62	0.47	nd
Dihydrocarvone	22.212	1217	8.63	9.13	0.34	0.38	nd	4.51	2.59	1.60
Pulegone	22.416	1224	4.19	3.50	nd	nd	6.23	nd	nd	nd
Linalyl acetate	22.797	1235	2.08	1.05	69.43	nd	nd	13.93	26.90	49.22
Piperitone oxide	23.700	1262	nd	nd	1.26	nd	0.21	nd	0.66	nd
Thymol	24.234	1277	nd	nd	nd	nd	0.28	0.56	1.54	1.05
Diosphenol	24.802	1293	nd	nd	0.52	nd	nd	nd	0.50	1.32
3-methylidenedecan-4-ol	26.431	1344	nd	0.15	nd	13.21	nd	nd	10.74	13.15
Humulene epoxide	26.865	1357	0.49	nd	0.56	7.80	0.53	0.88	9.02	0.41
1-[tert-butyl(dimethyl)silyl]-4-(2,2-dimethyl-6-methylidenecyclohexyl)butan-1-ol	27.125	1365	nd	0.76	nd	nd	nd	nd	nd	8.23
β-bourbonene	27.145	1366	nd	nd	nd	nd	0.43	nd	0.19	nd
7-(2-acetylcyclopropyl)-6,6-dimethylheptane-2,5-dione	27.826	1391	nd	nd	0.27	0.62	nd	nd	nd	0.83
5-(tetrahydro-2-furanylmethyl)-2-heptanol	28.272	1399	0.93	0.36	3.32	nd	2.79	nd	0.73	nd
β-caryophyllene	28.973	1422	nd	nd	0.91	nd	0.20	nd	2.63	nd
1-acetoxy-p-menth-3-one	29.341	1434	nd	nd	nd	0.90	1.59	6.64	8.47	2.53
Anhydroserricornin	29.798	1449	nd	0.05	1.09	5.49	nd	1.38	20.62	5.12
2-tridecyn-1-yl hexanoate	31.306	1497	nd	nd	nd	0.97	nd	0.50	0.93	0.85
3-(hydroxymethyl)-5-methoxyphenol	32.002	1521	nd	nd	nd	nd	nd	nd	nd	1.35
Cinerolone	33.069	1558	nd	nd	0.82	0.86	nd	nd	nd	nd
Spathulenol	33.296	1565	0.92	0.58	3.69	3.81	0.56	2.69	1.42	0.88
Caryophyllene oxide	40.067	1810	nd	nd	0.71	nd	nd	nd	0.69	nd

nd: not detected

They were exhibited better reducing power potential than α-tocopherol with high absorbance values. But the other EOs had low reduction powers compared to the standards.

The difference observed in the results is due to the component difference of EOs. The same taxons that grow in different areas often have different chemical components. Therefore, the increase in Fe^{3+} reducing activity over developmental period matched well with the increase in chemical contends.

3.2.1.3. Free radical scavenging activity on DPPH˙

The EOs of *Mentha* species were effective in reducing pink DPPH˙ to colorless 2,2-diphenyl-1-picryl hydrazine ($DPPH_2$) due to the presence of antioxidants in the medium.[28] The DPPH˙, initially purple in color, takes up hydrogen from the antioxidant substance, and then the yellow colored hydrazine compound is formed. It is observed that absorbance values decrease as color (purple to yellow). The DPPH˙ scavenging activities of the EOs were tested at 517 nm and compared with the standards used as a reference (Table 5).

Table 4. Identified chemical components EOs of wild *M. longifolia* (L.) Hudson subsp. *longifolia*, *M. spicata* L. subsp. *spicata* and *M. villoso nervata* Opiz in leaf (L) and flower (F) samples from the center of Tokat (K), Zile (Z), Yeşilyurt (Y) and Reşadiye (R)

Components	RT (min)	RI	*M. villoso nervata*		*M. spicata*		*M. longifolia* (L.)	
			K-3L	R-9F	Y-9L	Z-5L	R-5F	R-6L
3-octanol	13.531	965	nd	nd	nd	0.92	0.34	0.44
o-cymene	14.699	999	nd	nd	nd	0.33	0.51	0.32
Eucalyptol	14.991	1009	nd	nd	nd	0.23	0.23	0.19
Linalool oxide	16.293	1048	nd	nd	nd	nd	nd	nd
Linalool	17.105	1071	nd	nd	nd	0.81	nd	nd
β-linalool	17.127	1072	0.55	0.77	nd	nd	nd	0.24
Menthone	19.233	1132	19.41	2.58	10.60	1.00	0.31	0.40
α-terpineol	20.477	1168	nd	nd	0.21	0.52	nd	0.23
Isomenthone	19.634	1144	1.67	0.67	2.65	1.63	nd	nd
Menthol	19.833	1150	0.59	nd	1.12	nd	nd	nd
Dihydrocarvone	20.734	1175	0.50	0.70	0.74	1.20	nd	0.22
Pulegone	22.212	1217	6.18	1.97	1.87	21.52	nd	nd
Linalyl acetate	22.416	1224	nd	nd	nd	nd	nd	nd
Piperitone oxide	22.797	1235	56.19	68.92	46.82	2.08	47.60	nd
Thymol	23.700	1262	nd	1.95	nd	nd	0.92	1.04
Diosphenol	24.234	1277	nd	nd	0.60	nd	nd	1.06
3-methylidenedecan-4-ol	24.802	1293	0.88	nd	1.35	nd	nd	nd
Humulene epoxide	26.431	1344	nd	8.60	8.17	nd	11.68	nd
1-[tert-butyl(dimethyl)silyl]-4-(2,2-dimethyl-6-methylidenecyclohexyl)butan-1-ol	26.865	1357	nd	2.08	3.50	nd	9.70	3.75
β-bourbonene	27.125	1365	nd	nd	nd	nd	nd	0.31
7-(2-acetylcyclopropyl)-6,6-dimethylheptane-2,5-dione	27.145	1366	nd	nd	nd	0.64	nd	nd
5-(Tetrahydro-2-furanylmethyl)-2-heptanol	27.984	1391	nd	0.48	0.58	nd	0.70	0.95
β- caryophyllene	28.272	1399	nd	0.42	0.31	nd	nd	nd
1-acetoxy-p-menth-3-one	28.973	1422	nd	0.62	1.19	nd	nd	nd
Anhydroserricornin	29.341	1434	2.84	4.55	7.73	nd	6.67	9.30
2-tridecyn-1-yl hexanoate	30.575	1474	nd	1.40	1.83	nd	5.24	1.71
3-(hydroxymethyl)-5-methoxyphenol	31.306	1497	nd	nd	0.25	nd	1.18	0.37
Cinerolone	32.002	1521	nd	nd	nd	nd	nd	nd
Spathulenol	33.069	1558	nd	nd	0.38	1.19	0.51	0.61
Caryophyllene oxide	33.296	1565	3.83	1.73	1.44	8.32	2.05	1.89
Dihydro-β-agarofuran	32.015	1521	nd	0.39	0.53	nd	1.88	0.40

nd; not detected

Table 5. Comparisons of total antioxidant activity (A), reducing power (B), free radical scavenging activity (C), H_2O_2 scavenging activity (D), $O_2^{\bullet-}$ scavenging activity (E) metal-chelating activity (F) and inhibition of lipid peroxidation (G) of EOs at 100 µg ml^{-1}

No	Sample		Result of antioxidant assay						
			A, µmol α-toc/g	**B, 700 nm**	**C, %**	**D, %**	**E, %**	**F, %**	**G, %**
			***M. longifolia* subsp.*typhoides* (Briq.) Harley var. *typhoides* PH. Davis**						
1	K-2	L	17867±1178^{bcd}	0.109±0.003^{h-k}	26.92±0.00^{cd}	55.03±9.00^{abc}	31.84±11.71^{b-g}	36.40±9.58^{bcd}	74.25±3.89^{abc}
2	K-4	L	16303±361^{bcd}	0.117±0.016^{h-k}	16.43±1.78^{fg}	50.00±1.57^{a-f}	33.16±1.94^{b-g}	22.65±13.77^{cde}	53.75±22.27^{b-e}
3	K-4	F	12257±505^{ef}	0.125±0.001^{d-i}	23.08±1.09^{ef}	51.11±6.29^{a-f}	36.42±2.01^{a-e}	11.11±2.24^{gf}	77.75±6.01^{ab}
4	R-1	F	12308±914^{ef}	0.088±0.003^{klm}	20.00±1.09^{fg}	23.33±4.71^{ghi}	39.83±1.07^{a-b}	21.27±15.56^{def}	80.75±3.89^{bc}
5	R-2	L	15759±1274^{cd}	0.098±0.001^{klm}	29.62±0.54^{cd}	36.67±1.57^{c-i}	40.54±0.07^{a-b}	43.44±15.34^{bc}	81.75±6.72^{ab}
6	R-4	F	12257±505^{ef}	0.110±0.001^{h-k}	36.54±7.07^{bc}	40.00±6.29^{b-h}	43.38±1.40^{a}	29.47±5.70^{c-d}	90.75±4.60^{a}
7	R-7	L	17901±457^{bcd}	0.113±0.084^{h-k}	nd	46.67±9.43^{b-f}	30.65±3.88^{b-g}	23.70±12.57^{cde}	77.75±0.35^{ab}
8	Y-1	L	6069±710^{ijk}	0.154±0.006^{bc}	21.54±0.01^{ef}	53.33±6.29^{a-e}	37.32±0.20^{a-e}	12.96±9.20^{fg}	nd
9	Y-1	F	11390±144^{ef}	0.174±0.007^{a}	25.01±7.07^{ef}	23.33±1.57^{ghi}	40.44±5.82^{a-b}	57.62±0.97^{ab}	5.25±3.18^{h}
10	Y-2	L	7378±192^{ghij}	0.199±0.004^{a}	21.54±4.35^{ef}	23.33±1.57^{ghi}	37.32±1.14^{a-e}	17.35±13.02^{def}	16.75±19.45^{gh}
11	Y-2	F	24038±481^{a}	0.122±0.003^{d-i}	66.15±1.09^{a}	34.44±7.86^{d-i}	28.76±0.27^{c-g}	13.76±7.63^{fg}	92.75±8.84^{a}
12	Y-4	F	11050±240^{ef}	0.128±0.002^{d-i}	46.15±0.01^{b}	3.33±1.57^{j}	31.22±1.07^{b-g}	20.95±3.29^{def}	72.50±7.07^{bcd}
13	Y-10	L	11934±433^{ef}	0.148±0.001^{bcd}	14.62±1.09^{ef}	7.78±1.57^{j}	31.41±1.87^{b-g}	10.95±14.44^{gh}	69.25±11.67^{abc}
14	Y-10	F	7582±192^{ghij}	0.135±0.006^{c-g}	13.85±5.44^{ef}	22.22±9.43^{ghi}	28.71±0.07^{c-g}	15.56±1.95^{d-g}	87.50±4.95^{a}
15	Y-7	F	11526±481^{ef}	0.130±0.003^{d-h}	38.46±5.44^{bc}	32.22±1.57^{e-i}	32.73±2.41^{b-g}	10.88±00.41^{fg}	44.00±0.71^{def}
16	Y-11	F	5542±192^{jk}	0.153±0.001^{bc}	4.23±27.74^{i}	25.56±4.71^{f-i}	31.65±4.08^{b-g}	29.50±5.95^{cd}	nd
17	Y-12	L	4960±420^{klm}	0.165±0.051^{ab}	39.23±8.70^{bc}	37.78±9.43^{c-i}	32.36±1.74^{b-g}	9.37±6.81^{gh}	nd
18	Y-13	L	5831±120^{jkl}	0.143±0.013^{cde}	14.23±5.98^{fg}	57.78±6.29^{ab}	35.24±4.75^{a-f}	9.58±5.76^{gh}	nd
19	Y-14	F	3995±720^{n}	0.115±0.011^{h-k}	nd	54.94±1.57^{a-d}	36.80±4.95^{a-f}	12.22±3.97^{dg}	nd
20	Y-15	L	8432±481^{gh}	0.136±0.001^{c-f}	nd	50.00±7.86^{a-f}	35.43±1.40^{a-f}	29.74±7.78^{cd}	nd
21	Z-1	F	18938±577^{b}	0.151±0.006^{bc}	nd	40.00±9.43^{b-h}	35.86±4.01^{a-f}	29.50±13.36^{cd}	94.25±5.30^{a}
22	Z-2	L	8670±240^{gh}	0.129±0.004^{d-i}	nd	44.44±9.43^{b-g}	26.40±1.87^{e-g}	13.23±10.63^{d-g}	91.00±3.54^{a}
23	Z-2	F	8840±962^{gh}	0.139±0.001^{c-f}	nd	43.33±1.57^{b-g}	26.96±1.61^{e-g}	Nd	73.25±3.89^{abc}
24	Z-4	L	10506±274^{efg}	0.138±0.010^{c-f}	nd	51.11±9.43^{a-f}	27.72±0.54^{d-g}	18.68±3.22^{efg}	97.50±2.12^{a}
25	Z-7	L	19703±2284^{b}	0.105±0.001^{jkl}	13.46±4.90^{fg}	53.33±3.14^{a-e}	36.94±1.54^{a-f}	58.47±0.22^{bc}	82.75±1.06^{a}
26	Z-7	F	10387±601^{efg}	0.084±0.001^{m}	9.23±1.09^{gh}	37.78±6.29^{c-i}	35.10±3.61^{a-f}	5.82±7.93^{h}	77.50±16.26^{ab}
27	Z-10	F	18564±240^{bc}	0.181±0.005^{a}	12.69±0.54^{fg}	38.89±1.57^{b-h}	35.34±0.74^{a-f}	12.75±6.51^{fg}	87.00±6.36^{ab}
28	Z-11	F	14178±1346de	0.142±0.011^{c-f}	10.00±2.18^{fg}	18.89±4.71^{hi}	38.84±3.14^{abc}	58.99±0.67^{bc}	nd
			M. longifolia (L.)* Hudson subsp. *longifolia						
29	R-5	F	30073±890^{a}	0.078±0.005^{m}	4.23±0.54^{i}	nd	42.38±0.27^{c}	23.33±0.97^{def}	83.04±7.07^{abc}
30	R-6	L	15861±938^{cd}	0.137±0.008^{c-f}	nd	54.04±4.71^{a-d}	22.33±3.75^{g}	1.69±0.6^{h}	69.25±2.47^{bcd}
			M. spicata* L. *subsp. spicata						
31	Z-5	L	17153±890^{bcd}	0.177±0.002^{a}	14.62±7.61^{fg}	51.11±3.14^{a-f}	44.04±1.27^{c}	14.34±2.92^{fg}	82.5±7.78^{ab}
32	Y-9	L	12546±240^{ef}	0.123±0.003^{d-i}	nd	33.33±6.29^{d-i}	29.66±2.34^{b-g}	nd	78.5±4.24^{cd}
			***M. villoso nervata* Opiz**						
33	K-3	L	7939±216^{ghi}	0.095±0.006^{klm}	19.23±2.18^{ef}	56.67±4.71^{a-e}	33.11±2.68^{b-f}	26.35±4.04^{cde}	59.25±5.3^{d}
34	R-9	F	16303±1370^{bcd}	0.112±0.006^{e-k}	nd	38.89±7.86^{b-h}	25.54±2.14^{efg}	26.51±37.64^{cde}	87.75±1.77^{ab}
			Standards						
35	α-tocopherol		-	0.179±0.011^{h}	56.15±15.23^{d}	53.33±3.14^{b}	29.23±3.08^{cd}	49.21±1.20^{bc}	88.00±0.00^{abc}
36	BHA		158100±1252^{cd}	0.636±0.003^{b}	70.00±4.71^{a}	33.11±7.28^{c}	83.60±2.00^{abc}	91.92±4.90^{a}	80.00±2.83^{bcd}
37	BHT		89930±5049de	0.872±0.012^{a}	44.44±15.71^{bc}	33.21±7.22^{c}	55.56±0.90^{bc}	97.31±1.63^{a}	78.75±0.35^{cd}
38	Trolox		81260±8174de	0.414±0.006^{d}	72.22±1.57^{a}	41.11±1.81^{c}	80.11±2.54^{ab}	94.23±1.63^{a}	82.00±2.12^{bcd}
39	TBHQ		166600±1097^{cd}	0.539±0.004^{c}	32.22±1.57^{d}	40.30±0.80^{c}	56.77±2.02^{bc}	81.15±1.65^{abc}	42.25±3.89^{g}
	**: $p < 0.01$		**	**	**	**	**	**	**

TOC: α-tocopherol, **BHA:** buthylated hydroxyanisole, **BHT:** buthylated hydroxytoluene, **TBHQ:** tert-Butylhydroquinone, **nd:** not detected

The EOs showed lower performance than the standards. With the exception of the Z-2L, Z-2F, R-7L, Y-14F, Z-1F, Y-15L and Z-4L, all EOs exhibited a good inhibitory performance of the DPPH˙ (Table 5). Especially, Y-2F had significantly (p<0.01) better activity than α-tocopherol due to containing high rate menthone, piperitone oxide, linalool and caryophyllene oxide. The results indicated that some EOs are a free radical scavenger, and also phenolic composition capacity to scavenge the free radical may contribute to its antioxidant activity.[2,29] Furthermore, the EOs have previously been reported to have high free radical scavenging of *Mentha* species.[30]

3.2.1.4. H_2O_2 Scavenging activity

H_2O_2 is naturally formed and the most important source of ˙OH as a by-product of oxygen related metabolism in organisms. In the presence of Fe^{2+} and other transition elements, Fenton and Haber-Weiss reactions cause the formation of the ˙OH, which is the most active and harmful.[31] In this sense, removal of H_2O_2 is very important. Therefore, we evaluated EOs on reactive oxygen species using H_2O_2 scavenging test, p < 0.01. In Table 5, the EOs of K-2L, Y-13L, Y-14F, K-3L and R-6L exhibited higher H_2O_2 scavenging activity than α-tocopherol, BHT and TBHQ due to the high content of linalyl acetate, β-linalool, menthone and piperitone oxide molecules (Table 1-4).

3.2.1.5. Superoxide anion scavenging activity

The superoxide anion radical ($O_2^{\bullet-}$) is formed during the process of normal metabolism in all aerobic organisms and causes the formation of H_2O_2. H_2O_2 affects cells and their components due to strong oxidizing. The $O_2^{\bullet-}$ can damage proteins, lipids and nucleic acids. In this assay, $O_2^{\bullet-}$ is produced by the reduction of NBT and by the oxidation of NADH with the PMS-NADH system.[32] The conversion of yellow color to the formazan blue was determined with the reaction of NBT at 560 nm. The results showed that the EOs showed remarkable superoxide anion scavenging capacity (p < 0.01) and found to be comparable to the standards (Table 5). In this study, superoxide anion scavenging assay is successfully used to determine the antioxidant behavior of the EOs of the *Mentha* species, reaching the standard value of 41% (trolox). The EOs of R-4F, R-5F and Z-5L have higher superoxide anion scavenging activity than compared with α-tocopherol, BHA, BHT, trolox and TBHQ at 100 μg ml^{-1}. The high secondary content of the EOs may contribute to strong superoxide radical scavenging activities.[2]

3.2.1.6. Metal chelating activity

The transition metal ions are powerful oxidative catalysts that form free radicals in the living system. The free forms of iron, which display an important role in oxygen transport, ATP production and DNA synthesis in biological systems, are toxic in living cells. The reactive oxygen species (ROS) formed as a result of this toxicity can promote lipid oxidation or attack DNA molecules. The Fe^{2+} may facilitate ROS production in live systems. The removal of free-Fe^{2+} with a good chelating effect may be useful in the prevention of oxidative stress-related diseases since the Fe^{2+} is derived from effective prooxidants in food systems.[33] Hence, the Fe^{2+} chelating ability is important for antioxidant properties of natural materials. For this reason, metal chelating activities of the EOs were examined. The metal chelating activities were determined by measuring the absorbance of Fe^{2+}-ferrozine complex. The absorbance of a decrease in the assay indicates that the Fe^{2+} chelate without binding to the ferrous. The EOs interfered with the formation of ferrous (Table 5). The EOs of Z-11F, Y-1F and Z-7L exhibited significantly (p < 0.01) higher activity than TBHQ, α-tocopherol and BHT.

3.2.1.7. Inhibition of lipid peroxidation

Lipids are biomolecules that are most sensitive to the effects of free radicals. Oxidative degradation of polyunsaturated fatty acids is known as a lipid peroxidation. Lipid peroxidation is quite harmful and proceeds as a self-sustaining chain-reaction. The formations of lipid-free radicals (L˙) and lipid peroxide radicals (LOO˙) on cell membranes are considered to be an important feature of cell damage caused by reactive oxygen species. ROS disrupts the structure of polyunsaturated lipids, lead to the formation of malondialdehyde (MDA) and cause toxic stresses in the cells.[34] The formation of MDA is used as a biomarker to read the level of lipid peroxidation in the organism and the TBA-MDA complex. The low intensity of the absorbance indicates less lipid peroxidation. For this reason, the decrease in reaction color intensity of the EOs exhibits that it is effective in lipid peroxidation (Table 5). The inhibition levels of Y-2F, Y-1F, R-4F, Z-1F and Z-10F (*M. longifolia* subsp. *typhoides*) EOs were 92, 87, 90, 94 and 87%, respectively due to containing high levels of linalyl acetate, and they may contribute to antioxidant potential. The results are consistent with previous work.[35] Since piperitone oxide is major compound of *M. villoso nervata* Opiz, *M. longifolia* (L.) Hudson subsp. *longifolia*, *M. spicata* L. subsp. *spicata* leaf and flower EOs, it exhibits a high inhibition of lipid proxidation compared to the standards.

4. CONCLUSIONS

The results of the study were summarized as follows:

- **i.** The current study exhibited that the EOs of *Mentha* species (*M. longifolia* subsp. *typhoides* (Briq.) Harley var. *typhoides* PH. Davis; *M. villoso nervata* Opiz; *M. longifolia* (L.) Hudson subsp. *longifolia*; *M. spicata* subsp. *spicata*) contain high-value chemical composition such as linalool, menthone, isomenthone, pulegone, piperitoneoxide, 1-[tert-butyl(dimethyl)silyl]-4-(2,2-dimethyl-6-methylidenecyclohexyl) butan-1-ol, anhydroserricornin and caryophylleneoxide.
- **ii.** Almost all the EO samples exhibited antioxidant activity (total antioxidant, reducing power, inhibition of lipid peroxidation, metal chelating, H_2O_2, $DPPH^{\bullet}$ and $O_2^{\bullet-}$ scavenging assays) compared with standards.
- **iii.** The study of the relation between the chemical composition of the EOs and the antioxidant activities revealed the presence of different strong correlations with some major identified compounds in each case of study.
- **iv.** It could be concluded that EOs of *Mentha* species were effective as antioxidant activity due to the presence of several compounds in their chemical compositions. They could be used as flavoring agents and sources of antioxidants to make healthy food and healthy sensory acceptability. Further studies are required to research about the in vivo antioxidant activity of these EOs.

Acknowledgements: The authors are grateful to the Scientific Research Projects (PYO.FEN.1904.13.003; PYO.FEN.1904.13.006), Ondokuz Mayis University for their financial support.

Conflict of interest

All authors declare that there is no a conflict of interest with any person, institute, company, etc.

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ORCID

0000-0003-0133-5630 (T. Ozen)

0000-0002-3651-1641 (I. Telci)

0000-0002-4297-786X (F. Gul)

0000-0001-8946-647X (I. Demirtaş)