

## MINERAL CONTENT, ESSENTIAL OIL COMPONENTS AND BIOLOGICAL ACTIVITY OF TWO MENTHA SPECIES (*M. piperita* L., *M. spicata* L.)

Süleyman KIZIL<sup>1\*</sup> Nesrin HAŞİMİ<sup>2</sup> Veysel TOLAN<sup>3</sup> Ersin KILINÇ<sup>4</sup> Uyan YÜKSEL<sup>4</sup>

<sup>1</sup>Department of Field Crops, Faculty of Agriculture, Dicle University, 21280 Diyarbakır, TURKEY

<sup>2</sup>Department of Biology, Faculty of Art and Science, Batman University, 72100 Batman, TURKEY

<sup>3</sup>Department of Biology, Faculty of Art and Science, Dicle University, 21280 Diyarbakır, TURKEY

<sup>4</sup>Department of Chemistry, Faculty of Art and Science, Dicle University, 21280 Diyarbakır, TURKEY

\*Corresponding author: kizils@dicle.edu.tr

### ABSTRACT

The essential oil of mint species are widely used in food, pharmaceutical and cosmetic industries, growing throughout the world. Therefore, it is very important to know the chemical characteristics of the oil for economic use and enhanced performance of the end products. This study was carried out to determine mineral content, essential oil composition, antimicrobial and antioxidant activities of essential oil of *Mentha piperita* (L.) and *M. spicata* (L.) (Lamiaceae). Chemical compositions of hydro-distilled essential oils obtained from mint species leaves were analyzed by gas chromatography-mass spectrometry (GC-MS). For antimicrobial activity, the disc diffusion tests were carried out on *E. coli* line ATCC25922, *P. aeruginosa* line ATCC27853, *S. aureus* line 25923, *S. pyogenes* line ATCC19615 and *C. albicans* line ATCC10231, and the antioxidant activity was performed by using DPPH radical-scavenging method. It was determined that essential oil of *M. piperita* and *M. spicata* contains menthol and carvone as major components, respectively. Treatment of 5 µl, 10 µl 15 µl and 20 µl of the oil exhibited strong antimicrobial activity against *S. pyogenes*, *S. aureus* and *C. albicans* and *E. coli*, except *P. aeruginosa*. The antioxidant activity of essential oil of mint species lowered DPPH activity compared to ascorbic acid. The results demonstrated that mint species essential oil has clearly good antimicrobial activity against test organisms and acceptable antioxidant activity.

**Keywords:** *Mentha piperita*, *M. spicata*; essential oil; carvon, menthol; biological activity

### INTRODUCTION

Mint species have been exploited by man for more than two thousand years. Peppermint itself has only been used for 250 years (Hornok, 1992). The genus *Mentha* (Lamiaceae) is composed of 19 geographically widespread species and 13 named hybrids (Chambers and Hummer, 1994). *Mentha* species are widely used in conventional medicine, for their antispasmodic, antiseptic and emmenagogue effects (Edris et al., 2003); moreover, their essential oils are used in chewing gums, alcoholic beverages, cosmetics, perfumes, toothpastes and mouthwashes (Baytop, 1984). The plant is mainly used as salad, spice and for tea besides mint herbage used for wool dyeing (Trmic, 1991; Leung and Foster, 2003).

Peppermint (*M. piperita* L.), developed through the cross breeding of *M. aquatica* and *M. spicata* L., is native to Europe and it has become both cultivated and naturalised in the USA, India, China, the former USSR, Italy, France and Hungary. Its essential oil is considered industrially important (Aflatuni, 2005). Peppermint (*M. piperita*.) oil is one of the most popular and widely used essential oils, mostly because of its main components menthol and menthone (Hornok, 1992). The essential oil in the dried leaves of peppermint (2.5%) is mostly made up from menthol (50%), menthone (10 to 30%), menthyl esters (up to 10%) and further monoterpene derivatives (pulegone, piperitone,

menthofurane). Traces of jasmone (0.1%) improve the oil's quality remarkably (Saeidnia et al., 2005). Moreover, menthol has bactericidal effects and a spicy odour (Hornok, 1992).

*M. spicata* has formed from cross breeding of *M. longifolia* and *M. rotundifolia*. The leaves, herbs and essential oil of *M. spicata* were used much earlier than those of peppermint (Hornok, 1992). The essential oils extracted from *M. spicata*, containing mainly carvone (50-70%) and menthone, have shown strong insecticidal and mutagenic activity (Aflatuni, 2005; Hussain, 2009).

The present study is aimed at assessing herb quality with respect to its mineral contents and essential oil components and antimicrobial and antioxidant activities of essential oils of *Mentha piperita* and *M. spicata*, growing in semi arid climatic conditions.

### MATERIALS AND METHODS

**Plant Materials:** Plant has been grown at the Medicinal Plant Collection Garden of Department of Field Crops, Agriculture Faculty of Dicle University, Diyarbakır, Turkey. Clones of mint species were planted in sandy pool in high tunnel during January 2005-06 growing season from where, they were transplanted to field at 10-15 cm plant height on April 2006. The field plot arranged as 45x20 cm apart with 5 rows. No

chemical fertilizers were applied during experimentation to avoid long-term adverse impact on the soil organisms living in soil. Irrigation and weeding of the plot was done as and when needed. Plant harvest was done at full flowering period (June-July 2009). Dry herbage was determined by drying fresh herbage samples in cool dry shady place for one week.

**Mineral content of samples:** Mineral content of mint herbs were determined by Perkin Elmer Optima 2100 DV ICP OMS (USA).

**Essential oil Extraction:** Essential oils of dried mint herbs were isolated by hydro-distillation for 2.5 h using a Clevenger-type apparatus (v/w) according to standard procedure described in European Pharmacopoeia (1975) for determining the oil content (%). The isolated oils were stored in tightly closed vials at +4 °C until analysis.

**Gas chromatography/Mass spectrometry (GC/MS) Analysis:** The essential oils of mint samples were analysed by using a GC Clarus 600-MS Clarus 600 C (Perkin Elmer) equipped with an auto sampler. One microlitre of sample volume was injected using split method. Chromatographic separations were accomplished with a Elite 5-MS capillary column (5% Diphenyl)-Dimethylpolysiloxane, 0.25mm i.d.x30 m, film thickness 0.25 µm) with injections in the split mode with 20 split ratio. Analysis was carried out using helium as the carrier gas with flow rate of 1.0 mL/min. The column temperature was initially kept at 60 °C for 3 min than gradually increased to 130 °C at 4 °C min<sup>-1</sup> rate, held for 2 min, and finally raised to 240 °C at 20 °C min<sup>-1</sup>. The injection port temperature was 240°C. The ionization voltage applied was 70 eV with mass range m/z of 20–550 amu. The separated components were identified tentatively by matching with EI-MS results of National Institute of Standards and Technology (NIST), WILEY 8<sup>th</sup> edition and NBS mass spectral library data. The quantitative determination was carried out based on peak area integration.

#### Antimicrobial activity

##### Microbial strains

The essential oil was tested against microorganisms including *E. coli* ATCC25922, *P. aeruginosa* ATCC27853, *S. aureus* 25923, *S. pyogenes* ATCC19615, *C. albicans* ATCC10231. Bacterial strains were cultured overnight in Nutrient Broth (NB) at 37°C, with the exception of *C.albicans* (30°C).

##### Antimicrobial screening

The agar disc diffusion method was employed for the determination of antimicrobial activity of the essential oil in question (NCCLS, 1997). Briefly, a suspension of the tested microorganism (0.1 ml of 10<sup>8</sup> cells per ml) was spread on the solid media plates. Sterile Filter paper discs (6 mm in diameter) were impregnated with various volume and concentration (in DMSO) of the oil and placed on the inoculated plates. These plates, after standing at 4°C for 2 h, were incubated at 37°C for 24 h for bacteria and at 30°C for 48 h for the yeast. Imipenem (IMP) was used as a positive control. The diameters of the inhibition zones were measured in millimetres. All the tests were performed in triplicate.

Values are presented as means ± SD of three parallel measurements.

#### Antioxidant activity

##### DPPH assay

Hydrogen atoms or electrons donation ability of the corresponding oils was measured from the bleaching of purple coloured methanol solution of DPPH. This spectrophotometric assay uses stable radical 2,2'-diphenyl-1-picrylhydrazyl (DPPH) as a reagent (Cuendet et al., 1997; Burits and Bucar, 2000). Fifty microliter of the oil in methanol was added to 5 ml of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature the absorbance was read against a blank at 517 nm. The same procedure was repeated with the synthetic antioxidant, butylated hydroxyanisole (BHA) and ascorbic acid as positive controls. Inhibition free radical DPPH in percent (I%) was calculated in following way:

$$I\% = (A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}}) \times 100$$

where  $A_{\text{blank}}$  is the absorbance of the control reaction (containing all reagents except the test compound), and  $A_{\text{sample}}$  is the absorbance of the test compound. For the calculation of these values, Microsoft Excel software was used. Percent inhibition after 30 min was plotted against concentration, and the equation for the line was used to obtain the IC<sub>50</sub> value. A lower IC<sub>50</sub> value indicates greater antioxidant activity. Tests were carried out in triplicate. Values are presented as means ± SD of three parallel measurements.

## RESULTS AND DISCUSSION

#### Mineral Contents of samples

Minerals are of critical importance in the diet, even though they comprise only 4–6% of the human body (Ozcan 2004). Their excess or deficiency in organs and tissues leads to diseases. It is very important to know the possible influence of metals on pharmacological properties of herbal infusions (Queralt et al., 2005).

Mineral analysis of mint species showed that Ca content ranged from 4396 to 12150 mg kg<sup>-1</sup>, Cd from 0.210 to 0.220 mg kg<sup>-1</sup>, Cr from 1585 to 5410 mg kg<sup>-1</sup>, Cu from 1.76 to 11.52 mg kg<sup>-1</sup>, Fe from 0 to 531.5 mg kg<sup>-1</sup>, Mn from 2.37 to 70.82 mg kg<sup>-1</sup>, Se from 0 to 4.65 mg kg<sup>-1</sup>, and Zn from 0 to 12.64 mg kg<sup>-1</sup> (Table 1). Geographical origin of plants

**Table 1.** Microelement content of two *Mentha* species herbage.

Microelements	<i>M. piperita</i>	<i>M. spicata</i>
Ca	12150	4396
Cd	0.210	0.220
Co	Nd	nd
Cr	5.410	1.585
Cu	11.52	1.757
Fe	531.5	tr
Mg	3602	760.3
Mn	70.82	2.367
P	1102	289.1
Se	Nd	4.654
Zn	12.64	nd

nd: not detected; tr: trace

belonging to the same species can result in different concentrations of elements and their bioavailability, depending on soil features and environmental pollution (Queralt et al., 2005). The World Health Organization cites maximum permissible levels in raw plant materials for cadmium as 0.3 mg kg<sup>-1</sup>, for chromium as 2 mg kg<sup>-1</sup>, and for copper as 20 mg kg<sup>-1</sup> (Queralt et al., 2005; WHO, 2005). This study showed that Cd, Cr, Cu content of mint species were lower than that recommended by World Health Organization (WHO, 2005). Mint tea is widely used as herbal tea; therefore, mineral content of its herbs can meet daily elemental mineral demand of human body when consumed as herbal tea.

#### Essential oil analysis

Essential oils consist of complex chemical mixtures that vary widely in chemical composition. A total of 22 and 34 components, accounting for 95.59 and 99.84% of the total oil, were identified in the *M. piperita* and *M. spicata* essential oils. (+)-Menthol (38.06%), menthol (35.64%), neomenthol (6.73%) and cineole (3.62%) were the main components in the oil of *M. piperita*. In essential oil of *M. spicata*, the main components were carvone (50.33%) and D-Limonene (16.47%) (Table 2).

The chemical composition of *M. piperita* is characterized by the presence of oxygenated monoterpenes such as menthol, menthone, menthyl acetate, sabinene hydrate menthofurone and 1,8 cineole (Agarwal, 2008). Aflatuni (2005) reported that menthol content in *M. piperita* origins ranged from 9.8 to 26.2%. Moreover, menthol content of different peppermint origin varied from 10 to 63% and menthone content from 12 to 76%. The results of menthol content of this study are compatible with those reported by Aflatuni (2005) and European Pharmacopoeia (1997) as 30-55%.

*M. spicata* oil is rich in L-carvone. Major components of oil were determined as D-limonene (16.47%), 4-terpineol (3.78%), L-carvone (50.33) and borneol (3.93%). Major compound of *M. spicata* produced in Greece were beta-pinene (1.2-1.5%), myrcene (2.0-2.9%), 1,8 cineole (5.0-6.8%), dihydrocarvone (5.4-2.1%) and carvone (35.2-49.7%) (Agarwal, 2005).

Many scientific literatures revealed the antimicrobial, antifungal and antioxidant potential of essential oils (Mimica-Dukic et al. 2003; Gulluce et al. 2007; Hussain, 2009). In view of the multiple applications of essential oils, their characterization based on their chemical profiles, is of great importance. Moreover, seasonal variation, especially harvest time, affects also significantly biological activities of oils.

#### Antimicrobial activity

The antimicrobial activities of *M. piperita* and *M. spicata* essential oil against microorganisms were examined by the presence or absence of inhibition zones and zone diameter. As shown in Table 3, the essential oils of *M. piperita* and *M. spicata* had moderate antimicrobial activity (inhibition zone <20–12 mm) when 10, 15 and 20 µl were applied against all bacteria tested (except *P. aeruginosa*) and no antimicrobial activity against *P. aeruginosa*.

**Table 2.** Essential oil components of two *Mentha* species.

Oil Components	RT	<i>M. piperita</i>	<i>M. spicata</i>
Pinene	5.853	0.27	0.89
Camphene	6.308	-	0.10
Sabinen	6.975	0.20	0.81
laevo-beta-Pinene	7.129	0.44	1.47
1-Octen-3-ol	7.261	-	0.27
beta Myrcene	7.474	0.09	0.74
Alfa Phellandrene	7.797	0.10	0.79
Isoterpinolene	8.347	-	0.15
p-Cymene	8.611	0.51	1.22
D-Limonene	8.824	1.30	16.47
Cineole	8.882	3.62	0.07
alfa terpinene	9.719	-	0.29
4-Isopropyl-1-methyl-2-cyclohexen-1-ol trans	12.059	-	0.12
4-Isopropyl-1-methyl-2-cyclohexen-1-ol cis	12.689	-	0.31
D-isomenthone	13.115	0.61	-
Menthofurane	13.313	0.31	-
Isomenthone	13.452	0.27	-
Neo-menthol	13.658	6.73	-
4-Terpinenol	13.995	-	3.78
(+)-Menthol	14.105	38.06	-
Neoisomenthol	14.311	1.08	-
L(-)-Menthol	14.435	0.49	-
Myrtenal	14.501	-	0.52
p-Menth-8-en-2-ol	14.567	-	0.36
p-Mentha-6,8-dien-2-ol	15.998	-	1.12
1-Carvone	16.379	-	50.33
Borneol	17.494	-	3.96
Isomenthol acetate	17.113	3.38	-
Menthol	17.810	35.64	-
Thymol	17.861	0.50	1.47
Carvacrol	18.008	-	2.89
Carveol acetate	19.900	-	3.38
beta Bourbonene	20.634	0.21	1.56
(-)-beta-Elementene	20.832	-	0.84
Cis-Jasmone	21.030	-	0.95
Caryophyllene	21.866	0.48	1.67
(Z)-beta-Farnesene	23.274	-	0.55
beta Cubebene	23.714	-	0.56
Germacrene D	24.213	-	0.49
Spathulenol	25.988	0.11	0.25
Caryophyllene oxide	26.062	0.76	0.56
Viridiflorol	26.223	-	0.41
alfa Cadinol	26.883	-	0.49
<b>Total</b>		<b>95.59</b>	<b>99.84</b>

On the other hand, the essential oils showed strong antimicrobial activity (inhibition zone >20mm) against *C. albicans* when 15, 20 µl were applied and moderate antimicrobial activity in case of 5, 10 µl. The results obtained from disc diffusion method indicated that inhibition zones for *M. spicata* against *C. albicans*, *E. Coli*, *S. pyogenes* and *S. aureus* were 34.3 mm, 19.6 mm, 17.3 mm and 16.0 mm, and for *M. piperita* 26.3 mm, 15.3 mm, 16.3 mm and 15.0 mm, respectively. Table 3 shows that the essential oils of *M. piperita* (34.3 mm inhibition zone) and *M. spicata* (26.3 mm inhibition zone) are most effective against *C. albicans* when 20 µl was applied. The lowest activity was observed for *P. aeruginosa* with the smallest inhibition zones with 20 µl essential oils both of *M. piperita* (8.3 mm) and *M. spicata* (8 mm).

The essential oils of two mint species were lethal to both *S. aureus* and *E. coli*. The findings obtained are consistent with those of Mimica-Dukic et al. (2003). *M. piperita* oil was

**Table 3.** Antimicrobial activity of the essential oil of two *Mentha* species.

Test bacteria	Inhibition zone diameter (mm)							
	<i>Mentha spicata</i>				<i>Mentha piperita</i>			
	5 µl	10 µl	15µl	20µl	5 µl	10 µl	15µl	20µl
<i>S. pyogenes</i>	11±2.6	14±1.0	16.3±2.0	17.3±2.8	10.6±1.5	13.6±2	14.0±1.1	16.3±0.5
<i>S. aureus</i>	9.3±1.5	12.6±0.5	14.6±1.1	16.0±2.0	11.6±1.1	14.3±2.3	13.3±1.5	15.0±1.7
<i>E. coli</i>	11.3±0.5	14.6±2.0	16.0±2.0	19.6±2.6	10.0±1.0	12.0±1.7	14.3±1.1	15.3±2.0
<i>P. aeruginosa</i>	NZ*	NZ	NZ	8.0±0.1	NZ	NZ	NZ	8.3±0.1
<i>C. albicans</i>	13.0±1.4	15.5±2.1	22.5±6.3	34.3±5.1	12.0±0.5	15.0±0.4	23.0±0.1	26.3±1.5

\*No Zone

also reported to be cidal to *C. albicans* at 500 ppm (Tampieri et al., 2005), whereas *M. piperita* antimicrobial activity was recorded only to *S. aureus* and not to *E. coli* (Aridogan et al., 2002).

It is reported that peppermint oil exhibit strong activity against a wide range of human pathogenic Gram-positive and Gram-negative bacteria and fungal strains (Mimica-Dukic et al., 2003; Gulluce et al., 2007; Tassou, et al 1995; Lawrence, 1981). Moreover, these essential oils exhibit notable fungistatic and fungicidal activity, especially on different dermatomycetes and *Candida albicans* (Mimica-Dukic et al., 2003). Menthol has been reported to be responsible for the antimicrobial activity of *M. piperita* (Iscan et al., 2002). However, the antimicrobial activity, like many other biological activities, is closely related to the variability in the chemical composition of the essential oil present in different genotypes, cultivars and wild species of *Mentha* genus (Mimica-Dukic et al., 1998; Lawrence, 1981). In addition, it is well known that the quantitative chemical composition of the essential oils depends on various complex factors, both endogenous and exogenous. These factors are the persistence of chemotypes, influence of geographical and climatic conditions, collection time, plant phenophase, drying

conditions, mode of distillation, etc. (Mimica-Dukic et al., 1998; Malingre and Maarse, 1974).

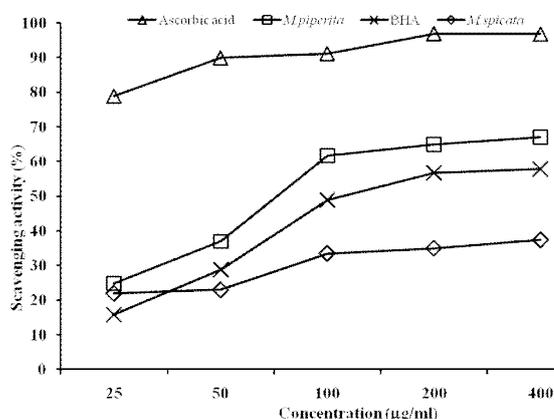
#### Antioxidant activity

The DPPH radical scavenging method was used to evaluate the antioxidant properties of *M. piperita* and *M. spicata* in comparison with those of known natural and synthetic antioxidants, ascorbic acid and BHA. Free radical scavenging capacities of the tested oil rose with increasing oil concentration and oil concentrations providing 50% inhibition (IC<sub>50</sub>) as shown in Table 4 and Figure 1. According to the results obtained from the study, the highest radical scavenging activity was observed in the following order; ascorbic acid > *M. piperita* > BHA > *M. spicata*. The free radical scavenging activity of the two mint species showed that the essential oils of *M. spicata* (IC<sub>50</sub>=77.40 µg/ml) is more effective than those of *M. piperita* (IC<sub>50</sub>=60.41 µg/ml).

**Table 4.** Antioxidant activity of some synthetic antioxidants and essential oil of two *Mentha* species.

Samples	DPPH IC <sub>50</sub> (µg/ml)
Ascorbic Acid	45.43±0.2.84
BHA	68.45±1.04
<i>M. piperita</i>	60.41±0.60
<i>M. spicata</i>	77.40±1.14

Essential oils with higher monoterpene abundance were reported to be almost ineffective. This approach is compatible with the poor performance given by the oils with similar patterns and by single monoterpene hydrocarbons (Ruberto and Baratta, 2000). *M. piperita* oil was reported to reduce DPPH to 50% (Mimica-Dukic et al., 2003). The most powerful scavenging compounds were reported to be monoterpene ketones (menthone and isomenthone) and 1,8-cineole (Mimica-Dukic et al., 2003). The amounts of menthone, isomenthone and 1,8-cineole in *M. piperita* and *M. spicata* used in the present study were 35.64%, 0.27% and 3.62%, and 0%, 0%, 0.07%, respectively (Table 2). Essential oil percentage of menthone, isomenthone and 1,8 cineole of the present study are consistent with those of Mimica-Dukic et al. (2003).



**Figure 1.** DPPH free radical scavenging activity in different concentrations of *M. piperita*, *M. spicata* essential oils, ascorbic acid and BHA.

Nickavar et al. (2008) reported that the *M. spicata* oil exhibited radical scavenging activity with the IC<sub>50</sub> as 87.89 µg/mL. The lower radical scavenging activity of *M. spicata* essential oils might be due to proportion of menthone and isomenthone. Similarly, in the present study, *M. spicata* showed lower scavenging activity (IC<sub>50</sub>= 77.40 µg/ml). Dorman et al. (2003) established that different *Mentha* isolates were capable of scavenging DPPH radical in the following decreasing order; *M. piperita*, *M. dalmatica* and *M. spicata*, respectively. Mata et al. (2007) stated that the ethanolic extract of *M. spicata* showed lower antioxidant activity than that of BHT.

The highest antioxidant properties of essential oils might be related to its phenolic contents like phenolic acids, rosmarinic acid and polyphenols as reported in a previous study (Mimica-Dukic et al., 1998). Therefore, the reason of the poor activity of these essential oils, probably, is due to its lack or low amount of phenolic contents; synergistic or antagonistic effect of its components (Candan et al., 2003). It has earlier been reported that plant phenols can behave as ROS (Reactive Oxygen Species) scavengers, metal chelators and enzyme modulators and prevent lipid peroxidation (Rodrigo and Bosco, 2006).

The results show a difference in the contents of the essential oil of two mint species, out of polyphenol, flavonoides and tannins. *M. piperita* is richest in these compounds and shows stronger antioxidant activity with respect to *M. spicata*. The present study confirmed the antioxidant activity of two mint species, as well.

## CONCLUSION

Mint species are used widely throughout the world as an important medicinal plant. Their oils are one of the most popular and widely used essential oils, mostly because of its main components such as menthol and carvone. Mineral contents of mint herbs are among acceptable limits.

(+)-Menthol (38.06%), neomenthol (6.73%) and cineole (3.62%) were the main components in the oil of *M. piperita*, whereas carvone (50.33%) and D-Limonene (16.47%) in *M. spicata* essential oil. The essential oils of two mint species showed strong antimicrobial activity against *C. albicans*. The free radical scavenging activity of mint species showed that the essential oil of *M. piperita* has higher antioxidant activity with respect to *M. spicata*.

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