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Screening Chemical Composition and Bioactive Properties of *Mentha x piperita* L. Essential Oil and Extract

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ABSTRACT

In recent years, medicinal and aromatic herbs, which contain variety of antimicrobial compounds and have no risk to human health in terms of antibiotic resistance, are increasingly used as alternatives to antibiotics. In the study, chemical composition, total phenolic content, antioxidant and antimicrobial activity of essential oil and extract of *Mentha x piperita* L. growing wild in Eskisehir were investigated. Carvone (55.8%), limonene (12.8%), 1.8-cineole (8.7%) and trans-dihydrocarvone (6.4%) were determined as the main constituents of the essential oil, while the major components of the extract were carvone (56.4%), 1.8-cineole (14.1%) and trans-dihydrocarvone (8.4%). The total phenolic contents of essential oil (2204.33 µg GAE/g) were significantly higher than of extract (744 µg GAE/g). The essential oil and the extract were inhibited 84.08% and 42.59% by the DPPH radical, 91.87% and 48.40% by the ABTS radical, respectively. The highest inhibition zone was observed against *S. aureus* (23 mm). However, minimum inhibition concentration (MIC) values of the essential oil were ranged between 2.5% and 10% (v/v), while MIC values of the extract were determined as 10% and >10% (v/v). The present study demonstrated that *M. piperita* could be used in pharmaceutical and food applications as a natural antioxidant and antimicrobial substance.

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Introduction

In recent years, consuming products with plant components providing natural antimicrobial effects has been increased by the awareness of consumers about healthy nutrition (Tajkarimi et al., 2010; Gyawali and Ibrahim, 2014; Calo et al., 2015; Sevindik et al., 2017). Plants are rich sources of phenolic compounds that have been reported to demonstrate high antioxidant activity (Hinneburg et al., 2006; Uribe et al., 2016; Mohammed et al., 2020). Plants contain natural compounds having antioxidant and antimicrobial effects such as saponins, alkaloids, coumarins, flavonoids, organic acids, phenolics, phytochemicals, terpenoids and thiosulfinates (Cowan, 1999; Tajkarimi et al., 2010; Gyawali and Ibrahim, 2014; Mohammed et al., 2021). The antimicrobial activity of these compounds varies depending on many factors, especially with their chemical structures and concentrations (Lai and Roy, 2004; Tiwari et al., 2009; Hayek et al., 2013; Pehlivan et al., 2021). Most of the current studies on plant origin antimicrobials have been focused on the antimicrobial or antioxidant effects of essential oils, extracts and their compounds (Boskovic et al., 2015; El-Maati et al., 2016; Reyes-Munguía et al., 2016; Rodriguez-Garcia et al., 2016; Zhang et al., 2016; Wafa et al., 2017; Mohammed et al., 2019).

Plant extracts and essential oils, which are effective in the formation of unique flavor and color, have been used in food industry, pharmaceutics, cosmetics and perfumery since ancient times (Hadian et al., 2012; Inan et al., 2012; Shiwakoti et al., 2016). Essential oils and extracts obtained from parts of the plants such as stem, leaf, bud, fruit, flower, and seed have antimicrobial effects on many microorganisms (Tajkarimi et al., 2010; Jayasena and Jo, 2013; Gyawali and Ibrahim, 2014; Calo et al., 2015). Besides, the presence of the hydroxyl groups in the phenolic compounds (caffeic acid, chlorogenic acid, *p*-coumaric acid, ferulic acid-7-*o*-glycoside, gallic acid, rosmarinic acid, etc.) contributes to their antioxidant and

antimicrobial properties (Cueva et al., 2010; Gyawali and Ibrahim, 2014).

Mentha is an important medicinal plant which belongs to Lamiaceae family with approximately 240 genera and 7200 species (Trevisan et al., 2017; Chagas et al., 2020). *Mentha x piperita* L. (peppermint), widely grown in Europe, Asia and America, is a plant commonly used worldwide (Tsai et al., 2013; de Sousa Barros et al., 2015; Uribe et al., 2016). It has been reported that peppermint has active ingredients such as caffeic acid, carotenes, flavonoids, polymerized polyphenols, tocopherols, tannins, betaine and choline (Sokovic et al., 2009). The essential oil of peppermint, which is reported to have antimicrobial activity against various microorganisms, is frequently used in the food, pharmaceutical and cosmetic industries. Besides, Yucel and Yucel (2019) reported that peppermint could be used as natural cleaning agent for the industrial wastes. Its leaf is commonly used as a therapeutic agent for cold, inflammation of the mouth, cramps, diarrhea, flatulence, nausea and vomiting (Trevisan et al., 2017). On the other hand, *M. piperita* has positive effects on human health such as antibacterial, antifungal, antiviral, antidiabetic, antispasmodic, antiulcer, anti-inflammatory, mildly anesthetic, anticancer, antimutagenic, cytoprotective, hypoallergenic, immunomodulatory and hepatoprotective effects (Shah and D'Mello, 2004; Grigoleit and Grigoleit, 2005; Bakkali et al., 2008; Raut and Karuppayil, 2014; Singh et al., 2015).

In the literature, there are many studies examining total phenolic contents, antioxidant and antimicrobial activity of *M. piperita*, grown in different geographical locations (Iscan et al., 2002; Yadegarinia et al., 2006; Kizil et al., 2010; Sujana et al., 2013; Singh et al., 2015; Zaidi and Dahiya, 2015; Okmen et al., 2017; Chagas et al., 2020). However, to the best of the knowledge of authors, there is no study investigating the bioactive properties of *M. piperita* growing wild in Eskisehir, Turkey. Therefore, the objective of the present study was to determine the chemical composition, total phenolic content, antioxidant and antimicrobial properties of the essential oil and the extract of *M. piperita* growing wild in Eskisehir.

Material and Methods

Plant Material

Mentha x piperita L. were gathered from Mihalliccik, Ucbasli Village/Eskisehir in 24.10.2015 (39°47'50.5"N, 31°39'42.8"E). Identification of the plant was performed by Prof. Dr. Ersin YUCEL. A voucher specimen, No. 18706, was deposited at the herbarium of the ANES (Herbarium of Anadolu University, Eskisehir).

The plant was washed under tap and distilled water, followed by drying on filter paper at room temperature. After drying, the samples were grained in a hammer mill. The materials were stored in tight plastic containers until use.

Production of the Essential Oil and the Extract of the Plant Material

For the isolation of essential oil, 100 g of plant material with 2 L of double distilled water was subjected to hydro-distillation for 3 h in a Clevenger-type apparatus (European Pharmacopoeia, 1975). The obtained essential oil was held at 4°C for further analysis.

For obtaining aqueous extract, powdered samples (20 g) were added distilled water (100 L) and followed by shaking in a rotary shaker at 220 × g for 24 h. Then the material was filtered and centrifuged at 5000 × g for 15 min. The obtained aqueous extracts were kept at 4°C for further analysis (Scur et al., 2016).

The Chemical Composition

Quantification of the chemical constituents was determined using Agilent 7890B GC System equipped with an HP innowax column (60 m length x 0.25 mm internal diameter; 0.25 µm film thickness) and flame ionization detector (FID) at Anadolu University, Plant, Drug and Scientific Research Application and Research Center, Eskisehir (USP, 1995). The oven temperature was kept at 60°C for 10 min and then increased from 60°C to 220°C at a rate of 4°C/min, held for 10 min and finally raised to 240°C at a rate of 1°C/min. The injector and detector temperatures were 250°C. Helium was used as a carrier gas at 0.7 mL/min. The sample diluted with hexane (1:10) was injected into the system as 1 µL with 40:1 split mode.

The constituents were identified by GC-MC Agilent 7890B GC 5977B Mass Selective Detector System equipped with a HP innowax column using the same conditions as in the GC analysis. An electron ionization system with an ionization energy of 70 eV was used over a scan range of 35–450 *m/z* for GC-MS detection and the ion source temperature was 230°C. The separated constituents were described by National Institute of Standards and Technology (NIST).

Total Phenolic Content

The total phenolic contents of *M. piperita* essential oil and extract were determined as described by Singleton and Rossi (1965). 2 g of sample was dissolved in hexane (1 mL) and phenolic compounds were extracted with 1 mL water:methanol solution (40:60, v/v) for 2 min. Then, the phases were separated by centrifugation at 3500 rpm for 10 min. The hexane phase was re-extracted in the same way. After that, methanolic extracts were diluted with water to a total volume of 2 mL.

6 mL of ultra-pure water and 500 µL of Folin-Ciocalteu reagent were mixed into a tube, followed by the addition of 100 µL methanolic extracts. Then, the mixture was kept at room temperature in the dark for 8 min. 1.5 mL of 20% (w/v) sodium carbonate solution was added to the reaction mixture and the mixture was held at room temperature in the dark for 1 h. The spectrophotometer (Agilent Technologies, Carry60 UV-Visible) was utilized for determining the absorbance of the solution at 765 nm. Standard gallic acid solution was used to construct the calibration curve and the results were stated in µg gallic acid equivalents (GAE)/g dry weight.

Antioxidant Activity

DPPH Radical Scavenging Activity

DPPH radical scavenging activity of the samples was evaluated according to the method of Naik et al. (2011). 1 mL of diluted methanol solutions of the samples (100 µg/mL) were mixed with 4 mL of a methanol solution of

DPPH (0.1 mM). After keeping the mixture in the dark at room temperature for 30 min, the absorbance was measured at 515 nm by spectrophotometer. Methanol was used as control. DPPH radical scavenging capacity was calculated by the following Eq 1:

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Ac-As}}{\text{Ac}} \times 100 \quad (1)$$

Where;
As is the absorbance of the sample
Ac is the absorbance of the control.

ABTS Radical Scavenging Activity

ABTS radical scavenging activity of the samples was determined as described by Re et al. (1999). $ABTS^+$ stock solution was prepared by mixing ammonium persulfate (2.45mM) with ABTS solution (7mM) and the mixture was kept in the dark at room temperature for 16 h. Then, until the absorbance value was measured by the spectrophotometer as 0.70±0.02 at 734 nm, the mixture was diluted with ethanol. Prepared $ABTS^+$ solution (3 mL) and ethanolic solution of the samples (0.3 mL) were mixed. After keeping in the dark at room temperature for 6 min, the absorbance was measured using the spectrophotometer at 734 nm. Ethanol was used as a blank. ABTS+ radical scavenging capacity was calculated using following Eq 2:

$$\text{ABTS radical scavenging activity (\%)} = \frac{\text{Ac-As}}{\text{Ac}} \times 100 \quad (2)$$

Where;
As is the absorbance of the sample
Ac is the absorbance of $ABTS^+$ solution.

Antimicrobial Activity

Bacterial Strains

The antimicrobial activity of the samples was tested against seven microorganisms, including *Bacillus subtilis* ATCC 6037, *Escherichia coli* ATCC 1103, *E. coli* O157:H7 ATCC 43895, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* Scott A, *Staphylococcus aureus* 6538P and *Salmonella* Typhimurium NRRL-B-4420 that were obtained from the Food Microbiology Research Laboratory, Food Engineering Department, Ege University, Izmir. Firstly, the bacterial strains were cultured on Tryptic Soy Broth (TSB, Oxoid, pH 7.3±0.2) at 37℃ for 24 h. Then the optimized bacterial cultures, equivalent to 0.5 McFarland (DEN-1 McFarland Densitometer, Grant-bio) turbidity standard, were used in the analyses.

Disc Diffusion Method

The preliminary antimicrobial activity of the samples was evaluated by the disc diffusion method described by Deng et al. (2014). Mueller Hinton Agar (MHA, Oxoid, pH 7.3±0.2) plates were supplemented with a bacterial suspension. Paper discs (diameter of 6 mm) impregnated 40 mg/mL of the essential oil or the extract dissolved in dimethyl sulfoxide (DMSO, Merck) were dropped on MHA and the plates were incubated at 37℃ for 24 h. After the incubation period, the diameter of the inhibition zones was determined using ruler. The paper discs impregnated with 10 µg/mL of antibiotics (ampicillin and gentamycin) and sterile water were used as positive and negative controls, respectively.

Broth Dilution Method

The minimum inhibitory concentration (MIC) value of the samples was determined by broth dilution method in 96 well "U" type sterile microplates (Deng et al., 2014). Firstly, 100 µL of Mueller Hinton Broth (MHB) medium was added to each well, followed by 100 µL of essential oil or extract dissolved in DMSO (5%) added to wells of the first row. Then, two-fold serial dilutions were carried out except for 11^{th} and 12^{th} wells and the final concentrations in the wells were; 10%, 5%, 2.5%, 1.25%, 0.625%, 0.313%, 0.156%, 0.078%, 0.039%, 0.020%, respectively. 100 µL of inoculum were added to wells except for 12^{th} well and the plates were incubated at 37℃ for 18 h. 20 µL of 0.5% 2,3,5-triphenyltetrazolium chloride (TTC, Merck) was used as an indicator of the microbial growth. After addition of TTC, the plates were allowed to incubate at 37℃ for 30 min. MIC was defined as the lowest concentration required to inhibit the visible growth of the test culture (no color formation). Minimum bactericidal concentration (MBC) was also defined as the lowest concentration no growth was observed on MHA.

Statistical Analyses

Experiments were performed in three replicates. Experimental data were evaluated by one-way ANOVA using the SPSS software version 20 and the means were separated by Independent-sample T-test at the confidence level of 95% (SPSS, 2011).

Results and Discussion

Chemical Composition of Mentha x piperita

In the present study, thirteen constituents were determined in *M. piperita* extract, which represented about 94.5% of the sample, while fourteen constituents were identified in *M. piperita* essential oil representing 92.3% of the oil (Table 1). The primary constituent of the essential oil of *M. piperita* was carvone (55.8%) and it was followed by limonene (12.8%), 1,8-cineole (8.7%) and trans-dihydrocarvone (6.4%). However, carvone (56.4%), 1,8-cineole (14.1%) and trans-dihydrocarvone (8.4%) were determined as the major compounds of *M. piperita* extract. Similarly, to our findings, carvone (49.27%) and limonene (37.18%) were found as the major components of essential oil of *M. piperita*, grown in Ceará (Brazil), by de Sousa Barros et al. (2015). In another study, the main constituents of *M. piperita* essential oil, collected from Tehran (Iran), were 1,8-cineole (28.03%), carvone (21.04%), pulegone (19.81%) and dihydrocarvone (18.62%) (Peyvandi et al., 2016). As in contrast with our results, the main components of essential oil of *M. piperita*, collected from Barij (Saudi Arabia), Jinan (China) and Amazonas (Brazil), were determined as menthol (36.9%, 30.69% and 33.8%), menthone (28.8%, 14.51% and 15.2%) and menthyl acetate (4.54%, 12.86% and 13.0%), respectively (Mahboubi and Kazempour, 2014; Sun et al., 2014; Chagas et al., 2020). In a study conducted by Verma et al. (2011), the chemical compositions of *M. piperita* and *M. spicata* essential oil, obtained from India, were examined. The main

components of *M. piperita* essential oil were menthol (36.51) and menthone (29.78%) while carvone (52.06%) and limonene (22.32%) were determined as major constituents of essential oil of *M. spicata*. In another study, the major constituents of *M. piperita* essential oil, purchased from a local market in New Delhi (India), were menthol (26.53%) and menthone (25.83%) while, the main component of *M. citrata* essential oil was determined as linalool acetate (26.69%) (Kumar et al., 2012). These studies have demonstrated that the chemical composition of plants might change depending on the plant type and geographical location the plant is grown in.

The Total Phenolic Content and Antioxidant Capacity of Mentha x piperita

Phenolic compounds are significant substances as radical scavengers, metal chelators, reducing agents, hydrogen donors and singlet oxygen quenchers. Due to the positive effects of phenolic compounds on health, the use of medicinal and aromatic herbs rich in phenolic compounds in food and pharmaceutical products has been increased in recent years (Proestos et al., 2006). Therefore, in the present study, the total phenolic contents of peppermint essential oil and extract were demonstrated as 2204.33 and 744 µg GAE/g, respectively (Table 2) ($P<0.05$). The findings of Zaidi and Dahiya (2015), who determined the total phenolic contents of *M. piperita* and *M. spicata* essential oil, grown in Delhi (India), as 12.63 and 9.41 µg GAE/5mg, respectively, were consistent with our results. In another study, the total phenolic contents of methanolic extracts of *M. piperita* (La Serena, Chile), dried using a vacuum oven at five different temperatures (50, 60, 70, 80 and 90°C), were found ranging between 11.56 and 27.12 mg GAE/g, which decreased with the increased drying temperature (Uribe et al., 2016).

The phenolic compounds significantly contribute to the antioxidant capacity of plants. To determine the antioxidant capacity of the plant materials, there are various spectrophotometric methods. In the present study, antioxidant activities of the samples were screened using two different methods. It was determined that, *M. piperita* essential oil and aqueous extract were inhibited 84.08% and 42.59% by the DPPH radical, respectively. Besides, the ABTS radical scavenging activity of essential oil and extract were 91.87% and 48.40%, respectively (Table 2).

As in the total phenolic contents of the samples, the antioxidant capacity of the essential oil was significantly higher than the extract of *M. piperita* ($P<0.05$). In a study performed by Singh et al. (2015), it was observed that essential oil and extracts (petroleum ether, chloroform, ethyl acetate, ethanol and aqueous) of *M. piperita*, grown in Benghazi (Libya), inhibited DPPH radical by 92.6%, 71.3%, 91.8%, 84.9%, 74.8% and 70.3%, respectively. In another study, crude methanolic extracts of *M. piperita*, collected from Sungai Petani (Malaysia), inhibited approximately 60% of the DPPH radical (Pramila et al., 2012). In another study, ABTS radical scavenging assay was used to determine the antioxidant activity of extracts (ethanol, methanol, aqueous) of *M. piperita*, collected from Mugla (Turkey). It was determined that, the extracts inhibited 79%, 26% and 88% of the ABTS radical, respectively (Okmen et al., 2017). All these results showed that the extraction method, solvent type and analyze method used affect the antioxidant activities of plants.

The antioxidant activity of plants is also affected by plant properties such as type, composition and concentration of the plant. In a study conducted by Sun et al. (2014), *M. piperita* essential oil, collected from Jinan (China), at a concentration of 1000 µg/mL inhibited 79.85% of the DPPH radical. In another study, essential oil of *M. piperita*, grown in Tehran (Iran), at a concentration of 200 µg/mL, inhibited 23.46% of the DPPH radical (Yadegarinia et al., 2006). Although the essential oil concentration used in our study (100 µg/mL) was lower compared to the essential oil concentration used in these studies, the antioxidant activities of *M. piperita* essential oil used in these studies were found lower when compared with our results. In another study, IC values (concentration required to scavenge 50% of the DPPH free radical) of essential oils of *M. piperita* and *M. spicata*, grown in Diyarbakir (Turkey), were determined as 60.41 and 77.40 µg/mL, respectively (Kizil et al., 2010).

Antioxidants which retard the progress of many chronic diseases as well as lipid peroxidation, could protect the human body from free radicals and reactive oxygen species effects (Gulcin et al., 2006; Gulcin et al., 2010; Sevindik, 2021). However, synthetic antioxidants that are widely used to protect foods against oxidative spoilage in the food industry, could lead to liver damage and carcinogenesis (Roy et al., 2020). Thus, in recent years, the tendency to consume products containing plant components with natural antimicrobial effects has been increased with revealing the role of foods containing chemical substances in diseases (Tajkarimi et al., 2010; Gyawali and Ibrahim, 2014; Calo et al., 2015). The results of the current study demonstrated that the essential oil and extract of peppermint could be used as an alternative to synthetic oxidants.

Diameter Inhibition Zone of Mentha x piperita

The preliminary antimicrobial activity of essential oil and extract of *M. piperita* were examined against seven bacteria using disc diffusion method. The inhibition effect of *M. piperita* essential oil was higher than extract for all test microorganisms except for *S.* Typhimurium. *M. piperita* essential oil demonstrated inhibitive effect with diameter inhibition zones (DIZ) ranging between 8.5 and 23 mm, depending on test culture used. Besides, the extract of *M. piperita* showed inhibition zones in the range of 6.5 - 11 mm on test bacteria except for *B. subtilis* (Table 3). The highest inhibition effect of *M. piperita* essential oil was observed against *S. aureus* (23 mm).

The antimicrobial activity of peppermint has shown a good correlation with the total phenolic content and antioxidant activity results found in the current study. In a study conducted by Okmen at al. (2017), the inhibition effect of ethanolic, methanolic and aqueous extracts of *M. piperita*, obtained from Mugla (Turkey), was investigated against different *Staphylococcus* spp. using disc diffusion method. It was determined that, the DIZ values of extracts were ranged between 7 and 21 mm depending on the test culture. In another study, the inhibition effects of different concentrations (0.1, 1 and 10 µL) of *M. piperita* L. essential oil, collected from Benghazi (Libya), were investigated against *S. aureus*, *Streptococcus pyogenes*, *E. coli* and *K. pneumonia* by agar diffusion method and the

most sensitive microorganism was found as *S. aureus* for all concentrations used (Singh et al., 2015). In a study performed by Tyagi and Malik (2011), essential oil of *M. piperita* (grown in New Delhi/India), showed inhibitive effect on *E. coli*, *Pseudomonas fluorescens*, *P. aeruginosa*, *B. subtilis* and *Candida albicans* with DIZ values in the range of 13-22 mm. In another study, antibacterial effects of extracts (ethanol, methanol, ethyl acetate, chloroform, hexane and petroleum ether) of *M. piperita*, grown in India, were examined against *B. subtilis*, *Streptococcus pneumonia*, *S. aureus*, *E. coli*, *Proteus vulgaris* and *Klebsiella pneumonia* using agar well diffusion method. DIZ values of the extracts were determined in the range of 2.4-15.3 mm and the highest inhibition zone was achieved by ethyl acetate extract against *B. subtilis* (Sujana et al., 2013). The results of the studies showed that the highest DIZ values against *S. aureus* was obtained from the essential oil of *M. piperita*, grown in Eskisehir. In the previous studies, the effect of chemical components of essential oil and extract of peppermint on cell membrane integrity of bacteria has been reported (Oussalah et al., 2006; Veras et al., 2011). The action mechanism of carvone on microorganisms has been associated with the destabilization of the phospholipid bilayer structure and inhibition of enzymes and proteins (Aggarwal et al., 2002)

Minimum Inhibition Concentration and Minimum Bactericidal Concentration of Mentha x piperita

The MIC values of *M. piperita* essential oil and extract were determined against seven microorganisms using broth dilution method. The MIC values of the essential oil were found ranging between 2.5% and 10% (v/v) (Table 3).

Table 1. Chemical composition of extract and essential oil of *Mentha x piperita* (%)

Constituents	Extract	Essential oil
Isoamyl alcohol	0.6	-
1,8-cineole	14.1	8.7
(Z)-3-hegzen-1-ol	0.5	-
Trans-sabinene hydrate	1.5	-
Terpinene-4-ol	1.1	-
α-pinene	-	0.6
β-pinene	-	1.4
Sabinene	-	0.9
Limonene	-	12.8
Myrcene	-	0.8
β-bourbonene	-	1.5
β-caryophyllene	-	2.0
Trans-dihydrocarvone	8.4	6.4
Cis-isodihydrocarvone	2.2	0.9
δ-terpineol	1.5	-
α-terpineol	3.1	1.1
Neodihydrocarveol	1.6	1.1
Carvone	56.4	55.8
Karyofillen oxide	-	0.5
Trans-carveol	0.7	-
Phenyl ethyl alcohol	0.6	-

Table 2. Total phenolic contents and antioxidant activity of extract and essential oil of *Mentha x piperita*

M. piperita	Total phenolic contents (µg GAE/g)	DPPH (%)	ABTS (%)
Extract	744.00 ± 29.82^{a}	42.59 ± 2.27^{a}	48.40 ± 1.04^{a}
Essential oil	2204.33 ± 95.45^{b}	84.08 ± 0.12^{b}	91.87 ± 0.16^{b}

*Values in the same column with different letters (a, b) are significantly different at P<0.05.

Table 3. Antimicrobial activities of extract and essential oil of *Mentha x piperita* against test microorganisms

Test Bacteria	MIC (v/v, %)		MBC (v/v, %)		Disc diffusion (mm)				
	EX	EO	EX	EO	EX	EO	Amp.	Gen.	SW
B. subtilis	>10	10	>10	10	6 ± 0	14.5 ± 0.5	29 ± 1.0	29.5 ± 0.5	6 ± 0
E. faecalis	10	5	>10	>10	7 ± 1	10 ± 0	21 ± 1.0	14.5 ± 0.5	6 ± 0
L. monocytogenes	10	2.5	>10	>10	8 ± 2	11.5 ± 1.5	28 ± 2.0	28.5 ± 1.5	6 ± 0
S. aureus	>10	10	>10	10	7 ± 1	23 ± 3.0	35.5 ± 0.5	22.5 ± 0.5	6 ± 0
E. coli	>10	5	>10	>10	8.5 ± 2.5	13 ± 3.0	20.5 ± 0.5	21.5 ± 1.5	6 ± 0
E. coli O157:H7	>10	5	>10	>10	6.5 ± 0.5	10.5 ± 0.5	11.5 ± 0.5	18 ± 0	6 ± 0
S. Typhimurium	>10	10	>10	10	11 ± 1	8.5 ± 1.5	18 ± 2.0	20 ± 0	6 ± 0

*EX: Extract; EO: Essential Oil; MIC: Minimum Inhibition Concentration; MBC: Minimum Bactericidal Concentration; Amp.: Ampicillin; Gen.: Gentamycin; SW.: sterile water

However, *M. piperita* extract had lower antimicrobial activity (10% and >10%). The most resistant microorganisms to the essential oil were *B. subtilis*, *S. aureus* and *S.* Typhimurium, while the essential oil demonstrated the highest antibacterial effect on *L. monocytogenes*. On the other hand, essential oil of *M. piperita* showed bactericidal effect at 10% concentration against *B. subtilis*, *S. aureus* and *S.* Typhimurium, while extract of *M. piperita* did not show bactericidal effect at concentrations under 10% against any test microorganisms (Table 3).

In a study conducted by Singh et al. (2015), the antimicrobial activity of *M. piperita* essential oil was examined against *S. aureus*, *S. pyogenes*, *E. coli* and *K. pneumonia*. MIC and MBC values of the essential oil were determined in the range of 0.4%-0.7% (v/v) and 0.6%-0.9% (v/v), respectively. In another study, it was observed that the MIC and MBC values of *M. piperita* essential oil against *Aeromonas* spp. were ranged between 2500 and10000 µg/mL (Chagas et al., 2020). In a study performed by Tyagi and Malik (2011), the antimicrobial effects of *M. piperita* essential oil were investigated against *E. coli* αDH5, *E. coli* ATCC 25922, *P. aeruginosa*, *P. fluorescens*, *B. subtilis*, *S. aureus*, *Penicillium digitatum*, *Aspergillus flavus*, *A. niger*, *Mucor* spp., *Fusarium oxysporum*, *C. albicans* and *Sacchromyces cerevisiae*. MIC and MBC or minimum fungicidal concentration (MFC) values of the essential oil were in the range of 1.13-2.25 mg/mL and 2.25-9 mg/mL, respectively. In another study, ethanol and methanol extracts of *M. piperita* had MIC values ranging between 3250 and 6500 µg/mL against *Staphylococcus* spp., while the aqueous extract did not show antimicrobial effect on test cultures (Okmen et al., 2017). These differences could be derived from geographical environment, plant properties, extraction method, test microorganism and analyze method used.

It is stated that the antimicrobial activity of essential oils and extracts is caused by chemical constituents containing hetero atoms such as oxygen and phenolic compounds (Dorman and Deans, 2000; Delaquis et al., 2002; Cueva et al., 2010; Gyawali and Ibrahim, 2014; Reddy and Al-Rajab, 2016). Various studies have reported the strong antimicrobial activity of carvone which has a hydroxyl group around the phenolic ring (Ceylan and Fung, 2004; Neri et al., 2009; Morcia et al., 2012). These groups disrupt the membrane structure of the cells by interacting with the cell membrane of the microorganism and cause the cellular components to leak out of the cell (Xue et al., 2013; Sengun and Ozturk, 2018).

Conclusion

The present study exhibited that *M. piperita* has significant antioxidant and antimicrobial potentials. It was determined that the main constituent of essential oil and extract was carvone. On the other hand, a significant correlation was observed between the total phenolic contents and antioxidant capacity (DPPH and ABTS). *M. piperita* samples exhibited antimicrobial activity to all test bacteria in various sensitivities, which could be caused by the major components and the total phenolic contents of the essential oil and the extract of *M. piperita* Also, bioactive and antimicrobial properties of the essential oil were higher than of the *M. piperita* extract. All these results showed that essential oil and extract of *M. piperita* could be recommended as antimicrobial and antioxidant agents for the food and pharmaceutical industries. Therefore, further studies should be focused on the use of *M. piperita* essential oil in food applications.

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