

Antibacterial Activities of *Mentha piperita* L. Extracts Against Bacteria Isolated from Soccer Player's Shoes and its Antioxidant Activities

Ahmet Sadan Okmen^{*1}, Gulden Okmen², Ali Arslan², Mustafa Vurkun²

¹Department of Basic Education, Class Teacher Department, Mugla Sitki Kocman University Education Faculty, Kotekli Mugla 48000, TURKEY

²Department of Biology, Mugla Sitki Kocman University Faculty of Science, Kotekli Mugla 48000, TURKEY

ABSTRACT

Objective: Microorganisms have an easier entry into the sportsman's epidermis, and these make diseases on athletes. The main scope of the study is to search the lack of information about the biological activities of *Mentha piperita* extracts against bacteria isolated from soccer player's shoes. **Materials and Methods:** The bacteria were isolated from soccer player's shoes from Balikesir Spor soccer team after the competition. The plant extracts were tested by disc diffusion assay for antibacterial activity. In addition, the different extracts of plant were screened by ABTS decolorization assay for antioxidant activity. **Results:** The highest inhibition zone in bacteria were determined on *Staphylococcus* sp. BFT12 (21 mm). MIC value was determined as 3250 µg/mL. The highest antioxidant activity of the plant was determined from aqueous extract of plant. This scavenging activity is about 88%. **Discussion/Conclusion:** The extracts of the plant have high antibacterial and antioxidant potential.

Keywords: Soccer Player, Bacteria, Medicinal Plant, *Mentha*, Antibacterial Activity, Antioxidant Activity.

INTRODUCTION

Microorganisms have an easier entry into the athlete's epidermis because skin has a suitable ground for bacterial growth.^{1,2} The microorganisms mostly attacks the feet because shoes create a warm, dark, and moist medium for bacterial improvement. *Staphylococcus aureus* is a kind of bacteria widely be found to on the skin.^{3,4} This kind of bacteria is the wide reason of many skin infections amongst athletes. These infections can be diffuse through direct or indirect contact with contaminated individuals. Direct contact with an contaminated individual is almost always the reason for *Staphylococ* infection. Indirect incurring to this infection can hold via handling infected sports equipment or objects.

Skin infections part of %10 percent of time-loss injuries in some sports and can

reason important illness. Skin infections can be diffuse from an athlete to other. Antibiotic-resistant bacteria cause skin infection outbreaks, especially in team sports. Nowadays antibiotic-resistant bacteria constitute a important health threat.^{7,5} Since 2002, wrestling, volleyball and sports teams in most frequent leather infections caused by antibiotic-resistant bacteria, including the football team outbreaks were reported.⁵⁻⁷ The clinical effectiveness of many existing antibiotics are threatened by the emergence of multidrug-resistant pathogens rapidly.⁸ Medical plants are rich sources of agents. These plants used medically in various lands are resource of much potent agents.^{9,79} According to World Health Organization, medicinal plants would be the very important resource to pro-

DOI: 10.5530/ijper.51.3s.5

Correspondence:

Ahmet Sadan Okmen,
Department of Basic
Education, Class Teacher
Department, Mugla Sitki
Kocman University
Education Faculty,
Kotekli Mugla 48000,
TURKEY
Phone no: +90 252 2111469
E-mail: sadanokmen@gmail.
com



www.ijper.org

vide a variety of drugs. Also, these plants should be searched to better understand their features, efficacy and safety.¹⁰ Many plants have been used due to their antimicrobial activities.⁷⁶

Peppermint (*Mentha piperita* L.), an aromatic plant of the *Lamiaceae* family, produces an essential oil rich in menthone (14- 32 %) and menthol (30- 50%).¹¹ *Mentha* is 50–90 cm high. This plant is used for allaying various diseases. The leaves of peppermint are commonly used as tea and flavoring. The plant essential oil is used many industry. It is also reported to possess antimicrobial, antiviral, antioxidant and anti-aging properties.^{11-15,77}

The biological activities of *Mentha* plant extracts against *Staphylococcus* species isolated from athlete's shoes has not been studied. In this study, *Mentha piperita* growing in Turkey was evaluated for antibacterial and antioxidant activities. The present study were aimed to detect the *in vitro* biological activities of different extracts of *Mentha piperita* against *Staphylococci*.

MATERIAL AND METHODS

Organisms

In this study, test bacteria obtained from previous studies by Dr. Ahmet Sadan Okmen, University of Mugla Sıtkı Kocman, TURKEY (Project number: 14/ 052). The extracts were individually tested against bacteria that Gram-positive bacteria isolated from athlete's shoes. Bacterial identities were made by traditionally techniques by Dr. Gulden OKMEN.^{16,17} Six bacteria were used in this study. All of bacteria are Gram-positive cocci. The bacteria were incubated in Mueller- Hinton Broth for 24 h at 37 °C (MHB; Merck).¹⁸

Plant materials

Mentha piperita leaves were obtained in February 2017 from Akhtar in Mugla. The identity of plant was diagnosed by Dr. Olcay Ceylan, University of Mugla Sıtkı Kocman. The voucher specimens were stored at herbarium (Herb no: MUH1254) of Biology Department. The identity of this plant was made according to the Flora of Turkey.¹⁹

Plant extraction

The leaves of *Mentha piperita* were washed 2-3 times with running water and sterile distilled water. These materials were air-dried, and then were pulverized in a agitator. The specimens were stocked at 4°C until required for analysis. Then the samples (50 g) were extracted with solvents (250 mL) using the Soxhlet. In this study was used methanol, ethanol and water as solvent. Extraction process was proceeded for 4 hs. All of extracts were

evaporated. After this the extracts were dissolved in their solvent. These extracts kept in small sterile opac bottles under refrigerator temperature until the experiments.

Antibacterial activity assay

The extract samples were individually tried against Gram-positive bacteria that isolated from athlete's shoes. The plant extracts were tested by disc diffusion method for antibacterial activity.²⁰ The concentration and quantity of plant extracts are 25 µL of 250 mg/mL. Solvents are methanol, ethanol and aqueous. Solvents are negative control. The bacteria were grown on Mueller-Hinton agar plates (MHA, Merck) at 37°C and the cultures set to 0.5 McFarland. The growth of bacteria were provided at 37°C in 24 h. The formed inhibition zones were measured as mm. The antibiotics in study are novobiocin (30 µg) and oxacillin (5 µg), and that were used as positive control.

Determination of minimum inhibitory concentration (MIC)

In this study, the MIC values of leaf extracts were found for antibacterial activity. The broth dilution method was done according to CLSI standards.^{21,22} In our study, final concentrations of extracts were 13000, 6500, 3250, 1625, and 812.5 µg/mL.

Non-enzymatic antioxidant activity assay

These experiments were done by ABTS scavenging activity.²³ The stock solutions contained ABTS•+ solution (7 mM) [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] and potassium persulfate (2.45 mM) solution. These solutions mixed in equal quantities and that allowed to react for 12 h at ambient temperature. The scavenging activities of extracts were made according to Re *et al.*²³ Then the absorbances were taken spectrophotometric method at 734 nm (Shimadzu UV-1201V, Japan). Trolox is reference standard of this study (6-hydroxy-2,5,7,8-tetramethylchroman- 2-carboxylic acid; Sigma). Results obtained from study were given as mM Trolox equivalents (TE)/g dry mass.

RESULTS

The biological activities of plant extracts were made against 6 *Staphylococcus* sp.-BFT isolates *in vitro* conditions. The results obtained from this study are given in Table 1. The highest inhibition zone was determined on *Staphylococcus* sp.-BFT12 for aqueous extract, and this zone is 21 mm. The ethanol extracts were inhibited growth of bacteria and the inhibition zones were between 7- 16 mm. Also antibacterial effects of two extracts used against bacteria absent. These bacteria

Table 1: Antibacterial activities of *Mentha piperita* extracts against bacteria isolated from athlete's shoes

Bacteria	Inhibition zone diameters (mm)							
	Extracts			Antibiotics		Solvents		
	E	M	A	N	O	E (25 µL)	M (25 µL)	A (25 µL)
<i>Staphylococcus</i> sp. BFT8	9	10	(-)	34	17	(-)	(-)	(-)
<i>Staphylococcus</i> sp. BFT9	9	9	14	28	20	(-)	(-)	(-)
<i>Staphylococcus</i> sp. BFT12	16	20	21	34	25	(-)	(-)	(-)
<i>Staphylococcus</i> sp. BFT23	16	14	14	46	26	(-)	(-)	(-)
<i>Staphylococcus</i> sp. BFT28	7	(-)	(-)	36	30	(-)	(-)	(-)

Table 2: Minimum inhibitory concentrations of *Mentha piperita* extracts (µg/mL)

Bacteria	Ethanol	Methanol	Aqueous
<i>Staphylococcus</i> sp. BFT8	3250	6500	(nt)
<i>Staphylococcus</i> sp. BFT9	6500	6500	(-)
<i>Staphylococcus</i> sp. BFT10	3250	6500	(-)
<i>Staphylococcus</i> sp. BFT12	3250	3250	(-)
<i>Staphylococcus</i> sp. BFT23	3250	6500	(-)
<i>Staphylococcus</i> sp. BFT28	3250	(-)	(nt)

(-): no inhibition (nt): not determined

Table 3: Non-enzymatic antioxidant activities of *Mentha piperita* extracts (250 mg/mL)

Ethanol		Methanol		Aqueous	
TE	Scavenging activity (%)	TE	Scavenging activity (%)	TE	Scavenging activity (%)
2.4	79	1.7	26	2.5	88

TE: mM Trolox equivalents (TE)/g dry mass

are resistant to extracts. The lowest inhibition zone was measured as 7mm. Antibiotics and solvents were used as control.

Another biological activity test is MIC, and broth dilution method was used in this test. MIC values belong to plant leaf extracts were given in Table 2. In our study, the extracts were used from 13000 to 812.5 µg/mL for MIC studies. A lot of bacteria have shown the lowest sensitivity to *Mentha piperita* leaf ethanol extract. Minimal inhibitory concentration of this extract was determined as 3250 µg/mL.

The antioxidant capacities of plant leaves were screened by ABTS scavenging activity. The results of ABTS scavenging studies are provided in Table 3. The results were given as percent ABTS, and trolox were used as reference. The aqueous extract of plant leaves shown 88 % ABTS scavenging capacity at 250 mg/mL concentration. The

antioxidant activities of plant extracts were in the order of *Mentha piperita* (aqueous) > *Mentha piperita* (ethanol) > *Mentha piperita* (methanol) (Table 3).

DISCUSSION

Nowadays, antibiotic resistance of pathogens increased and it's undesirable side effects of antibiotics. Researchers were suggested the use of plant extracts as antibiotics or alternatives for the treatment of various infectious diseases. *In vitro* antimicrobial potential of the three extracts from *Mentha* was assessed quantitatively by Kirby- Bauer method.

The highest inhibition zone was found against *Staphylococcus* sp.-BFT12 for *Mentha piperita* aqueous extract. This zone was 21 mm (Table 1). Antimicrobial activities of peppermint oils have also been previously investigated by different groups.²⁴⁻³² The presence of some of the

secondary metabolites reported by the earlier workers from the leaves such as eudesmanoids, isoflavone glycosides and essential oils may be the cause of the antibacterial activity of this plant.^{33,34} It is evident from the literature that the phenols, tannins, terpenoids, flavonoids and flavonoid glycosides are active against a wide range of microbes.³⁵⁻³⁹ Some researchers had posted that both Gram positive bacterial species tested were sensitive to peppermint essential oil with the inhibition zone 17 and 13 mm, respectively.⁴⁰ There is evidence in the literature that Gram-positive bacteria are more sensitive to plant oil and extracts than Gram-negative bacteria.⁴¹⁻⁴³ The effect of components of essential oil on cell membrane integrity of Gram positive and Gram negative bacteria has been previously reported.^{44-45,78} In Gram-positive bacteria, volatile oil and hydrophobic components may contact directly with the phospholipid bilayer of the cell membrane. Some researchers reported that these components cause increase in ion permeability, leakage of vital intracellular constituents or impairment of the bacterial enzyme systems.^{46,47} Foregoing reports also discussed the antibacterial activity of the mint essential oils against *S. aureus*, *E. coli* and *Klebsiella* spp.⁴⁸⁻⁵⁰ *M. piperita* essential oils contained monoterpenes and oxygenated terpenes.⁵¹ Earlier studies report that the presence of active monoterpene constituents, such as *b*-pinene has the membrane damaging effects on microbes.⁵² Mahboubi and Haghi examined the antibacterial effect of *M. spicata* essential oil by broth microdilution and disk diffusion methods and reported that the essential oil had high antibacterial activity against *S. aureus*, and other bacteria.⁵³ The mechanism of antibacterial activity of carvone is not completely understood in great detail. It has been demonstrated that the mechanism of action of carvone on the growth microorganisms includes the destabilization of the phospholipid bilayer structure, interaction with membrane enzymes and proteins, and its act as a proton exchanger reducing the pH gradient across the membrane.⁵⁴ These reports also supports the our results. According to present study, the plant extracts owned antibacterial activity, and showed minimal inhibitory concentration at 3250 µg/mL (Table 2). Iscan *et al.* tested peppermint oil and its components menthol and menthone against 21 human and plant pathogens and found moderate inhibitory activity against the human pathogens.⁵⁵ *Staphylococcus aureus* was inhibited by 0.63 mg/mL of oil. The differences in the biological activities with the related one may be due to different geographical region, age of the plant, various method followed for extraction of oil, plant variety, seasonal conditions etc. Previous studies of *Arbutus pavarini* reported that

methanolic extract of plant demonstrated 20 mm as inhibition zone and the MIC value is 4.86 mg/mL.⁵⁶ Whereas, MIC value was measured as 3250 µg/mL in our study, and it is showed that our MIC results are better than results of other investigators.^{56,57}

Excessive production of free radicals has been caused damage to biological materials, caused physiological and pathological abnormalities, and various diseases.⁵⁸⁻⁶¹ The results obtained from antioxidant activity of plant extracts are summarized in Table 3. *Mentha piperita* aqueous extract disclosed 88 % inhibition at 250 mg/mL (Table 3). Chloroform extract and peppermint oil showed almost equal antioxidant potency (about 90 %).⁴⁰ It is clear from the data that the concentration of 150 ppm of *Mentha piperita* essential oil gave a percentage inhibition of DPPH ($81 \pm 1.2\%$) nearly of the same concentration of vitamin C which was $90 \pm 1.8\%$.⁵¹ The most powerful scavenging compounds were reported to be monoterpene ketones and 1,8-cineole.⁶² The former investigations reported that *M. piperita* oil composition are found menthol and menthone as major compounds.⁶³⁻⁷⁰ The diversity in antioxidant capacities with the reported that one may be attributed to different procedures followed or a different geographical environment, plant type, seasonality, physiological age of the plant, and the method of oil isolation.⁴⁰ The antioxidant capacity of mints greatly depends on the presence of phenolics compounds. The major phenolic constituents of mints especially contained that rosmarinic acid and flavonoids.^{71,72} Researchers were determined both flavonoid content and total phenolic content in this plant.⁷³ Thus, the biological activities of mint species oils are originated from presence of phenolics and alkaloids. A correlative relationship has been reported between the phytochemicals such as tannins and flavanoids and the free radical scavenging activity and antibacterial activity.⁷⁴ Our results are in concert with the results of various researches.

CONCLUSION

In conclusion, medical plants might be considered as precursors for antimicrobial and antioxidants drugs. Our study results suggested that *Mentha piperita* has significant antibacterial activity and it could be many useful in the detection of novel agents of plant origin. In antioxidant activity study, *Mentha piperita* aqueous extracts can be considered good source of natural compounds with significant antioxidant activity. The present study result proved that the *Mentha piperita* can be used as a potential source of antibacterial and antioxidant compounds. Therefore it is essential to research farther by the identification of biologically active compounds,

characterization and purification of the extracts of this plant. *Mentha piperita* leaves could be a possible alternative to chemicals as it can be harnessed as antibacterial, antioxidant and flavouring agent as spice.

ACKNOWLEDGEMENT

I express my thanks to Assoc. Prof. Dr. Ibrahim Erdemir (Balikesir University, School of Physical Education and Sports) and Dr. Olcay Ceylan (University of Mugla Sitki Kocman, Department of Biology).

CONFLICT OF INTEREST

None

ABBREVIATION USED

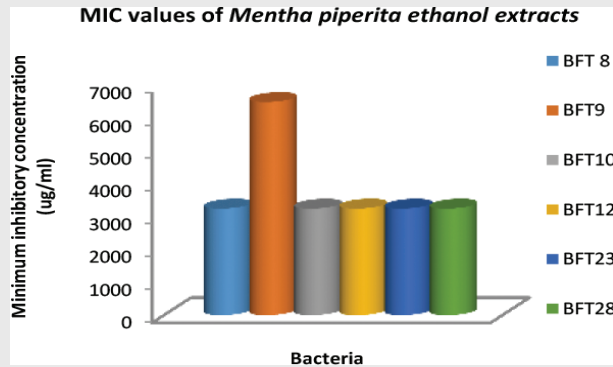
CLSI: Clinical and Laboratory Standards Institute; MIC: Minimum inhibitory concentration; ABTS: 2,2'-azino-bis(3-ethyl benzothiazoline-6-sulfonic acid); TE: Trolox equivalent; MHA: Mueller-Hinton Agar; DPPH: 2,2-diphenyl-1-picrylhydrazyl; h: hour; mg/mL: milligram/milliliters; ug/mL: microgram/milliliters; nm: nanometer; °C: Celsius degree; mm: millimeter.

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PICTORIAL ABSTRACT



SUMMARY

- The highest antibacterial activity was determined at aqueous extract for *Staphylococcus* BFT12. In this study, all of the bacteria were shown sensivity for 3250 µg/mL concentration of this plant. The highest antioxidant activity was shown at aqueous extract. Our results suggest that *Mentha piperita* has significant antibacterial and antioxidant activity and it could be very useful in the discovery of novel antibacterial and antioxidant agents of plant origin

ABOUT AUTHORS



Assist. Prof. Dr. Ahmet Sadan Okmen: He works at the Faculty of Education in Mugla Sitki Kocman University. He is Sports Science Association member. He participated in many meeting and has many publications. He gives many courses at undergraduate and graduate degree. He made Ph D. degree at Ataturk University. He also worked as a reviewer for various national and international journals.



Assoc. Prof. D. Gülten Okmen: She works Mugla Sitki Koçman University in Biology Department. She is member of the European Association of Biotechnology and Biologists Association. She has a lot of scientific meetings and publications. She teaches a lot of curses at the bachelor and graduate degree. She done Ph. D degree at Ankara University, Department of Biology. She is working about Archaeal, Cyanobacterial and Microbial Biotechnology. She also worked as a potential reviewer for various national and international journals.



MSc. Ali Arslan: He is MSc. student in the scope of Microbial Biotechnology at Mugla Sitki Kocman University. He works about Archaea. He has a lot of scientific meetings and article. He is pursuing his MSc. at Mugla Sitki Kocman University.



Msc. Mustafa Vurkun: He is Msc. student at Mugla Sitki Kocman University and works about Microbial Biotechnology. He works about Methicillin resistance *Staphylococcus aureus* (MRSA). He has a lot of scientific meetings and article. He is pursuing his MSc. at Mugla Sitki Kocman University.

Cite this article: Okmen AS, Okmen G, Arslan A, Vurkun M. Antibacterial Activities of *Mentha piperita* L. Extracts Against Bacteria Isolated from Soccer Player's Shoes and its Antioxidant Activities. Indian J of Pharmaceutical Education and Research. 2017;51(3)Suppl:S163-69.