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ANTİBACTERİAL ACTİVİTİES OF *MELİSSA OFFİCİNALİS* L. EXTRACTS AGAİNST VARİOUS *MİCROCOCCUS* SPECİES ISOLATED FROM FOOTBALL PLAYER'S SHOES AND ITS ANTİOXİDANT ACTİVİTİES

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ABSTRACT

Objective / Purpose: The bacteria have an easier entry into the sportsman's epidermis. Particularly Staphylococcus aureus, commonly determined on the skin or in the nose. The purpose of the study is to search the lack of knowledge about the antibacterial effects of Melissa officinalis extracts against bacteria isolated from football player's shoes and its antioxidant effects. Materials and Methods: These bacteria obtained from previous studies by Dr. Ahmet Sadan Okmen. The bacteria were isolated from soccer player's shoes from Balıkesir Spor soccer team after the competition. Additionally, M. officinalis (bract) was obtained commercially from herbalists in Mugla. Antibacterial activities of the extracts were tested against eight bacterial strains. In antibacterial activity studies, the plant extracts were tested by disc diffusion assay. In addition, the plant extracts were studied by DPPH radical scavenging activity. Results: The highest antibacterial activities in bacteria were determined on M. sedentarius BFT28 (18 mm) for M. officinalis. The different extracts possessed antibacterial activity, and showed MIC effect at 3250 µg/mL. The highest antioxidant activity of M. officinalis was determined from methanol extract of plant by DPPH assay. This ratio is about 87 %. Conclusion / Discussion: Different extracts of M. officinalis have antibacterial and antioxidant potential. The extracts from Melissa officinalis can be used for foot health.

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Introduction

The microorganisms most commonly attack feet because shoes create a warm, dark, and moist environment for bacterial growth. *Staphylococcus aureus* is a type of bacteria commonly found on the skin [1, 2]. This type of bacteria is the common cause of many skin infections among athletic populations. These infections can be spread through direct or indirect contact with infected individuals. Direct contact with an infected individual is almost always the cause for *Staphylococ* infection. Indirect exposure to this infection can occur through touching infected objects like towels, sheets, wound dressings, clothes, shoes, workout area, or sports equipment. Skin infections account for up to 10 % of time-loss injuries in some sports and can cause serious illness. Skin infections can be spread from one athlete to another.

Outbreaks of skin infections caused by antibiotic-resistant bacteria have been increasingly reported in sports teams including football, basketball, wrestling, volleyball and rowing teams. Antibiotic-resistant bacteria currently pose a significant health threat. Since the summer of 2002, outbreaks of skin infections caused by antibiotic-resistant bacteria have been reported in sports teams including wrestling, volleyball, and most frequently, football teams [3-5]. The clinical effectiveness of many existing antibiotics is being threatened by rapid emergence of multidrug resistant pathogens [6].

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Medicinal plants are the main sources of natural antimicrobial and antioxidants. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy [7].

The leaves of *Melissa officinalis* have been used in folk medicine especially in Turkey and Iran, for the treatment of some disease [8]. *Melissa officinalis*, otherwise known as lemon balm or balm, is a small perennial herb with a distinctive lemon smell and small white flowers [9]. Lemon balm, member of the family Lamiaceae (formerly Labiatae) is a perennial bushy plant and is upright, reaching a height of about 1 m. The soft, hairy leaves are 2 to 8 cm long and either heart-shaped [10]. Flowers white or pale pink consisting of small clusters of 4 to 12 blossom in the summer [11]. It is native to southern Europe and northern Africa, Caucasus and northern Iran [12], the Eastern Mediterranean region and Western Asia, as well as tropical countries (Brazil) [13].

It was commonly used for its anti-angiogenic [14], antioxidant [15], antimicrobial [16-22], anticancer [23-25], anti-*Herpes* and anti-viral [23-27], anti- tumor, anti- Alzheimer [28,29], anti-diabetic[30], anti-inflammatory [31]. These biological activities have been attributed to the essential oil [32-34] flavonoids and phenolic acids [35, 36] such as rosmarinic acid [37] and caffeic acids [38], phenylpropanoid heteroside [39], and triterpene [40].

The antimicrobial activity of medicinal plants extracts against Gram positive bacteria isolated from football player's shoes has not been studied, that the *in vitro* antimicrobial activity of leaf of *Melissa officinalis* growing in Turkey was evaluated using disc diffusion method. The present study were aimed to determine the *in vitro* antibacterial activities, antioxidant activities of different extracts from *Melissa officinalis* against Gram-positive bacteria.

Material and Methods

Organisms

The extracts were individually tested against Gram-positive bacteria isolated from athlete's shoes. These bacteria obtained from previous studies by Dr. Ahmet Sadan Okmen, Mugla Sitki Kocman University, TURKEY (Project number: 14/052). Bacterial identifications were studied by conventionally methods by Dr. Gulten OKMEN [41,42]. Six bacteria were used in this study. All of bacteria are Gram-positive cocci. The bacteria were grown for 24 hour at 37 °C in Mueller- Hinton Broth (MHB; Merck) [43]. All of bacteria stored in microbiological collection at the Laboratory of Microbial Biotechnology (Faculty of Science, University of Mugla Sitki Kocman).

Plant Materials

Melissa officinalis (bract) was obtained commercially from herbalists on Mugla (in February 2017). The identity was confirmed by Dr. Olcay Ceylan, Department of Biology, Mugla Sitki Kocman University. The voucher specimens were deposited at Herbarium of Department of Biology, Mugla Sitki Kocman University. The identification of these specimens was carried out using the Flora of Turkey [44].

Plant extraction

Melissa officinalis bracts were washed thoroughly 2-3 times with flowing water and once with sterile distilled water. These materials were air-dried, and then the dried materials were pulverized in a blender. All samples were stored at room temperature until initial sample preparation, after which they were stored at 4°C until required for analysis. Then the air dried and powdered samples (50 g) were extracted with methanol, ethanol and aqueous (250 mL) using the Soxhlet apparatus. All the experiments were carried out for 4 hours. All of extracts were evaporated and then the extracts were dissolved in their solvent and then kept in small sterile opac bottles under refrigerated conditions until used.

In vitro Antibacterial activity assay

The extracts were individually tested against Gram-positive bacteria isolated from athlete's shoes. Kirby-Bauer method applied for antibacterial activity. The concentration and quantity of extracts were used as 25 µL of 300 mg/mL. Methanol, ethanol and aqueous were used as solvent in this study. The bacteria were grown on Mueller-Hinton agar plates (MHA, Merck) at 37°C [45]. The cultures adjusted 0.5 McFarland. After incubation, the inhibition zones were measured. Solvents used as negative control. Novobiocin (30 µg) antibiotic used as positive control.

Determination of minimum inhibitory concentration (MIC)

Another antibacterial activity is MIC. The MIC was evaluated on extracts of bracts for antibacterial activity. The MIC was taken as the lowest concentration that inhibits growth after incubation. The broth dilution assay was done as described in the CLSI standards [46, 47]. This test was performed at final concentrations of each extract (13000; 6500; 3250; 1625; and 812.5 µg/mL).

Non- enzymatic antioxidant activity assay

The antioxidant activities were determined using DPPH as a free radical. Extract (0.1 mL) was added to 3.9 mL of a 0.1 mM methanol DPPH solution. After incubation for 30 minutes, absorbance of extract was determined at 515 nm using spectrophotometer. DPPH in methanol was used as control [48]. DPPH radical scavenging activity was determined using the following formula: DPPH radical scavenging activity (%) = [Abs (control) – Abs (extract)] × 100.

Results

The results of antibacterial activities were measured as zone of inhibition in mm for all the materials used as follows. The antibacterial activities of plant extracts were evaluated in vitro against 8 Gram positive bacteria isolates. The antimicrobial activities of the different extracts of the plant studied are shown in Table 1 and compared with standard antibiotic disc.

Table 1: Antibacterial activities of Melissa officinalis extracts against Gram-positive bacteria isolated from football player's shoes

Bacteria	Inhibition zone diameters (mm)						
	Extracts			Antibiotic	Solvents (25 µL)		
	E	M	A	N	E	M	A
M. sedentarius BFT8	13	12	10	34	(-)	(-)	(-)
M. sedentarius BFT9	14	12	10	28	(-)	(-)	(-)
M. luteus BFT10	16	10	9	27	(-)	(-)	(-)
M. varians BFT12	14	14	15	34	(-)	(-)	(-)
M. luteus BFT15	12	13	9	42	(-)	(-)	(-)
M. luteus BFT22	12	14	10	40	(-)	(-)	(-)
M. sedentarius BFT23	(-)	(-)	(-)	46	(-)	(-)	(-)
M. sedentarius BFT28	12	18	9	36	(-)	(-)	(-)

(-): No inhibition; N: Novobiocin (30 µg); E: Ethanol; M: Methanol; A: Aqueous

The highest antibacterial activity was shown on BFT 28 (18 mm) for M. officinalis. Results show that, the all of extracts inhibit the growth of 7 bacteria and the inhibition zones ranged between 9- 18 mm. In addition to, all of extracts did not determine any antibacterial effects against used one bacterium. This bacterium (BFT 23) was found resistant to all of extracts. The lowest activity was found as 9 mm. Novobiocin used as positive control. Solvents used as negative control. Data of antibacterial activities of the extracts are demonstrated in Table 1.

In Table 2, MIC values of different extracts belong to bracts of M. officinalis were summarized. The lowest sensitivity to M. officinalis have shown in bacteria on ethanol extract. This extract of plant possessed antibacterial activity, and showed minimal inhibitory concentration effect at 3250 µg/mL.

Table 2: Minimum inhibitory concentrations of Melissa officinalis extracts (µg/mL)

Bacteria	Ethanol	Methanol	Aqueous
M. sedentarius BFT8	6500	6500	-
M. sedentarius BFT9	6500	6500	-
M. luteus BFT10	6500	6500	-
M. varians BFT12	3250	6500	-
M. luteus BFT15	6500	6500	-
M. luteus BFT22	6500	6500	-
M. sedentarius BFT23	(nt)	(nt)	(nt)
M. sedentarius BFT28	3250	6500	-

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

Table 3 show the per cent of DPPH radical scavenging capacity with trolox as reference. The plant extracts showed 87 % inhibition at 300 mg/mL concentration for methanol solvents. The antioxidant activity by DPPH assay were in the order of M. officinalis (methanol) > M. officinalis (aqueous) > M. officinalis (ethanol) (Table 3).

Table 3: Non-enzymatic antioxidant activities of Melissa officinalis extracts (300 mg/mL)

Ethanol		Methanol		Aqueous	
TE	**Scavenging activity (%)**	**TE**	**Scavenging activity (%)**	**TE**	**Scavenging activity (%)**
1.8	62.3	2.4	87.3	2.0	70.9

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

Discussion

The traditional use of plants as medicines, increasing antibiotic resistance of pathogens and undesirable side effects of antibiotics suggested the use of *Melissa officinalis* extracts as antibiotics or alternatives for the treatment of various infectious diseases. *Melissa officinalis* extracts investigated in the present study exhibited varying degree of inhibitory effect against the selected Gram positive bacteria. In this study, the highest antibacterial activity was shown as 18 mm against *Staphylococcus* sp.-BFT 28 for *Melissa officinalis* methanol extract (Table 1). Our results are in concert with the results of various researches [16, 17, 49-54]. In Gram-positive bacteria, cell wall allows the essential oil and hydrophobic constituents to be in direct contact with the phospholipid bilayer of the cell membrane. Researchers reported that where they bring about their effect, causing either an increase in ion permeability and leakage of vital intracellular constituents, or impairment of the bacterial enzyme systems [55, 56]. These reports also support our results. In some studies, *S. aureus* was found resistant to the different extracts of *Melissa* [7, 57]. The biological activity and medicinal value of plants are usually due to their phytochemical profiles, whose composition is totally dependent on geographical and environmental factors. The extracts of *M. officinalis* have been known to contain a number of antimicrobial compounds. Phytochemical screening of this plant has shown the presence of flavonoids, caffeic acid, rosmarinic acid, vanillic acid, *p*-coumaric acid, phenolic substances and tannin [58, 60, 61]. These differences may be attributed to the genotypic variation, climatic conditions, using different bacteria, different activity assay and different extract concentrations [57].

According to our results, the plant extracts possessed antibacterial activity, and showed minimal inhibitory concentration effect at 3250 µg/mL (Table 2). A lot of researchers tested *Melissa* extracts against different pathogens and found moderate inhibitory activity against the pathogens. *Staphylococcus aureus* was inhibited by 10 mg/mL of extract [62, 63]. Our results are better than these studies. Whereas, our results are in concert with the results of various researches [49, 54]. The differences in the antimicrobial activities with the reported one may be due to different geographical environment, age of the plant, different method followed for isolation of oil, cultivar type, seasonality, etc.

Excessive production of free radicals has been noted to cause damage to biological material leading to several physiological and pathological abnormalities an essential event in the etiopathogenesis of various diseases [64-67]. The results of DPPH scavenging assay of plant extracts are shown in Table 3. *Melissa officinalis* methanol extract showed 87 % inhibition at 300 mg/mL concentration (Table 3). The results show that methanol was the best solvent for extracting the DPPH radical scavenging components from the plant samples. Mencherini, *et al*. [40] demonstrated that the major component of the ethanol extract of *M. officinalis* and rosmarinic acid had free radical scavenging and antimicrobial activities. Herodez, *et al*. [60] with use of HPLC, showed that combination ethanol extraction *Melissa officinalis* include kaempferol methyl ether and three other combinations ursolic acid, rosmarinic acid methyl ester, carnosic acid that strong antioxidant. De Sousa *et al*. [29] performed the study on antitumoral and antioxidant activities of *Melissa* essential oil. *Melissa* essential oil has been shown to have antioxidant properties that increase with dose and it is the mono and sesquiterpenes components that have the strongest antioxidant properties [68, 69]. Also *Melissa* contains caffeic acid and flavonoids which have antioxidant properties [70]. A study on mice has shown rosmarinic acid, contained in *Melissa*, to protect the liver from damage with its antioxidant action [71]. These studies indicate that *Melissa* has a strong antioxidant property. Some researchers reported that DPPH scavenging activities of the plant extracts were found as 80 % [50, 57]. The differences in antioxidant activities with the reported one may be attributed to different procedures followed or a different geographical environment, cultivar type, seasonality, physiological age of the plant, harvesting stages, harvesting hours, drying methods and the method of oil isolation [72, 73]. The antioxidant potential of mints greatly depends on the presence of phenolics.

Conclusion

Melissa is recognised as safe and side effects are very rare and generally mild when they do occur [74]. The results of this study show that the various extracts of *Melissa* can be used as natural sources in the pharmaceutical industry due to their strong antimicrobial and antioxidant activities. Our results suggest that *Melissa officinalis* has significant antibacterial activity and it could be very useful in the discovery of novel antibacterial agents of plant origin. The results in this study using DPPH method to evaluate the antioxidant showed that the *M. officinalis* methanol extracts can be considered good sources of natural compounds with significant antioxidant activity. These results, also, offer a scientific basis for the traditional use of extracts of plant. *M. officinalis* bracts could be a possible alternative to chemicals as it can be harnessed as antibacterial, and antioxidant agent. However, *in vivo* studies are needed to confirm the health-promoting potential of this plant.

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