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ORIGINAL ARTICLE **OPEN ACCESS**

Antibacterial activities of different plant extracts against different *Micrococcus* species isolated from soccer shoes

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ABSTRACT
Athlete shoes encourage the development of microorganisms because they are a warm, dark and humid environment. 10% of sports injuries are caused by skin infections. Skin infections are known to cause serious illnesses. Skin infections caused by antibiotic-resistant bacteria are now reported in team sports. The therapeutic properties of medicinal plants and their antimicrobial effects are known to originate from the secondary metabolites in the structures. In our study, bacterial isolates from athletes' shoes were examined for their morphological and biochemical characteristics and diagnosed. Antibacterial activity studies were performed by disk diffusion method. The minimum inhibitor concentration was determined by broth dilution method. In this study, the highest antibacterial activity was determined by Hypericum perforatum (24 mM) against Micrococcus sedentarius BFT9. Another antibacterial activity study is the minimum inhibitory concentration. The lowest value obtained from this study was 812.5 µg/mL. As a result, secondary metabolites obtained from plants need to be investigated in more detail by in vitro and in vivo studies.
Key Words: Sports shoes, Foot hygiene, Indirect infection, medicinal plant, Antibacterial activity.

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INTRODUCTION

Microorganisms are often found on feet, because they provide a warm, darkness, and wet environment that encourages the growth of microorganisms. *Staphylococcus aureus*, is a genus of Gram positive bacteria found on the skin or in the nose [1,2]. This bacterium is often among athletes and is the main cause of most skin diseases. Staphylococcal diseases can be spread by direct or indirect contact with an infected individual. Indirect exposure to staphylococcal infections can occur through contact with infected objects such as towels, bed sheets, wound dressings, clothes, shoes, exercise areas or sports requisites.

Bacterial infections of skin in athletes can consist as follicules, abscess, boils and cellulites. Today, outbreaks of skin infections induced by multiple antibiotic-resistant bacteria are reported in athletes such as basketball player, soccer player, wrestler, volleyball player and rower. Antibiotic resistant bacteria are still an important health risk. Since 2002, skin infections begin by antibiotic-resistant bacteria have been noted in sports teams [3-5]. Impetigo infects athletes such as wrestlers, footballers and rugby players [6]. *Staphylococcus* and *Streptococcus* may infect the follicule of athlete and cause furunculosis [6-9]. In one study, *S. aureus* was cultured in 22 % of the furuncle of the wrestler [10]. In an epidemiological study, furunculosis developed in 25 % of soccer players and 20 % of basketball players [11]. Pitted keratolysis is a unique bacterial infection, affecting moist and closed-legged athletes [7,12,13]. Several microorganisms, including *Corynebacterium* and *Micrococcus* species [12], may cause the situation.

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections have begun to be investigated in the society, apart from health facilities [14-17]. Nowadays there are researches on football, wrestling and fencing, but the number of studies is lack [10,18-20].

The risk of illness can be greatly reduced, depending on whether the athlete follows proper hygiene practices. Athletes should be informed and trained about the hygiene and personal hygiene of the

sporting goods they use. This lack of information has prompted scientists in recent years to search for new cures in the treatment of these diseases. Medical plants are a rich source of biologically active compounds, and are a very effective and powerful source of medicine [21]. According to the WHO (World Health Organization), medical plants will be a good resource of medicines. For this reason, the characteristics, safety and effectiveness of plants must be well understood and researched [22].

Lavandula L., a member of the *Labiateae* family, is naturally distributed in many parts of the world [23,24]. *L. stoechas* has two subspecies in Anatolia, subsp. *stoechas* and subsp. *cariensis* [25]. *Plantago major* L. plant is a plant of the Plantaginaceae family [26], which can grow in the soil up to 3500 m above sea level [27]. *Arbutus andrachne* L. belongs to the family of Ericaceae, is distributed in the coastal regions of Anatolia and has edible fruits. *Hypericum perforatum* L., a representative of the family Hypericaceae, has been used as a medicinal plant for many years [28]. *Anthemis* L. (Asteraceae) is referred to in Turkey as 81 taxa covering 51 species, 29 of which are endemic to Turkey. *Allium sphaerocephalon* herbaceous, large and annual plant. It lives on sun-exposed rocky slopes, sandy ground, vineyards and dry bush habitats [29]. The *Urtica dioica* plant belongs to the family *Urticaceae* and is a plant that grows at a height of 0 to 1800 m. It is also known for its distraught, thorny nettles, thorny thorns, gooseberry names.

In the literature, it has been found that there is little work to be done on the exposure of athletes to indirect infections [30]. Hygiene is also an important factor in performance and efficiency of the athlete. Considering the money spent on raising the athlete and the transfer fees paid to the athletes, hygiene is an important precaution. For this reason, more detailed researches on hygiene and sports subjects should be done. For this reason, our aim in the study is to make antibacterial activity tests *in vitro* using extracts of various medical plants grown in Turkey. This study will determine the antibacterial effects of different plant extracts against different *Micrococcus* species isolated from athlete shoes and contribute to the lack of information on this subject.

MATERIAL AND METHODS

Sample Collection

Organism

In this study, bacteria were isolated from totally 28 soccer players (swabs from the shoes after rivalry). positive for Gram positive cocci were included. Isolates were gathered from soccer players after game at Balikesir Spor soccer team (U-16 and U-17) in Balikesir, Turkey in 2014 “It was obtained from Dr. Ahmet Sadan Okmen's previous work”. Identification of all bacteria was carried out by Assoc. Prof. Dr. Gulten Okmen (University of Mugla Sitki Kocman).

Plant materials

The plant samples were collected from April-July 2014 in the provinces of Mugla, Kusadasi, Hatay and Hakkari in Turkey. Nine plants were analyzed in our study. The species including; *Hypericum perforatum* L. subsp. *veranese* (Schrant) H. Lindb.; *Plantango major*, *Arbutus andrachne*, *Anthemis chia* L., *Anthemis sp., Lavandula steochas, Crepis sancta, Urtica dioica, Allium sphaerocephalon.* The identity was confirmed by Dr. Olcay CEYLAN, Mugla Sitki Kocman University. The materials were stored at the Herbarium of Department of Biology [25].

Identification of Organism

Pure cultures developed on Nutrient Agar (Merck) for 24h at 37˚C were subjected to biochemical assays for identification. The identification of isolates was carried out by Assoc. Prof. Dr. Gülten Okmen using traditional methods [31-33].

Plant Extraction

Firstly, the plant material has been completely washed with sterile distilled water and 2-3 times flowing water, then fresh plant materials are air dried. The dried plants were then pulverized in a agitator. All of dried plants were stocked at 4˚C and the samples to analysis were prepared at room temperature. Then dried and pulverized samples were extracted with methanol by Soxhlet apparatus and the extracts were all evaporated after 4 hours' duration. The extracts were then dissolved in their own dissolver and stored in 25 mL sterile opaque bottles at the refrigerator temperature until further use.

In vitro antibacterial activity tests

The selection of the bacteria used in the study was made randomly and It was 8. The extracts obtained from medical plants have been tested against bacteria. Antibacterial activity tests were performed by Kirby-Bauer method. Methanol extracts were used for extractions. Bacteria were inoculated on Mueller-Hinton agar plaque (MHA, Merck) and they were incubated at 37˚C [34]. All bacterial broth cultures were set to 0.5 McFarland and used as active cultures in experiments. The experiments will be carried out in three parallel form. After incubation, the inhibition zones formed are recorded in mm. In our experiments, methanol was used as a negative control and antibiotics were used as positive controls. These antibiotics are oxacillin (5μg), vancomycin (30μg), and erythromycin (15μg).

Detection of Minimum Inhibitory Concentration (MIC)

MIC test was applied to plant extracts as other antibacterial activity test. These tests were performed with broth dilution experiments as explained in CLSI standards [35,36]. These tests were accomplished on each extract. The final concentrations of extracts are 6500, 3250, 1625, 812.5, and 406.25 µg/mL.

RESULTS

Bacteria used in the study are 8, all Gram positive and cell morphology is coccus. In addition, all of the isolates have catalase enzyme and mannitol and glucose fermentation are negative. Biochemical properties of isolates are given in Table 1. Isolated bacteria; *Micrococcus sedentarius* BFT8, *Micrococcus sedentarius* BFT9, *Micrococcus luteus* BFT10, *Micrococcus varius* BFT12, *Micrococcus luteus* BFT15, *Micrococcus luteus* BFT22, *Micrococcus sedentarius* BFT23 and *Micrococcus sedentarius* BFT28.

Table 1. Morphological and biochemical characteristics of bacteria isolated from athletic shoes.

Bacteria	Gram Reaction	Colony morphology	Cell Morphology	Catalase activity	Mannitol fermentation	Yellow pigment presence	Oxidase activity	Simons' citrate	Glucose fermentation
M. sedentarius BFT8	(+)	Yellow/Round/ Flat/Domed	Cocci	+	-	+	-	-	-
M. sedentarius BFT9	(+)	Yellow/Round/ Flat/Domed	Cocci	+	-	+	-	-	-
M. luteus BFT10	(+)	Yellow/Round/ Flat/Domed	Cocci	+	-	+	+	-	-
M. varians BFT12	(+)	Yellow/Round/ Rough /Domed	Cocci	+	-	+	-	-	+
M. luteus BFT15	(+)	Light Yellow/Round/ Flat/Domed	Cocci	+	-	+	+	-	-
M. luteus BFT22	(+)	Yellow/Round/ Flat/Domed	Cocci	+	-	+	+	-	-
M. sedentarius BFT23	(+)	Yellow/Round/ Flat/Convex	Cocci	+	-	+	-	-	-
M. sedentarius BFT28	(+)	Yellow/Round/ Flat/Domed	Cocci	+	-	+	-	-	-

When the data obtained from this study were taken in consideration, the results of antibacterial activity were recorded as inhibition zone (mm). The antibacterial activity results are summarized in Table 2.

Table 2. Antibacterial activities of different plant extracts against different *Micrococcus* sp. isolated from athletic shoes.

	Inhibition zone diameter (mm)																
	Plant extracts (100 mg/mL)													Antibiotics			Negative control
Bacteria	HP (f)	PM (l)	UD (l)	AA (l)	AA (f)	As (f)	As (l)	AS (f)	AS (r)	AC (l)	CS (l)	LS (l)	LS (f)	O	V	E	M
M. sedentarius BFT8	20	-	-	-	11	-	-	-	-	-	-	10	11	17	25	32	-
M. sedentarius BFT9	24	-	-	-	10	-	-	-	-	-	10	10	-	20	28	40	-
M. luteus BFT10	20	-	-	-	-	-	-	-	-	-	-	-	-	21	19	42	-
M. varians BFT12	21	-	-	-	16	-	-	-	-	-	-	-	-	25	24	13	-
M. luteus BFT15	18	-	-	-	-	-	-	-	-	-	-	-	-	19	25	15	-
M. luteus BFT22	20	-	-	-	-	-	-	-	-	-	-	-	-	26	30	40	-
M. sedentarius BFT23	21	15	-	-	-	-	-	-	-	-	-	-	-	26	27	13	-
M. sedentarius BFT28	22	-	-	-	10	-	-	-	-	-	-	10	10	30	37	27	-

HP: *Hypericum perforatum* L. subsp. *veranese* (Schrant) H. Lindb.; PM: *Plantago major*; UD: *Urtica dioica*; AA: *Arbutus andrachne*; As: *Anthemis* sp.; AS: *Allium sphaerocephalon;* AC*: Anthemis chia* L.; CS: *Crepis sancta L.;* LS*: Lavandula stoechas;* f: flower; l: leaf; r: root; (-): No Inhibition Zone; O: Oxacillin (5 µg); V: Vancomycin (30 µg); E: Erythromycin (15µg); M: Methanol (25µL)

The results showed that the methanol extracts inhibit the bacterial growth and the inhibition zones are between 10 and 24 mm. Additionally, the 5 plants have not antibacterial activity. Bacteria were found to be resistant to these extracts. The lowest inhibition zone in this study was found as 10 mm. The antibiotics and methanol were used as control. *Hypericum perforatum* flower extracts showed

antibacterial activity against all bacterial species in the research. Leaf extracts of *Plantago major* plant showed activity against 1 isolate, while leaf extracts of *Urtica dioica* and *Arbutus andrachne* showed no bactericide activity. *Arbutus andrachne* flower extracts have different antibacterial activities on 4 bacteria. It has been determined that *Anthemis* sp., *Allium sphaerocephalon* and *Anthemis chia* extracts do not exhibit antibacterial activity against any bacteria. *Crepis sancta* and *Lavandula steochas* plant extracts showed antibacterial activity similar to bacteria (Table 2).

In this part of our study, broth dilution method was also used to determine the minimum inhibitory concentration as antibacterial activity. In Table 3, the MICs of extracts are summarized. The MIC values of the plant extracts were tested to a concentration from 6500 µg/mL to 406 µg/mL. As a result of the studies, the lowest minimum inhibitory concentration was obtained from different plants as 812,5µg/mL. These are *Hypericum perforatum* (flower), *Arbutus andrachne* (flower), *Crepis sancta* (leaf) and *Lavandula stoechas* (leaf) (Table 3).

Table 3. Minimum inhibitory concentrations of different plant extracts against different *Micrococcus* sp. isolated from athletic shoes.

	Plant Extracts (µg/mL)												
Bacteria	**HP (f)**	**PM (l)**	**UD (l)**	**AA (l)**	**AA (f)**	**As (f)**	**As (l)**	**AS (f)**	**AS (r)**	**AC (l)**	**CS (l)**	**LS (l)**	**LS (f)**
M. sedentarius BFT8	1625	NT	NT	NT	1625	NT	NT	NT	NT	NT	NT	812,5	1625
M. sedentarius BFT9	812,5	NT	NT	NT	1625	NT	NT	NT	NT	NT	812,5	1625	NT
M. luteus BFT10	1625	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
M. varians BFT12	1625	NT	NT	NT	1625	NT	NT	NT	NT	NT	NT	NT	NT
M. luteus BFT15	812,5	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
M. luteus BFT22	812,5	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
M. sedentarius BFT23	1625	1625	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
M. sedentarius BFT28	1625	NT	NT	NT	812,5	NT	NT	NT	NT	NT	NT	1625	3250

HP: *Hypericum perforatum* L. subsp. *veranese* (Schrant) H. Lindb.; PM: *Plantango major*; UD: *Urtica dioica*; AA: *Arbutus andrachne*; As: *Anthemis* sp.*;* AS: *Allium sphaerocephalon;* AC*: Anthemis chia* L.; CS: *Crepis sancta L.;* LS*: Lavandula stoechas;* f: flower; l: leaf; r: root; NT= Not Tested

DISCUSSION

The number of plants used for therapeutic purposes worldwide is around 21,000. In our country, only about 500 of 9000 plant species are used for therapeutic purposes [37]. Plant oils and extracts have been used for different purposes for hundreds of years [38]. Biologically active compounds have proven to be abundant sources, many of compounds are used to develop new pharmaceutical drugs [39]. This study confirms that different organs of many plants have antibacterial activities.

As a result of this study, it was found that only one plant extract exhibited antibacterial activity against all of Gram-positive bacteria, *Hypericum perforatum* L. The extracts of this plant were found to be effective in the 18-24 mm inhibition zone interval against test organisms (Table 2). *Hypericum* extract has been reported to exhibit bactericidal activity against a number of bacterial strains, including *Staphylococcus aureus*, *Proteus vulgaris*, *Escherichia coli* and *Pseudomonas aeruginosa* [40]. Keles *et a*l., [41] has been reported that *H. perforatum* extract has a wide range of inhibitor activity. The *H. perforatum* extract has been reported to exhibit an inhibitory effect of 14-16 mm against *Staphylococcus aureus* and *Streptococcus agalactiae*, respectively. These results also support the results obtained from our study. Ranccic *et al*. [42] reported that *H. perforatum* extract had a 5mm inhibition zone against *S. aureus*, our results are better than this study in the literature. This difference between studies can be attributed to the phytochemical differences between plant species, the antibacterial effects of plant groups being different. In addition, the cell wall of Gram-positive bacteria permits the passage of volatile oils and hydrophobic substances since it is in directly contact with the phospholipid bilayer of the membrane. This study confirms that above-ground organs of plants have antibacterial activity.

As a result of our study, the extracts of flowers and roots of *Allium sphaerocephalon* plant were found to have no antibacterial activity against any test bacteria (Table 2). In Redzi'c [43] research, he used disk diffusion method for *in vitro* antimicrobial activity and he has screened fresh leaf extracts against five bacteria and two fungal strains, and found a weak bactericidal effect. Our results did not show the presence of antibacterial activity. These results support our work. Abuhamdah *et al*., [44] reported that *A. andrachne* extract did not inhibit 3 test bacteria. This result is supported to our studies (Table 2). Antibacterial activity studies with *U. diocia* revealed no antibacterial activity against bacterium (Table 2).

Ertürk and Demirkol [45] reported that *U. diocia* have not antibacterial activity against *S. aureus*. This supports our results.

As a result of our studies, the lowest minimum inhibitory concentration was obtained as 812.5 µg/mL of different plant extracts (Table 3). Dordevic *et* al. [46] reported a minimum inhibitory concentration, the value is 3.13 mg/mL for *H. annulatum* and *H. elegans* essential oils against *S. aureus*. Another study reported that the volatile oil isolated from the aerial parts of *H. rumeliacum* exhibited moderate activity against all tested bacteria, while the MIC value was between 3.80-17.20 mg/mL [47]. The MIC value of *Arbutus andrachne* was found to be 812.5 µg/mL in our study (Table 3). In a study with *Arbutus pavarii*, the minimum inhibitor concentration was reported as a higher value (4.86 mg/mL) [48]. These outcomes are better than the results of Alsabri *et al*. [48]. According to the study results of Aligiannis *et a*l. [49] for classification of plant materials on MIC results, all plant extracts showing the lowest MIC value of 812.5 µg/mL which we find are considered as weak inhibitors against test pathogens.

CONCLUSION

As a result, most of the plants used in the study showed antibacterial activity against test bacteria. This study is thought to be useful for the find of new drugs of plant origin. *In vitro* and *in vivo* studies should be investigated in more detail to ensure that the plants have the best antibacterial activity in their search for new drugs. In addition, the investigation of the chemical composition of plant extracts with activity, and all fractions should be tested in laboratory conditions to do multidirectional research is useful.

CONFLICT OF INTEREST: The authors declare that there is no conflict of interest.

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