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ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF DIFFERENT PLANTS EXTRACTS AGAINST *STAPHYLOCOCCUS AUREUS* ISOLATED FROM SOCCER PLAYER'S SHOES AND KNOWLEDGE AND APPLICATIONS ABOUT FOOT HYGIENE OF THE SOCCER PLAYERS

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Abstract

Background: Microorganisms most commonly attack the feet. Bacteria have an easier entry into the athlete's epidermis. *Staphylococcus aureus*, commonly found on the skin or in the nose. The purpose of the study was to research the lack of knowledge on the antibacterial and antioxidant effects of different plants extracts and to report existing knowledge on hygiene of sports equipment and that of soccer players.
Materials and Methods: The bacteria were isolated from soccer player's shoes (n=28) from Balıkesir Spor soccer team after the competition. Additionally, ten plants were collected from Mugla, Hatay, and Hakkari in Turkey. In antibacterial activity studies, the plant materials were tested by disc diffusion assay. Furthermore, the different plants extracts were studied by ABTS decolourization assay.
Results: The highest antibacterial activity was shown on *S. aureus*- BFT2 (22 mm) for *Hypericum perforatum* L. subsp. *veranese* (Schrant) H. *Lindb*. The different extracts possessed antibacterial activity, and showed MIC effect at 812.5 µg/mL. The antioxidant activities by ABTS assay were in the order of *Arbutus andrachne* (flower) > *Arbutus andrachne* (leaf) > *Anthemis* sp. (flower) > *Crepis sancta L.* (leaf) > *Allium sphaerocephalon* (root) > *Plantago major* (leaf) > *Lavandula steochas* (flower) > *Anthemis* sp. (flower) > *Urtica dioica* (leaf) > *Lavandula steochas* (leaf) > *Anthemis* sp. (leaf) > *Hypericum perforatum* L. subsp. *veranese* (Schrant) H. *Lindb* (flower) > *Anthemis chia* L. (leaf).
Conclusion: Different plant extracts have antimicrobial and antioxidant potential.

Key words: Soccer player, hygiene, *Staphylococcus aureus*, medicinal plant, antibacterial, antioxidant

Introduction

Sports medicine topics tend to focus on physiological (cardiovascular, neural, muscular etc.,) problems of athletes, yet the most common sports injuries are dermatologic in nature (Adams, 2006). This article will highlight bacterial infections common to shoes of soccer players. Bacteria have an easier entry into the athlete's epidermis due to sweat saturation (which causes super saturation and vulnerability of the stratum corneum), skin trauma/abrasions, and occluding athletic gear that can provide a warm, moist environment for bacterial growth (Adams, 2001; Adams, 2006).

The microorganisms most commonly attacks the feet because shoes create a warm, dark, and moist environment for bacterial growth. *Staphylococcus aureus*, often referred "*staph*", is a type of bacteria commonly found on the skin or in the nose of healthy people (Powell, 1994; Adams, 2002). Approximately 30% of people have *staph* in their noses and do not have any symptoms (Kluytmans *et al.*, 1997). This type of bacteria is the common cause of many skin infections among athletic populations. *Staph* infections can be spread through direct or indirect contact with infected individuals, but *staph* infections are never spread through the air. Direct contact with an infected individual is almost always the cause for *staph* infection. Indirect exposure to *staph* 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. Bacterial infections of the skin in soccer players can occur as abscesses, furuncles, folliculitis, and cellulitis. 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 (CDC, 1962; Decker *et al.*, 1986; Nguyen *et al.*, 2005).

MRSA which stands or Methicillin-Resistant *Staphylococcus aureus* is *staph* that is resistant to commonly used antibiotics such as penicillins and currently available cephalosporins. In the early 1960s, an antibiotic-resistant strain of S. *aureus* known as MRSA was described (Barrett *et al.*, 1968; Rihn *et al.*, 2005a). Methicillin-resistant S. *aureus* has acquired the mecA gene (Ma *et al.*, 2002; Naimi *et al.*, 2003), and is resistant to β-lactam antibiotics, including penicillins and cephalosporins (Fridkin *et al.*, 2005; Crawford *et al.*, 2007; Daum, 2007) although resistance to other classes of antibiotics, such as fluoroquinolones and tetracyclines, is increasing (Frazee *et al.*, 2005; Fridkin *et al.*, 2005; Gorwitz *et al.*, 2006) Until recently, MRSA was thought to be exclusively a hospital-acquired infection (Fridkin *et al.*, 2005; Turbeville *et al.*, 2006; Daum, 2007). In the mid- to late 1990s, however, MRSA infections started to be detected in the community outside the typical health care settings (Naimi *et al.*, 2003 Turbeville *et al.*, 2006; Daum, 2007; Klevens *et al.*, 2007), being diagnosed in soccer players participating in football (CDC, 2003; Begier *et al.*, 2004; Rihn *et al.*, 2005b), wrestling, (Lindenmayer *et al.*, 1998) and fencing (CDC, 2003;), where as many as 70% of team members required hospitalization and intravenous antibiotic therapy (CDC, 2003; Rihn *et al.*, 2005a). When a MRSA infection occurs in the community it is called community-associated MRSA, or CA-MRSA. Infections caused by CA-MRSA appear to be more common than those caused by *Staph* in the past, particularly in amateur and professional athletic teams.

If proper hygienic practices are followed, this risk can be greatly reduced. There are different steps that soccer players can take to help prevent MRSA. Athletes can be educated and informed about hygiene and hygiene of sports equipments used in training and competition. However we know that hygiene comes from family and goes on the school. Therefore, the education of hygiene should start at kindergarten, elementary and secondary school. Because of the lack of hygenie, scientists have turned within quest for new approaches to the treatment of these infections recently.

Medicinal plants represent a rich source of are antimicrobial agents. These plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava *et al.*, 1996). According to World Health Organization, medicinal plants would be the best

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source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Nascimento *et al.*, 2000). Many plants have been used due to their antimicrobial traits.

The antimicrobial activity of medicinal plants extracts against *Staphylococcus aureus* isolated from athlete's shoes has not been studied, before that the *in vitro* antimicrobial activity of various parts of different plants growing in Turkey was evaluated using disc diffusion method. Additionally, antioxidant activities of medicinal plants extracts have not been reported. This work attempts to contribute to this lack of knowledge about the antimicrobial and antioxidant effects of different plants extracts and to search the knowledge about hygiene and hygiene of sports equipment that is used in the competition by athletes, of soccer players and what they do about the hygiene.

Material and Methods

Questionnaire

The study included 28 male professional (division III) soccer players (age: 16.86±0.86 years). All of the participants were fully informed of the goals and methodology of the test and provided signed consent. The participants agreed with the testing process and the use of the data for further research. Prior to participation in the study, the players were interviewed about their medical records and completed an questionnaire about hygiene and sports equipment.

Sample Collection

Organisms

A total of 28 soccer players (swabs from the shoes after competition) positive for Gram positive cocci were included. Specimens were collected from soccer players after competition at Balikesir Spor soccer team (U-16 and U-17) in Balıkesir, Turkey in 2014. Specimens were collected aseptically, transported immediately to the Microbial Biotechnology Laboratory of the Department of Biology, Mugla Sitki Kocman University, Turkey, the tests were performed. They were stored at 4°C and analysed within 24 hours.

Plant materials

The plants were collected in May between July 2013 from Mugla, Hatay and Hakkari in Turkey. Ten plants were used in this study. These species including; *Hypericum perforatum* L. subsp. *veranese* (Schrant) H. Lindb., *Plantago major*, *Urtica dioica*, *Arbutus andrachne*, *Anthemis sp.*, *Allium sphaerocephalon*, *Anthemis chia* L., *Crepis sancta* L., and *Lavandula steochas.* The identity was confirmed by Dr. Olcay CEYLAN, Department of Biology, Mugla Sitki Kocman University. The plant materials were deposited at the Herbarium of Department of Biology, Mugla Sitki Kocman University. The identification of these specimens was carried out using the Flora of Turkey (Davis, 1975).

Isolation of Organisms

The spread-plate method was used for isolating pure cultures. The organisms were isolated from soccer player's shoes in Balikesir Spor (U-16 and U17) soccer team. Samples (28 swabs) were aseptically collected and spread on agar plates using a drigalski spatula. The plates were incubated at 37°C for 24 hours. The species include; six *S. aureus*. The bacteria were grown for 24h at 37°C in Mueller- Hinton Broth (Merck).

Identification of Isolated Organisms using Conventional Tests

Isolates were incubated at 37°C for 18- 24 hours on Mueller- Hinton Broth (Merck). Bacterial identifications were studied by conventionally methods by Dr. Gulten OKMEN. The identification of microorganisms was based on such tests as: Gram reaction, colonial morphology, cell morphology, and biochemical tests. Gram staining was carried out on presumptive isolates. Single colonies of Gram positive cocci were then tested with catalase test, coagulase test, pigment production, and growth on Mannitol salt agar (MSA). Sequel testing of the isolates was further performed beginning with MSA, followed by finally Tube Coagulase Test, to evaluate the performance of individual tests. Results were confirmed using manual for determinative bacteriology (Cowan and Steel, 1965; Monica, 1991).

Enumeration of Total Bacteria Number

Plate count method was used to estimate the total number of bacteria on a solid medium containing Plate Count Agar. Total aerobic bacterial counts of swabs were determined by the incubation of all the inoculated plates at 37°C, and colonies were counted using colony counter (Funke GERBER) at 24 h after inoculation (Atlas, 2004).

Determination of Bacterial Flora

In this study, samples were taken from 28 athlete's shoes from Balikesir Spor soccer team (U-16 and U-17), TURKEY. Bacteria were isolated from these samples. To identify purified isolates to genus or species level, basic tests, namely Gram's stain, morphology, catalase, pigment production, and growth on MSA, were performed following the criteria described in the *Bergey's Manual of Systematic Bacteriology* (Holt *et al.*, 1994).

Plant extraction

The plant materials were washed thoroughly 2-3 times with running water and once with sterile distilled water. Fresh plant materials were air-dried, and then the dried materials were powdered in a blender. All samples were stored at ambient temperature until initial sample preparation, after which they were stored at 4°C until required for analysis. The air dried and powdered samples were extracted with methanol

(0.1g / mL) using the Soxhlet apparatus. All experiments were continued for 4 hours. All of extracts were evaporated for and then the extracts were dissolved in their solvent and then kept in small sterile opac bottles under refrigerated conditions until used.

Antibacterial activity assay

The extracts of plants were individually tested against *Staphylococcus aureus.* Kirby-Bauer method applied for antibacterial activity. The plant materials were tested by disc diffusion assay. The concentration and quantity of extracts were used as 40 µL of 100 mg/mL. Methanol was used in this study. The bacteria were maintained on Mueller-Hinton agar plates (MHA, Merck) at 37°C (Bauer *et al.,* 1966). The cultures adjusted 0.5 McFarland. The experiments were performed in triplicate. Bacteria were incubated at 37°C in 24 hr. After incubation, the inhibition zones formed and then the values of zone were measured. Methanol used as negative control. Oxacillin (5 µg), Vancomycin (30 µg), and Erythromycin (15 µg) antibiotics used as positive control.

Determination of minimum inhibitory concentration (MIC)

The MIC was evaluated on plant extracts as antimicrobial activity. The MIC was taken as the lowest concentration that inhibits growth after incubation. The broth dilution assay was performed as described in the CLSI standards (CLSI, 2003; CLSI, 2006). This test was performed at final concentrations of each extract (6500; 3250; 1625; 812.5; and 406.25 µg/mL).

***In vitro* Antioxidant Activity**

The experiments were carried out using an improved ABTS decolourisation assay (Re *et al.*, 1999). The stock solutions included 7 mM $ABTS^{\bullet+}$ [2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)] solution and 2.45 mM potassium per sulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 hr at room temperature in the dark. The solution was then diluted by mixing 1mL $ABTS^{\bullet+}$ solution with 10 µL methanol. Absorbance was measured 15 min after the initial mixing of 10 µL of the methanolic extracts with 1 mL of $ABTS^{\bullet+}$ solution. Then the absorbance was taken at 734 nm using the spectrophotometer (Shimadzu UV–1201V, Japan). Trolox (6-hydroxy-2,5,7,8-tetramethychroman- 2-carboxylic acid; Sigma Chemical Co. St. Louis, MO, USA) was used as a reference standard. Results are expressed in mM Trolox equivalents (TE)/g dry mass. The scavenging capability of $ABTS^{\bullet+}$ radical was calculated using the following equation:

$$ABTS\ scavenging\ effect\ (\%) = (1- A_1/A_0) \times 100$$

where A_0 is the initial concentration of the $ABTS^{\bullet+}$ radical cation (s) and A_1 is absorbance of the remaining concentration of $ABTS^{\bullet+}$ radical cation (s) in the presence of the extract.

Table 1: Properties of yellow bacteria isolated from athlete's shoes

Isolates	Bacteria frequency (%)	Catalase activity	Mannitol fermentation	Yellow pigment production	Numbers of total bacteria in shoes
Gram (+) cocci	69	+	-	+	
Gram (-) cocci	23	+	-	+	
Gram (+) bacil	2	NA	-	+	1140
Gram (-) bacil	6	NA	-	+	
S. *aureus*	11	+	+	+	

(-): Not fermented (+): Fermented NA: No activity

Table 4: Trolox equivalents of antioxidant activities of different plants extracts

Plants extracts (100mg/mL)	HP (flower)	PM (leaf)	UD (leaf)	AA (leaf)	AA (flower)	As (flower)	As (leaf)	AS (flower)	AS (root)	AC (leaf)	CS (leaf)	LS (leaf)	LS (flower)
TE	0,59	1,58	0,86	1,88	2,25	1,02	0,79	1,86	1,65	0,26	1,81	0,85	1,52

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 steochas*; TE: Trolox equivalent (mM)

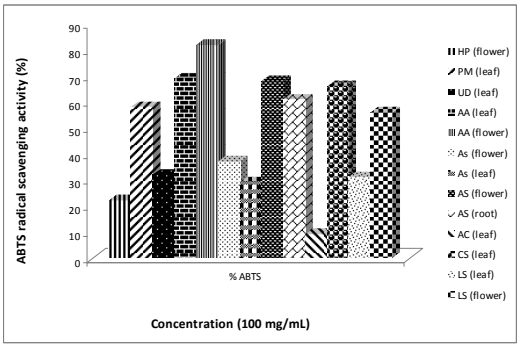

Figure 1: Non-enzymatic antioxidant activities of different plants extracts
HP: *Hypericum perforatum* L. subsp. *veranese* (Schrant) H. *Lindb.*; PM: *Plantago major*; UD: *Urtica dioica*; AA: *Arbutus andrachne*; As: *Anthemis sp.;* AS: *Allium sphaerocephalon;* AC*: Anthemis chia* L.; CS: *Crepis sancta L.;* LS*: Lavandula steochas*

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Table 2: Antibacterial activities against isolated *S. aureus* of different plants extracts

Bacteria	Inhibition Zone Diameter (mm)													Antibiotics			Negative control
	Plant extracts (100 mg/mL)																
	HP (flower)	PM (leaf)	UD (leaf)	AA (leaf)	AA (flower)	As (flower)	As (leaf)	AS (flower)	AS (root)	AC (leaf)	CS (leaf)	LS (leaf)	LS (flower)	O	V	E	M
S. *aureus* BFT1	12	-	-	-	10	-	-	-	-	10	10	-	-	-	17	-	-
S. *aureus* BFT2	22	10	-	-	15	-	14	-	-	-	-	10	-	7	20	24	-
S. *aureus* BFT3	19	-	-	-	15	-	11	-	-	-	-	-	10	9	21	27	-
S. *aureus* BFT4	18	-	-	-	-	-	-	-	-	-	-	-	-	-	27	23	-
S. *aureus* BFT5	17	12	-	-	10	-	13	-	-	-	-	11	-	-	27	23	-
S. *aureus* BFT6	16	-	-	-	12	-	-	-	-	10	-	11	-	8	17	25	-

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 steochas* (-): No inhibition; O: Oxacillin (5 µg); V: Vancomycin (30 µg); E: Erythromycin (15µg); M: Methanol (25µL)

Table 3: Minimum inhibitory concentrations of different plants extracts (µg/mL)

Bacteria	HP (flower)	PM (leaf)	UD (leaf)	AA (leaf)	AA (flower)	As (flower)	As (leaf)	AS (flower)	AS (root)	AC (leaf)	CS (leaf)	LS (leaf)	LS (flower)
S. *aureus* BFT1	6500	NT	NT	NT	1625	NT	NT	NT	NT	3250	1625	NT	NT
S. *aureus* BFT2	3250	1625	NT	NT	1625	NT	812.5	NT	NT	NT	NT	812.5	NT
S. *aureus* BFT3	3250	NT	NT	NT	1625	NT	1625	NT	NT	NT	NT	NT	812.5
S. *aureus* BFT4	812.5	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
S. *aureus* BFT5	1625	1625	NT	NT	1625	NT	3250	NT	NT	NT	NT	1625	NT
S. *aureus* BFT6	1625	NT	NT	NT	1625	NT	NT	NT	NT	1625	NT	1625	NT

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 steochas*; NT= Not Tested

Results

Participants (age: 16.86±0.86 years) filled out a Questionnaire about the hygiene and the life style of soccer players. It shows that their mother's and father's education level are primary school (57%) and senior high school (43%) respectively. Most of the participants live in city centre (72%). They do exercise 6 days in a week (80%) regularly and have played soccer for 3-7 years (47%) and for 7-11 years (47%). Fifty percent of the participants haven't been educated about hygiene.

Firstly, bacteria isolated from the 28 athlete's shoes after competition were performed Gram staining. Gram-positive cocci separated and the biochemical tests were performed. Especially, biggest problem in contact sports is antibiotic-resistant S. aureus and these bacteria were studied for identification. In this study, total 1140 bacteria were isolated from 28 player's shoes. Gram positive cocci were inoculated on MSA medium, 58 yellow pigment-producing bacteria have been selected randomly at the end of the incubation period. 69% of these bacteria are Gram-positive cocci. 11% of Gram-positive cocci are *S. aureus*. The other bacteria include; 23% Gram negative cocci, 2% Gram positive bacilli, 6% Gram-negative bacilli. Catalase and mannitol fermentation tests were applied to yellow pigment-producing bacteria. Then, catalase-positive and mannitol fermenting- bacteria were selected. At the end of biochemical processes were isolated 6 *S.* aureus strains. Further studies were carried out with these six bacteria (Table 1).

The results of antibacterial activities were measured as zone of inhibition in mm for all the materials used as follows. The antibacterial activities of plants extracts were evaluated *in vitro* against 6 *Staphylococcus aureus.* Results of antibacterial activities of methanol extracts of used plants against the test bacteria are shown in Table 2. The highest antibacterial activity was shown on S. *aureus* BFT2 (22 mm) for *Hypericum perforatum* L. subsp. *veranese* (Schrant) H. *Lindb*.

Results show that, the methanol extracts of 8 plants inhibited the growth of bacteria and the inhibition zones ranged between 10- 22 mm. In addition to, the extracts of 5 plants did not determine any antibacterial effects against used 6 bacteria. These bacteria were found resistant to all of extracts. The lowest activity was found as 10 mm. 3 antibiotics used as positive control. These include; oxacillin, vancomycin, and erythromycin. Methanol used as negative control. Data of antibacterial activities of the extracts are demonstrated in Table 2.

Antibacterial activity studies have been tested against pathogens by using broth dilution method. In Table 3, MIC values of methanol extracts belong to leaves, flowers, and root of ten plants were summarized. MIC values for plant extracts were applied from 6500 to 406 µg/mL. S. *aureus* BFT4 have shown the lowest sensitivity to *Hypericum perforatum* flower extract. S. *aureus* BFT2 have shown the lowest sensitivity to two extracts. These include; *Anthemis* sp. leaf and *Lavandula steochas* leaf extracts. However, S. *aureus* BFT3 have found the lowest sensitivity to *Lavandula steochas* flower extract. These extracts of different plants possessed antibacterial activity, and showed minimal inhibitory concentration effect at 812.5 µg/mL.

ABTS free radical scavenging method was used for antioxidant activity. The results of ABTS scavenging assay of different plants extracts are shown in Table 4 and Figure1. Table 4 and Figure1 show the per cent of ABTS radical scavenging capacity with trolox as reference. *Arbutus andrachne* flower extracts showed 82% inhibition at 100 mg/mL concentration. Whereas, the lowest activity was found by *Anthemis chia* (Table 4 and Figure1). The antioxidant activity by ABTS assay were in the order of *Arbutus andrachne* (flower) > *Arbutus andrachne* (leaf) > *Anthemis* sp. (flower) > *Crepis sancta* (leaf) > *Allium sphaerocephalon* (root) > *Plantago major* (leaf) > *Lavandula steochas* (flower) > *Anthemis* sp. (flower) > *Urtica dioica* (leaf) > *Lavandula steochas* (leaf) > *Anthemis* sp. (leaf) > *Hypericum perforatum* (flower) > *Anthemis chia* (leaf) (Table 4 and Figure1).

Discussion

This study confirms that the leaf, root and flower of different plants possess antimicrobial and antioxidant activities. The properties commonly found in the plants, and have been reported to posses multiple biological effects including antimicrobial and antioxidant activities. In this study, the antibacterial activity for methanolic extract was also high against the one tested pathogens, results indicated the polarity of the solvent plays an important role in the extraction of the active ingredient and consequently on its antimicrobial activity.

http://www.ingentaconnect.com/content/iafp/jfp/1995/00000058/00000003/art00010In this study, the highest antibacterial activity was showed as 22 mm against S. *aureus* BFT2 for *Hypericum perforatum* flower extract (Table 2). 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 (Ratledge and Wilkinson, 1988; Wendakoon and Sakaguchi, 1995). Dua *et al.* (2013) reported that antibacterial activities of *Foeniculum vulgare* Miller seeds were found as 11 mm inhibition zone against *S. aureus* (Dua *et al.*, 2013). This report also supports the results we obtained from our study.

In this work, the inhibition zone was not produced by some of extracts against test organisms (Table 2). These include; *Urtica dioica* (leaf), *Arbutus andrachne* (leaf), *Anthemis* sp. (flower), *Allium sphaerocephalon* (flower), and *Allium sphaerocephalon* (root). Abuhamdah *et al.* (2013) reported that the extract of *A. andrachne* was not inhibited three test bacteria. This research supports the results we obtained from our study. According to our results, the extracts from different plants possessed antibacterial activity, and showed minimal inhibitory concentration effect at 812.5 µg/mL (Table 3). These include; *Hypericum perforatum* (flower), *Anthemis* sp. (leaf), *Lavandula steochas* (leaf), and *Lavandula steochas* (flower). Previous antibacterial studies of *Arbutus pavarii* indicate that methanolic extract exhibited antibacterial effect against *S. aureus*, with zone of inhibition of 20 mm, and the minimum inhibitory concentrations were 4.86 mg/mL (Alsabri *et al.*, 2013). In this study, MIC value was generally measured as 812.5 µg/mL, and our results are better than those of Sharma *et al***.** (2004) and Alsabri *et al*. (2013).

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 (Sakanaka *et al.*, 2005; Alothman *et al.*, 2009; Hasan *et al.*, 2012; Keser *et al.*, 2012). The results of ABTS scavenging assay of different plant extracts are shown in (Table 4 and Figure1). *Arbutus andrachne* (flower) methanol extract showed 82% inhibition at 100 mg/mL concentration (Table 4 and Figure1). The antioxidant activity by ABTS assay were in the order of *Arbutus andrachne* (flower) > *Arbutus andrachne* (leaf) > *Anthemis* sp. (flower) > *Crepis sancta* (leaf) > *Allium sphaerocephalon* (root) > *Plantago major* (leaf) > *Lavandula steochas* (flower) > *Anthemis* sp. (flower) > *Urtica dioica* (leaf) > *Lavandula steochas* (leaf) > *Anthemis sp* (leaf) > *Hypericum perforatum* (flower) > *Anthemis chia* (leaf). *A. andrachne* was found to be the highest among 51 other medicinal plant species in Jordan that have antioxidant content (Tawaha *et al.*, 2007). Phytochemical studies have shown that the leaf extract contains phenolic antioxidant compounds, such as flavonoids (Mazza and Miniati, 1993; Males *et al.*, 2006), tannins, phenolic

glycosides, anthocyanins, gallic acid derivatives *etc.* (Hertog *et al.*, 1992; Ayaz *et al.*, 2000; Kivcak *et al.*, 2001a; Kivcak *et al.*, 2001b; Pabuccuoglu *et al.*, 2003; Fiorentino *et al.*, 2007).

Conclusion

Medicinal plants had good antibacterial action on all test organisms, further investigation is necessary for possible use of the active component in chemotherapy. Our results suggest that *Hypericum perforatum* has significant antibacterial activity and could be very useful in the discovery of novel antibacterial agents of plant origin. Further phytochemical studies are required to determine and isolate compounds responsible for the antibacterial effects of these species. In conclusion, medicinal plants might be considered as a potential source of metabolites which could be developed as precursors for antimicrobial and antioxidants drugs. The results in this study using ABTS method to evaluate the antioxidant showed that the *Hypericum perforatum* and *Arbutus andrachne* extracts can be considered good sources of natural compounds with significant antioxidant activity. Consumption of microbiologically safe plants should be encouraged for their rich antioxidants.
Soccer players and their families should be educated about hygiene, dermatological problems and bacteria damages in sports. Mother's and father's education level should be increased and they informed about cleaning, hygiene and sanitation. Result of that the best treatment for bacterial and dermatological conditions is prevention.

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References

1. Adams, B.B. (2006). Sports Dermatology. New York, Springer.
2. Adams, B.B. (2001). Adolescent medicine: state of the art reviews. Sports Derm., 12: 305-322.
3. Powell, F.C. (1994). Sports dermatology. J. Eur. Acad. Dermatol. Venerol., 3(1): 1-15.
4. Adams, B.B. (2002). Dermatologic disorders of the athlete. Sports Med., 32(5): 309-321.
5. Kluytmans, J., van Belkum, A. and Verbrugh, H. (1997). Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clin. Microbiol. Rev., 10(3): 505-520.
6. CDC. (1962). Centers for Disease Control. Staphylococcal infections in wrestlers: Iowa. MMWR Morb Mortal Wkly Rep., 11:152.
7. Decker, M.D., Lybarger, J.A., Vaughn, W.K., Hutcheson, R.H. and Jr, Schaffner W. (1986). An outbreak of staphylococcal skin infections among river rafting guides. Am. J. Epidemiol., 124(6): 969-976.
8. Nguyen, D.M., Mascola, L. and Brancoft, E. (2005). Recurring methicillin-resistant *Staphylococcus aureus* infections in a football team. Emerg. Infect. Dis. 11(4): 526-532.
9. Barrett, F.F., McGehee, R.F. and Jr, Finland M. (1968). Methicillin-resistant *Staphylococcus aureus* at Boston City Hospital: bacteriologic and epidemiologic observations. N. Engl. J. Med., 279(9): 441-448.
10. Rihn, J.A., Michaels, M.G. and Harner, C.D. (2005a). Community-acquired methicillin resistant *Staphylococcus aureus*: an emerging problem in the athletic population. Am. J. Sports Med., 33(12): 1924-1929.
11. Ma, X.X., Ito, T., and Tiensasitorn, C. (2002). Novel type of staphylococcal cassette chromosome mec identified in community-acquired methicillin-resistant *Staphylococcus aureus* strains. Antimicrob. Agents Chemother., 46(4): 1147-1152.
12. Naimi, T.S., LeDell, K.H., and Como-Sabetti, K. (2003). Comparison of community-and health care-associated methicillin-resistant *Staphylococcus aureus* infection. JAMA. 290(22): 2976-2984.
13. Fridkin, S.K., Hageman, J.C., and Morrison, M. (2005). Methicillin-resistant *Staphylococcus aureus* disease in three communities. N. Engl. J. Med., 352(14): 1436-1444.
14. Crawford, S.E., Boyle-Vavra, S., and Daum, R.S. (2007). Community associated methicillin-resistant *Staphylococcus aureus*. In: (Hooper DC, Scheld M, eds). Emerging Infections. Vol 7. Washington, DC: ASM Press, pp 153-179.
15. Daum, R.S. (2007). Clinical practice. Skin and soft-tissue infections caused by methicillin-resistant *Staphylococcus aureus*. N. Engl. J. Med., 357(4): 380-390.
16. Frazee, B.W., Lynn, J., Charlebois, E.D., Lambert, L., Lowery, D. and Perdreau-Remington, F. (2005). High prevalence of methicillin-resistant *Staphylococcus aureus* in emergency department skin and soft tissue infections. Ann. Emerg. Med., 45(3): 311-320.
17. Gorwitz, R.J., Jernigan, D.B., Powers, J.H., and Jernigan, J.A. (2006). Strategies for clinical management of MRSA in the community: summary of an experts' meeting convened by the Centers for Disease Control and Prevention, http://www.cdc.gov/ncidod/dhqp/ar_mrsa_ca.html.
18. Turbeville, S.D., Cowan, L.D. and Greenfield, R.A. (2006). Infectious disease outbreaks in competitive sports: a review of the literature. Am. J. Sports. Med., 34(11): 1860-1865.
19. Klevens, R.M., Morrison, M.A., and Nadle, J. (2007). Invasive methicillin resistant *Staphylococcus aureus* infections in the United States. JAMA., 298(15): 1763-1771.
20. CDC. (2003). Centers for Disease Control and Prevention. Methicillin resistant *Staphylococcus aureus* infections among competitive sports participants: Colorado, Indiana, Pennsylvania, and Los Angeles County, 2000-2003. MMWR Morb Mortal Wkly Rep., 52(33): 793-795.
21. Begier, E.M., Frenette, K., and Barrett, N.L. (2004). A high-morbidity outbreak of methicillin-resistant *Staphylococcus aureus* among players on a college football team, facilitated by cosmetic body shaving and turf burns. Clin. Infect. Dis., 39(10): 1446-1453.
22. Rihn, J.A., Posfay-Barbe, K., and Harner, C.D. (2005b). Community-acquired methicillin-resistant *Staphylococcus aureus* outbreak in a local high school football team unsuccessful interventions. Pediatr. Infect. Dis. J., 24(9): 841-843.

23. Lindenmayer, J.M., Schoenfeld, S., O'Grady, R., and Carney, J.K. (1998). Methicillin resistant *Staphylococcus aureus* in a high school wrestling team and the surrounding community. Arch. Intern. Med., 158(8): 895-899.
24. Srivastava, J., Lambert, J., and Vietmeyer, N. (1996). Medicinal plants. An expanding role in development, World Bank Technical Paper. 320: Washington.
25. Nascimento, G.G.F., Lacatelli, J., Freitas, P.C., and Silva, G.L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Braz. J. Microbiol.*, 31: 886-891.
26. Davis, P.H. (1975). Flora of Turkey and the East Aegaean Islands, Vol. 5. Edinburgh University Press, Edinburgh, pp 228-251.
27. Cowan, S.T. and Steel, K.J. (1965). Manual for the Identification of Medical Bacteria. London: Cambridge University Press.
28. Monica, C. (1991). Medical Laboratory manual for Tropical countries. VOL 11. ELBS, 60-63.
29. Atlas, R.M. (2004). Handbook of Microbiological Media, CRC Press, New York.
30. Holt, J.G., Krieg, N.R., Sneath, P.H.A. and Williams, S.T. (1994). Bergey's manual of determinative bacteriology. Williams and Wilkins, Baltimore.
31. Bauer, A.W., Kirby, W.M., Sherris, J.C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Path., 45: 493-496.
32. CLSI (Clinical and Laboratory Standards Institute). (2003). Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically; Approved Standard M7-A 6th edn. National Committee for Clinical Laboratory Standards, Wayne, Philadelphia.
33. CLSI (Clinical and Laboratory Standards Institute). (2006). Performance Standards for Antimicrobial Susceptibility Testing. 16th Informational Supplement M100-S16. National Committee for Clinical Laboratory Standards, Wayne, Philadelphia.
34. Re, R., Pellegrini, N., Protrggente, A., Pannala, A., Yang, M. and Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol. Med. 26: 1231-1237.
35. Ratledge, C. and Wilkinson, S.G. (1988). An Overview of Microbial Lipids. In: Microbial Lipids, (Ratledge C, Wilkinson SG. eds). Academic Press, London, pp 3-22.
36. Wendakoon, C.N. and Sakaguchi, M. (1995). Inhibition of amino acid decarboxylase activity of *Enterobacter aerogenes* by active components in spices. J. Food Prot., 58: 280-283.
37. Dua, A., Gaurav, Garg. and Mahajan, R. (2013). Polyphenols, flavonoids and antimicrobial properties of methanolic extract of fennel (*Foeniculum vulgare Miller*). Eur. J. Exp. Biol., 3(4): 203-208.
38. Abuhamdah, S., Abuhamdah, R., Al-Olimat, S. and Chazot, P. (2013). Phytochemical investigations and antibacterial activity of selected medicinal plants from Jordan. European. J. Med. Plant., 3(3): 394 - 404.
39. Alsabri, S.G., El-Basir, H.M., Rmeli, N.B., Mohamed, S.B., Allafi, A.A., Zetrini, A.A., Salem, A.A., Mohamed, S.S., Gbaj, A. and El-Baseir, M.M. (2013). Phytochemical screening, antioxidant, antimicrobial and anti-proliferative activities study of *Arbutus pavarii* plant. J. Chem. Pharma. Res., 5(1): 32 - 36.
40. Sharma, N., Maiti, S.K. and Koley, K.M. (2004). Studies on the incidence of subclinical mastitis in buffaloes of Rajnandgaon district of Chhattisgarh state. Vet. Practitioner., 5(2): 123 - 124.
41. Sakanaka, S., Tachibana, Y. and Okada, Y. (2005). Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha - cha). Food Chem., 89: 569 - 575.
42. Alothman, M., Bhat, R. and Karim, A.A. (2009). Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chem., 115(3): 785 - 788.
43. Hasan, M., Alam, M.N., Wahed, T.B., Sultana, F. and Jamiuddin, A. (2012). *In vitro* antioxidant potential of the methanolic extract of *Bacopa monnieri* L. J. Pharm. Sci., 9(3): 285 - 292.
44. Keser, S., Celik, S., Turkoglu, S., Yilmaz, O. and Turkoglu, I. (2012). Hydrogen Peroxide Radical Scavenging and Total Antioxidant Activity of Hawthorn. Chem. J., 2(1): 9 - 12.
45. Tawaha, K., Alali, F., Gharaibeh, M., Mohammad, M. and El-Elimat, T. (2007). Antioxidant activity and total phenolic content of selected Jordanian plant species. Food. Chem., 104: 1372 - 1378.
46. Mazza, G. and Miniati, E. (1993). Anthocyanins in fruits, vegetables, and grains. CRC Press Inc., Boca Raton.
47. Males, Z., Plazibat, M., Vundac, V.B. and Zuntar, I. (2006). Qualitative and quantitative analysis of flavonoids of the strawberry tree - *Arbutus unedo* L. *(Ericaceae)*. Acta. Pharm., 56: 245 - 250.
48. Hertog, M.G.L., Hollman, P.C.H. and Venema, D.P. (1992). Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. J. Agric. Food. Chem., 40: 1591 - 1598.
49. Ayaz F.A., Kucukislamoglu, M. and Reunanen, M. (2000). Sugar, Non-volatile and Phenolic Acids Composition of Strawberry Tree (*Arbutus unedo* L. var.*ellipsoidea*) Fruits. J. Food. Comp. Anal., 13(2): 171 - 177.
50. Kivcak, B., Mert, T. and Denizci, A.A. (2001a). Antimicrobial activity of *Arbutus unedo* L. Journal of Pharmaceutical Sciences. J. Pharma. Sci., 26: 125 - 128.
51. Kivcak, B., Mert, T.D., Emirci, B. and Baser, K.H.C. (2001b). Composition of the essential oil of *Arbutus unedo*. Chem. Nat. Com., 37(5): 445 - 446.
52. Pabuccuoglu, A., Kivcak, B., Bas, M. and Mert, T. (2003). Antioxidant activity of *Arbutus unedo* leaves. Fitoterapia., 74: 597 - 599.
53. Fiorentino, A., Castaldi, S., D'abrosca, B., Natale, A., Carfora, A., Messere, A. and Monaco, P. (2007). Polyphenols from the hydroalcoholic extract of *Arbutus unedo* living in a monospecific Mediterranean woodland. Biochem. Syst. Ecol., 35: 809 - 811.