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ORIGINAL ARTICLE

Inhibitory Effect of *Ziziphus zizyphus* L. Extract on Staphylococcus Genera

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ABSTRACT
Staphylococcus aureus, a gram positive, non-motile, catalase and coagulase positive, facultative anaerobe coccus is a common type of bacteria that normally lives on the skin and nasal passages of healthy people. Medicinal plants are natural resources, yielding valuable herbal products which are often used in the treatment of various ailments. The aim of this study was to evaluation of antimicrobial effects of Ziziphus zizyphus L. essential oils against Staphylococcus spp. fourteen clinical isolates of Staphylococcus cultured from patients. The disc diffusion method was used for determination of antimicrobial activity of essential oil. Results showed that this inhibitory effect is dose-dependent, to wit, by increasing the concentration of the extract in the culture media, reduction in growth was obviously revealed. In conclusion, can be state that Ziziphus zizyphus L. essential oils have inhibitory effect against staphylococcus spp.
Key words: *antimicrobial activity, Ziziphus zizyphus L., essential oils, staphylococcus spp.*

INTRODUCTION

Staphylococcus aureus, a gram positive, non-motile, catalase and coagulase positive, facultative anaerobe coccus is a common type of bacteria that normally lives on the skin and nasal passages of healthy people. When it enters the body through a cut or other medical devices, it can cause local or serious infections [1]. Methicillin Resistant S. aureus (MRSA) has become one of the major causes of nosocomial and community pathogens causing significant morbidity and mortality because there are multi drug resistant pathogens that are resistant to all penicillins, so the option antibiotics for treatment of MRSA infections are limited to antibiotics such as vancomycin, tigecycline, lincozolid and mupirocin [2]. The patterns of antimicrobial susceptibility of S. aureus have been changed worldwide and it has been reported increasingly to be less effective. Development of mupirocin [3] and vancomycin [4] microbial resistance in MRSA has increased in settings with extensive use of these agents. Microbial resistances to conventional antibiotics and adverse effects of these agents have led to find new sources as antimicrobial agents. Medicinal plants have a long history of use as traditional medicines for treatment of different kinds of ailments especially for infectious diseases.

Medicinal plants are natural resources, yielding valuable herbal products which are often used in the treatment of various ailments [5]. From ancient times, plants are rich source of effective and safe medicines. In recent years there has been focus on plants with antimicrobial activity. There are many published reports on the effectiveness of traditional herbs against Gram-positive and Gram negative microorganisms and as a result, plants are still recognized as the bedrock for modern medicine to treat infectious diseases [6]. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world [7, 8, 9]. Some foods contain naturally occuring substances showing antimicrobial activity. Some spices are known to contain cinnamic aldehyde, allicin in garlic and allicin in onion. These substances can be use for protection against microorganisms [10]. It has been reported that the higher plants have shown to be a potential source for the new antimicrobial agents [11]. The antimicrobial compounds from plants may inhibit bacterial growth by different mechanisms than those presently used. Antimicrobials therefore, may have a significant clinical value in treatment of resistant microbial strains [12]. Besides, the antimicrobial activity of plant oils and extracts has formed the basis of many applications including raw and processed food preservation, pharmaceuticals, alternative medicine, and natural therapies [13]. Frankel et al. and Mau et al. also reported that the use of herbal drugs increased instead of synthetic drugs [14, 15].

Although several researchers as Dıgrak et al. in 2001, Sarac and Ugur in 2007, Poyrazoglu et al. in 2009, Karatas and Ertekin in 2010 and so on were investigated about antibacterial activity of Turkish plants, ethnobotanical and pharmaceutical studies on these plants are inadequate [16, 17, 18, 19]. The aim of this study was to evaluation of antimicrobial effects of essential oils against Staphylococcus spp.

MATERIALS AND METHODS

Bacterial cultures and preparation of Ziziphus zizyphus L. extracts

Fourteen clinical isolates of Staphylococcus cultured from patients and S. aureus ATCC 25923 were used in all experiments. Methicillin resistant S. aureus directed detected on CHROMagarTM MRSA (CHROMagar Paris, France). Bacterial suspensions were made in Brain Heart Infusion (BHI) broth to concentration of approximately 108 CFU/ml using standard routine spectrophometrical method. Subsequent dilutions were prepared from the above suspensions, which were then used in the tests.

Disc diffusion method

The disc diffusion method was used for determination of antimicrobial activity of essential oil. Briefly, using a sterile cotton swab, above microbial suspensions was spread on the Mueller Hinton Agar (MHA) plates. Sterile paper discs (6 mm in diameter) were impregnated with 10, 15, 20 l of each oil and were placed on the inoculated plates. After remaining at 4 °C for 2 h, plates were incubated for 24 h at 37 °C. The diameters of the inhibition zones were measured in millimeters. All tests were performed in triplicate as NCCLS protocol in 2009.

RESULTS AND DISCUSSION

Chemical composition of *A. millefolium L.* essential oil is showed in table 1 [20].

Table 1: Chemical composition of *Ziziphus zizyphus L.* essential oil

Compound	abundance
α-pinene	4.4
Sabinene	16.9
B-pinene	Trace
Myrcene	1.9
p-cymene	Trace
Limonene	0.7
1,8-cineole	60.5
(E)-β-Ocimene	0.3
γ-Terpinene	Trace
Terpinolene	Trace
α-Terpineol	14.1
(E)-Caryophyllene	0.6
(E)-β-Farnesene	0.5
γ-Muurolene	Trace

Biochemical test such as catalase, oxidase, coagulase and OF was carried out to proven the genera and data are showed in the table 2.

Table 2: results obtained from different biochemical tests

Genera	Coagulase test	Hemolysis	Pigmented colonies	Mannitol salt agar	Maltose
Staphylococcus aureus	+	+	+	+	+
S. intermedius	+	+	-	(d)	±
S. hyicus	(d)	-	-	-	-
S. epidermidis	-	(d)	-	-	+
S. saprophyticus	-	-	(d)	(d)	+
S. aureus ssp. anaerobious	+	+	-	0	+
S. capare	-	(d)	-	(d)	(d)
S. gallinarum	-	(d)	(d)	+	+
S. arlettae	-	-	+	+	+
S. lentus	-	-	(d)	+	(d)
S. equorum	-	(d)	-	+	(d)
S. simulans	-	(d)	-	+	±
S. delphini	0	+	-	(+)	+
S. chromogenes	-	-	+	(d)	(d)

d: 11-89% positive
+: 90% and more positive
-: 90% and more negative
0: unknown

Inhibitory effect of *Ziziphus zizyphus* L. extract was determined by different concentrations of this herbal extract and results showed that this inhibitory effect is dose-dependent, to wit, by increasing the concentration of the extract in the culture media, reduction in growth was obviously revealed (table 3).

Table 3: Anti-staphylococcal activity of *Ziziphus zizyphus* L. essential oils by disc diffusion method

Genera	***Ziziphus zizyphus* L.** Inhibition Zone Diameters (mm*)		
	10%	**20%**	**30%**
S. aureus	8.65	9.21	10.18
S. intermedius	8.84	9.42	10.04
S. hyicus	8.97	9.15	10.36
S. epidermidis	8.64	8.86	9.52
S. saprophyticus	9.23	9.38	9.81
S. aureus ssp. anaerobious	9.48	9.61	9.73
S. capare	7.74	8.30	9.16
S. gallinarum	8.61	9.53	9.24
S. arlettae	9.19	9.78	9.69
S. lentus	8.71	9.00	10.14
S. equorum	9.26	9.34	10.30
S. simulans	8.51	8.68	9.23
S. delphini	7.98	8.70	9.46
S. chromogenes	8.73	8.54	9.35

Staphylococci are among the most commonly encountered pathogens in clinical practice [21] S. aureus is a major cause of nosocomical infections, food poisoning, osteomyelitis, pyoarthritis, endocarditis, toxic shock syndrome, and a broad spectrum of other disorders [21, 22, 23]. In recent years, there has been an alarming increase in nosocomial staphylococcal infections by strains with multiple drug resistance [24, 25, 26].

Obtained results revealed that essential oil exhibited variable levels of antibacterial activity against all tested bacterial strains. According to the literature data [27, 28] Gram-positive bacteria seemed to be more sensitive to the examined essential oil than Gram-negative bacteria. Furthermore, the essential oil obtained from *A. collina s.l.* in the most of the cases exhibited stronger antibacterial activity than *A. pannonica* oil (in some of tested Staphylococcus aureus and Streptococcus strains). This could be due to the presence of high ratio of chamazulene in the essential oil. On the other hand, stronger bacteriostatic activity of *A. pannonica* was observed on Streptococcus hyicus and one strain of Streptococcus agalactiae in comparison to A. collina oil This could be explained by notable amounts of 1,8-cineole (40.40%), camphor (11.10%) and borneol (3.22%) in the essential oil. All of the three substances are confirmed as strong antimicrobials on a different range of bacteria [28, 29, 30].

Gram-positive bacteria are known to be more susceptible to essential oils than Gram-negative bacteria [34]. *P. aeruginosa* was least susceptible to the essential oils. The weak antibacterial activity against Gram-negative bacteria was ascribed to the presence of their cell wall, lip polysaccharide [31]. B. subtilis was the most susceptible micro-organism to the rosemary essential oil. Concerning the activity of pure active compounds, the most susceptible bacteria to thymol was B. subtilis (23.0 mm) and the most resistant was *P. aeruginosa* (11.5 mm).

The essential oil of *Achillea distans* W. et K. flower heads was analyzed by GC and GC-MS. Altogether 43 components in concentrations more than 0.1% were identified representing 93.5% of the oil composition. The main constituents were 1,8-cineole (16.8%), trans-thujone (9.8%), sabinene (8.2%), borneol (7.5%), beta-pinene (6.5%), and camphor (5.8%). The oil showed moderate activity against Staphylococcus aureus and Candida albicans, and weak activity against Salmonella typhimurium, Proteus vulgaris, and Escherichia coli [32].

In one study the screening of the antimicrobial activity of yarrow essential oil was conducted by a disc diffusion test against Gram-positive (Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Streptococcus faecalis), Gram-negative (Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis) and fungal organisms (*Aspergillus niger, Aspergillus fumigatus, Candida albicans)*. The activity was more pronounced against Gram-negative and fungal organisms than against Gram-positive bacteria. A. clavennae oil was found to possess antimicrobial activity against *Klebsiella pneumoniae, Pseudomonas aeruginosa* and all fungal organisms [33].

CONCLUSION

From the obtained results it is obvious that the chemical composition of the essential oil has important impact on both antioxidant and antimicrobial effects of A. millefolium obtained from different biological sources. The presence of chamazulene increases the antibacterial activity, whereas the antioxidant and scavenging effects of essential oil are related to some other substances, such are camphor and borneol.

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