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International journal of Advanced Biological and Biomedical Research

Volume 2, Issue 2, 2014: 440-445

Invitro Evaluation Of Some Iranian Plants Against *Staphylococcus aureus* Isolation Of Urinary Tract Infection

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Abstract

In this study, Invitro evaluation of some Iranian plants against *Staphylococcus aureus* Isolation of Urinary Tract Infection by microtiterplate method. All 12 strains of *Staphylococcus aureus* isolated from urine culture of hospitalized patients during the years 2012- 2013. Minimum Inhibitory Concentration (MIC) of plant extract and essential oil against bacteria were determined using micro dilution broth method at six different concentrations. The result showed that the antibiotic susceptibility of *S.aureus* isolates was evaluated for 6 antimicrobial. Antibiotic susceptibility of *S.aureus* isolates was evaluated for 6 antimicrobial and more resistance were to oxacillin(83.3%), ceftazidime(66.6%) and penicillin(50%). The highest MIC values of extract were found to be 2.5 mg/ml against *S.aureus* and one of MIC value for *S.aureus* was 0.62mg/ml. This study also confirm the antimicrobial potential of investigated plants and their usefulness in treatment of resistance microorganisms gram-positive.

Key word: Extract plant, Essential oil, Antibacterial activity, Minimum Inhibitory Concentration (MIC), *Staphylococcus aureus*

Introduction

Development of microbial resistance to antibiotics is a global concern. Isolation of microbial agents less susceptible to regular antibiotics and recovery of increasing resistant isolates during antibacterial therapy is rising throughout the world which highlights the need for new principles. The aromatic medicinal plants are probably natural alternatives for antibiotics. The aromatic plants and essential oils extract from these plants are important growth promoter due to their antimicrobial and stimulating effects on animal digestive system. *Staphylococcus aureus* is a major nosocomial pathogen that causes a range of diseases, including endocarditis, osteomyelitis, pneumonia, toxic-shock syndrome, food poisoning, carbuncles, and boils and its role in causing diseases such as sepsis and abscesses was first described by Ogston in the late nineteenth century (Ogston, 1882). *Myrtus communis*, the myrtle belongs to the family of the Myrtaceae, and is a beautiful evergreen bush or small tree with small elliptic, fragrant, deep green leaves and pure

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white flowers which is widespread around the Mediterranean basin, but also extends to eastern Iran and Afganistan (Bonafé 1979). Essential oils have been found to be antibacterial (Kivanc and Akgul, 1986), antifungal (Pandey *et al*., 1996) and therapeutic in cancer treatment (Crowell, 1999). *Cuminum cyminum a* member of the family Apiaceae. The seeds are used to flavour foods and liquors and the oil is utilized as a perfume and cosmetic. It possesses emmanagogue and carminative activity and stimulates the production of maternal milk. *Zataria multiflora* locally known as "SAATAR belongs to a family Labiatae possessing fragrant odor like lemon and thyme. The medicines of plant origin are used for a variety of diseases (Shiba et al., 2005). The interest in use of plants and their antimicrobial activity has revived due to the problems associated with the current use of antibiotics(Shiota et al.,2004). Dill (*Anethum graveolens*), is either a perennial or annual herb. It is the sole species of the genus *Anethum*. In Arab countries, dill seed, called *ain jaradeh* (grasshopper's eye), is used as a spice in cold dishes such as fattoush and pickles. In Arab countries of the Persian Gulf, dill is called *shibint* and is used mostly in fish dishes. Coriander (*Coriandrum sativum*), also known as cilantro, Chinese parsley or dhania. Coriander, like many spices, contains antioxidants, which can delay or prevent the spoilage of food seasoned with this spice. A study found both the leaves and seed to contain antioxidants, but the leaves were found to have a stronger effect. In this study, Invitro evaluation of some Iranian plants against *Staphylococcus aureus* Isolation of Urinary Tract Infection by microtiterplate method

Materials and Methods

A cross-sectional study was carried out based on reports of bacteria isolates from the urinary tract infection of Hospital- Zabol-Iran from 2012-2013. All samples that were collected aseptically from 50 patients were plated right after the collection. Identification of all causative microorganisms was performed by standard microbiologic methods. Susceptibility testing was performed using disk diffusion method. The result were interpreted according to the guide lines of the Clinical and Laboratory Standards Institute.

Plant materials:

The seed *Cuminum cyminum* and leaf *Myrtus communis L, Anethum graveolens dhi*, *Coriandrum sativum* and *Zataria multiflora Bioss* was collection in the region of Iran (Sistan and Kerman, south-eastern, Iran) and plant in Zabol university herbarium received approval and dried at room temperature .Samples were crashed and transferred into glass container and preserved until extraction procedure was performed in the laboratory.

Distillation of essential oil:

The seed *Cuminum cyminum* and leaf *Zataria multiflora Bioss*, *Coriandrum sativum* and *Anethum graveolens dhi* was ground prior to the operation and then 300 g of ground rosemary was submitted to water distillation for 4 h using a Clevenger apparatus. The distilled essential oil was dried over anhydrous sodium sulfate, filtered and stored at 4°C.

Preparation of extracts:

The leaf *M.communis* L was properly dried and pulverized into a coarse powder. Each of 20 g grinded powders was soaked in 60 ml ethel acetate, separately for one day (shaking occasionally with a shaker). After one day of dissolving process, materials were filtered (Whatman no. 1 filter paper) .Then the filtrates were evaporated using rotary evaporator. At last, 0.97 g of dried extracts were obtained and then stored at 4^0C in air tight screw-cap tube.

Minimum Inhibitory Concentration (MIC) of extract and essential oil:

The broth microdilution method was used to determine MIC. All tests were performed in Mueller Hinton broth supplemented with Tween 80 at a final concentration of 0.5% (v/ v). Briefly, serial doubling dilutions of the extract were prepared in a 96-well microtiter plate ranged from 20mg/ml to 0.3mg/ml. To each well, 10 µl of indicator solution (prepared by dissolving a 10-mg extract in 2 ml of DMSO) and 10 µl of Mueller Hinton Broth were added. Finally, 10 µl of bacterial suspension (10^6 CFU/ml) was added to each well to achieve a concentration of 10^4 CFU/ml. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and then they were placed in an incubator at 37°C for 18–24 hours. The color change was then assessed visually. The lowest concentration at which the color change occurred was taken as the MIC value. The average of 3 values was calculated providing the MIC values for the tested extract. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate the visible growth. The microorganism growth was indicated by turbidity (Bokaeian et al., 2013).

Statistical Analysis:

All experiments and measurement were repeated at least three times. Statistical analyses were performed using SPSS and Excel 2010 software. All experimental results were analyzed using mean descriptive statistics and the correlation-coefficient. A value of $P<0.05$ was regarded as statistically significant.

Result:

Antibiotic susceptibility of *S.aureus* isolates was evaluated for 6 antimicrobial and *S.aureus* were more resistance to oxacillin(83.3%), ceftazidime(66.6%) and penicillin(50%)(Table1). The highest MIC values of extract of *M.communis* was found to be 2.5 mg/ml against *S.aureus* and one of MIC value for *S.aureus* was 0.62mg/ml. (Table 2). Among the essential oil, the least MIC value was observed by the *Z.multiflora* against *S.aureus* (1.25 mg/ml) (Table2).

Discussion:

Plants products have been used for relieving pain in humans diseases. In the study result show that *S.aureus* were more resistance to oxacillin(83.3%), ceftazidime(66.6%) and penicillin(50%). The study of Zarifian, *Staphylococci* isolates were highly resistant against ceftazidime (94%), followed by penicillin (91%), ampicillin (82%), cefotaxime (65%), erythromycin (60%), and oxacillin (43%) (Zarifian et al., 2012). The another study, the isolated *S. aureus* strains showed low resistance to vancomycin (1.5%), amikacin (2.3%) and gatifloxacin (3.8%) (Gamal Fad et al., 2010). The study of Onanuga, the isolates were highly resistant to ampicillin (91.7%), clindamycin (78.3%), cephalexin (75%), methicillin (71.7%) and vancomycin (68.3%) but had very low resistance to gentamicin (3.3%), ciprofloxacin (3.3%), ofloxacin (3.3%), sparfloxacin (3.3%) and pefloxacin (10.0%) (Onanuga et al., 2005). In the study, the highest MIC values of extract were found to be 2.5 mg/ml against *S.aureus* and one of MIC value for *S.aureus* was 0.62mg/ml. The study of Shahidi Bonjar, the least MIC, as 0.62 mg/ml, belonged to *Myrtus communis* seeds against *S. aureus*, *Bacillus cereus* and *B. bronchiseptica*(Shahidi Bonjar, 2004). The study of Mahboubi, the antifungal evaluating showed that myrtle oil exhibited good antifungal activity against *A. niger, A. parasiticus,* six isolates of *Aspergillus flavus*(Mahboubi and Ghazian Bidgoli, 2010). The previous study *S.aureus* was carried out from nose and throat area of patients and *Myrtus communis L* essential oil and ethanol extract have a significant effect on concentration of 5 mg/ml whereas 10 mg/ml was belonging to high Minimum Bacterial Concentration (MBC) for both treatments(Saeedi et al.,2012) . The previous study, ethyl acetate crude extract of *Myrtus communis L* had a maximum inhibitory effect on *Pseudomonas aeroginosa* and *Serratia marcescens*(Saeidi et al., 2013). The study of Bokaeian, the result show that the levels of MIC and MBC of ethanol extract were observed ranges from 2.5 and 5 mg/ml in radius respectively against *Morganella morganii* (Bokaeian et al., 2013). The study of Alem, the Minimum Bactericidal Concentration of Myrtle for most tested microorganisms was similar to the Minimum Inhibitory Concentration. i.e. 0.5 mg/ml. for S. aureus, 2.5 mg/ml for *P. mirabilis* and *P. vulgaris*, 15 mg/ ml for *Klebssiela* and *S. typhi*, 20 mg/ml for *P. aeruginosa*(Alem et al., 2008). The study of Bonjar, the

least MIC value was observed by the methanolic extract of Myrtus communis seeds against S. aureus, Bacillus cereus and B. bronchiseptica(Bonjar, 2004). The study of Shiri, the least MIC and MBC value was observed by the ethanol extract of *M. comminus L.* against penicillin, oxacillin, trimethoprim, sulfamethoxazole resistance *S. aureus* (1.25 and 2.5 mg/mL) (Shiri et al., 2014). This plant is found to be rich in polyphenolic compounds such as flavonoids and tannins (Sissi et al., 1967; Martin et al., 1999). Phytocemical studies about *M. communis* showed also presence of essential oil (Ozek et al., 2000). In the study among the essential oil, the least MIC value was observed by the *Z.multiflora* against *S.aureus* (1.25 mg/ml). The study Eftekhar, the antibacterial activity of *Z.multiflora Bioss* essential oil was measured against 10 ESBL producing urinary isolated of *K.pneumoniae* as well as six ATCC bacterial standards and the result showed inhibition zones of 18.3-30.3 mm for the ATCC standards and 20.7-29.7 mm for the 10 clinical isolates (Eftekhar et al., 2011). Carvacrol and thymol are the antimicrobial components of Z. multiflora essential oil exhibited strong inhibitory effect against broad spectrum of microorganisms including clinical isolates of *C. albicans* (Mahboubi *et al*., 2008), *S. pneumonia*, *E. faecalis*, *S. agalactiae*, *S. pyogenes*, *S. sanguis*, *S. salivarius*, *S. mutans* (Mahboubi and Feizabadi, 2009). According to the finding of this study, the extract and essential oil showed relatively high antimicrobial activity against the *Staphylococcus aureus*. The present study suggests that the extract and essential oil of this plants are a potential source of natural antibacterial agenst.

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Table 1: Antimicrobial susceptibility of 12 strains of *S.aureus* (%)

	CN	SXT	V	CAZ	P	OX
S	58.3	50	50	25	25	0
I	8.3	8.3	41.6	8.3	25	16.6
R	33.3	41.66	8.3	66.6	50	83.3

CAZ= Ceftazidime, CN= cefixime, SXT= trimethoprim-sulfamethoxazol, P= penicillin, OX= oxacillin,V= Vancomycin.

Table2: Antimicrobial susceptibility, MIC extract and essential oil plants aganist *S.aureus*(mg/ml)

Bacterial Cod	MIC for Extract plant of M.communis(mg/ml)	MIC for essential oil Z.multiflora	MIC for essential oil of A.graveolens dhi	MIC for essential oil C.cyminum	MIC for essential oil C.sativum	Resistance pattern
1	1.25	2.5	2.5	NO	5	A_1, A_2, A_3, A_4, A_5, A_6,
2	1.25	10	10	NO	5	A_1, A_2, A_4, A_5, A_6,
3	0.62	2.5	NO	20	NO	A_1,A_2,A_4, A_5, A_6,
4	2.5	2.5	5	20	NO	A_4, A_6
5	2.5	2.5	NO	NO	NO	A_4, A_6
6	1.25	2.5	NO	10	NO	A_6
7	2.5	1.25	NO	NO	10	-
8	2.5	1.25	NO	NO	10	-
9	2.5	1.25	NO	5	NO	A_6
10	2.5	1.25	10	10	20	A_2, A_4, A_5, A_6,
11	1.25	1.25	10	10	20	A_1, A_2, A_4, A_5, A_6,
12	1.25	1.25	NO	5	NO	A_4, A_5, A_6,

A_1= cefixime , A_2= trimethoprim-sulfamethoxazol, ,A_3= Vancomycin, A_4= Ceftazidime A_5= penicillin, A_6= Oxacillin .NO= any aticities