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Antibacterial Activity of Certain Iranian Medicinal Plants Against Methicillin-Resistant and Sensitive *Staphylococcus aureus*

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Abstract: Bacterial resistance to antibiotics is a serious global problem and includes strains of beta-lactam-resistant *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA). Novel antimicrobials and/or new approaches to combat the problem are urgently needed. The aim of this study was to investigate the antimicrobial activity of alcoholic and aqueous extract of 23 medical plants species of Golestan province on clinical and standard stains of MRSA and MSSA. Twenty three medicinal plants were collected from their natural habitat in Golestan province in north of Iran. Their ethanolic and aqueous extract obtained by percolation methods. Antibacterial effects were assessed by disk diffusion method and the Minimum Inhibitory Concentration (MIC) of the extracts was determined by the micro broth dilution against 14 clinical and standard strains of methicillin resistant and sensitive of *Staphylococcus aureus*. The ethanolic and aqueous extract of 8, 3 plants showed best anti *staphylococcal* effect, respectively. The ethanolic extract of *Artemisia*, *herbaalba*, *Nigella sativa*, *Punica granatum*, possed the most outstanding *in vitro* antibacterial activity which the maximum inhibition zone was 22.4-18 mm, respectively and the lowest MIC values was measured in *Punica granatum*, as 0.01 mg mL^{-1} against MRSA The results showed that ethanolic extract had better antibacterial effect than aqueous extract and anti *staphylococcal* activity of Ethanolic extract of plants against MRSA was better than MSSA strains. Ethanolic and aqueous extract of *Punica granatum* had the best antibacterial activity against the tested microorganisms. The result obtained from these plants might be considered sufficient for further studies.

Key words: Antibacterial effect, *Staphylococcus aureus*, medicinal plant, ethanolic and aqueous extract

INTRODUCTION

Staphylococcus aureus is a major *Human pathogen* responsible for a wide spectrum disease. It is an important cause of community and hospital acquired infections (Edine *et al*., 2004).

It is an organism commonly found in the nose and on the skin of healthy people. It can cause infection with clinical manifestations ranging from pustules to sepsis and death. Approximately 25 to 30% of the population is colonized in the nose with *staph* bacteria at a given time (North Dakota Department of Health, 2005).

According to WHO reports increase of antibacterial resistance is a growing problem in many countries (North Dakota Department of Health, 2005). And *S. aureus* strains acquired infections in the hospital are often resistant to antibiotics and soon after methicillin was introduced in to clinical use, methicillin-resistant *S. aureus* (MRSA) strains were reported over the last three decades and it was problem in hospital through the world (Akinyemi *et al*., 2005).

S. aureus first acquired resistance to methicillin and other penicillin-based antibiotics in the 1960 in England and emerged in the United States in the mild 1980 s (North Dakota Department of Health, 2005). MRSA strains are frequently resistant to many different classes of antimicrobial drugs and Many investigators have reported an increase in the incidence of MRSA during recent years (Edine *et al*., 2004). Most of which originated from wounds (North Dakota Department of Health, 2005). MRSA is responsible for approximately 25% of nosocomial infections (Shopsin and Kreswirth, 2001). Also recent study of isolates obtained in the United States reports that 41-43% of *S. aureus* isolates are methicillin-resistant (Drew *et al*., 2000).

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The data were collected from January 1999 through December 2002, in Europe show that MRSA prevalence varied from <10% in northern Europe to >10% in southern and western Europe (Edine *et al*., 2004).

Study in Iran show that 9.9% of all *S. aureus* isolated from patients and health care workers was MRSA and the rate of MRSA and antibacterial resistance was higher in the *S. aureus* isolated from clinical infection (25%), compared with those from carries (7.9%) and in another study in Iran prevalence of MRSA strains in health care workers was 11.8%. The control of MRSA has become a significant problem in hospital (Mansouri and Khaleghi, 1997).

Medical plants have been used for centuries as remedies for human disease because they contain components of therapeutic value and source of both traditional and modern medicine and by increasing resistance of pathogenic bacteria to antibiotics in last few decades, many new compounds as a substitute for non-effective antibiotic. Compounds and extracts of the plants could be part of these substitutes. In this study we assessed efficacy of 23 medical plants that obtained in Golestan province in north of Iran against 14 standard and local isolated MRSA and MSSA strains.

MATERIALS AND METHODS

Plants: Fresh plant materials of 23 plants species commonly used in folk medicine in Iran were collected from natural habitat in Golestan Province in north of IRAN. Mature plants and their parts were collected from different places during the months of April-May 2005. Their botanical identities were determined and authenticated Samples were deposited in the Department Herbarium. The plants used in study were as follows (Table 1).

Extraction

Ethanol extract: Plants parts were dried, ground to find texture and after which it, 70% ethanol were added to 50 g of dried plants powder in decanter for extended periods and the resultant extracts were obtained in period of 24 h. The resultant extracts were concentrated, under reduced pressure finally each samples were diluted with propylene glycol for provide 4 concentrations: 200, 100, 50 and 25 mg mL^{-1} (Dulgar and Gonuz, 2004; Mashhadian and Rakhshandeh, 2004; Murphy, 1999).

Aqueous extract: Hundred millilitre of hot sterile distilled water, 70-80°C, was added to the 30 g powder samples which were allowed to soak for 24 h in water bath at 45-50°C. The extracts were filtered by using filter- paper and the resultant extracts were transformed to sterile glass dishes. Finally obtained 4 dilutions: 1, 1/2, 1/4 and 1/8 (Mashhadian and Rakhshandeh, 2004).

Bacterial strains: The *S. aureus* strains used in this study were clinical isolates from patients presenting of *S. aureus* associated disease. The isolates were identified by standard method. The MRSA isolated were identified by screening tests were done on Mueller Hinton Agar (MH agar) supplemented with 4% NaCl and the oxacillin disc contain 1 mg mL^{-1} placed on it for isolating MRSA (Roberts *et al*., 2002).

Table 1: Ethonbotanic data of studied plants

Scientific name	Family	Activity	Plants part used
Eucalyptys global	Myrtaceae	Effective against *E. coli-pesudomonas.* Sp. *S.aureus, klebsiella*	Leaves
Menta piperita	Lamiaceae	General anti septic	Leaves
Rosmarinus officinalis	Lamiaceae	Effective against *E. coli*, *S. aureus*	Mature plants
Hypericum perforatum	Hypericaceae	General	Mature plants
Nigella sativa	Ranuculaceae	Effective against *P. pyogens S. aureus*, *Sviridans*	Fruits
Juniperus communis	Copressaceae	Anti septic	Fruits
Urtica dioica	Urticaceae	Effective against *E. coli*, *Proteus, Kleseilla*, *Salmonella*	Leaves
Brasica napus	Brasicaceae	Treatment of wound	Fruits
Matricaria chammomila	Astraceae	Acts of anti septic, anti fangi, anti bacterial	Flower
Cuminum cyminum	Apiaceae	Used for cuogh, anti bacterial	Sead
Thymus vulgaris?	Lamiacea	Anti bacterial, anti fangi	Mature plants
Allium sativum	Alliaceae	Anti viral, anti biotic	Mature plants
Berberis vulgaris	Berberidaceae	Effective against *N. menangiridis*	Root
Peganum hermala	Zygophyllaceae	Anti bacterial	Mature plants
Echinacea purpurea	Asteraceae	Use for cuogh treats inflomations of urinory tract system	Root
Artemisia herbaalba	Asteraceae	Anti bacterial, anti fungi	Mature plants
Tamarix aphylla	Tamariaceae	Anti bacterial, anti fungi	Flower
Punica granatum	Punicaceae	Treatment for wound	Fruits
Artemisia dracunculus	Asteraceae	Treatment for wound	Mature plants
Salvia tomentosa	Lamiaceae	Acts as anti septic	Mature plants
Thymus carmanicus	Labiate	Anti bacteria	Mature plants
Artemisia absinthium	Astraceae	Anti septic	Mature plants
Gossypium herbaceum	Gossypiceae	General	Sead

Finally we obtained 8 MRSA strains and 4 MSSA strains from patients. Two standard strains, ATCC 25923 (MRSA) and PTCC 1341 (MSSA), that obtained from Scientific and Technological Research center in Iran, also were used in this study.

The organisms were maintained on agar slope at 4°C and sub-cultured for 24 h before use.

Bacterial susceptibility testing

Disk diffusion method: sterile paper Blank disk previously soaked in different concentration of ethanolic extract (200, 100, 50, 25 mg mL^{-1}) with the final amount of extract in disks: 4, 2, 1 and 0.5 mg per disk and disks previously soaked in different dilution of aqueous extract with specific amount of it were prepared too. Mueller Hinton Agar plates were cultured with a standardized inoculums(1-2×10^8 cfu mL^{-1} equal to 0.5 macfarland) of each bacterial strain then the blank disks contain specific amount of extracts were carefully placed at the labeled seeded plate (Nostro, 2000).

The plates were incubated aerobically at 37°C and examined for zones of inhibition after 24 h. The inhibition zones were measured with a ruler and compared with the control disks (disk containing only propylene glycol that used as diluents of ethanolic extract and disk containing vancomycin 30 mg as positive control) (El Astal *et al*., 2005). Each test was repeated 3 times and means inhibition zone were recorded. Inhibitory zone ≥12 mm used as good inhibitory effect of extract (Nostro *et al*., 2000).

Micro dilution broth method: The ethanolic extracts of plants that showed an inhibition zone≥12 mm in disk diffusion method were chosen to assay the Minimum Inhibitory Concentration (MIC) with the Broth micro dilution method. Two fold serial dilutions of extracts were obtained with propylene glycol at a final concentration ranging from 200 to 0.01 mg mL^{-1}. Hundred microliter of each diluted extract and 100 mL of each bacterial suspension on Mueller Hinton broth (final inoculums of 10^5 bacteria mL^{-1}), were added in to each Elisa wells. The bacterial suspensions were used as negative control and vancomicin 30 mg were used as positive control. The OD of each well, were determine in 630 nm, by Elisa reader instrument, after 24 h of incubation at 37°C. the lowest concentration of the extracts in the wells that don't showed any addition on OD after this time, signed as the MIC value (National Committee for Clinical Laboratory Standards, 1993; Thornsberry and Dougal, 1983). Each test was assayed in triplicate.

RESULTS

The results of antibacterial activity of the ethanolic extracts of these plants revealed that, the high concentration only 8 ethanol extracts (4 mg mL^{-1}) of the 23 plants had good inhibitory effect against *Staphylococcal* strains with inhibition zones between 12.2 to 22.5 mm and the largest zone belong to punica granatum (22.5 mm). Seven out of 8 plants with good anti

Table 2: Inhibition zone (mm) of ethanolic and aqueous extract of 23 plants at various concentration on MRSA

		Diometer of inhibition zone (mm)										
Test extracts	conc. (mg mL^{-1})	*Nigella sativa*	*Eucalyptus global*	*Hypericum perforatum*	*Brasica napus*	*Cuminum cyminum*	*Urtica dioica*	*Juniperus communis*	*Matricaria chammomila*	*Rosmarinus officinalis*	*Menta piperita*	*Artemisia herbaalba*
Ethanolic	4.0	19.0	17.0	13.0	11.5	11.5	7.6	7.5	10.6	8.5	7.5	22.5
	2.0	15.0	15.0	14.5	10.5	10.0	8.4	7.5	8.6	8.0	0.0	18.0
	1.0	13.5	14.5	18.5	9.5	9.0	8.7	7.5	0.0	8.0	0.0	15.0
	0.5	13.0	11.5	20.0	8.0	8.5	9.0	8.0	0.0	0.0	0.0	11.5
Aqueous	4.0	1.0	14.0	7.6	7.0	0.0	0.0	0.0	8.0	8.5	7.0	12.0
	2.0	0.0	11.0	2.0	0.0	0.0	0.0	0.0	7.5	7.5	0.0	11.5
	1.0	0.0	9.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.2
	0.5	0.0	7.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.4

Table 2: Continued

	Diometer of inhibition zone (mm)											
Test extracts	*Peganum hermala*	*Berberis vulgaris*	*Tamarix aphylla*	*Thymus vulgaris*	*Artemisia absinthium*	*Allium sativum*	*Gossypium herbaceum*	*Echinaceae purpurea*	*Punica granatum*	*Thymus carmanicus*	*Salvia tomentosa*	*Artemisia Dracunculus*
Ethanolic	18.0	12.5	12.2	10.5	9.0	7	7.5	0	17.7	11.2	8.4	8
	15.0	11.5	11.6	8.2	8.5	7	9.0	0	16.1	9.4	0.0	7
	13.0	10.0	11.0	7.5	8.5	7	9.0	0	14.6	8.1	0.0	0
	11.0	9.0	10.0	0.0	8.5	7	9.0	0	13.6	6.0	0.0	0
Aqueous	7.4	8.4	2.0	0.0	0.0	0	0.0	0	16.7	0.0	0.0	0
	0.0	0.0	0.0	0.0	0.0	0	0.0	0	15.3	0.0	0.0	0
	0.0	0.0	0.0	0.0	0.0	0	0.0	0	12.4	0.0	0.0	0
	0.0	0.0	0.0	0.0	0.0	0	0.0	0	10.6	0.0	0.0	0

Table 3: Inhibition zone (mm) of ethanolic and aqueous extract of 23 plants at various concentration on MSSA

		Diometer of inhibition zone (mm)										
Test extracts	Conc. (mg mL^{-1})	*Nigella sativa*	*Eucalyptus global*	*Hypericum perforatum*	*Brasica napus*	*Cuminum cyminum*	*Urtica dioica*	*Juniperus communis*	*Matricaria chammomila*	*Rosmarinus officinalis*	*Menta piperita*	*Artemisia herbaalba*
Ethanolic	4.0	14.0	15.5	10.5	8.0	8.5	7.2	7.0	8.8	7.0	8.5	11.0
	2.0	12.5	14.5	14.0	8.0	8.5	8.0	7.5	0.0	7.0	0.0	10.0
	1.0	11.0	13.0	15.0	8.0	8.5	8.6	7.7	0.0	6.5	0.0	9.0
	0.5	10.0	10.5	15.5	8.0	8.0	8.9	8.0	0.0	6.5	0.0	8.0
Aqueous	4.0	0.0	11.0	7.6	7.6	0.0	8.2	0.0	7.5	7.5	7.5	9.0
	2.0	0.0	9.3	0.0	0.0	0.0	7.4	0.0	0.0	0.0	0.0	8.0
	1.0	0.0	8.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.5
	0.5	0.0	7.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.0

Table 3: Continued

	Diometer of inhibition zone (mm)											
Test extracts	*Peganum hermala*	*Berberis vulgaris*	*Tamarix aphylla*	*Thymus vulgaris*	*Artemisia absinthium*	*Allium sativum*	*Gossypium herbaceum*	*Echinaceae purpurea*	*Punica granatum*	*Thymus carmanicus*	*Salvia tomentosa*	*Artemisia. dracunculus*
Ethanolic	20.0	15.5	12.8	9.0	8.0	7.0	9.0	0	16.2	9.0	6.8	7
	16.0	13.5	12.0	7.5	8.0	7.3	8.5	0	14.4	8.4	0.0	0
	14.5	12.0	11.2	6.0	8.5	7.6	9.0	0	12.0	7.8	0.0	0
	11.5	10.0	10.0	0.0	8.5	7.6	9.5	0	10.8	7.0	0.0	0
Aqueous	0.0	7.0	0.0	0.0	0.0	0.0	0.0	0	13.0	0.0	0.0	0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	12.0	0.0	0.0	0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	11.4	0.0	0.0	0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	9.8	0.0	0.0	0

Table 4: MIC value of effective ethanolic plants extracts on MRSA and MSSA

	MIC values (mg mL^{-1})							
Bacterial strains	*Eucalyptus global*	*Peganum hermala*	*Punica granatum*	*Tamarix aphylla*	*Berberis vulgaris*	*Nigella sativa*	*Hypericum Perforatum*	*Artemisia Herbaalba*
MRSA$_{(n=s)}$	0.18	0.02	0.01	0.78	0.39	0.04	0.18	0.39
MRSAst	0.09	0.02	0.02	0.04	0.04	0.02	0.18	0.04
MSSA$_{(n=4)}$	0.39	0.02	0.02	0.02	0.39	0.04	0.02	0.39
MSSAst	0.39	0.02	0.04	0.02	0.04	0.02	0.02	0.02

Table 5: Percent activity of effective ethanolic plants extract on MRSA and MSSA

	Percent activity plants extract							
Bacterial strains	*Punica granatum*	*Eucalyptus global*	*Artemisia Herbaalba*	*Peganum hermala*	*Tamarix aphylla*	*Berberis vulgaris*	*Nigella sativa*	*Hypericum Perforatum*
MRSA	100	100	100.0	100	88.8	88.8	88.8	100.0
MSSA	100	100	40.0	100	100.0	100.0	80.0	60.0
Total	100	100	78.5	100	92.8	92.8	85.7	85.7

Table 6: Percent activity of effective aqueous plants extract on MRSA and MSSA

	Percent activity plants extract		
Bacterial strains	*Punica granatum*	*Eucalyptus global*	*Artemisia herbaalba*
MRSA	100	100	66
MSSA	100	60	0
Total	100	78	42

Staphylococcal effect, means *Hypericum perforatum*, *Nigella sativa*, *Peganum harmalla*, *Punica granatum*, *Eucalyptys global*, *Berberis vulgaris* and *Tamarix aphylla* showed good antibacterial activity against all MRSA and MSSA strains but *Artemisia herbaalba* had very strong antibacterial activity against MRSA but low activity against MSSA strain (Table 2 and 3).

Although the best inhibition action of these plants were seen in 4 mg mL^{-1} but in *Hypericum Perforatum* the best effect were seen in 0.5 mg mL^{-1} (Table 2 and 3) among 23 medical studied plants only The aqueous extract of 3 plants: *Punica granatum*, *Eucalyptys global* and *Artemisia herbaalba* have good inhibition zone against both MRSA and MSSA and *Punica granatum* had the best antibacterial activity and having inhibition zone 16.7 mm (Table 2 and 3).

Data in Table 2 and 3 clearly illustrates that ethanolic extracts of 8 plants and aqueous extract of 3 plants had a

broad action against most of the tested microorganisms. All of the other extracts at one or more tested concentrations showed some activity against one or more microorganisms.

The results of MIC tests in Table 4 were shown that: the highest MIC values were measured as 0.78 mg mL^{-1} in *Berberis vulgaris* against MRSA with 12.5 mm DIZ (Diameter Inhibition Zone) and the lowest MIC values were measured in *Punica granatum*, as 0.01 mg mL^{-1} against MRSA (Table 4).

Percent activities of each effective plant extract were calculated. Ethanolic extract of *E. global*, *P. granatum*, *P. hermala* and aqueous extract of *P. granatum* had a 100% activity on MRSA and MSSA (Table 5 and 6).

DISCUSSION

The potencies of traditional antibiotics are decreasing steadily since drug-resistant bacteria are globally increasing.

MRSA poses and increasingly serious health care problem in many parts of the world. Several studies have reported increased morbidity and mortality associated with MRSA compared to Methicillin-suceptible *S. aureus* (MSSA) infections.

Medical plants have been used for a wide variety of purposes for many thousand of years in countries. The increasing use of plant extracts in the pharmaceutical industries suggests that, in order to find active compounds of medical plants is very important.

Recently, the antimicrobial activity of various plant extracts has been studied on the growth of many microorganisms.

Duglar and Gonuz (2004) observed antimicrobial activity of ethanolic extracts of 16 Turkish plants against nine bacterial species using the disk diffusion method. Of the 16 plant tested, ten showed antimicrobial activity and *S. aureus* is more susceptible to the extracts. Although, the results of the study on 6 Nigerian medical plants indicated that both ethanol and aqueous extracts of four out of six medical plants showed good activity against MRSA strains (Akinyemi *et al*., 2005) and the antibacterial activity of aqueous, ethanolic and phenolic extracts from three Palestinian medical plants in addition to their commercial oils against ten pathogen microorganisms, among the 10 tested microorganisms *S. aureus* was, the most susceptible microbe to most extracts of the three plants studied (El Astal *et al*., 2005). We here report a comparative study on the antimicrobial properties of two different extracts of 23 plants in order to choose that which gives the most efficient anti microbial compounds.

The results obtained in this study indicate a considerable difference in antimicrobial activity between ethanolic and aqueous extracts.

Both aqueous and ethanol extract of *P. granatum*, *A. herbaalba* and *E. global* were effect on MRSA strains. Ethanolic and aqueous extract of *P. granatum* had the best antibacterial activity against microorganisms. This activity was more pronounced against MRSA strains than MSSA.

The results obtained from ethanolic and aqueous extracts were compared, it was determined that the ethanolic extracts have higher antibacterial activity and MRSA strains were more susceptible to the extracts ($p<0.05$).

The plants differ significantly in their activity against tested microorganisms (MRSA and MSSA). This differences may attributed to fact that photochemical properties and differences a many species and for the evaluation of plants which are naturally grown in Golestan province are potential useful resources, additional studies will be beneficial from medicinal and economic stand point.

In conclusion, ethanolic, especially the extracts of *P. granatum*, can be used for protection against MRSA and MSSA strains.

It seems important to recommend that, further studies using isolated constituents instead of whole extract must be done in this field.

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