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Inhibition of Three Isolates of *Staphylococcus aureus* Mediated by Plants used by Iranian Native People

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The resistance of *Staphylococcus aureus*, a gram-positive bacterium causing several pathogenic disorders, to antibacterials, is increasing worldwide. To investigate for pant antibacterials effective against *S. aureus*, 180 methanolic plant extracts of 72 plant families, having ethnopharmacological usage by Iranian Native People, were tested against three isolates of *S. aureus* in an *in vitro* bioassay. Seventy-nine plant samples in 41 families exhibited anti-*S. aureus* activity. Bioactivities were determined by measuring Diameter of inhibition zones. The extracts with the broadest spectra of activity, were used to evaluate Minimum inhibitory concentrations (MIC). The least MICs belonged to *Myrtus communis* and *Terminalia chebula* seeds as 0.93 and 0.46 mg ml^{-1}, respectively. The active extracts were well stable in dimethyl sulfoxide: methanol (1:1, v/v) solvent and dried forms at room temperature with no reduction of activity up to 18 months.

Key words: Antibacterial, plant extracts, folkloric medicine, *Staphylococcus aureus*

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INTRODUCTION

Staphylococci are members of the normal flora of the human skin, respiratory and gastrointestinal tracts. Pathogenic, invasive *S. aureus* tends to produce several disorders such as focal suppuration (abscess), pneumonia, meningitis following skull fracture, empyema, endocarditis, chronic osteomyelitis subsequent to an open fracture, pulmonary infection, or sepsis with suppuration in any organ. Serious multiple skin infections (furunculosis) may occur in adolescents and in patients receiving prolonged courses of corticosteroids. *S. aureus* also causes disease through the elaboration of toxins, without invasive infection as in toxin shock syndrome, which in severe cases may lead to onset of cardiac and renal failure[1]. *S. aureus* developing resistance to antibacterials is a problem worldwide. Based on WHO reports[2], increase of antimicrobial resistance is a growing global problem. Since the semi synthetic penicillins arrived in the 1960s it was not before staphylococcal resistance emerged and for the 15 years methicillin-resistant *S. aureus* (MRSA) has been troubling hospital services all over the world[3,4]. Contact spread of infection has assumed importance in hospitals, where a large portion of the staff and patients carry antibiotic-resistant *S. aureus* in the nose or on the skin. Reports on isolation of pathogenic *S. aureus* less susceptible to regular therapies and recovery of increasing resistant isolates during antibacterial therapy is increasing in literature[5-16]. To minimize the increasing rate of resistance throughout the time, it is a necessity to have continuous research for new, safe and effective antimicrobials as alternative agents to rotate or replace with no effective ones. Plants and microorganisms are potent candidates for this purpose. In this regard, many workers have reported antibacterials from plant origins. McCutcheon *et al.*[11] tested 100 methanolic extracts of the plants, used by British Colombian Native People, against 11 bacterial isolates. They found 85% of the plants were active at least against one of the bacteria. Mansouri[18] tested ethanolic extracts of 11 plants against 489 samples of *S. aureus* and noticed *Myrtus communis* leaves had the greatest activity, inhibiting the growth of 99% of the isolates. Adedayo *et al.*[19] reported antibacterial potency of *Senna alata* flower extract against *S. aureus*, *S. faecalis, Micrococcus luteus, Bacillus subtilis* and *Pseudomonas putida.* Mansouri *et al.*[20] evaluated antibacterial activity of crude extract of *Myrtus communis* against 10 laboratory strains of bacteria. They noticed that the crude extract inhibited the growth of all tested bacteria except *Campylobacter jejuni.*

At the present study, in a three year survey, a procedure was set to screen and evaluate anti-*S. aureus* activity of a vast number of plants which are used by Iranian Native People (INP) as antiseptic, anti-inflammatory and in treatment of infectious diseases and dermatophytes. According to the information gathered about their ethnopharmacological usages, the plant organs used in this study are as used in INP.

MATERIALS AND METHODS

Plant material and extraction procedure: One hundred eighty plant species belonging to 72 families, being used by INP, were collected from South-East regions of Iran and identified by Mrs. P. Rashid Farrokhi in the Herbarium of Plant Systematic Laboratory of the College of Agricultural Sciences, Bahonar University of Kerman, Iran, where voucher specimens of plants were deposited. Sixteen species, used in INP but not grown in Iran, were obtained from the mentioned Herbarium. The fine powder of air dried specimens were extracted three times with methanol at 70°C for 4 h and the extracts were then concentrated under reduced pressure to yield a dense residue. Sample were transferred to glass vials and lyophilized overnight before use.

Test organisms and agar well diffusion bioassay: From Persian Type Culture Collection, Tehran, Iran (PTCC), three registered isolates of *S. aureus* (PTCC No. 1112, International No. ATCC 6538P), (PTCC No. 1113, International No. ATCC 9144, NCIB6571, NRRL B-314, NCTC6571) and (PTCC No. 1337, International No. ATCC 29737) were obtained. The bacteria were rejuvenated on Mueller-Hinton-Agar medium (MHM, Merk, Germany) and subcultured as needed. For bioassays, suspension of approximately $1.5x10^8$ cells ml^{-1} in sterile normal saline were prepared as described by Forbes *et al.*[21] and about 1.5 ml of it was uniformly seeded on MHM in 12x1.2 cm glass Petri dishes, left aside for 15 min and excess of suspension was then drained and discarded properly. Wells of 6 mm in diameter and about 2 cm apart were punctured in the culture media using sterile cork borers. As a precaution not to miss trace amounts of antimicrobials, a relatively high concentration of 20 mg ml^{-1} of each extract was prepared in dimethyl sulfoxide (DMSO): methanol (1:1, v/v) solvent and administered to fullness in each well. Culture plates, were incubated at 37°C for 48 h and then bioactivity was determined by measuring Diameter of inhibition zones (DIZ) in mm. All samples were tested in triplicate. Controls included incorporated growth medium without test compounds and where applicable, solvent controls were included, although no antibacterial activity noted in the solvent employed for the test.

Determination of minimum inhibitory concentration: Minimum Inhibitory Concentrations (MIC) were

determined for the most active plant extracts using two-fold dilution series of 15-0.46 mg ml^{-1} in DMSO: methanol (1:1, v/v) solvent and tested as mentioned earlier against the three isolates of *S. aureus*.

Determination of shelf life or stability of the crude extracts: To measure the stability of the bioactive extracts in both solubilized and dry states, 20 mg ml^{-1} of each was prepared in DMSO: methanol (1:1, v/v) solvent and 20 mg dry samples in small vials. The samples kept at room temperature and tested for antibacterial activity against *S. aureus* (PTCC No. 1112) (the most sensitive isolate) at 14 days intervals up to 18 months.

RESULTS

From 180 plant samples in 72 families used by INP, 79 samples in 41 families showed anti-*S. aureus* activity (Table 1). Umbelliferae with 12, Labiatae and Compositae each with 5, had most number of active samples per plant family. All other 101 plant samples did not show any inhibitory effects, hence are not listed in the Table 1. At 20 mg ml^{-1} concentration, the largest DIZ (>20 mm) belonged to *Myrtus communis*, *Cinnamomum zeylanicum*, *Terminalia chebula*, *Zingiber officinale* and *Pimpinella anisum*. Twenty-eight samples had the broadest spectra of activity, being effective against all three isolates. Seventeen samples were active against two and 34 samples were effective against only one isolate. The MIC values determined against the three isolates of *S. aureus* via two fold serial dilutions (Table 2). The lowest MICs found in *T. chebula*, *Eucalyptus globolus* and *Citrus medica* as 0.46 mg ml^{-1} and in *M. communis*, *Glycyrrhiza glabra, Lawsonia inermis* as 0.92 mg ml^{-1}, respectively. All of the active extracts were well stable at room temperature in both DMSO: methanol (1:1, v/v) solvent and dry state up to 18 months and did not show any reduction of activity against *S. aureus* as compared to the activities of the starting day.

Table 1: Evaluation of antibacterial activity, indicated by diameter of inhibition zones (DIZ, mm), of plants used in Iranian Native Medicine against 3 strains of *S. aureus*. Plant species, families, organs used and bacterial isolates are indicated. Plants are listed according to their relative spectra of activity

c	b	a	OT	Plant family	Plant species
24	20	28	LE	Myrtaceae	*Myrtus communis*
21	17	22	SE	Myrtaceae	*Myrtus communis*
22	20	20	SB	Lauraceae	*Cinnamomum zeylanicum*
21	21	18	RS	Combretaceae	*Terminalia chebula*
18	23	15	US	Combretaceae	*Terminalia chebula*
19	18	17	FR	Anacardiaceae	*Rhus coriaria*
20	18	15	ST	Liliaceae	*Smilax china*
23	15	15	RH	Zingiberaceae	*Zingiber officinale*
25	11	11	SE	Umbelliferae	*Pimpinella anisum*
15	15	16	WP	Caryophyllaceae	*Dianthus coryophyllus*
20	14	12	WP	Ranunculaceae	*Ranunculus asitaticus*
20	12	13	WP	Solanaceae	*Capsicum annum*
17	14	14	FR	Berberidaceae	*Berberis vulgaris*
17	16	11	RO	Papilionaceae	*Glycyrrhiza glabra*
16	16	12	LE	Rosaceae	*Rubus idaeus*
16	16	12	SE	Convolvulaceae	*Cuscuta epithymum*
14	13	16	LE	Euphorbiaceae	*Chrozophora verbasafalia*
16	13	12	LE	Anacardiaceae	*Semicarpus anacardium*
16	13	12	SE	Umbelliferae	*Trachysermum copticum*
14	14	13	SG	Leguminosae	*Alhagi camelorum*
15	13	12	SE	Zingiberaceae	*Amomum subulatum*
17	11	12	RO	Polygonaceae	*Rheum ribes*
17	11	10	FL	Nymphaceae	*Nymphaea alba*
13	13	12	WP	Labiatae	*Thymus vulgaris*
16	10	10	WP	Labiatae	*Salvia officinalis*
12	12	12	FR	Zingiberaceae	*Alpinia officinarum*
14	11	10	SE	Myristicaceae	*Myristica fragrans*
12	12	10	FR	Umbelliferae	*Anethum graveolens*
16	0	17	LE	Lythraceae	*Lawsonia inermis*
20	12	-	FR	Umbelliferae	*Heracleum persicum*
16	16	-	LE	Ephedraceae	*Ephedra intermedia*
18	10	-	SE	Umbelliferae	*Cuminum cyminum*
18	10	-	SE	Umbelliferae	*Trachyspermum ammi*
18	10	-	SG	Papilionaceae	*Alhagi camelorum*
10	10	9	FL	Violaceae	*Viola odorata*
10	15	-	SE	Cucurbitaceae	*Citrullus colcocynthis*
10	15	-	FR	Cucurbitaceae	*Citrullus colcocynthis*
12	12	-	FR	Umbelliferae	*Cuminum cyminum*

Table 1: Continue

c	b	a	OT	Plant family	Plant species
10	10	-	LE	Rutaceae	*Ruta graveolens*
10	10	-	FL	Boraginaceae	*Echium amoenum*
10	9	-	LE	Theaceae	*Camellia sinensis*
9	10	-	FL	Compositae	*Calendula officinalis*
9	10	-	FR	Berberidaceae	*Berberis integerrima*
17	-	-	SE	Umbelliferae	*Trigonella foenum graecum*
17	-	-	FR	Cesalpinaceae	*Cassia fistula*
9	-	11	LE	Myrtaceae	*Eucalyptus globolus*
9	-	10	SG	Leguminosae	*Astragalus gossypinus*
18	-	-	SE	Zygophyllaceae	*Peganum harmala*
17	-	-	FL	Punicaceae	*Punica granatum*
18	-	-	LE	Polygonaceae	*Rumex acetosa*
16	-	-	LE	Tamaricaceae	*Tamarix gallica*
16	-	-	SE	Rutaceae	*Citrus medica*
15	-	-	FR	Rhamnaceae	*Ziziphus spini-christi*
12	-	-	RO	Umbelliferae	*Foeniculum vulgare*
10	-	-	FL	Malvaceae	*Althaea officinalis*
10	-	-	FL	Compositae	*Marticaria chamomilla*
10	-	-	LE	Rhamnaceae	*Ziziphus spini-christi*
10	-	-	SE	Portulaceae	*Portulaca oleracea*
10	-	-	SE	Cruciferae	*Raphanus sativus*
10	-	-	ST	Compositae	*Tussilago farfara*
10	-	-	RO	Malvaceae	*Althea officinalis*
10	-	-	RO	Graniaceae	*Bieberstenia multifida*
10	-	-	LE	Compositae	*Echinops cephalotes*
10	-	-	FL	Compositae	*Anthemis nobilis*
10	-	-	WP	Colchicaceae	*Colchicum luteum*
10	-	-	SE	Umbelliferae	*Coriandrum sativum*
10	-	-	FL	Papaveraceae	*Papaver rusticum*
9	-	-	LE	Labiatae	*Nepeta menthoides*
9	-	-	LE	Umbelliferae	*Apium celleri*
9	-	-	LE	Labiatae	*Origanum majorana*
9	-	-	FL	Salicaceae	*Salix aegyptica*
9	-	-	LE	Urticaceae	*Urtica atlantica*
9	-	-	FL	Violaceae	*Viola odorata*
9	-	-	FL	Juglandaceae	*Juglans rejia*
9	-	-	RO	Labiatae	*Nepeta menthoides*
9	-	-	LE	Cruciferae	*Lepidium campestre*
9	-	-	SE	Rosaceae	*Rosa gallica*
9	-	-	SE	Umbelliferae	*Petroselinum sativum*
8	-	-	SE	Ranunculaceae	*Nigella sativa*

OT: Organs tested, as FL: Flower, FR: Fruit, LE: Leaves, PE: Petals, RH: Rhizome, RO: Roots, SB: Stem Bark, SE: Seeds, SG: Stem Gum, ST: Stem and WP: Whole Plant. a: *S. aureus* (PTCC No. 1112), b: *S. aureus* (PTCC No. 1113), c: *S. aureus* (PTCC No. 1337)

Table 2: Antibacterial inhibitory actions, indicated as diameter of inhibition zones (DIZ, mm), of the most active plant extracts determined in two fold serial dilutions against three isolates of *S. aureus*. Concentrations at which DIZs are indicated in bold represent the MIC of the extract on the respective bacterium. Plants are listed according to their relative spectra of activity

	Mg (ml^{-1}) DIZ (mm) on three isolates of *S. aureus*																	
	0.46			0.93			1.87			3.75			7.5			15		
Plant species	c	b	a	c	b	a	c	b	a	c	b	a	c	b	a	c	b	a
Myrtus communis	0	0	0	**9**	0	**8**	11	0	12	10	**10**	15	22	14	17	26	18	22
Terminalia chebula	0	0	**9**	0	0	12	0	**10**	14	**13**	13	15	11	16	17	14	17	17
Terminalia chebula	0	0	0	0	0	0	**9**	0	**10**	10	**10**	12	11	12	13	13	15	16
Cinnamomum zeylanicum	0	0	0	0	0	0	0	0	0	**11**	**11**	**10**	13	14	14	17	19	19
Zingiber officinale	0	0	0	0	0	0	0	0	**10**	**10**	0	14	10	**11**	17	12	13	20
Pimpinella anisum	0	0	0	0	0	0	0	0	**10**	**10**	0	14	0	0	18	8	**9**	22
Dianthus coryophyllus	0	0	0	0	0	0	0	0	0	0	**10**	**12**	**13**	11	12	15	14	14
Smilax china	0	0	0	0	0	0	0	0	0	0	0	**10**	**9**	**11**	14	12	14	17
Glycyrrhiza glabra	0	0	0	0	0	**10**	0	0	11	0	0	11	0	0	13	**9**	**14**	15
Rhus coriaria	0	0	0	0	0	0	0	0	0	0	0	0	**10**	**9**	**10**	13	14	15
Rheum ribes	0	0	0	0	0	0	0	0	**10**	0	0	12	0	0	13	**10**	**9**	15
Nymphaea alba	0	0	0	0	0	0	0	0	0	0	0	**9**	0	0	12	**9**	**9**	15
Amomum subulatum	0	0	0	0	0	0	0	0	0	0	0	0	0	**10**	**11**	**9**	12	14

Table 2: Continue

	Mg (ml^{-1}) DIZ (mm) on three isolates of *S. aureus*																	
	0.46			0.93			1.87			3.75			7.5			15		
Plant species	c	b	a	c	b	a	c	b	a	c	b	a	c	b	a	c	b	a
Thymus vulgaris	0	0	0	0	0	0	0	0	0	0	0	0	0	**10**	**11**	**10**	13	11
Trachysermum copticum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**11**	**9**	**9**	12
Salvia officinalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**9**	**8**	**8**	13
Ranunculus asitaticus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**10**	**11**	**17**
Capsicum annum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**11**	**10**	**16**
Berberis vulgaris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**10**	**12**	**15**
Rubus idaeus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**10**	**14**	**14**
Cuscuta epithymum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**9**	**14**	**14**
Chrozophora verbasafalia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**13**	**10**	**11**
Semicarpus anacardium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**9**	**11**	**14**
Alpinia officinarum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**9**	**10**	**10**
Myristica fragrans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**9**	**8**	**11**
Anethum graveolens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**8**	**10**	**10**
Lawsonia inermis	0	0	0	0	0	**9**	0	0	11	**10**	0	12	13	0	14	16	0	15
Cuminum cyminum	0	0	0	0	0	0	0	0	**10**	0	0	10	0	0	13	0	**10**	16
Alhagi camelorum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**8**	0	**11**	12
Trigonella foenum graecum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**10**	0	0	13
Cassia fistula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**11**	0	0	15
Eucalyptus globolus	0	0	**9**	0	0	**8**	0	0	9	0	0	**8**	0	0	**8**	**9**	0	8
Peganum harmala	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**9**	0	0	14
Citrus medica	0	0	**9**	0	0	10	0	0	10	0	0	10	0	0	11	0	0	14

DISCUSSION

According to Brooks *et al.*[1] Penicillin G-resistant *S. aureus* strains from clinical infection constitute over 90% isolates in the USA. In the USA, naficillin-resistant *S. aureus* accounted for only 0.1% of isolates in 1970 but in mid-1980s constituted 10-30% of isolates from nosocomial infections in some hospitals. Accordingly, the increasing prevalence of drug-resistant *S. aureus* is a major concern worldwide. Smith *et al.*[4] express that the emergence of *S. aureus* resistance threatens to return us to the era before the development of antibiotics. In view of rapid emergence of drug resistance among *S. aureus*, although the origins of drug-resistant bacteria have been determined, in most countries it is generally difficult to manage the resistance upraise properly. Consequently, the potencies of traditional antibiotics are decreasing steadily leading to reduced useful-period of drugs. This situation implies heavy duties upon Health organizations of all nations to consider appropriate surveillance, proper reactive measures and put emphasis on research for new and safe antimicrobials especially from natural origins as plants and microorganisms for replacement with invalidated traditional antimicrobials or to be used in antibiotic rotation programs[22-24].

Although the nature and number of active antibacterial principles involved in each extract of the present research are not clear, but the broad spectra of activity of several plant extracts against *S. aureus* isolates opens new avenues for future studies. Since S. *aureus* (PTCC No. 1112) had the broadest spectra of activity among the three isolates, it may be used as a suitable isolate in preliminary screening programs when large numbers of samples are used. Evaluation suggested for the most active extracts (Table 2) against MRSA and vancomycin-resistant enterococci (VRE) isolates, two of the most problematic bacteria-in terms of their occurrence and impact on the clinical outcomes of hospitalized patients. The results of such studies form the basis for further investigation to isolate pure compounds, elucidate the structures and evaluate them against wide range of drug-resistant isolates with the goal to find new drugs for therapeutic usages to serve all humans.

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