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Antibacterial activity of eight Iranian plant extracts against methicillin and cefixime restistant *Staphylococcous aureus* strains

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ABSTRACT

Objective: To assess the antibacterial activity of eight Iranian plant extracts against *Staphylococcus aureus (S. aureus)* strains which were isolated from infected patients. **Methods:** The studied strains were isolated from urine, stool, blood and wound of infected patients and identified by biochemical tests. In further, the antibacterial activity of 8 ethanolic local plant extracts including *Querqus brantii (Q. brantii)*, *Ziziphus spina-christi* (*Z. spina-christi*), *Peganum harmala* (*P. harmala*), *Oliveira decumbens* (*O. decumbens*), *Galium tricornutum* (*G. tricornutum*), *Vitex pseudo negundo* (*Vi. pseudo negundo*), *Salvia officinalis* (*S. officinalis*) , *Vaccaria pyramidata* (*V. pyramidata)* were then evaluated using agar disc diffusion method. **Results:** A total of 9 isolates were isolated and identified as *S. aureus*. Antibacterial profile of the strains showed that the strains were resistant to methicillin and cefixime. The highest antibacterial activity against the studied strains were belong to *Q. brantii*, *P. harmala*, *Z. spina-christi* and *O. decumbens vent* extracts with 11-40 mm, 15-28 mm, 8-26 mm and 10-20 mm of diameters, respectively. Intermediate antibacterial activity was exhibited by *G. tricornutum* and *Vi. pseudo negundo* against some of the studied strains with 7-20 mm and 7-13 mm of diameters, respectively. Noteworthy, out of 9 studied strains; 6 and 2 of them were resistant to the *G. tricornutum* and *Vi. pseudo negundo* extracts, respectively. The *S. officinalis* and *Va. pyramidata*, however, showed no antibacterial activity against the studied strains. **Conclusions:** The *Q. brantii*, *P. harmala*, *Z. spina-christi* and *O. decumbens* extracts were shown to possess strong antibacterial activity against methicillin and cefixime resistant *S. aureus* strains and can be considered as the promising natural antibiotics for treating the studied strains.

1. Introduction

Staphylococcus aureus(S. aureus) is one of the most important pathogens that causes conjunctivitis, blepharitis, keratitis[1], suppuration, abscess formation, a variety of pyogenic infection, and even fatal septicemia. *S. aureus* which can induce bacteremia (associated with 80% mortality in the preantibiotic era), proved to be susceptible to the earliest antimicrobial substance; however *Staphylococcal* resistance rapidly developed as the level of antibiotic consumption increased[2]. Methicillin-resistant *S. aureus* (MRSA) emerged in the 1980s as a major clinical and epidemiologic problem in hospitals. Presently, hospitals of all sizes are facing the MRSA problem[3]. Today, MRSA is common in many areas of the world. The frequencies of infections and outbreaks due to MRSA have continued to increase. MRSA is often multidrug resistant and therapeutic options are limited[4, 5]. Therefore, novel antimicrobial agents are needed to address this problem[2].

As a result, plants remain the most common source of antimicrobial agents. Their usage as a traditional health remedies is the most popular for 80% of the world population in Asia, Latin America and Africa and is also reported to have minimal side effects[6]. The antimicrobial compounds from plants may inhibit microbial growth by different mechanisms compared with presently used antimicrobial agents and may have significant clinical value in treatment of resistant microbe[7]. Many studies indicate that in some plants there are many substances such as peptides, unsaturated long chain aldehydes, alkaloidal constituents and some essential oils that may have antibacterial activity[8-11]. These plants then emerged as compounds with potentially significant therapeutic application against human pathogens, including bacteria, fungi or viruses[12].

In Khuzestan, the southern west province of Iran, plants

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are widely being used as folklore remedies for infections. The aim of this study was to analyze the antimicrobial activity of the selected parts of eight local plants on MRSA strains from urine, stool, blood and wounds of the infected patients.

2. Materials and methods

2.1. Sampling and Identification

S. aureus strains were isolated from urine, stool, blood and wounds of infected patients at one of the hospitals in Khouzestan, Iran from November 2007 to May 2008. The isolates were identified by routine biochemical tests[13] prior to antibiotic susceptibility pattern analysis according to National Committee for clinical laboratory standards[14]. The methicillin-resistant isolates were then selected for further analysis.

2.2. Plant collection and identification

Eight plant species commonly used in folk medicine in Iran, particularly in Khuzestan, were tested for their antibacterial activity against MRSA isolates. The studied plants were collected from Ahvaz, Izeh and Behbahan (Three large cities in the Khouzestan province) (Table 1). The identities of the collected samples were further confirmed by our plant taxonomy research group. Voucher specimens were deposited at the Department of Biology, Shahid Chamran University, Iran.

2.3. Preparation of extracts

Plant extracts were prepared as described previously by Seyyednejad *et al*[15] with slight modifications. Briefly, the desired part of each plant (Table 1) was shade dried and crushed into powder using electric blender (Black & Decker, USA). 10 mL of 80% ethanol (v/v) was added to 1 g of the powder followed by vigorous vortexing for 1 min. The suspension was left at room temperature for 15 min prior to centrifugation at 3 000 rpm for 15 min. This process was repeated three times and the supernatant was collected. Finally the supernatant was incubated at room temperature until complete evaporation of ethanol. The precipitates were then scratched into powder form using sterilized scalpel. The desired concentration of plant extract was then prepared by dissolving the powder into sterilized distilled water.

2.4. Determination of antibacterial activity

Antibacterial activity of the ethanolic extracts was evaluated by paper disc diffusion method[16]. Stock culture of test bacteria were grown in TSB medium at 37 ℃ for 22 h. Final cell concentrations were adjusted to 10^8 cfu/mL with reference to the McFarland turbidometer[17]. 1 mL of this inoculum was added on the surface of each plate containing Mueller-Hinton agar (MHA, Oxoid) by sterile cotton swab and allowed to remain in contact for 1 min. Five concentrations of each extracts were prepared as follows 0.05, 0.15, 0.25, 0.35, 0.45 g/mL. Sterile 6 mm filter paper discs were placed on these cultures and immediately 40 μL of each concentration from each extract was loaded[18]. The plates allowed to remain 1 hour at room temperature in order to diffuse the extract across the surface and then were incubated at 37 ℃ for 24 h. The inhibition zone around each disc was measured in millimeter and the assay was carried out three times for each extract. 80% ethanol (v/v) impregnated disc was used to check whether it has any inhibitory effect on bacterial growth.

3. Results

3.1. Isolation, identification and antibiotic susceptibility patterns of S. aureus strains

A total of 86 *S. aureus* strains were isolated and identified according to routine biochemical tests[13], 9 of which were resistance to methicillin and cefixime according to antibiotic susceptibility pattern (Table 2) and selected for further analysis.

3.2. Preliminary evaluation of antimicrobial activity

The results obtained from the analysis of antibacterial activity exhibited by studied plant extracts are summarized in Table 3. Among all of the studied extracts, the *Querqus brantii (Q. brantii), Peganum harmala (P. harmala), Ziziphus spina-christi (Z. spina-christi)* and *Oliveira decumbens* (*O. decumbens*) extracts showed the highest antibacterial activity against MRSA isolates. However, the *Galium tricornutum* and *Vitex pseudo negundo* exhibited intermediate antibacterial activity at high concentrations against some of the studied strains. On the other hand, *Vaccaria pyramidata* and *Salvia officinalis* extracts showed no antibacterial activity. Ethanol impregnated disks did not have a zone of inhibition due to the volatile nature of ethanol, so it was not considered as a factor that it might affected the results[19].

The antibacterial activities of the *Q. brantii*, *P. harmala* and *Z. spina-christi* extracts were increased in accordance with their concentrations. Among these three extracts, *Q. brantii* showed the highest antibacterial activity against the studied strains with the widest halo zone of 40 mm diameter for the concentrations 0.35 g/mL and 0.45 g/mL.

4. Discussion

Infections caused by MRSA have increased over the past decade. The ability of *S. aureus* to cause a disease may be due to the production of large number of enzymes, toxins and other substances, some of which may play an important role in their capacity to cause conjunctivitis. The increasing occurrence of *S. aureus* resistant not only to methicillin but to a wide range of antimicrobial agents including vancomycin[20] and cefixime (this study) has made therapy more difficult[21].

Antibacterial effects of *Q. brantii* and *Z. spina-christi* extracts against some enteric pathogens were previously measured in our laboratory[22,23]. In this study, however, these extracts showed higher antibacterial activity against MRSA strains. Similarly, antibacterial activity of *Q. brantii* extract on some other gram-negative bacteria[24] and *Z. spina-christi* fruit extract on few gram positive and negative bacteria were previously reported[25].

The phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds are secondary metabolits of plants that serve as defense mechanisms against predation by many microorganisms, insects and herbivores[6]. The antibacterial activity of *Q. brantii* and *Z. spina-christi* detected in this study could probably be due to the presence of cyclopeptide alkaloids, several flavonoids in leaves of *Z. spina-christi*[26, 27] and tannins in fruits of *Q. brantii*[24, 28].

A large number of reports concerning antibacterial properties of *P. harmala* can be found in the literature. The antibacterial activity of *P. harmala* was attributed to

Table 1
List of plants and their parts used in the study with reference to their source.

Scientific name (family)	Vernacular name (in source)	Part used	Source
Querqus brantii (Fagaceae)	*Jaft*	Seeds	Izeh
Ziziphus spina-christi (Rhamnaceae)	*Sedr, Konar*	Leaves	Ahvaz
Peganum harmala (Nitrariaceae)	*Esfand*	Seeds	Behbahan
Oliveira decumbens (Umbelliferae)	*La´al kouhestan*	Aerial par	Behbahan
Galium tricornutum (Rubiaceae)	*Jeghjeghak*	Fruits	Izeh
Vitex pseudo negundo (Lamiaceae)	*Hendeh bid*	Seeds	Behbahan
Salvia officinalis (Lamiaceae)	*Maryam goli*	Aerial part	Behbahan
Vaccaria pyramidata (Caryophyllaceae)	*Shirpanir*	Seeds	Izeh

Table 2
Antibiotic susceptibility patterns.

S. aureus strains	Antibiotics							
	STX	ME	CP	CFM	TY	T	NFX	VAN
S1	23	R	28	R	20	R	32	16
S2	19	R	29	R	24	R	32	17
S3	21	R	29	R	22	26	33	21
S4	26	R	33	R	25	24	34	17
S5	R	R	R	R	R	R	15	18
S6	R	R	10	R	17	R	15	18
S7	22	R	34	R	28	16	33	21
S8	20	R	20	R	17	18	16	20
S9	26	R	17	R	15	R	17	18

STX: Sulfamethoxazole; ME: Methicillin; CP: Ciprofloxacin; CFM: Cefixime; TY: Tylosin; NFX: Nafcillin; VAN: Vancomycin.

Table 3
Antibacterial activity of the studied plant extracts at various concentrations against methicillin and cefixime resistant *S. aureus* strains.

S. aureus strains	Inhibition zones (mm)* of plants extracts at various concentrations (g/mL)																			
	Q. brantii					*Z. spina-christi*					*P. harmala*					*O. decumbens vent*				
	0.05	0.15	0.25	0.35	0.45	0.05	0.15	0.25	0.35	0.45	0.05	0.15	0.25	0.35	0.45	0.05	0.15	0.25	0.35	0.45
S1	17	21	25	21	21	11	13	14	15	17	17	17	18	21	22	R	R	10	11	11
S2	16	19	25	20	22	10	13	14	15	16	15	15	16	18	20	R	R	R	10	10
S3	14	19	21	22	25	12	16	18	20	22	20	20	20	20	24	R	11	12	15	16
S4	16	19	21	25	32	12	20	23	24	26	23	23	24	26	28	R	R	10	14	14
S5	11	13	20	21	23	10	12	16	16	17	15	15	16	17	21	R	10	11	12	13
S6	12	20	24	27	27	8	12	14	14	16	16	16	16	18	21	R	R	7	11	11
S7	18	21	22	24	27	12	16	18	20	22	16	16	17	19	21	R	R	10	11	14
S8	27	31	32	40	40	12	17	20	22	24	20	20	21	26	28	R	12	13	17	20
S9	20	25	30	31	32	10	15	17	19	21	17	18	20	22	24	R	12	16	17	18
	G. tricornutum					*Vi. Pseudo negundo*					*S. officinalis*					*Va. Pyramidata*				
	0.05	0.15	0.25	0.35	0.45	0.05	0.15	0.25	0.35	0.45	0.05	0.15	0.25	0.35	0.45	0.05	0.15	0.25	0.35	0.45
S1	R	R	R	R	8	R	R	R	R	7	R	R	R	R	R	R	R	R	R	R
S2	R	R	R	R	R	R	7	7	7	9	R	R	R	R	R	R	R	R	R	R
S3	R	R	R	R	R	R	R	R	7	10	R	R	R	R	R	R	R	R	R	R
S4	R	7	7	11	12	R	7	9	11	11	R	R	R	R	R	R	R	R	R	R
S5	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
S6	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
S7	R	7	12	12	12	R	R	7	7	10	R	R	R	R	R	R	R	R	R	R
S8	R	10	13	17	20	R	10	10	12	13	R	R	R	R	R	R	R	R	R	R
S9	R	R	R	R	R	R	R	8	9	10	R	R	R	R	R	R	R	R	R	R

R: Resistant, *: the discs were 6 mm of dimeter.

the presence of harmine[29] or harmaline and harmalol [30] or all of these indole alkaloids[31]. In a similar study, the antibacterial activity of *P. harmala* against *S. aureus* strains was determined[32]. Present study, however, showed that *P. harmala* seed extract has antibacterial activity against methicillin and cefixime resistant *S. aureus* strains as well (Table 3).

Jan *et al*[33] reported the antibacterial activity of *G. tricornutum* against *S. aureus*. Moreover, antifungal activity of *Vi. pseudo negundo* extract was previously reported[34]. The terpenoides from *S. sclarea* were reported to possess anti-biofilm and antibacterial activity on *Staphylococci* including MRSA strains[35,36]. In our study, however, the *G. tricornutum* and *Vi. pseudo negundo* extracts showed

relatively low antibacterial activity against some of the studied strains in concentrations of 0.15 g/mL and above. On the other hand, *S. sclarea* showed no antibacterial activity against the studied strains.

To the best of our knowledge, this study was the first report on antibacterial activity of *O. decumbens* vent and *V. pyramidata* extracts. In spite of possessing antibacterial agents such as flavonoids and alkaloids, *Va. pyramidata*[37,38] in our study showed no antibacterial effects against the studied strains. On the other hand, the entire studied MRSA strains were susceptible to *O. decumbens vent* extract at concentrations of 0.35 g/mL and 0.45 g/mL.

Survival of studied MRSA strains in the presence of *Va. pyramidata* and *S. sclarea* could probably be due to the cell membrane permeability or other genetic factors[12]. Moreover, the type of compounds responsible for antibacterial activity (eg, flavonoids, alkaloids, tannins and terpenoids) could be another factor that resulted in the survival of the studied MRSA strains against these extracts. For instance, different terpenoids had different effects on *S. aureus* strains [35].

In conclusion, the isolated *S. aureus* strains were resistance to methicillin and cefixime. *Q. brantii* extract had the highest antibacterial activity against the studied strains followed by *P. harmala* and *Z. spina-christi* and *O. decumbens* respectively. While the *G. tricornutum* and *Vi. pseudo negundo* extracts were active against some of the studied strains, the *Va. pyramidata* and *S. sclarea* extracts had no antibacterial activity on the studied strains.

Conflict of interest statement

We declare that we have no conflict of interest.

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