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Original Article

Microbiology Section

Invitro Antibacterial Activity of the *Prosopis Juliflora* Seed Pods on Some Common Pathogens

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ABSTRACT

Introduction: *Prosopis juliflora* is probably the most widespread species of genus *Prosopis* and it is a good source of compounds that have been shown to be pharmacologically active. This plant has been used as a traditional treatment for several diseases.

Aim: To investigate the in-vitro antibacterial activity of the *P. juliflora* seed pods from Bushehr, South West of Iran.

Materials and Methods: In the present study, the antibacterial activity of *P. juliflora* seed pods extract was tested against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa*. The minimum inhibitory concentration (MIC) of the extract was determined for each test microorganism.

Results: *P. juliflora* seed pods extract exhibited antibacterial activity against all four test organisms. The MIC of the extract was 0.312 mg/ml and 0.078 mg/ml for *S. aureus* and *S. epidermidis,* respectively and 1.25 mg/ml for both *E.coli* and *P.aeruginosa*.

Conclusion: *P. juliflora* seed pods from Bushehr, South West of Iran could be an appropriate source of antibacterial compounds that makes it a promising candidate for further studies.

Keywords: Leguminosae, Natural products, Seed pods extract

INTRODUCTION

Bacterial contamination causes serious infections globally which are associated with a high rate of mortality in human. Antimicrobial agents are frequently used in the treatment of bacterial infections; however, multiple drug resistance in human pathogens has been developed due to use of commercial antimicrobial drugs commonly used to cure such diseases [1]. Hence, discovery of new antibacterial substances either synthetic or natural with minimal side effects is necessary. Increasing popularity of plant-based traditional medication has led researchers to consider the natural compounds, of plant origin, as a valuable source for medicines [2]. Natural products of the plants have been used to treat various illnesses [3]. Plants contain a group of chemicals that protect them against various microbes and some of their extracts have been used against many types of infections. Even today plants are the almost exclusive source of drugs for the majority of people in the world [4-6]. However, there are only a handful of plants that have been comprehensively investigated for their potential value as a source of drugs [6]. Genus *Prosopis* includes 44 species that are mainly distributed in the dry regions of Asia, Africa and America, from Western North America to the Patagonia and offers large phenotypic diversity of the morphological features. *Prosopis juliflora* (family Leguminosae, subfamily Mimosoideae) is probably the most widespread species, being a major source of fuel and fodder [3,7].

P. juliflora has been used as a traditional treatment for catarrh, cold, diarrhea, dysentery, flu, hoarseness, inflammation, measles, sore throat, and hepatic and ocular problems [6,8]. Wounds can be treated with the decoction obtained from leaf and seed extracts [6,9]. The syrup, made from ground pods of *P. juliflora*, is used for nourishment of underweight kids or those suffering from retardation in motor development. The syrup may raise lactation as well [6]. Tea prepared from *P. juliflora* is believed to be useful in healing of digestive disorders and skin wounds. *P.juliflora* is a good source of piperidine alkaloids. Several alkaloids including juliflorine, julifloricine and julifloridine, juliprosine, juliprosinene and juliflorinine, 3' oxojuliprosopine, sceojuliprosopinol, 3-oxojuliprosine and 3'-oxo-juliprosine have been isolated from various parts of *P. juliflora* and have shown to be pharmacologically active [6]. Although a lot of work has been carried out on the medicinal applications of *P. juliflora*, there is still little data on the utilization of seed pods. The aim of the present study was invitro investigation of antibacterial activity of the *P. juliflora* seed pods from Bushehr, south west of Iran. This study therefore provides insight into antibacterial activity of *P. juliflora* seed pods against some microorganisms causing skin, respiratory tract, gastrointestinal and urinogenital tract infections.

MATERIALS AND METHODS

This study was carried on in microbiology laboratory of Department of Microbiology and parasitology, Faculty of Medicine, Bushehr University of Medical Sciences, Bushehr, Iran from July 2013 to December 2013.

Collection of Plant Material and Extraction Procedure

Seed pods were detached from the plant *P. juliflora* in Bahmani region of Bushehr. The identification was confirmed by Jahad Keshavarzi Research Center, Bushehr, Iran. *P. juliflora* seed pods were washed thoroughly with distilled water, dried partially on paper towel, then, kept in an oven at 60°C for proper drying. It was then crushed to fine powder in a mixer grinder. Thirty grams of powdered prosopis seed pods were weighed and dissolved in 30 ml of double distilled water, 180 ml methanol and 90 ml of ethyl acetate. The suspension was gently mixed for 3 hours in a shaker with 110 rpm. The extract was filtered through filter paper and then, using a separatory funnel, the organic phase was separated from the aqueous phase during the three repeats [10].

Bacterial Strains and Maintenance Procedure

The American Type Culture Collection (ATCC) strains of *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 14990), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922) were examined as test microorganisms. These microorganisms were purchased from the Iranian Research Organization for Science and Technology, Tehran, Iran. To stock the bacterial strains, they were cultured on brain heart agar (Merck, Darmstadt, Germany) and incubated at 37°C. Next, the grown colonies were picked up from the culture medium and suspended

in skim milk (Merck, Darmstadt, Germany) containing 10% glycerol (Merck, Darmstadt, Germany) and maintained at –20° C [11].

Antibacterial Activity Assay

Fresh cultures of mentioned test microorganisms were prepared by culturing on tryptic soy agar (Merck, Darmstadt, Germany). Antibacterial activity of the extract against each microorganism was investigated by the following protocol: the extract with a concentration of 2.5 mg/ml was added in a tube containing Mueller Hinton broth (Merck, Darmstadt, Germany) and was tested on a bacterial concentration of 5×10^5 colony forming units (CFU)/ml. A tube of Mueller Hinton broth with the same microbial concentration, but without the extract, was planted as the positive control. Another tube of the Mueller Hinton broth containing the same concentration of the extract, but lacking the bacterium, was prepared as the negative growth control. The test tube and control tubes were incubated at 37ºC. After 24 hour incubation, antibacterial effect of the extract in the test tube was recognized by lack of turbidity (like the negative growth control) which indicated the inhibition of microbial growth [12-14].

Minimum Inhibitory Concentration (MIC)

The MIC of the extract was evaluated via broth dilution method [13-16]. The MIC of extract for *S. aureus, S. epidermidis*, *P. aeruginosa*, and *E. coli* was measured as follows: the extract was added as serial dilutions to several tubes containing Mueller Hinton broth in order that the concentrations organized from 2.5 to 0.001 mg/ml. The bacterial concentration in these tubes was 5×10^5 CFU/ml. In addition, control tubes were prepared as aforesaid. The test and control tubes were incubated at 37ºC for 24 hour. Finally, the lowest concentration of extract that inhibited the bacterial growth was measured as MIC.

RESULTS

[Table/Fig-1] shows the results of antibacterial effect of the extract of *P. juliflora* seed pods against *S. aureus* (ATCC 25923), *S. epidermidis* (ATCC 14990), *E. coli* (ATCC 25922), and *P. aeruginosa* (ATCC 27853). In the step of antibacterial activity assay, a concentration of 2.5 mg/ml of extract showed activity against all four test bacteria. In the next step of the study, the MIC of the extract for each test organism was determined [Table/Fig-1]. The MIC of extract was 0.312 mg/ml and 0.078 mg/ml for *S. aureus* and *S. epidermidis*, respectively. The MIC was 1.25 mg/ml for both *E. coli* and *P. aeruginosa*. The MIC for gram-positive bacteria was found to be lower than the MIC for gram-negative bacteria.

Microorganisms	Antibacterial effect of extract	MIC(mg/ml)
S.aureus ATCC 25923	+	0.312
S.epidermidis ATCC 14990	+	0.078
E.coli ATCC 25922	+	1.25
P.aeruginosa ATCC 27853	+	1.25

[Table/Fig-1]: Antibacterial effect and MIC of the extract of *Prosopis juliflora* seed pods
* +: Shows effect

DISCUSSION

P. juliflora seeds contain nutritional compounds and can be considered as an alternate protein source to protein-energy-malnutrition (PEM) among the economically weaker people [17]. The plant *P. juliflora* produces several compounds including alkaloid, tannin, phenolics, steroids, terpenes, flavonoid, proteins, sugars, and fatty acids [18-21]. Some of these compounds may exhibit therapeutic activities such as antibacterial activity [19,21]. For example, juliprosinene and juliflorinine isolated from *P. juliflora* exhibit antibacterial effect on bacteria such as *E. coli*, *S. aureus*, *Klebsiella pneumoniae*, and *Shigella sonnei* [21]. The antibacterial substances may protect *P. juliflora* against microorganisms [6]. In this study, due to the availability of *P. juliflora* in our region (Bushehr, south west of Iran), we decided to test the *P. juliflora* seed pods extract against selected microorganisms for antibacterial effect. Crude extract of *P. juliflora* seed pods was examined on *S.aureus*, *S. epidermidis*, *P. aeruginosa*, and *E. coli*. These organisms have been tested in many antibacterial studies [12,15,16].

Results of the present study revealed that the extract of *P. juliflora* seed pods was effective against all tested organisms, but the MIC for gram-positive species was lower than that of for gram-negative species [Table/Fig-1]. Based on the results of this study, gram-positive organisms were more susceptible to the extract of *P. juliflora* seed pods than gram-negative organisms. The strongest activity (MIC of 0.078 mg/ml) was shown against *S. epidermidis*. Higher susceptibility of gram-positive bacteria to other extracts has also been reported in some previous investigations [11,15]. The less susceptibility of gram-negative bacteria to antibacterial substances in such studies may be associated with their outer membrane and lipopolysaccharide molecules which provide the barrier against easy penetration of some antimicrobial molecules. Gram-positive bacteria do not have this type of outer membrane and cell wall construction [22-24].

In the study conducted by Singh et al., the antibacterial effect of alkaloid rich fractions of *P. juliflora* taken from different parts of the plant including leaf, pod and flower was investigated on gram-positive and gram-negative bacteria. They collected the plant material from the Shekhawati regions of Rajasthan, India. According to their study, the leaf extract showed the highest antibacterial properties but other parts including pod and flower also exhibited the antibacterial activity with the potential to inhibit antibiotic-resistant strains. On the contrary, the root and stem extracts did not show zone of inhibition against any of the tested bacteria [6]. In our study, seed pods of *P. juliflora* exhibited antibacterial activity. Therefore, our results are in accordance with the results of the study done by Singh et al., [6].

In the study performed by Taheri et al., antibacterial effect of hydro-alcoholic extract obtained from leaf of a *Prosopis* sp. from Chabahar, south east of Iran was investigated. These authors did not find any antibacterial effect in non-heated extract. Strikingly, they found that extract shows antibacterial activity after heating by an autoclave. Hence, they supposed that some compounds might cleave by heat and produce new materials with antibacterial properties [25]. In contrast, the results of our study indicated the antibacterial effect of extract without use of autoclave. Differences between their results and our results may be due to some factors such as possible dissimilarities in plant species, solvents and the part of plant used for extraction, or geographical regions.

In the investigation done by Aqeel et al., antimicrobial activity of julifloricine isolated from *P. juliflora* has been shown [26]. In our study, we performed the primary experiments on *P. juliflora* seed pods collected from our region (Bushehr). In next studies, further works will be needed to isolate the active components and investigate their antibacterial activities against wide series of bacterial strains.

CONCLUSION

P. juliflora seed pods from Bushehr, south west of Iran could be an appropriate natural source for antibacterial components and it is a suitable candidate for purification of crude extracts and next *in vivo* investigations.

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