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Research Article

ANTIBACTERIAL ACTIVITY OF RAW AND PROCESSED MESKIT (*PROSOPIS JULIFLORA*) PODS' EXTRACTS

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ABSTRACT

The methanolic crude extracts of raw and processed *Prosopis juliflora* pods were assessed for their antibacterial activity using well-diffusion method on *Escherichia coli*, *Kelbsiella spp.*, *Staphylococcus aureus*, *Streptococcus spp.*, and *Bacillus sp*. Tests showed that raw pods' extract has higher zone of inhibition compared with soaked and roasted pods' extracts with diameter ranged between 13 and 20 mm against all tested bacteria except for *Klebsiella spp.* There was with more inhibition of Gram-positives than Gram-negatives. However, roasted pods extract inhibited *Streptococcus spp* and *Bacillus* only with an inhibition zone of 10 and 6 mm, respectively. Dilution experiments showed that the minimum inhibitory concentration (MIC) of raw pods' extract was as follows; *S. aureus* (250 mg/ml), *Streptococcus spp*. (62.5 mg/ml) *Bacillus spp*. (125 mg/ml) and *E-coli* (125 mg/ml). Results indicated that the MIC of 62.5 mg/ml of raw pods' extract is equivalent to effects of Gentamicin (30 mg) and Kanamycin (30 mg) against *Streptococcus spp*. It was concluded that the raw and soaked *P. juliflora* pods extracts could be a potential source for antibacterial agents. However, roasting of *Prosopis julilfora* pods extremely reduced the strength of antibacterial activity.

INTRODUCTION

Bacterial contamination causes serious infections worldwide which are associated with high rate of mortality in humans and animals. A wide range of antimicrobial agents are frequently used in the treatment of bacterial infections. However, multiple drug resistance in human pathogens has been developed due to the misuse of commercial antimicrobial drugs commonly used to cure infections (Saga; 2009). Natural plants products have been used to treat various illnesses (Pasiecznik *et al*., 2001). Plants have always been a source of natural product for the treatment of various diseases (McDonald, and Paterson, 2006). Plants develop several chemical compounds to protect themselves from various microbes. Some of their extracts were used by early human civilization against many forms of disease and infection. Even today plants are the almost exclusive source of drugs for the majority of the world population. People in developing countries utilize traditional medicine for their primary health care needs. Despite having a wide historical background, there are only a handful of plants that have been exhaustively studied for their potential value as a source of drugs. Considering the risks of antibiotic resistance in humans (Manero *et al*., 2006) and the occurrence of residues in foods of animal origin, the European Union has banned antibiotic use in livestock as feed additives (Jouany *et al*., 2007). Therefore, there is a real growing demand for new natural anti microbiological agent to replace current widely-used antibiotics. Consequently, the scientific community initiated efforts to exploit natural products as feed additives, since many natural compounds and plant extracts have some of the benefits of antibiotics (Gibbons *et al*., 2005).

Prosopis juliflora tree has been used as a folk remedy for catarrh, cold, diarrhea, dysentery, excrescences, flu, inflammation, measles, sore throat and in healing of wounds (Hartwell, 1971). Decoction prepared from leaf and seed extracts are used for wound healing, as a disinfectant and also to treat scurfy. Tea made from *P. juliflora* is believed to be good for digestive disturbances and skin lesion.

Many plants of genus *Prosopis* (Leguminosae) are known to be medical value. *P.juliflora* is rich source of piperidine alkaloids. Many alkaloids such as juliflorine, juliforcine and julifloridine, juliprosine, juliprosiene and juliflorinine have been isolated

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from leaves and have proven to be pharmacologically active (Singh *et al*., 2011).

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. Many research efforts were carried out to find out the various phytochemicals of this invasive plant and the mechanisms of action as well as bioactivity of the various phytochemicals. Several alkaloids have been isolated from leaf extracts having pharmacological properties (Ahmed *et al*., 1989, Aqeel *et al*., 1989; Swaapnil and Verma, 2011). Apart from alkaloids, other important compounds isolated from *P.Juliflora* including flavones glycoside Patulitrin, Prosogerin D, Procyanidin, ellagic acid, tannin and polystyrenes (Rastogi and Mehrotra, 1993).

The current study aimed at investigating the antibacterial activity of raw and processed *Prosopis juliflora* pod extracts on some pathogenic bacteria.

MATERIALS AND METHODS

Prosopis juliflora pods collection and processing

Dry *P. juliflora* pods were collected during the fruit production season and stored in a cool dry shed. The pods were then chopped to lengths of 0.5- 1.0 cm to ensure good processing. *P. juliflora* pods were roasted at 150°C for 30 minutes using a locally-made roasting device made up of a 40 kg steel container rotated by an electric motor and heated by gas flame. For soaking, ten kg of chopped pods were added to a 30 liter capacity bucket containing 20 liters of tap water. The mixture was left for 24 hours with frequent manual stirring. The pods were washed and allowed to dry for 2 days under the sun with frequent turning over

One hundred gram of dried *P. juliflora* pods were ground by blender and soaked in 500 ml (1:5 v/w) methanol and then incubated for 24h at room temperature with gentle shaking. The organic extracts were filtered through a muslin cloth to remove large plant tissues and then rotary evaporated at 60°C for fast evaporation (RE100, Heidolph, Germany). The final dried crude extracts were weighed and dissolved in distilled water. The extracts were then centrifuged to make sure all debris precipitates out of solution. The supernatants were filtered through vacuum filtration using polystyrene non-pyrogenic Corning 500 ml Filter unit system 0.22 mm (Corning-USA) and then stored in the refrigerator.

Bacterial cultures

The stock for bacterial cultures including *Klebsiella spp*., *Escherichia coli*, *Stayphlococcus aureus, Streptococcus sp., and Bacillus spp.* were provided by the Department of Animal and Veterinary Sciences, Sultan Qaboos University, Oman. All the microbes were streaked onto individual nutrient agar plates and incubated at 37° C for 24 h prior to the assay.

Antibacterial assay

Well-diffusion method

One ml of each bacterial culture was aseptically spread on Mueller-Hinton agar plates using a sterile glass spreader. Holes in the agar were made by cutting with a sterile serological tube (6 mm) diameter. Each well was filled with 30 µl filter sterilized crude extracts of raw, roasted and soaked pods. Sterile distilled water was used as a control.

Minimum inhibitory concentration (MIC)'s method

To determine the minimum inhibitory concentration (MIC) of the crude extracts of raw, roasted or soaked pods, serial dilutions were made. The methanolic crude extracts raw and processed Meskit pods were serially diluted to final concentrations (mg/ml) of 500, 250, 125 and 62.5. Several wells were made in each plate and filled with 30 µl of different concentrations. Plates were incubated at 37°C for 24 hrs. The sensitivity of the test organisms to the crude extracts and the standard antibiotics was indicated by clear zone around wells. The inhibition measured with a transparent ruler and expressed as the degree of sensitivity (NCCLS, 1993) after measuring the diameter of inhibitory zones formed around each hole in mm. All plates were incubated in the incubator at 37°C for 24h.

RESULTS AND DISCUSSION

The well-diffusion test for *P. juliflora* methanolic crude extract on two tested Gram- negative bacteria (*E.coli and Klebsiella spp.)* and three Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus spp*. and *Streptococococcus spp*.) indicated an inhibition of all tested bacteria except *Klebsiella spp*. (Table 1 and Figure 3). Raw pod extracts showed the widest zone of inhibition on most tested bacteria with 20 and 13 mm zone of inhibition against *Streptococcus spp*. and *Bacillus spp*. respectively, whereas soaked pods revealed intermediate inhibition. The tests on Gram-positive bacteria (*S. aureus, Streptococcus spp*. and *Bacillus spp*.) showed higher sensitivity than for Gram-negative bacteria (*E. coli* and *Klebsiella spp*.) (Table 1 and Fig.3) The findings generally indicated that Gram-positive organisms were more susceptible to the extract of *P. juliflora* pods than gram negative organisms. The strongest activity (MIC of 62.5 mg/ml of raw pods extract) was shown against *Streptococcus spp*. higher susceptibility of Gram-positive bacteria to other extracts has also been reported in some previous studies (Tajbakhsh *et al*., 2008, and 2011). 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 antimicrobial molecules. Gram-positive bacteria do not have this type of outer membrane and cell wall construction (Nikaido., 1994; Willey *et al*., 2008).

Tajbakhsh *et al* (2011) investigated the *In vitro* antibacterial activity of *Prosopis juliflora* pods on Gram positive and Gram-negative common pathogenic bacteria. The pods of *P. juliflora* exhibited antibacterial activity. In India, Singh *et al* (2011) at investigated the antibacterial effect of alkaloid rich fractions of *P. juliflora* taken from different parts of plant including leaf, pods and flower. According to their study, the leaf extract showed the highest antibacterial properties but other parts including pods and flower also exhibited an antibacterial activity with the potential to inhibit antibiotic-resistant bacterial strains. Stem and root extracts did not show a zone of inhibition against any of the tested bacteria (Singh *et al*., 2011).

Table.1 Minimum inhibition concentration (MIC) activity of *P. juliflora* raw, roasted and soaked pod extracts as assayed by well-diffusion method and compared to different standard antibiotics against different pathogenic bacteria.

Type of antibacterial substance	mg/ml	Bacteria				
		Staphylococcus aureus	Streptococcus sp.	Bacillus sp.	Escherichia coli	Kelbsiella sp.
RPE	500	14	20	13	13	0
	250	6	17	10	12	0
	125	0	14	6	7	0
	62.5	0	10	0	0	0
ROPE	500	0	10	6	0	0
	250	0	0	0	0	0
	125	0	0	0	0	0
	62.5	0	0	0	0	0
SPE	500	0	20	13	10	0
	250	0	16	12	8	0
	125	0	13	10	6	0
	62.5	0	8	0	0	0
Antibiotics	TE_{10}	18	20	23	20	18
	E_{15}	16	15	20	20	15
	K_{30}	18	10	28	15	7
	Gen_{30}	16	22	16	20	18
	Met_{30}	20	16	7	15	16

RPE; Raw pods' extract; ROPE: Roasted pods' extract; SPE: Soaked pods' extract.TE_{10}: Tetracycline (10µg); E_{15}: Erythromycine (15 µg); K_{30}: Kanamycin (30 µg); Gen_{30}: Gentamicin (30 µg); Met_{30}: Methicillin (30 µg).

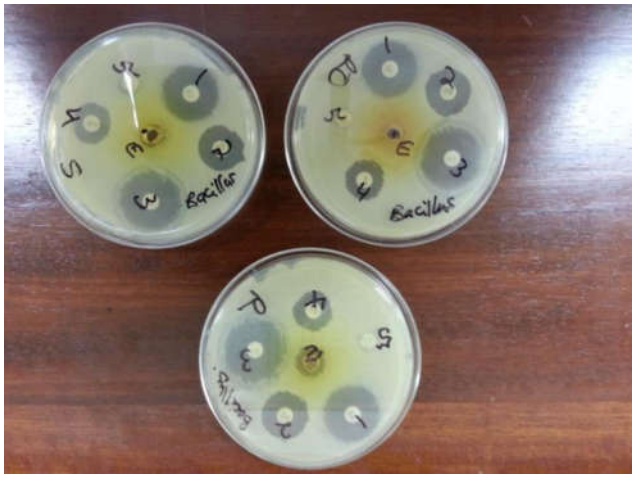

Figure 1 The inhibitory effect of methanolic crude extract of (R) raw, (RO) roasted and (S) soaked pods against *Bacillus sp*. Compared to different standard antibiotics.

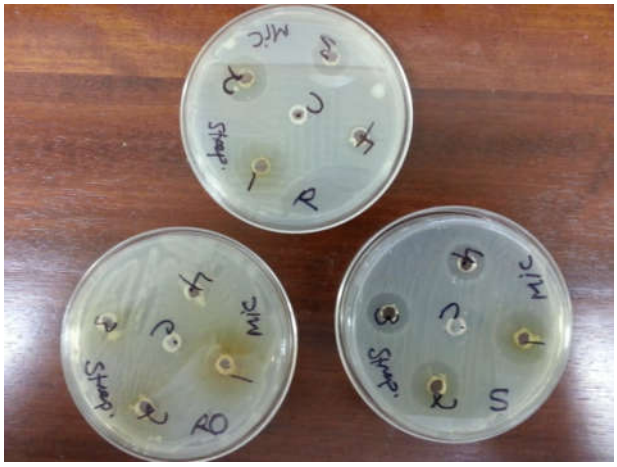

Figure 2 MIC activity of methanolic crude extract of (R) raw, (RO) roasted and (S) soaked pods against *Streptococcus sp*.

Elisabetsky and Costa-Campos, (2006) reported that alkaloids are used by the plants in defense mechanism against pathogens and predators.

Overall, *P. juliflora* methanolic crude extracts of raw and soaked pods were effective to inhibit growth of most tested bacteria, whereas the roasting of the pods had lower activity of bacteria's inhibition.

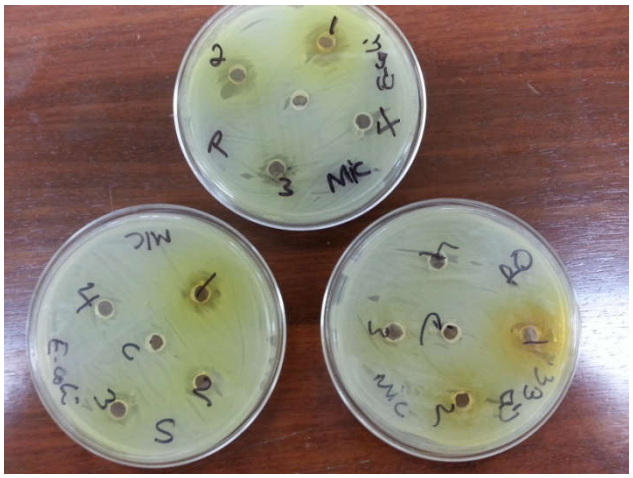

Figure 3 MIC activity of methanolic crude extract of (R) raw, (RO) roasted and (S) soaked pods against *E.coli sp.*

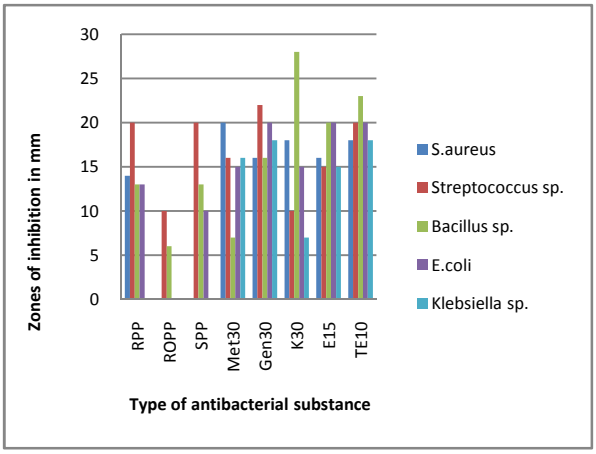

Figure 4 Comparison of the antibacterial activity of *Prosopis juliflora* pods' extracts with standard antibiotics

RPP: Raw prosopis pods; ROPP: Roasted prosopis pods; SPP: Soaked prosopis pods; Met_{30}: Methicillin (30 µg); Gen_{30}: Gentamicin (30 µg);); K_{30}: Kanamycin (30 µg) E_{15}: Erythromycin (15 µg) ;TE_{10}: Tetracycline (10 µg)

The inhibitory effects of methanolic crude extract of raw, roasted and soaked pods were compared with different standard antibiotics. Findings, indicated that zone of inhibition against *Bacillus spp* bacteria (Fig. 1). Minimum inhibitory concentration (MIC) activity of the crude extracts of raw pods on *S. aureus*, *Streptococcus spp*., and *Bacillus sp*., was 250 and 62.5 and 125 mg/ml, respectively (Fig. 1, 2 and 3). However, results showed less sensitivity for *Bacillus spp*. while *Klebsiella spp.* was resistance (Table 1) and Figures (2 and 4), and showed the lowest sensitivity compared to other bacteria (Fig.. 3). Also, zone of inhibition for raw pods' extract against *Bacillus spp*., was 13 mm at 500 mg/ml concentration and the MIC was seen at the concentration of 125 mg/ml (Fig. 4). MIC of the crude extracts of roasted pods showed activity only against *Streptococcus spp*., and *Bacillus spp.* (Table 1). For *Streptococcus spp.*, and *Bacillus spp*., the inhibition stopped at the concentration of 62.5 mg, while for *E.coli* the inhibition stopped at the concentration of 62.5 mg/ml of soaked extract at 500 mg/ml. However, MIC activity of methanolic crude extract of roasted pods against *E. coli*, *Klebsiella spp* and *S.aureus*., was not clear.

Comparing the concentration obtained from crude extracts of raw, roasted and soaked pods and the activity of different standard antibiotics. Data indicated that the minimum inhibitory concentration (MIC) of *P.juliflora* raw pods' extract was as follows: *S. aureus* 250 mg/ml, *Streptococcus spp.* 62.5 mg/ml , and *E. coli* 125 mg/ml (Table 2). The MIC of 125 mg/ml, *Bacillus spp*. was close to Methicillin (30 µg), and the MIC of 62.5 mg/ml against *Streptococcus spp*. and similar to Kanamycin (30 µg) (Table 1). Moreover the MIC activity of 500 mg/ml of roasted pods extract against *Streptococcus spp*. close to Kanamycin (30 µg), (Table 1). This shows the potential of *Prosopis juliflora* pods extracts to control the growth of pathogenic bacteria. It could also be concluded that the antibacterial compound extracted from *P. juliflora* pods might be a good source in inhibiting bacterial growth.

Both raw and soaked pods' extract were effective with higher effect on Gram-positive bacteria (*S. aureus, Streptococcus spp*., and *Bacillus spp*) than Gram-negative bacteria (*Klebsiella spp*.). While roasted pods extract was less effective on both gram positive and negative bacteria, mainly because roasting might cleave some compounds that have antibacterial activity. These results are consistent with the bacterial structure. The difference is sensitivity between Gram-negative and positive bacteria to inhibition by pods extracts is supported by other researchers (Shelef, 1983; Saadoun and Hameed, 1999; Saadoun *et al*., 2008; Saadoun *et al*., 2014). Overall, *P.juliflora* proved to have strong antimicrobial activity and can be considered to have a broad spectrum of action.

CONCLUSION

It can be concluded that the raw and soaked *Prosopis juliflora* pod extracts has an antibacterial attitudes; however roasting the pods had extremely reduced the strength of the antibacterial inhibition. It is recommended that this attitude needs further investigation for the possibility of being used in the medical field.

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