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Investigation of Phytochemical Profile and Antibacterial Potential of Ethanolic Leaf Extract of *Prosopis juliflora* DC.

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ABSTRACT

Prosopis juliflora DC. is commonly known as "mesquite". This tree is native to tropical America, but is naturalized in many countries including Egypt and India. The preliminary phytochemical screening of the leaves revealed the presence of tannins, acids, glycosides, flavonoids and alkaloids. *In vitro* antibacterial studies on the ethanolic leaf extracts were carried out on ten medically important bacterial strains (*Salmonella typimurium*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli*, *Psudomonas* sp. *Staphylococcus epidermis*, *Micrococcus luteus*, *Staphylococcus aureus*, *Streptococccus* sp. and *Bacillus subtilis*) procured from the Microbial Type Culture and Collection, Chandigarh, India, using the agar disc diffusion method. The bacterial strains were exposed to the following four different concentrations of extracts: 50mg/ml, 100mg/ml, 200mg/ml and 300mg/ml solvent. The results of our antibacterial assay revealed that the extract showed good inhibitory activity against all the tested pathogens compared with standard antibiotics like streptomycin and penicillin. The inhibitory activities were found to be dose dependent.

INTRODUCTION

Man has used plants to treat common infectious diseases, and some of the traditional medicines are still included as part of the habitual treatment of various maladies (Heinrich *et al.,* 2004; Rios *et al.,* 2005). Scientific interest in medicinal plant has burgeoned in recent times due to increased efficiency of new plant derived drugs and rising concerns about the side effects of modern medicine. It is a well known fact that intensive use of antibiotics often followed the development of resistant strains (Ahmad *et al*., 1987). The continuing emergence of drug resistant organisms and the increasing evolutionary adaptations by pathogenic organisms to commonly used antimicrobials have reduced the efficacy of antimicrobial agents currently in use. This propensity of drug resistance requires the search for new, effective and safe drugs. Therefore, the search for new drugs from plants continues to be a major source of commercially consumed drugs. Even most synthetic drugs have their origin from natural plant products (Sofowara, 1982). The scientific analysis of medical plants has led to the discoveries of many important drugs (Pelletier et al., 1817; Ahmad et al., 1992).

Prosopis juliflora is an evergreen tree with a large crown and an open canopy, growing to a

height of 5-10 m. Stem green-brown, sinuous and twisted, with axial thorns situated on both sides of the nodes and branches. Bark somewhat rough; dull red. The root system includes a deep taproot. Leaves compound; leaflets in 13-25 pairs, oblong (3 x 1.7 mm) and dark green, bipinnate with 1 or sometimes 2 pairs of rachis, almost pendulous. Flowers lateral to the axis with a tubular, light greenish-yellow, 1.5 mm wide calyx with hooded teeth; corolla light greenish-yellow, composed of 5 petals with 3 mm wide pubescent along its edges. Fruit a non-dehiscent pod, straight, linear, falcate to annular, with a coraceous mesocarp in 1 segment or divided into several segments; seeds compressed, ovoid, hard, dark brown, with mucilaginous endosperm surrounding the embryo; cotyledons flat, rounded, epigenous when germinating.

Larger branches and trunks yield a high quality timber, comparable in colour, finish and physical attributes to Indian rosewood and other commercial hardwoods. While also used for posts and poles, the wood, called 'wooden anthracite' in some areas, is almost unsurpassable as a fuel. Fruit pods are high in sugar and protein and are a rich food source for man and beast. Prosopis honey is of the highest quality and exudates gum is comparable to gum arabic. Prosopis products have added value if processed, such as a 30-fold increase by turning firewood to finished timber, and even more if manufactured into furniture.

This study aimed at investigating the phytochemical and antibacterial properties of the ethanolic leaf extract from this tree against ten bacterial isolates in order to validate or otherwise prove the claims of the herbalists who use it as an antimicrobial remedy. This study will also hopefully expose new frontiers by improving on the current applications of the plant extract. It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumoural and antimicrobial agents (Chung *et al.* 1995; Vlietinck *et al.* 1995). The selection of crude plant extracts for screening programs has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products (Kusumoto *et al.* 1995).

MATERIALS AND METHODS

Collection of plant materials

Mature healthy leaves were collected from the tree found in the Centre of Biodiversity and forest studies, Madurai Kamaraj University, Madurai, India. The collected plant materials were botanically authenticated by the Director, Centre for Biodiversity and Forest Studies, Madurai Kamaraj University, Madurai, India.

Preparation of plant extract

The leaves were washed in tap water, shade dried for 10 days and made into a fine powder of 40 mesh size using the laboratory mill. Following that, 100g of the powder was filled in the thimble and extracted using 500 ml of distilled ethanol in soxhlet apparatus for 8 – 10 hours. The extract was filtered through Whatman No.1 filter paper to remove all unextractable matter, including cellular materials and other constitutions that are insoluble in the extraction solvent. The entire extract was concentrated to dryness using rotary flash evaporator under reduced pressure. The dried extract was redissolved in ethanol to yield solutions containing 50, 100, 200 and 300mg of leaf extract per ml solvent.

Test Organisms

The extract was tested on the following five Gram positive bacteria: *Staphylococcus epidermis*, *Micrococcus luteus*, *Staphylococcus aureus*, *Streptococccus* sp. and *Bacillus subtilis*. Five Gram negative bacteria were also tested, including *Salmonella typimurium*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli*, *Psudomonas* sp. All the strains were procured from the Microbial Type Culture and collection, Chandigarh, India.

Phytochemical Investigation

Phytochemcial analysis of the extract was conducted following the procedure of Indian Pharmacopeia (1985). By this analysis, the presence of several phytochemicals like flavonoids, tannins, glycosides, Alkaloids and phenolic acids were confirmed.

Anti bacterial Screening

The four different concentrations of the leaf extracts were tested for antibacterial activity using agar disc diffusion assay according to the method of Bauer et al., 1966. The strains of microorganisms obtained were inoculated in conical flask containing 100 ml of nutrient broth. These conical flasks were incubated at 37º C for 24 h and were referred to as seeded broth. Media were prepared using Muller Hinton Agar (Himedia, Mumbai, India), poured on Petri dishes and inoculated with the test organisms from the seeded broth using cotton swabs. Sterile discs of six millimeter width had been impregnated with 20 µl of test extract and introduced onto the upper layer of the seeded agar plate. The plates were incubated overnight at 37ºC. Antibacterial activity was assigned by measuring the inhibition zone formed around the discs. The experiment was done three times and the mean values were presented. Streptomycin (10µg/disc) and penicillin (10µg/disc) were used as standards.

The preliminary phytochemical analysis of the leaf extract revealed the presence of tannins, flavonoids, alkaloids, glycosides and acids as presented in Table 1. The results obtained from the disc diffusion assay showed that there has been an increasing effect on bacterial growth inhibition with increasing concentration of the extract. And the extract showed good inhibitory activity on almost all the bacteria tested. It has been found that among all the tested organisms, the Gram positive bacterial strain, *Staphylococcus aureus* was found to be more susceptible to the plant extract by showing inhibition zone ranging from 9.9 – 16.8 mm and the gram negative strain *Psuedomonas aeroginosa* was least susceptible with the inhibition zone ranging from 8.1 – 13.8 mm. The antimicrobial activity in terms of zone of inhibition was presented in Table 2. The observed activity may be due to the presence of potent phytoconstituents in the leaf extracts.

Table 1. The phytochemical profile of the leaf extract.

Phytochemcials	Presence/Absence
Sugar	-
Tannin	+
Alkaloid	+
Flavonoid	+
Saponin	-
Steroid	-

Terpenoid	-
Glycoside	+
Ester	-
Resin	-
Acid	+

Table 2. Antibacterial activity of ethanolic leaf extract of *Prosopis juliflora*.

Sl.No.	Bacterial strains used	Zone of Inhibition in mm					
		Streptomycin	Penicillin	50mg/ml	100mg/ml	200mg/ml	300mg/ml
1.	*Salmonella typhimurium*	16.80±0.81	19.70±0.35	08.55±0.30	11.15±0.67	13.05±0.66	14.81±0.20
2.	*Pseudomonas aeroginosa*	10.30±0.33	16.90±0.47	08.05±0.19	11.50±0.55	12.65±0.36	13.75±0.28
3.	*Klebsiella pneumonia*	12.10±0.25	17.60±0.71	10.78±0.28	12.10±0.66	13.60±0.48	15.20±0.36
4.	*Escherichia coli*	14.70±0.60	10.10±0.25	09.82±0.05	11.70±0.54	12.95±0.50	14.82±0.48
5.	*Pseudomonas* sp.	18.70±0.15	21.60±0.19	08.77±0.55	11.78±0.70	13.04±0.62	14.70±0.57
6.	*Staphylococcus epidermis*	24.10±0.19	22.10±0.33	08.65±0.60	11.05±0.47	13.55±0.88	15.30±0.55
7.	*Micrococcus luteus*	20.80±0.61	19.10±0.55	08.85±0.59	11.70±0.45	12.60±0.45	15.20±0.77
8.	*Staphylococcus areus*	22.80±0.25	24.40±0.35	09.88±0.68	12.20±0.76	14.85±0.66	16.75±0.48
9.	*Streptococcus* sp.	24.10±0.50	20.80±0.45	09.62±0.55	11.80±0.44	13.56±0.80	15.80±0.67
10.	*Bacillus subtilis*	19.50±0.25	22.60±0.40	08.74±0.56	10.78±0.77	14.77±0.55	16.55±0.80

*All the values are mean ± standard deviation of three determinations.

DISCUSSION AND CONCLUSION

Antibiotics provide the main basis for the therapy of bacterial infections. However, the high genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. Thus, there has been a continuing search for new and more potent antibiotics (Heisig, 2001). According to World Health Report of Infectious diseases 2000, overcoming antibiotic resistance is the major issue of the WHO for the next millennium. Hence the last decade witnessed an increase in the investigation of plants as a source of human disease management (Prashanth *et al*. 2001). *Prosopis juliflora* showed notable antibacterial activity and so this plant can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals that address hither unmet therapeutic needs. Such screening of various natural organic compounds and identifying active agents is the need of the hour, because successful prediction of lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development.

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