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In vitro antimicrobial activity and phytochemical analysis of *Prosopis spicigera*

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ABSTRACT

This study was performed to evaluate the antimicrobial activity of aerial parts of methanolic extract of *Prosopis spicigera*. The methanolic extract of *Prosopis spicigera* were shown to process an antimicrobial activity against two gram positive and two gram negative human pathogenic bacteria *viz.Bacillus substilis, Staphylococcus aureus, Escherichia coli and pseudomonas aeruginosa* by using disc diffusion method. The extract showed antimicrobial activity at all concentrations selected but only the extract with the concentration of 300 µg/ml showed maximum antimicrobial activity against all the organisms except *pseudomonas aeruginosa* which are comparable with standard control, amikacin. The phytochemical analysis showed the presence of alkaloids, carbohydrates, fats, tannins, gum, fixed oil & mucilage, lignin, terpinoids, flavonoids, saponins and sterols. It is concluded that the antimicrobial activity showed by the plant due to the presence of these phytochemicals. Further studies are highly needed for future drug development.

Keywords: Soxhlet extractor. Pathogenic bacteria. Amikacin. Disc diffusion. Methanolic extract.

1. INTRODUCTION

The medicinal plants are of great interest to human health. Plant based medicines have been a part of traditional healthcare in most parts of the world for thousands of years. [1, 2] Medicinal plants have been a valuable source of natural active constituents that products for maintain human health and treatment of many human disease. [3] According to WHO (World Health organization) 80% of developed countries use traditional medicine [4] that medicinal compounds obtained from medicinal plants. It is cheaper than modern medicine. [5] Thus it is important to characterize the different types of medicinal plants for their antioxidant and antimicrobial potential. [6-8] Scientific investigations of medicinal plants have been initiated in many countries because of their contributions to health. The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller. Thus, any phytochemical investigation of a given plant will reveal only a very narrow spectrum of its constituents. Historically pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents. Random screening as tool in discovering new biologically active molecules has been most productive in the area of antibiotics. [9, 10] Even now, contrary to common belief, drugs from higher plants continue to occupy an important niche in modern medicine. On a global basis, atleast 130 drugs, all single chemical entities extracted from higher plants, or modified further synthetically, are currently in use, though some of them are now being made synthetically for economic reasons [11]. *Prosopis spicigera Linn.* (Syn. *Prosopis cineraria* (L.) Druce.) Belonging to the family Fabaceae, is a moderate sized evergreen thorny tree, with slender branches armed with conical throns and with light yellowish-green foliage. Prosopis cineraria tree occurs in the dry and arid regions of India. It is one of the chief indigenous trees of the plains of the central and southern India. Leaves are eaten as a fodder by cattle. Smoke of leaves good for eyes. The stem is often rich in tannin sacs and gum passages; they are used as fodder due to presence of rhamnose, sucrose and starch. Stem portion and wood are generally used as good fuel for the tribal people. The Bark is thick, dark brown in color and

hard. It is available in the form of single quill and pieces. Liver-warts and lichens are located on the surface of bark. Stem bark is recommended for snake bite. The flowers are small in size and yellowish in colour appear from March to May after the new flush of leaves. Flowers are mixed with sugar and used during pregnancy as safeguard against miscarriage. Fruits are legume and sweet in taste. Fleshy pods are sickle shape which are10 to 20 cms long and contain sweetish mucilaginous pulp. Pods are mature in May-June before the onset of the rain. Seeds are dark brown in color packed in brown pulp. Seeds contain fixed oil, those are major part of cattle feed [12]. The literature search reveals that still no work have been done on this plant. And nobody has isolated this crude extracts from ethanol solvent and analyse the crude extract. With this knowledge the present study was intended to determine the anti microbial activity of the extract of *Prosopis spicigera* using ethanol and aqueous solvents.

2. MATERIALS AND METHODS

2.1. Collection of the plant material

Healthy aerial parts of the Prosopis spicigera (stem, leaves) were collected from the Thoothukudi Dist, Tamil Nadu, India, in the month of December 2012. The collected plant materials was identified and authenticated by Dr. V. Chelladurai, Retired Research officer-botany, Central Council For Research In Ayurveda and Sidha (C.C.R.A.S). Govt.of India, Tiruneveli. The collected plant material was free form disease and also free from contamination of other plants. Herbarium of the plant was prepared and preserved in the department of pharmacognosy, Dr. Samuel George Institute of Pharmaceutical Sciences, Markapuram, Prakasam dist. Andhra Pradesh.

2.2. Preparation of plant extract

2.2.1. Solvent extraction

10 g of dried leaves powder was extracted with 100 ml of ethanol kept on a rotary shaker at 190-220 rpm for 24 h. There after it filtered through 8 layers of muslin cloth and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was evaporated to make final volume one-fourth of the original volume (16). It was stored at 4 °C in airtight bottles for further studies [13].

2.2.2. Preliminary phytochemical screening

The extract was subjected to preliminary phytochemical screening to identify for the presence of different chemical groups of compounds. The extract was carried out qualitatively for the presence of Alkaloids, carbohydrates, fats, tannins, gum, fixed oil & mucilage, lignin, terpinoids, flavonoids, saponins and sterols by using standard method given by Brindha et al., [14].

2.2.3. Microorganisms

Four bacteria, two from Gram-positive group (*Bacillus substilis, Staphylococcus aureus*) and rest two from Gram-negative group (*Escherichia coli and pseudomonas aeruginosa*) were obtained from the stock culture of Microbiology Research Laboratory, NRI Medical College, Mangalagiri, Guntur dist.

2.2.4. Screening of antimicrobial activity

Screening of antimicrobial activity was done by Agar well diffusion method [15]. Two organism of gram positive and two for gram negative bacteria were used for this study. The organisms were sub-cultured on Muller Hinton Agar medium, incubated at 37˚C for 24 hrs and store at 4˚C in the refrigerator to maintain stock culture [16]. About 20 ml of sterilized Muller Hinton Agar was poured into sterile petriplate. Then the test culture were swabbed on the top of the solidified media and allowed to dry for 10 min. There are three different concentrations at 100, 200 and 300µg/ml in that order of crude extract. The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Negative control was prepared using respective solvent. Amikacin (50µg/ml) was used as positive control. Then the plates were incubated for 24 hrs at 37˚C. The zone of inhibitions was measured by measuring scale in millimeter (mm) [17]. The sensitivity of the microorganisms of plant extract was determined by measuring the size of inhibitory zones on agar surface around the discs [18].

3. RESULT AND DISCUSSION

The use of antimicrobial has increased steadily since the discovery of penicillin. Many drugs have been developed since then, few of which were considered potentially toxic.

Table - 1: Phytochemical investigation of methanolic extract of *Prosopis spicigera*

Compounds	Methanol
Alkaloids	+
Carbohydrates	+
Fat	–
Tannins	+
Gum & Mucilage	–
Fixed oil	–
Lignin	–
Terpinoides	+
Flavonoides	+
Saponins	+
Sterol	+

Table - 2: Antibacterial activity of methanolic extract of *Prosopis spicigera* in different strains

Drug	Conc. (µg/ml)	Zone of inhibition			
		B.subtilis	***S.aureus***	***P.aeruginosa***	***E.coli***
Prosopis spicigera	100	10	5	0 (NI)	8
	200	12	8	0 (NI)	9
	300	16	13	0 (NI)	13
Control	-	0 (NI)	0 (NI)	0 (NI)	0 (NI)
Amikacin	50	20	19	18	19

A number of factors contribute to antibiotics resistance including misuse and overuse of antibiotics in humans, animal and agriculture; patient's needs them; and failure to finish an antibiotic prescription. Therefore the Ayurveda medicines have increased now a day's [19]. The bio active compounds obtained from medicinal plants have been used to treat various diseases caused by micro organisms. The most important of these bio active principles are alkaloids, flavonoids, phenolic compounds and tannins that may develop in plants as self defence against pests and pathogens [20]. The phytochemical screening of methanolic extract of *Prosopis specigera* revealed the presence of alkaloids, carbohydrates, fats, tannins, gum, fixed oil & mucilage, lignin, terpinoids, flavonoids, saponins and sterols (Table 1). Therefore the methanolic extract has been selected for investigating anti microbial activity.

The anti microbial activity of P.specigera suggests that the extract contains the effective active phytochemical responsible for elimination of microorganisms. The in vitro anti microbial activities of methanolic extract of *P.specigera* were found to have maximum activity against all organisms except *pseudomonas aeruginosa*. The extract revealed (Table 2.) antibacterial activity at all concentrations selected, but only the extract with the concentration of 300µg/ml showed maximum anti microbial activity against the organism which are comparable with the standard control, Amikacin. The study compares the anti microbial properties obtained by a plant and which is easily available to common man. It may have fewer side effects. The present study exhibited the anti microbial effect of methanolic extract justified the medicinal use of *P.specigera* and further study is required to find out the active components of medicinal value.

4. CONCLUSION

It is concluded based on the findings of the present study that the aerial part of *Prosopis specigera* shows higher anti microbial activity against the bacterial pathogens such as *Bacillus subtilis*, *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Eschrichia coli*. Phytochemical analysis showed that the anti microbial activity of *P. specigera* was due to presence of phytochemical compounds. The extract of *P. specigerea* showed the maximum zone of inhibition at the concentration of 300µg/ml for anti bacterial activity against bacterial pathogens. The present study justifies the claimed uses of areal parts of the *P. specigera* in the traditional system of medicine to treat various infectious disease caused by microbes.

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