Research Article **ISSN: 2321 – 0915**

Asian journal of Phytomedicine and Clinical Research

Journal home page: www.ajpcrjournal.com

INVITRO ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF BARK OF *PROSOPIS SPICIGERA*

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ABSTRACT

Medicinal plants are value added for the content and chemical composition of their active components. Infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health, despite the tremendous progress in human medicine. In this paper, Hot and cold methanolic extracts from the stem bark of *Prosopis spicigera*, was tested for its possible presence of significant Antibacterial activity in an attempt to validate some of its folkloric uses. The antibacterial activity was evaluated by employing 24hr culture of 4 different test organisms, viz., *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Proteus vulgaris.* The hot and cold methanol extracts are screened for antibacterial activity by cup plate method using ciprofloxacin. The observations in the present study of in vitro antibacterial evaluation of *P.spicigera* forms a primary platform for further phytochemical and antibacterial studies.

KEYWORDS

Prosopis spicigera, Antibacterial activity, Cup plate method

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INTRODUCTION [1-9]

Antimicrobial activity of natural extracts and pure compounds can be detected by observing the growth response of various micro-organisms to samples that are placed in contact with them. Several methods for detecting activity are available, but since they are not equally sensitive or not based upon the same principle, results will be profoundly influenced by the method.

The impact of infectious diseases is particularly large in developing countries due to the relative unavailability of medicines and the emergence of

widespread drug resistance. Research on new antimicrobial substances must therefore be continued and all possible strategies should be explored. Besides small molecules from medicinal chemistry, natural products are still major sources of innovative therapeutic agents for various conditions, including infectious diseases. Only a minute portion of the available diversity among fungi, marine fauna and flora, bacteria and plants has yet been explored and ample opportunities lie theoretically ahead.

Attention has been drawn to the antimicrobial activity of plants and their metabolites due to the challenge of growing incidences of drug-resistant pathogens. Some plants have shown the ability to overcome resistance in some organisms and this has led to researchers' investigating their mechanisms of action and isolating active compounds. Particular focus is on establishing the effect of the plant(s) extracts in terms of their microstatic and microcidal action and the spectrum of organisms affected. This has enabled exploitation of plants for the treatment of microbial infections and in the development of new antimicrobial agents. Results comparability is largely dependent on the techniques employed in the investigations and conclusive results can only be obtained if methods are standardized and universal. The present study was undertaken to elucidate the significance of *Prosopis spicigera* locally known as ghaf, jand, jandi, and khejri for its antibacterial activity.

MATERIALS AND METHODS

Preliminary phytochemical studies

Solvent purification - methanol

A mixture of 500ml Methanol (National Scientific products Pvt Ltd., Guntur, AP.), 25ml furfural and 60ml of 10% NaOH solution was refluxed in a 2lit.Round bottom flask fitted with a double surface condenser for 6-12hrs. A resin was formed which carried down all the acetone present. The methanol was then fractionated through an efficient column. The first 5ml which might contain a trace of formaldehyde was rejected.

Plant material and Preparation of extracts

The species for the proposed study that is *Prosopis spicigera,* barks were collected from the campus of SIMS College of Pharmacy , Guntur, Andhra Pradesh. The barks of *Prosopis spicigera* were washed properly with water to remove mud (or) dust and it was dried completely under shade. The dried stems were then grinded by means of iron motor and pestle and were sieved through sieve no.12, to get the coarse powder, which was used for extraction with solvent. The dried and powdered plant material was subjected to both hot and cold methanol extraction shown in Figure No.1 and 2.

Extractive values

The yield and % yield of Hot & Cold methanol extracts of powdered bark of *Prosopis spicigera* were reported was show in Table No.1.

Standard Antibacterial Agent used for comparison of Antibacterial activity

The hot and cold methanol extracts are screened for antibacterial activity by cup plate method using ciprofloxacin (Ciprozol-500) in dimethyl sulphoxide (DMSO, 1 mg/ml) as a standard drug for the comparison of antibacterial activity. The sample extracts were prepared by dissolving each of 100,200,300,400,500 mg in 10ml of DMSO (i.e. 10, 20, 30, 40,50mg/ml) respectively and screened by cup plate method [10].

RESULTS AND DISCUSSION

Preliminary Phytochemical results of Hot and Cold Methanol Extracts of the Bark of *Prosopis spicigera* reveals the presence of Alkaloids, carbohydrates, steroids, glycosides, saponins, tannins, flavanoids was show in Table No.2.

IR spectra, showed sharp absorbance band at 2921.82, 2853.06, 1612.52, 1517.42, 1374.32, 1244.48, 1108.81, 1059.66, 822.48, 774.78 cm^{-1} and the following functional groups are present, CH-stretching (alkane), CH-stretching (alkane), N-H bending, N=N stretching, N-H bending, C=C stretching(aromatic), C-O stretching phenols, S=O stretching, C-N vibration, S=O stretching, C-O stretching alcohols, C-O stretching alcohols, C-H

bending (aromatic) was show in Table No.3,4 and Figure No.3, 4.

Results of the Antibacterial screening of Hot and Cold Methanol extracts of *Prosopis spicigera* bark revealed significant antibacterial activity against all tested bacterial strains with zones of inhibition ranging from 1.0 – 4.7 cm in diameter. The maximum antibacterial activity of Hot and Cold methanol extracts was exhibited against *B.subtilis*, when compared with standard drug. The hot methanol extract of *P.spicigera* bark showed more significant activity against all tested bacterial organisms than that of the cold methanol extracts. The highest zone was against the *B.subtilis*, while the least zone was against *P.vulgaris*.

This piece of work is preliminary screening to suggest that bark of *P.spicigera* has antibacterial activity. The present study of in vitro antibacterial evaluation of *P.spicigera* forms a primary platform for further phytochemical and antibacterial studies.

Table No.1: Extractive values of hot and cold methanol extracts of *Prosopis spicigera* bark

S. No	Extracts	Yield (gms)	% Yield (w/w)
1.	Hot Methanol Extract	5.3	2.65
2.	Cold Methanol Extract	5.9	2.95

Table No.2: Qualitative Phytochemical Analysis of Hot & Cold Methanol Extracts of Bark of *Prosopis Spicigera*

S. No	Phytoconstituents	Hot Methanol Extract	Cold Methanol Extract
1	Alkaloids	+	+
2	Carbohydrates	+	+
3	Reducing Sugar	+	+
4	Glycosides	+	+
5	Steroids	+	+
6	Terpenes	-	-
7	Lignin	-	-
8	Saponins	+	+
9	Tannins	+	+
10	Flavonoids	+	+

(+) - Present, (-) - Absent.

Table No.3: Preliminary TLC(Thin Layer Chromatography) Method Development of Hot & Cold Methanol Extracts of *Prosopis Spicigera*

Extract	Solvent system	colour of the spot	R_f Value
Hot Methanol Extract	Toulene: Methanol (86:14) Toulene: Ethylacetate: Diethyl Amine (70:20:10)	Light brown Light brown	0.36 0.6
Cold Methanol Extract	Ethyl acetate: Methanol: Water (77: 13: 5.8))	Light brown	0.58

Table No.4: Different Bacterial strains used for the determination of antibacterial activity

Name	***S.aureus* (G+)**	***B.subtilis* (G+)**	***E.coli* (G-)**	***P.vulgaris* (G-)**
Growth Medium	Nutrient broth medium	Nutrient broth medium	Nutrient broth medium	Nutrient broth medium
Growth condition	Aerobic	Aerobic	Aerobic	Aerobic
Incubation temp	37°±1°	37°±1°	37°±1°	37°±1°
Incubation time	24 hr	24 hr	24 hr	24 hr
Subculture	3 times for 3 days .			
Source	Microbiology lab, SIMS college of Pharmacy, Guntur, AP.			

Table No.5: Zone of inhibition of the hot and cold methanol extracts of *Prosopis spicigera*

Extracts tested	Conc. (mg/ml)	Diameter of the zone of inhibition in (cm)			
		***S.aureus* (G+)**	***B.subtilis* (G+)**	***E.coli* (G-)**	***P.vulgaris* (G-)**
Hot methanol extract	10	2.4	1.5	1.6	1.0
	20	1.3	1.0	1.7	1.7
	30	1.6	1.9	2.0	1.7
	40	1.3	1.2	2.0	1.8
	50	2.2	2.3	2.0	1.9
Cold methanol Extract	10	1.5	2.0	1.4	1.3
	20	1.4	1.4	1.6	1.3
	30	1.9	1.2	1.3	1.6
	40	1.4	1.9	1.7	1.9
	50	1.6	1.4	1.4	2.0
Ciprofloxacin	10	3.7	4.7	3.3	2.5
	20	3.8	3.5	3.3	3.3
	30	4.0	3.6	3.6	3.5
	40	3.6	3.7	3.5	3.6
	50	3.6	3.5	3.4	3.6

Table No.6: IR Spectra of Cold Methanolic Extract of *P.spicigera*

Wave Number(cm-1)	Corresponding functional group
2921.82	CH-stretching (alkane)
2853.06	CH-stretching (alkane)
1612.52	N-H bending N=N stretching
1517.42	N-H bending C=C stretching(aromatic)
1374.32	C-O stretching phenols S=O stretching
1244.48	C-N vibrations S=O stretching
1108.81	C-O stretching alcohols
1059.66	C-O stretching alcohols
822.48 774.78	C-H bending (aromatic)

Figure No.1: TLC of Hot Methanol Extract of *P.S* Bark

Figure No.2: TLC of Cold Methanol Extract of *P.S.* Bark

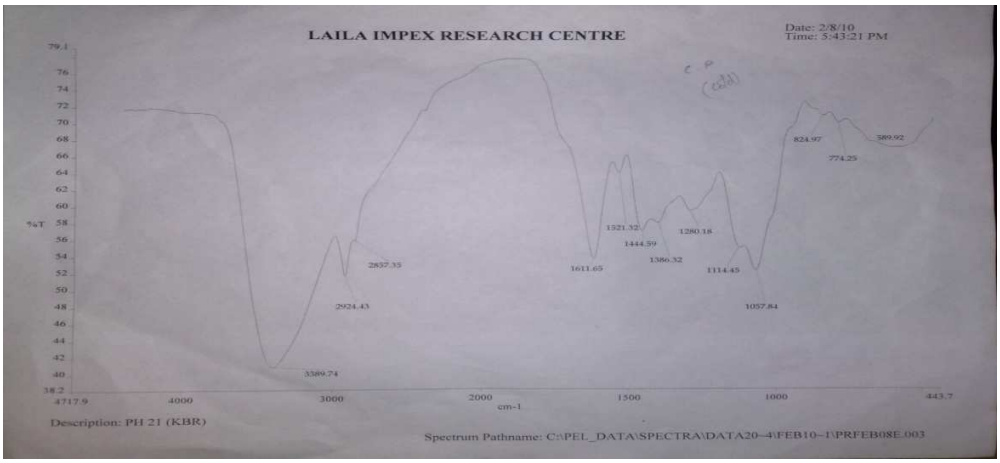

Fig 3: IR Spectrum of Cold Methanol Extract of *P.S.* Bark

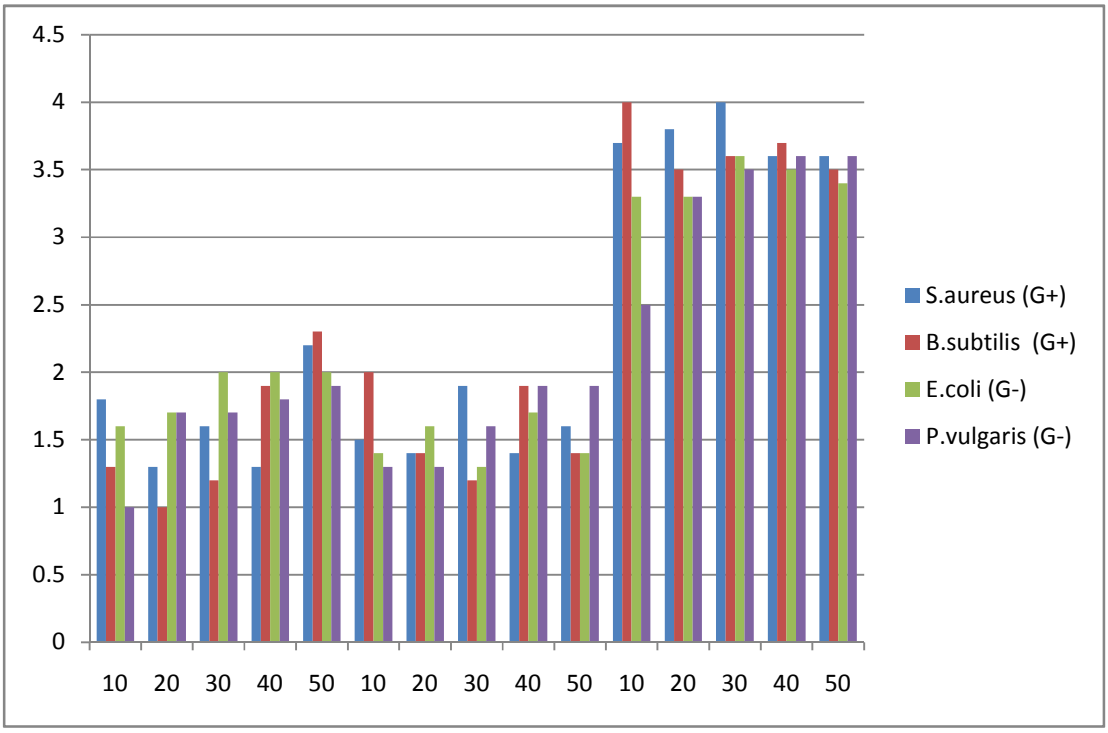

Figure No.4: Antibacterial activity of the hot and cold methanol extracts of *Prosopis spicigera* Bark

CONCLUSION

In conclusion, the hot methanol extract of *P.spicigera* bark showed more significant activity against all tested bacterial organisms than that of the cold methanol extracts. These promissory extracts open the possibility of finding new clinically effective antibacterial compounds.

CONFLICT OF INTEREST

The authors do not have a direct financial relation with the commercial identities mentioned in this paper that might lead to a conflict of interests.

ACKNOWLEDGEMENT

The authors are sincerely thankful to the management of SIMS College of Pharmacy, Mangaldasnagar, Guntur, for providing the facilities to carry out this research work. Also,We are very grateful to Prof. Dr. S.M. Khasim , H.O.D, Botany, Acharya Nagarjuna University, Guntur,AP, for giving suggestions and Authentification regarding the plants to carry out our project work.

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