



## ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF DRIED PODS OF *PROSOPIS CINERARIA*

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Accepted on: 29-02-2012; Finalized on: 25-04-2012.

### ABSTRACT

The antibacterial activity and phytochemical screening of dried unripe pods of *Prosopis cineraria* was evaluated against four gram negative bacteria viz *Escherichia Coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi* which causes severe diseases in human beings. The polar and non polar extracts were prepared and studied for the antimicrobial activity using agar well diffusion method. Result showed promising antibacterial activity of methanolic extract against all the pathogens. The phytochemical analysis showed the presence of tannins, alkaloids, flavonoids and glycosides in the dried unripe pods of *Prosopis cineraria*. The study supports the traditional use of *Prosopis cineraria* in curing diseases and results revealed that unripe pods of *Prosopis cineraria* possesses antibacterial activity and antimicrobial phytoconstituents.

**Keywords:** Dried unripe pods of *Prosopis cineraria*, Antibacterial activity, Phytochemical analysis.

### INTRODUCTION

Plants and plants products used as medicine from the ancient time. It is estimated by the World Health Organization that approximately 75-80% of the world's population uses plant medicines either partly or entirely as medicine. Interest in plant derived drug increases mainly due to the increasing use, and misuse, of existing antibiotics which increases the development of resistance in microbes. This poses the need for search and development of new drugs to cure diseases<sup>1</sup>.

Western Thar Desert is rich in varieties of plants. *Prosopis cineraria*, one of boon plants of Western Rajasthan provide valuable natural resources to the people. It belongs to member of a family Leguminosae, is a tree that is mainly found in the Thar Desert of Western Rajasthan. It is native to arid portion of Western and South Asia. *Prosopis cineraria* is one of the highly valued plant in the Indigenous System of Medicine. It is also known to possess anthelmintic, antibacterial, antifungal, antiviral, anticancer and several other pharmacological properties<sup>2</sup>. Traditionally dried pods of *Prosopis Cineraria* are commonly used as a food. The dried pods locally called "Kho-Kha" or "Sangari" are eaten as a vegetable. Dried pods also form rich animal feed, which is liked by all livestock. They are also used as famine food and known even to prehistoric man. Though unripe pods of *Prosopis cineraria* is found in profusion and contain nutritional qualities like protein, sugar, carbohydrate, fat but still no literature was found on its antimicrobial properties<sup>3</sup>. The present study was therefore undertaken to evaluate the antibacterial property of the dried pods of *Prosopis cineraria* against four clinical isolates with preliminary phytochemical screening.

### MATERIALS AND METHODS

#### Collection of Dried Unripe Pods

Dried unripe pods of *Prosopis cineraria* were collected from the market Jodhpur, Rajasthan and identified with the help of Flora of Rajasthan<sup>4</sup>.

#### Preparation of Extracts

Dried pods were powdered and 25 grams of powder was used for extraction using soxhlet apparatus. The extraction was made with different solvents on the basis their increasing of polarity viz petroleum ether, chloroform, and methanol. The resultant extracts were placed in rotary evaporator to evaporate the solvents and kept in vacuum desiccators for further study.

Aqueous extract was prepared by reflux method in which 20gm of powder was heated with 200ml of distil water at 50°C under reflux for 48 hours<sup>5</sup>.

#### Bacterial Culture

The antibacterial activity was tested against four clinical isolates. It includes four Gram negative bacteria *Escherichia Coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*. These isolates were taken from the Ramdeo laboratory, of Jodhpur, Rajasthan and were identified microscopically and on biochemical basis<sup>6</sup>. Identified isolates were stored in 20% glycerol at -20°C and subculture on the nutrient agar at 37°C for 24 hours before use.

#### Inocula Preparation

Bacterial strains were inoculated into 10 ml of sterile nutrient broth, and incubated at 37°C for 24 hours. The concentration of inocula was set to 0.5 McFarland's standards<sup>7</sup>.



### Antibacterial Susceptibility Assay

The antimicrobial susceptibility was determined by using Agar Well Diffusion Method<sup>8</sup>. The entire nutrient agar surface was seeded with the inoculums suspension and allowed to dry for 5 minutes. The wells of 6mm were created and 70µl of the each extract was poured into it. The plates were kept in refrigerator for about 15 minutes for proper diffusion of the extract and then were incubated at 37°C for 24 hours. At the end of incubation zone of inhibition was measured in millimeters. This exercise was done in triplicates to ensure reliability.

### Phytochemical screening

Preliminary Phytochemical analysis of the dried pods were undertaken and tested for various antimicrobial phytoconstitutents<sup>9, 10</sup>.

#### Alkaloids

About 200gm of plant material was boiled with 2% HCl on a steam bath and filtered. 1 ml portion of the filtrate was measured into four test tubes. Each of the 1 ml filtrate was treated with 2 drops of the following reagents for the presence of alkaloids.

- 1) Mayer's reagent (Potassium mercuric iodide solution): gives creamy-white colored precipitate.
- 2) Dragendorff's reagent (Potassium bismuth iodide solution): gives red precipitate.
- 3) Wagner's reagent (Iodine potassium iodide solution): gives reddish-brown precipitate.
- 4) Hager's reagent (Picric acid and Picrolonic acid): gives yellow colored precipitate.

#### Tannins

About 200mg of powdered plant material was boiled in 10ml of distil water. The mixture was filtered.

To the 2 ml of the filtrate, 2ml of FeCl<sub>3</sub> solution was added and observed for greenish to black color.

#### Saponin

About 100mg of sample was boiled with 5 ml of distil water. The mixture was filtered and 1ml of the filtrate was shaken vigorously and observed for froth formation.

#### Flavonoids

Two methods were used to determine the presence of flavonoids in plant sample:

1. About 200 mg of plant material was dissolved in diluted NaOH and then HCL was added to it. A yellow solution turns to colorless, show the presence of flavonoids.

2. To the 200 mg of plant material and 10 ml ethanol was added. The mixture was boiled for few minutes. A small piece of magnesium ribbon was added to the filtrate with addition of few drops of conc. HCl. Pink tomato red color indicates the presence of flavonoids.

#### Steroids and Terpenoids

A quantity (9 ml) of ethanol was added to 1 gm of the extract and refluxed for a few minute and filtered. Each of the filtrates was concentrated to 2.5 ml in a boiling water bath. Distilled water, 5 ml was added to the concentrated solution, the mixture was allowed to stand for 1 hour and the waxy matter filtered off. The filtrate was extracted with 2.5 ml of chloroform using a separating funnel. To 0.5 ml of the chloroform extract in a test tube, 1 ml of concentrated sulphuric acid was carefully added to form a lower layer. A reddish brown interface shows the presence of steroids.

To another 0.5 ml of the chloroform extract was evaporated to dryness on a water bath and heated with 3 ml of concentrated sulphuric acid for 10 minutes on a water bath. A grey color indicates the presence of terpenoids.

#### Glycosides

Keller-kiliani test –To 2ml of the filtrate, 1ml of Glacial acetic acid, FeCl<sub>3</sub> and conc. H<sub>2</sub>SO<sub>4</sub> was added. A green Blue color indicates the presence of glycosides.

## RESULTS AND DISCUSSION

Preliminary screening of antimicrobial potential was evaluated by using Agar well diffusion method on different extracts of dried unripe pods of *Prosopis cineraria* presented in Table 1 respectively. Methanolic extract shows significant results on all pathogens whereas no activity was recorded by petroleum ether extract. Among the tested four gram negative bacteria, *K.pneumoniae* was more susceptible to methanol, chloroform and aqueous extracts. Maximum zone of inhibition 34.0±1.0 was observed against *K.pneumoniae* in methanolic extract, where as 19.0±1.0 in chloroform extract and 6.3±0.57 in aqueous extract. Aqueous and chloroform extract of the unripe pods of *Prosopis cineraria* did not showed any activity against *E.coli*, *P.aeruginosa* and *S.typhi*.

**Table 1:** Antibacterial activity of different extracts of dried unripe pods of *Prosopis cineraria*

Clinical Isolates		Zone of Inhibition (mm)				
		Aqueous	Methanol	Chloroform	Petroleum Ether	Positive Control
1.	<i>E.coli</i>	0.0	29.3±0.57	0.0	0.0	10.0±0.57
2.	<i>K.pneumoniae</i>	6.3±0.57	34.0±1.0	19.0±1.0	0.0	12.0±1.0
3.	<i>P.aeruginosa</i>	0.0	19.6±0.57	0.0	0.0	15.0±1.0
4.	<i>S.typhi</i>	0.0	23.3±0.57	0.0	0.0	16.6±0.57

Data given are mean of three replicates ± Standard error.



The minimum zone of inhibition  $19.6 \pm 0.57$  was showed by methanol extract in *P.aeruginosa*. Standard antibiotic, Chloramphenicol was taken as a positive control. It was observed that methanol the extract showed better results against all pathogens in comparison to standard antibiotic. Earlier the antimicrobial properties were reported by researchers from stem bark<sup>11</sup> and leaflets<sup>12</sup> of *Prosopis cineraria* and even from root, stem, bark, pods of the different species of *Prosopis* i.e. *Prosopis julifera*<sup>13</sup> and *Prosopis Africana*<sup>14</sup>. Therefore, this was the first attempt when dried unripe pods of *Prosopis cineraria* were screened for antimicrobial potential.

Table 2 showed the presence of antimicrobial phytoconstituents tannins, alkaloids, flavonoids and glycosides in the dried unripe pods of *Prosopis cineraria*.

### CONCLUSION

So, it may be concluded that antimicrobial active chemical was supposed to belongs to these categories. Considering the medicinal purpose further studies are required to find out more activities of unripe pods of *Prosopis cineraria* and to isolate, purify and identify the compounds responsible for antibacterial activity.

**Table 2:** Phytochemical analysis of dried unripe pods of *Prosopis cineraria*

Compounds	Results
Tannins	+
Alkaloids	+
Saponin	-
Steroids	-
Flavonoids	+
Terpenoids	-
Glycosides	+

+ Presence, - Absence

**Acknowledgement:** The authors are grateful to Prof. Swarnjeet Kaur, Head, Department of Botany, Jai Narain Vyas University, Jodhpur for providing basic research facilities.

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