Research Article

# Preliminary Phytochemical and Antimicrobial Screening of *P. cineraria* Leaf Extract

**Narware Neha[1*], PK. Mishra[1] and PN. Shrivastava[2]**

[1]Department of Zoology, Govt. J.H. P.G. College, Betul, Madhya Pradesh, India.
[2]S.S.L. Jain. P.G. College Vidisha, Madhya Pradesh, India.

**ABSTRACT**

In the present study, plant *Prosopis cineraria* belong to the family Leguminosae and Sub-family Mimosaceae, commonly known as, Khejri or Shami was reported to have antimicrobial activity against *S. aureus* and *E. coli* and results reported preliminary phytochemical screening of the leaf extracts of *P. cineraria* with good percentage yield viz. 3.89, 3.81 and 3.16% in chloroform, ethanol and water, respectively and extracts possess alkaloids, glycosides, saponins and flavonoids. The results of antimicrobial activity of leaf extracts of *P. cineraria* described that the maximum zone of inhibition (12±0.1 mm) against *S. aureus* was found at 1.25 mg/disc of leaf ethanolic extract followed by leaf water extract (10±0.1mm) and leaf chloroform extract (8±0.1), respectively which was compared with the inhibition zone of *S. aureus* (26±0.1 mm) with standard antibiotics streptomycin 10µg/disc. Besides this, antimicrobial activity of *P. cineraria* leaf extract was tested against *E. coli* and it was noticed that the maximum zone of inhibition was found at 1.25 mg/disc leaf ethanolic extract (10±0.1 mm) followed by leaf water extract (8±0.1 mm) and leaf chloroform extract (7±0.1 mm), respectively which was compared with the inhibition zone (28±0.1 mm) of *E. coli* with standard antibiotics streptomycin 10µg/disc.

**Keywords:** Antimicrobials, *Prosopis cineraria, Staphylococcus aureus, Escherichia coli.*

## INTRODUCTION

Plants are the most common source of antimicrobial agents. Their usage as traditional health remedies is the most popular for 80% of world population and is reported to have minimal side effects. In recent years, pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants, to produce more cost effective remedies that are affordable to the population. The rising incidence in multidrug resistance amongst pathogenic microbes has further necessitated the need to search for newer antibiotic sources. *Prosopis cineraria* belong to the family Leguminosae and Sub-family Mimosaceae, commonly known as, Khejri or Shami. Leaves and pods are extensively used as fodder for cattle, camels and goats. *Prosopis* species have also been extensively used in indigenous system of medicine as folk remedy for various ailments like leprosy, dysentery, bronchitis, asthma, leucoderma, pile, muscular tremors and wandering of the mind (Kirtikar and Basu, 1984, Duke 1983). It is also known to possess anthelmintic, antibacterial, antifungal, antiviral, anticancer and several other pharmacological properties. Leaf paste of *Prosopis cineraria* is applied on boils and blisters, including mouth ulcers in cattle and leaf infusion on open sores on the skin. The smoke of the leaves is considered good for eye troubles (Chopra et al., 1958; Chopra et al., 1956; Nadkarni, 1954; Usmanghani et al. 1974).

### Experimental

In the present study, whole plants and their parts used were collected from surroundings of Betul and identified and authenticated by Taxonomist Dr. P. N. Shrivastava, Dept. of Botany, S. S. L. Jain P. G. College, Vidisha (M.P.) which were procured in the Herbarium Record (Se. No. 48) at Dept. of Botany, S. S. L. Jain P. G. College, Vidisha (M.P.). The sample brought into the laboratory for shade drying at room temperature. The powdered materials of the plant in 40-60 mesh size was prepared by using mortar and pestle. The powdered material was poured in Soxhlet apparatus for extraction and applied different solvent viz. chloroform, ethanol and water. The obtained crude extracts were further purified and subjected to phytochemical screening to testify the presence of the phytoconstituents viz. Tannins, Saponins, Sesquiterpene, Alkaloids and Flavonoids.

In the present study, the average number of viable *Staphylococcus aureus* and *Escherichia coli* (M.T.C.C No 739 and 96) organism per ml of the stock suspension was obtained in a vials and were maintained at 4°C and sub-cultured it in Agar media, regularly till the completion of experiment in the Microbiology Lab., Govt. J.H.P.G. College, Betul and determined for the means of the surface viable counting technique (Miles and Misra 1938). Approximately, 108-109 colony forming units per ml were used. Each time, a fresh suspension was prepared and the experimental conditions were maintained constant so that suspensions with very close viable counts were obtained. For the present study, agar cultures of the testing microorganism were prepared as described by Mackeen et al. (1997) and three to four colonies were selected and transferred to 5 ml broth with a loop and the broth cultures were incubated for 24 hours at 37°C. The extracts of the plants were dissolved in dimethylsulfoxide (DMSO) with a magnetic stirrer. For screening, sterile 6-mm diameter filter paper discs were impregnated with 125 mg/disc of the plant extracts and then placed in Muller Hinton Agar medium. The inoculum for *Staphylococcus aureus* and *Escherichia coli* was prepared from broth culture. Results of the extracts of the selected plants were recorded by measuring the zones of growth inhibition of *Staphylococcus aureus* and *Escherichia coli* surrounding the disc by Disc Diffusion technique and comparison was done with the standard antibiotics streptomycin (10 µg) was placed as controls.

A minimum inhibitory concentration (MIC) is the lowest concentration of an anti-microbial that inhibits the growth of a micro-organism after 18-24 hrs. The extracts of plants were subjected to the serial broth dilution technique to determine their minimum inhibitory concentration. Standard antibiotics streptomycin was used as standard to compare with obtained values. A 10 µl of 107 (CFU) bacterial cultures were added to the tubes and were incubated at 37°C for 18 hr. MIC was determined by visual observation. The MIC of the extracts that showed no detectable growth was taken as the minimum inhibitory concentration. However, a minimum inhibitory concentration (MIC) is the lowest concentration of an antibiotic required to kill a microorganism. The MIC was determined by sub-culturing 10µL of the test dilutions from MIC tubes on fresh Muller-Hinton agar plates. Plates were incubated at 18 to 24 hr. The highest dilution that yielded no single bacterial colony on the plates was recorded as MIC. In the present study, MIC was calculated for each extract of selected plant against *Staphylococcus aureus* and *Escherichia coli*.

## RESULTS AND DISCUSSION

In the present study, the percentage yields of the extract were obtained from the parts used of the plant and the highest percentage yield (3.89%) of *Prosopis cineraria* leaf extract was found in chloroform, followed by 3.81%, in ethanol and minimum 3.16% was found in water (Table 1). Similarly, highest percentage yield (13.46% w/w) and lowest (1.16%w/w) percentage yield of *Prosopis cineraria* hydro alcoholic extract of leaf and stem bark was noticed by Robertson, Narayanan and Nargis (2012), respectively. In the preliminary phytochemical study of the *Prosopis cineraria* extract was found to be positive for alkaloid, glycosides, saponins, flavonoids whereas the study of Tarachand et al. (2012) reported the extract positive for alkaloid, protein, carbohydrates, flavonoids, glycosides, saponins and tannins in alcoholic and water extracts of *Prosopis cineraria*. Antimicrobial activity of leaf extracts of *Prosopis cineraria* were tested against *Staphylococcus aureus* bacterial strain. It was observed that the maximum zone of inhibition against *S. aureus* was found when applied 1.25 mg/disc, leaf ethanolic extract (12±0.1 mm) followed by leaf water extract (10±0.1mm) (Fig. 1) and leaf chloroform extract (8±0.1), respectively which was compared with the inhibition zone of *S. aureus* (26±0.1 mm) by applying standard antibiotics streptomycin 10µg/disc (Fig.3). Besides this, antimicrobial activity of *Prosopis cineraria* leaf extract was tested against *Escherichia coli* bacterial strain, it was observed that the maximum zone of inhibition was found when applied 1.25 mg/disc leaf ethanolic extract (10±0.1 mm) (Fig.2) followed by leaf water extract (8±0.1 mm) and leaf chloroform extract (7±0.1 mm), respectively which was compared with the inhibition zone (28±0.1 mm) of *E. coli* when applied standard antibiotics streptomycin 10µg/disc (Table 3 and Fig. 4). Similarly, Valmurugan et al. (2010) have discussed antibacterial activity of various stem bark extracts against certain pathogenic microorganisms and maximum zone of inhibition in case of *S. aureus* was noticed 10, 13 and 13 mm in chloroform, water and methanolic extract of *P. cineraria*, respectively.

## CONCLUSION

The results of the present study indicate that herbal extracts of *P. cineraria* plant can be used as potential antimicrobial agent.

**Table 1: Percentage yields of *P. cineraria* extract by Soxhletion**

| Whole Plant materials | Solvent used for extraction | % yield of plant extract |
|---|---|---|
| *Prosopis cineraria* Leaf | Pet. ether | 4.16% |
| | Chloroform | 3.89% |
| | Ethanol | 3.81% |
| | Water | 3.16% |

**Table 2: Phytochemical screening test of *Prosopis cineraria* extract**

| S. No. | Phytochemical constituents | Chloroform extract | Ethanol extract | Water extract |
|---|---|---|---|---|
| 1 | Alkaloid | + | + | + |
| 2 | Glycosides | + | + | + |
| 3 | Saponins | + | + | + |
| 4 | Flavonoids | + | + | + |
| 5 | Phenolics/Tannins | + | + | _ |
| 6 | Proteins/Amino acids | _ | _ | _ |
| 7 | Steroids | _ | _ | _ |
| 8 | Carbohydrates | + | + | _ |

+ (Present), - (Absent)

**Table 3: Minimum Inhibitory Concentration (MIC) values of leaf extracts of *P. cineraria*.**

| Microbial strain | Maximum Zone of Inhibition (mm.) Minimum Inhibitory Concentration (1.25mg/Disc) | | | |
|---|---|---|---|---|
| | Chloroform extract | Ethanolic extract | Water extract | Standard drug* |
| *Staphylococcus aureus* (MTCC No. 739) | 08±0.1 | **12±0.1 (Fig.1)** | 10±0.1 | 26±0.1(Fig.3) |
| *Escherichia coli* (MTCC No. 96) | 07±0.1 | **10±0.1 (Fig.2)** | 08±0.1 | 28±0.1 (Fig.4) |

Values in mean ± S.E., Streptomycin 10µg/disc

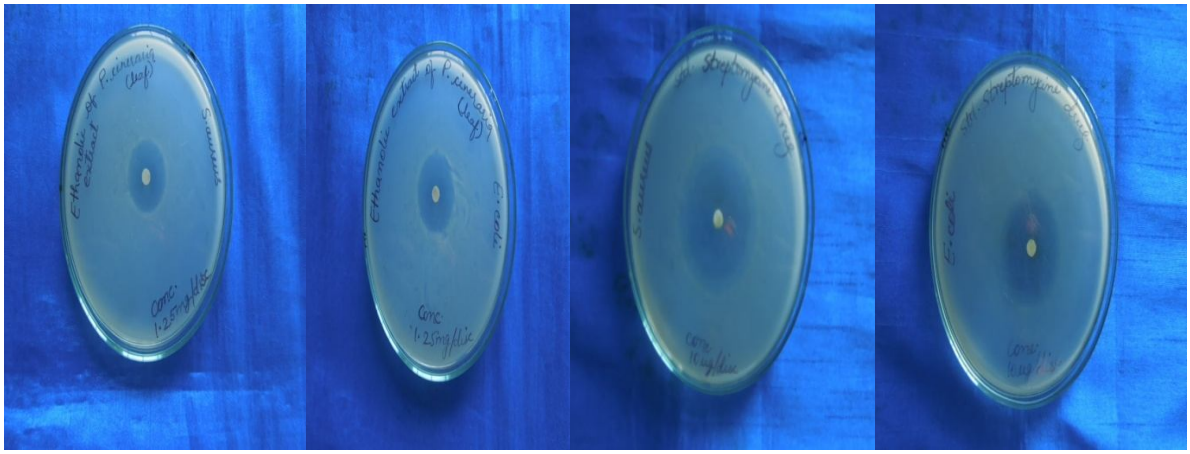

**Fig. 1- 4: Maximum zone of inhibition**

## REFERENCES


1. Chopra RN, Chopra IC, Handa KL and Kapoor LD. In: Chopra's Indigenous drugs of India. U.N. Dhur and Sons Pvt. Ltd. Calcutta. 1958;521
2. Chopra RN, Nayar SL and Chopra LC. Glossary of Indian Medicinal Plants. New Delhi: Council of Scientific and Industrial Research. 1956;123.
3. Duke JA. Handbook of energy crops. http://www.hort. purdue. edu/ewcrop /duke_energy /Prosopis cinereria.html. 1983.
4. Kritikar KR and Basu BD. Indian medicinal plants, 2nded, 1987;910-912.
5. Mackeen MM, Ali AM, El–Sharkawy SH, Salleh MY, Lajis NH and Kawazu K. Antimicrobial and cytotoxic properties of some Malaysian traditional vegetables. Int J Pharmacogn. 1997; 35:237-243.
6. Miles AA and Misra SS. Estimation of Bacterial power of blood J Hyg. 1938;38:732.

7. Nadkarni AK. In: Indian Materia Medica. Popular Book Depot, Bombay. 1954;1011.
8. Robertson S, Narayanan N and Nargis NR. Toxicity evaluation on hydroalcoholic extracts of leaf and stem bark of Prosopis cineraria. Intern J of Pharmacy and Pharmaceutical Sciences. 2012;4(3):113-118.
9. Tarachand A, Bhandari BK, Sharma KA and Nagar N. Physicochemical and Preliminary phytochemicals screening of pods of Prosopis cineraria (L.) Druce. Pelagia Research Library, Der Pharmacia Sinica. 2012;3(3):377-381.
10. Usmanghani K, Saquib QN, Jewers K, Nagler MJ, Zirvi KA, Amir F and Kottee FH. Pahlavi Med J. 1974;5:1.
11. Velmurugan V, Arunachalam G and Ravichandran V. Antibacterial activity of stem barks of Prosopis cineraria (Linn.) druce. Archives of Applied Science Research. 2010;2(4):147-150.