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ANTIMICROBIAL ACTIVITY OF *PROSOPIS CINERARIA* (LEAF) METHANOL & CHLOROFORM EXTRACT AGAINST SELECTED BACTERIAL SPECIES

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ABSTRACT

Western Thar Desert is rich in varieties of plants. *Prosopis cineraria*, one of boon plants of Western Rajasthan give valuable natural possessions to the people. It belongs to member of a family Leguminosae, is a tree that is mostly found in the Thar Desert of Western Rajasthan. It is native to arid portions of Western and South Asia. *Prosopis cineraria* is one of the exemplary attempts were made to isolate successive extracts with methanol & chloroform of *selected* valued plant in the native System of Medicine. Therefore, in the present investigation *is P. cineraria(Leaf)* and Antimicrobial activity were performed against selected bacterial species. Methanolic extract of *P. cineraria* leaf extract shows maximum inhibition zone against *Proteus vulgaris*(AI=2.5 for 10mg/disc) and *Staphylococcus aureus* (AI= 2.09 for 5mg/disc). Therefore *Prosopis cineraria* can be used for bioactivity guided fractionation to work as potential source of therapeutics and /or antibiotics in future for curing of various ailments against multi drug resistant bacteria. Further, this weed will work as novel targets for antibiotics in future.

KEYWORDS: ***Prosopis cineraria*** **(L.) Druce, Antimicrobial activity.**

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INTRODUCTION

Prosopis cineraria is one of the mainly general tree of the Indian desert belonging to family Mimosacease and locally famous as Khejri. This is a favorite tree for agro-forestry and is a trendy renewable source of fuel, fodder, timber and vegetables needed general population [1]. It is an vital factor of desert environment of India as biomass manufacturer and as leguminous tree it enrich arid region soil, fix atmospheric nitrogen and provide a green exposure. The undeveloped and mature pods are energy rich safe to eat and have high nutritional value [2]. *P. cineraria* is well adopt to arid and semi arid situation of the Indian desert, maybe due to their well developed and unreserved tap root system which arrive at up to a length of 20 m, often reaching out the soil water resources [3]. In Rajasthan, there is a general observe to produce the green pods, which are boiled, dehydrated and sell as vegetable either separately or in mixture with an additional local plant crop to yield *Pach-kutta,* a mixture of five variety. Green pods generally called as *sangria* or *hangri* and the developed ones, called *khokha,* are used as vegetables. During India's infamous Rajputana famine (1868-69), many lives were secure by the sweetish bark as a food. It was a land into flour to make cakes. Wood used for boat frames, houses, post and tool handles. The reduced form of unimproved trees confines its use as wood with 31% soluble potassium salts and the wood ash may serve as a potash foundation. Pods and leaves give expensive fodder in the dry time of year. Bark and leaf galls are regularly used for tanning. The gum exudate from the trunk is indicative of gum Arabic. report to be sharp, demulcent and pectoral, *ghaf* is a folk therapy for a variety of ailments. The flowers are mixed with sugar and administer to stop miscarriage. The vestiges are rub over the skin to eliminate hair. The bark careful antihelminthic, refrigerant and tonic that are used for asthma, bronchitis, dysentery, leucoderma, leprosy, muscle tumors, piles and wandering of the mind. Smoke from the leaves is used for eye troubles, but the fruit is said to be indigestible, inducing biliousness, destroying nails and hairs. Little information is available about disease incidence on *P. cineraria.* Recently heavy mortality of standing age old trees have been recorded due to their attacks of root and wood rotting fungi (*Gonoderma sp., Fomes sp., Phillinus sp.*) mainly in areas which accept less than 250 mm rainfall [4]. 2-20% mortality was practical in Rajasthan by the mutual activity of insect and fungal organisms [5, 6].

MATERIALS AND METHODS

(1) Collection and Drying of the Plant Material

The plant *Prosopiscineraria*(L.) Druce. Chosen for the present study. Various plant parts were collected in the month of July 2013, from Ramchandrapura, Jaipur. The leaves were washed in tap water, shade dried for 10 days and then homogenized to fine powder of 40 mesh sizes using the electric blender and stored in airtight bottles.


(ii) Preparation of extract

The powdered Material (Leaf) of *Prosopis cineraria* (500gm) was subjected to hot continuous extraction in a soxhlet extractor, successively with different known solvents in increase order of polarity *viz* petroleum ether or DCM(Di-chloromethane) (60-80^{o} C), Benzene, Ethyl acetate, Chloroform, Alcohol. Finally, the powdered material was macerated with water for 24 hrs to obtain aqueous extract. Each time before extracting with next solvent, the powdered material was dried in hot air oven below 500C. Each extract was then concentrated by distilling off the solvent by evaporation to a water bath. All the extracts were stored in refrigerator for qualitative analysis.

(III) Culture of test microbes

For the cultivation of bacteria, Nutrient Agar Medium (NAM) was prepared by using 20 gm Agar, 5 gm Peptone, 3 gm beef extract and 3 gm NaCl in 1 liter distilled water and sterilized at 15 lbs pressure and 121°C temperature for 25-30 minutes. Agar test plates was prepared by pouring approximately 15 ml of NAM into the Petri dishes (10 mm) under aseptic conditions. A saline solution was prepared (by mixing 0.8 % NaCl) in distilled water, followed by autoclaving and the bacterial cultures were maintained on this medium by regular sub-culturing and incubation at 37°C for 24-48 hours. To prepare the test plates, in bacteria, 10-15 ml of the respective medium was poured into the Petri plates and used for screening. For assessing the bactericidal efficacy, a fresh suspension of the test bacteria was prepared in saline solution from a freshly grown Agar slant. Crushed powder of leaf of the Prosopic cineraria was soxhlet extracted with use of different solvent . Later, each of the homogenates was filtered and the residue was re-extracted twice for complete exhaustion, the extract was pooled individually. Each filtrate was concentrated to dryness in vitro and stored at 4°C in a refrigerator, until screened for antibacterial activity.

(IV) Bactericidal assay

For both, bactericidal in vitro Disc diffusion method was adopted (Gould and Bowie, 1952), because of reproducibility and precision. The different test organisms was proceeded separately using a sterile swab over previously sterilized culture medium plates and the zone of inhibition was measured around sterilized dried discs of Whattman No.1 paper (6 mm in diameter), which was of three different concentration

- A = 1mg of test extract/disc
- B = 5mg of test extract/disc
- C = 10mg of test extract/disc

And tetracycline as reference drugs (standard disk) separately. Such treated discs was air-dried at room temperature to remove any residual solvent, which might interfere with the determination, sterilized and inoculated. These plates was initially placed at low temperature for 1 h so as to allow the maximum diffusion of the compounds from the test disc into the agar plate and later, incubated at 37°C for 24 h in case of bacteria, after which the zones of inhibition could be easily observed. Replicate of each test extrac was examined and the

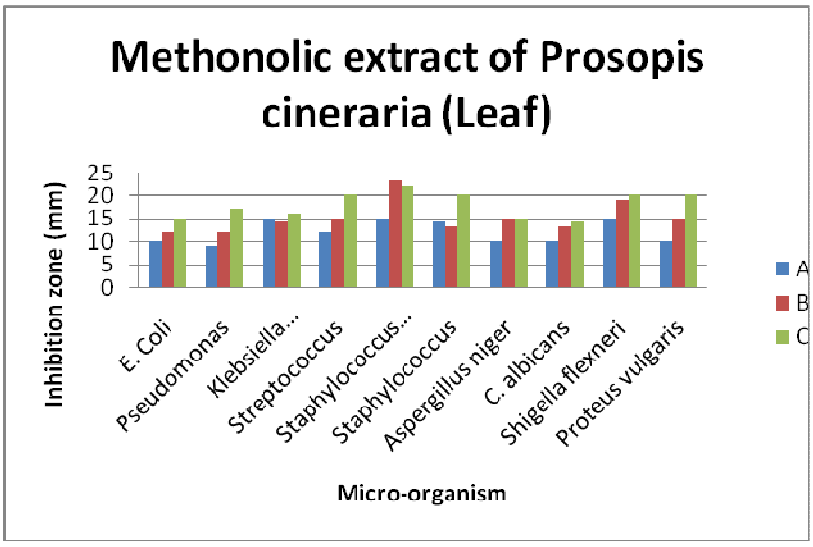
mean values were then referred. The inhibition zone (IZ) in each case was recorded and the activity index (AI) was calculated as compared with those of their respective standard reference drugs (AI = Inhibition Zone of test sample / Inhibition zone of standard).

OBSERVATIONS & RESULTS

The present study concluded that *Prosopis cinerari* shows antimicrobial activity against the pathgenic bacteria and could be useful in curing the diseases of human as well as the animals to some extent.

Table 1
Showing the inhibition zone and activity index of Methanol extract of Prosopis cineraria (leaf) against selected test microorganisms

Micro-organisms	A		B		C		Standard
	I.Z. (mm)	A.I.	I.Z. (mm)	A.I.	I.Z. (mm)	A.I.	I.Z. (mm)
E. coli	10	0.5	20	1	15	0.75	20
Pseudomonas aeruginosa	9	0.34	12	0.46	17	0.65	26
Klebsiella pneumoniae	15	1.36	14	1.27	16	1.45	11
Streptococcus sps.	12	0.66	15	0.83	20	1.11	18
Staphylococcus aureus	15	1.36	23	2.09	22	2	11
Staphylococcussps.	14	1.73	13	0.56	20	0.86	23
Aspergillus niger	10	0.66	15	1	15	1	15
C. albicans	10	0.52	13	0.68	14	0.73	19
Shigella flexneri	15	0.75	19	0.95	20	1	20
Proteus vulgaris	10	1.25	15	1.87	20	2.5	8



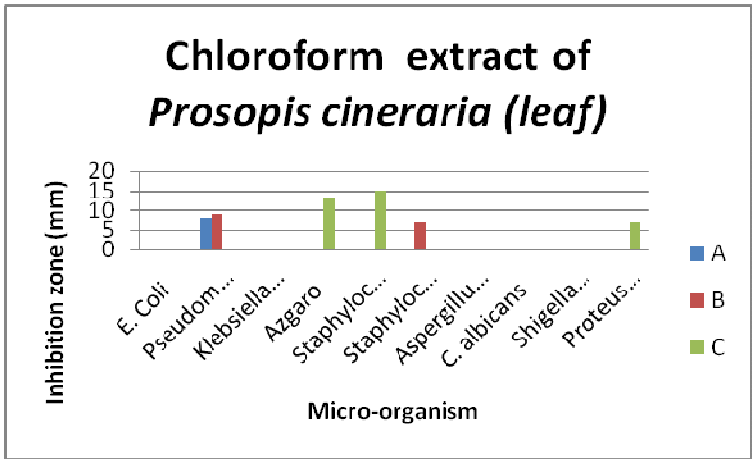
Graph 1
Antimicrobial activity of Prosopis cineraria (Leaf) Extracts with Chloroform

While screening, the Chloroform extract of *Prosopis cineraria (leaf)* showing moderate activity against selected test micro-organisms. The maximum efficacy was shown *Staphylococcus* and Azgaro

- A = 1mg/disc; B = 5 mg/disc; C= 10mg/disc
- I.Z. = Inhibition zone; A.I. = Activity index

Table 2
Showing the inhibition zone and activity index of Chloroform extract of Prosopis cineraria (leaf) against selected test microorganisms

Micro-organisms	A		B		C		Standard
	I.Z. (mm)	A.I.	I.Z. (mm)	A.I.	I.Z. (mm)	A.I.	I.Z. (mm)
E. coli	Nil		Nil	Nil	Nil	Nil	20
Pseudomonas	8	0.30	9	0.34	Nil	Nil	26
Klebsiella pneumoniae	Nil	Nil	Nil	Nil	Nil	Nil	11
Streptococcus	Nil	Nil	Nil	Nil	13	0.72	18
Staphylococcus aureus	Nil	Nil	Nil	Nil	15	1.36	11
Staphylococcus	Nil	Nil	7	0.30	Nil	Nil	23
Aspergillus niger	Nil	Nil	Nil	Nil	Nil	Nil	15
C. albicans	Nil	Nil	Nil	Nil	Nil	Nil	19
Shigella flexneri	Nil	Nil	Nil	Nil	Nil	Nil	20
Proteus vulgaris	Nil	Nil	Nil	0.86	7	0.87	8



Graph 2
Figures showing Antimicrobial activity of Prosopis cineraria (Leaf)extract with Methanol against selected test organisms

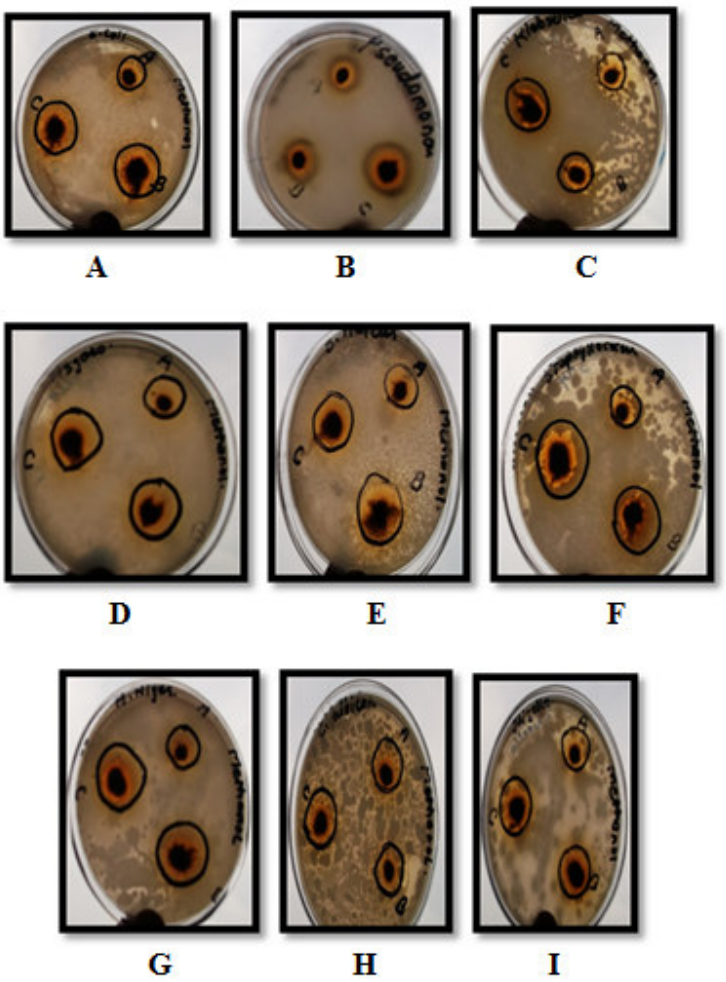


Figure 1

Antimicrobial activity of Prosopis cineraria (Leaf) with chloroform extract A (E.coli) B (Pseudomonas), C (Klebsiella pneumoniae), D (Azgaro), E (Staphylococcus aureus), F (Staphylococcus,) G (Aspergillus niger), H (C. albicans) and I (Shigella flexneri) J (Proteus vulgaris)

Figures 2 Showing Antimicrobial activity of Prosopis cineraria (Leaf)extract with Chloroform against selected test organisms

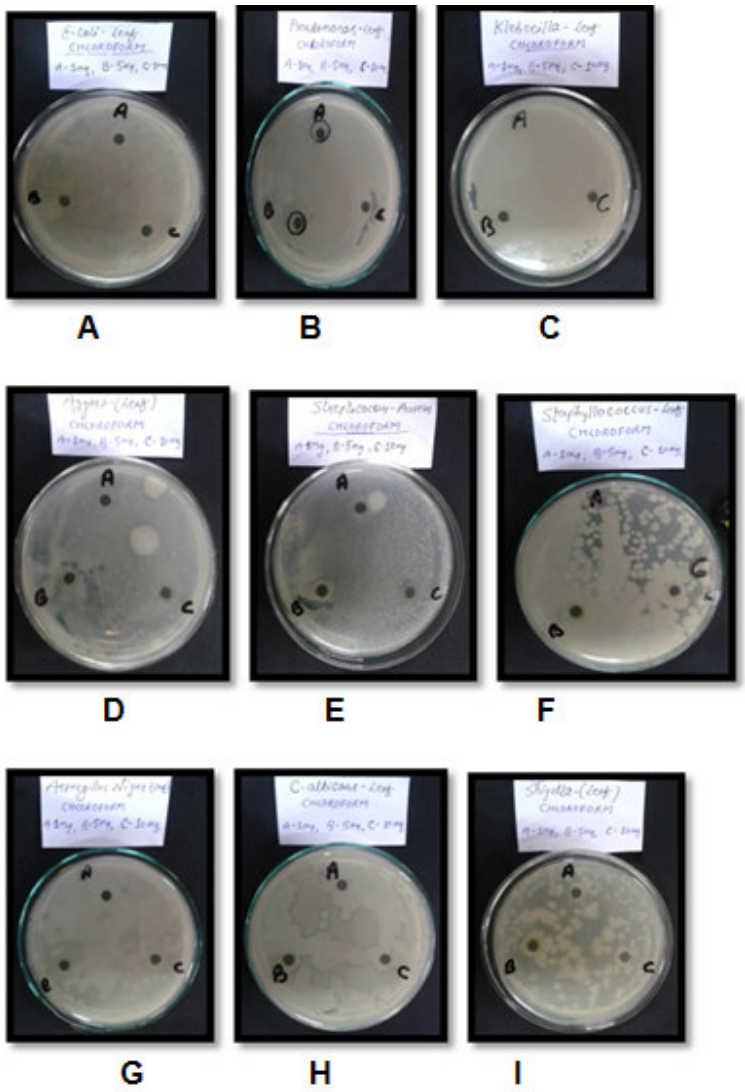


Figure 2

Antimicrobial activity of Prosopis cineraria (Leaf) with chloroform extract A (E. coli) B(Pseudomonas sps.), C (Azgaro sps.), D (Klebsiella pneumoniae), E (Staphylococcus aureus), F (Staphylococcus sps.) G (Aspergillus niger), H (C. albicans) and I (Shigella flexneri).

CONCLUSION

In present attempt were made to isolate successive extracts with methanol & chloroform of *Prosopis cineraria(Leaf)* and Antimicrobial activity were performed against selected bacterial species. Methanolic extract of *P. cineraria* leaf extract shows maximum inhibition zone against *Proteus vulgaris*(AI=2.5 for 10mg/disc) and *Staphylococcus aureus*(AI= 2.09 for 5mg/disc). Therefore *Prosopis cineraria* can be use for bioactivity guided fractionation to work as potential source of therapeutics and /or antibiotics in future for curing of various ailments against multi drug resistant bacteria.

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