Antimicrobial Activity of Bauhinia Racemosa Against Clinical Pathogens

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Abstract- *Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Clinical microbiologists have great interest in screening of plants for their antimicrobial activities as potential new therapeutics. The antimicrobial activities of plant extracts may be found in variety of different components which includes aldehyde and phenolic compounds. The combinations of these compounds can be synergistic and often found having greater antimicrobial activity than the purified individual constituents. In the present study, antibacterial activity of mandharai was determined against pathogens. Two methods were adopted to evaluate the efficacy of its antibacterial activity. Ethanol extracts were found to have maximum activity by both methods on comparison with that of aqueous extracts of this plant. The bioactive constituents such as saponins, tanins and alkaloids were put up together along with ethanol proves this plant to be a good antimicrobial agent.*

Keywords- Medicinal plant, Mandharai, Phytochemical analysis, RAPD

I. INTRODUCTION

Healing powers in plants is an ancient thought. Plant derived substances have recently become of great interest owing to their versatile applications[1]. Medicinal plants have been used for centuries as remedies for human diseases and offer a new source of biologically active chemical compound as antimicrobial agent. Medicinal plants are the richest bio-resources of drugs of traditional medicinal systems, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceuticals, intermediate and chemical entitled for synthetic drugs[2].

Recently the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led authors to investigate the antimicrobial activity of medicinal plants[3]. Out of the several hundred thousand medicinal plant species around the globe, only a small portion has been investigated both phytochemically and pharmacologically[4].

II. COLLECTION OF CLINICAL SAMPLES

Clinical samples like pus swabs were collected with the help of sterile cotton swabs. They were transported in Amie's transport media to the laboratory and were processed as per the standard protocols.

Processing of clinical samples:

The swabs were inoculated onto blood agar, nutrient agar and Mac Conkey agar, mannitol salt agar, eosin ethylene blue agar and incubated at 37°C for 24 hours. After 24 hours the colonies were picked up and preliminary tests like gram staining, motility, catalase and oxidase tests were performed. Further these isolates were identified as per standard biochemical methods.

III. COLLECTION OF MEDICINAL PLANTS

The whole plant of *Bauhinia racemosa* (Mandharai) was collected and was processed for its evaluation of antimicrobial activity against isolated organisms.

Processing of the Plant:

The whole plants along with its leaves stems, root parts were selected and they were shade dried in a clean environment for few days. The dried plant parts were collected and were powdered and stored in a clean container for further screening and extraction procedures.

IV. PLANT EXTRACTION METHODS

Three solvents were used for the preparation of the extracts - distilled water, acetone, and ethanol at four different concentrations. Plant powder was weighed and mixed with the three solvents to attain four different concentrations viz., 20%, 40% 60% and 80% respectively. These extracts were further used for the study.

V. EVALUATION OF ANTIBACTERIAL ACTIVITY OF PLANT EXTRACTS

The evaluation of antibacterial activity of the plant was performed by two different methods, which included-

1) Radial diffusion in two layers of perforated agar(RDAP)
2) Disc diffusion method(DDM)

1) Radial Diffusion In Two Layers Of Perforated Agar (RADP) By Using Plant Extract (Grove and randal.1990)[5].
The radial diffusion in two layers of perforated agar procedure was performed using Plate count agar (PCA). An initial layer of this medium (15ml) was poured into the plates. 0.25ml of fresh cultures was spread plated on to the initial layer of agar using a sterile bent glass rod. After 10 minutes, second layer of PCA was added and was allowed to solidify. The wells in the medium were made with a well borer (6mm diameter). These wells were filled with 75µl of plant extract and the plates were incubated at 37°C for 24 hrs.

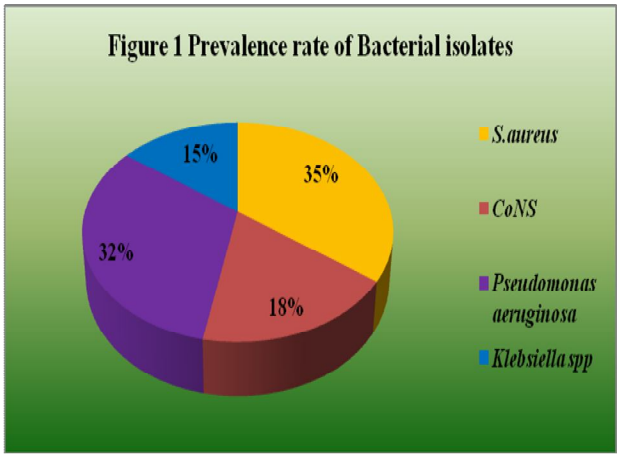
2) Disc Diffusion Method (DDM) (NCCLS 2000)[6]
Sterilized filter paper discs were soaked with 30µl of plant extracts. Discs with the extracts were dried in an oven. A lawn culture of the bacterial isolates was made onto Muller Hinton agar (MHA). Discs impregnated with the plant extracts were placed on to the surface of MHA and the plates were incubated at 37°C for 24 hrs. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the disc.

VI. PHYTOCHEMICAL SCREENING

Qualitative phytochemical analysis of the plant Mandharai was determined as per the standard qualitative method by Khandelwal, 2011[7]

VII. RESULTS

A total of 25 pus swabs were collected with the help of sterile cotton swabs. A total of 34 bacterial isolates were obtained with the following prevalence rate- *S.aureus* 12/34 (35%), CoNS 6/34(18%), *Pseudomonas aeruginosa* 11/34(32%) and *Klebsiella* spp5/34 (15%).



VIII. EVALUATION OF ANTIBACTERIAL ACTIVITY OF PLANT EXTRACTS

1) Radial Diffusion in Two Layers of Perforated Agar (RADP) By Using Plant Extract:

S. aureus gave a zone size ranging from 5-13mm with different concentrations of plant extract with ethanol, 3-15mm with acetone and 3-10mm with with distilled water. *S. aureus* gave a maximum zone size of 15mm at 80% concentration of plant extract with acetone. CoNS gave a zone size ranging from 3-9mm with different concentrations of plant extract with ethanol, 2-11mm with acetone and 1-6mm with distilled water. CoNS gave a maximum zone size of 11mm at 80% concentration of plant extract with acetone. *P.aeruginosa* gave a zone size ranging from 9-20mm with different concentrations of plant extract with ethanol, 8-16mm with acetone and 1-5mm with distilled water. *P.aeruginosa* gave a maximum zone size of 20mm at 80% concentration of plant extract with ethanol. *Klebsiella spp* gave a zone size ranging from 4-9mm with different concentrations of plant extract with ethanol, 3-10mm with acetone and 2-8mm with distilled water. *Klebsiella spp* gave a maximum zone size of 10mm at 80% concentration of plant extract with acetone. (Table 1)

Table 1: RADP of Mandhari

Plant Extract	Concentration	Organism (Zone size in mm in diameter)			
		S.aureus	*CoNS*	*P.aeruginosa*	*Klebsiella spp*
Ethanol	20%	5mm	3mm	9mm	4mm
	40%	9mm	4mm	13mm	6mm
	60%	11mm	7mm	16mm	8mm
	80%	13mm	9mm	20mm	9mm
Acetone	20%	3mm	2mm	8mm	3mm
	40%	10mm	5mm	12mm	4mm
	60%	12mm	7mm	13mm	6mm
	80%	15mm	11mm	16mm	10mm
Distilled water	20%	3mm	1mm	1mm	2mm
	40%	5mm	3mm	3mm	6mm
	60%	7mm	4mm	3.5mm	7.2mm
	80%	10mm	6mm	5mm	8mm

2) Disc Diffusion Method (DDM)

S.aureus gave a maximum zone size of 21mm at 80% concentration of plant extract with acetone. CoNS gave a maximum zone size of 20mm at 80% concentration of plant extract with ethanol. *P.aeruginosa* gave a maximum zone size of 14mm at 80% concentration of plant extract with acetone. *Klebsiella spp* gave a maximum zone size of 15mm at 80% concentration of plant extract with ethanol. (Table 2)

Table 2: Disc diffusion of Mandhari

Extract	Concentration	Organism			
		S.aureus	*CoNS*	*P.aeruginosa*	*Klebsiella spp*
Ethanol	20%	8mm	7mm	4mm	3mm
	40%	12mm	12mm	6mm	5mm
	60%	17mm	18mm	10mm	10mm
	80%	21mm	20mm	13mm	15mm
Acetone	20%	4mm	4mm	5mm	3mm
	40%	9mm	5mm	8mm	7mm
	60%	15mm	9mm	10mm	12mm
	80%	18mm	13mm	14mm	14mm
Distilled water	20%	3mm	2mm	3mm	2mm
	40%	7mm	5mm	7mm	4mm
	60%	11mm	8mm	9mm	5mm
	80%	12mm	10mm	11mm	6mm

PHYTOCHEMICAL SCREENING

Phytochemical analysis of all the extracts revealed the presence of tannins, saponins, flavonoids, steroids and terpenoids in varying amounts in *Mandharai.*(Table 3)

S.No	Table 3: Phytochemical compound	Results
1.	Tanins	+
2.	Saponins	+
3.	Flavonoids	+
4.	Alkaloids	-
5.	Proteins	-
6.	Steroid	+
7.	Quinones	-
8.	Terpenoid	+
9.	Cardio glycosides	-
10.	Phenols	-

IX. DISCUSSION

Plants and plant products have been used extensively throughout history to treat medical problem[8, 9, 10]. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries and moreover the use of herbal remedies has risen in the developed countries in the last decades[11]. In this connection, plants continue to be a rich source of therapeutic agent. Recently the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics led authors to investigate the antimicrobial activity of medicinal plants[12, 13].

In our study, mandharai showed phenols, terpenoids and flavonoids which are identified as antimicrobial agents that contribute the microbicidal activity to these plants which coincides with the previous results of El –Khatiba and Khaleel (1995)[14] and also by El-Hossary *et al*.(2000)[15] .

In previous studies, they tested leaf extracts obtained with three solvents of different polarities and discovered that ethanolic extract was the most efficient against the pathogens especially enterobacteriaceae which coincides with our results showing maximum efficacy with ethanolic extract of mandharai but in contrast staphylococcus showed maximum zone of inhibition followed by other pathogens.

X. CONCLUSION

Our study suggests that medicinal plants particularly ethanol extracts can be used for treating both gram positive and gram negative bacterial infections. The traditional preference of ethanol extract is due to various reasons. Mainly the presence of bioactive constituents like, saponins,tannins and alkaloids. Plant extracts are more bacteriostatic than bactericidal and hence they can be a safe alternative to synthetic medicine against multi drug resistant pathogens.

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