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Nidhi Patearia Mishra, Archana Verma
Department of Botany,Govt. (Auto.) Girls P.G. College of Excellence, Sagar (M.P.)

Evaluation of Antimicrobial, Antifungal and Phytochemical Properties of *Bauhinia variegata*

Nidhi Patearia Mishra, Archana Verma

ABSTRACT

Medicinal plants have played an essential role in the development of human culture. Plant products have been part of phytomedicines since time immemorial. The phytochemical research based on ethno pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants. These can be derived from any part of the plant like bark, leaves, flowers, seeds, etc i.e., any part of the plant may contain active components. In the present work, qualitative phytochemical analysis was carried out of *Bauhinia variegata* of Caesalpiniaceae in three different solvents viz. ethanol, methanol and water. During the antimicrobial study it was observed that the ethanolic and methanolic extracts of leaves and stem of *Bauhinia variegata* showed best results against *S.aureus* (19mm) and *P.aeruginosa* (19mm) respectively among the bacterial strains and in fungal strains it gave good results in *C.albicans* (17.25mm) and *C.albicans* (16mm). The preliminary phytochemical screening of leaves and stem of *Bauhinia variegata* showed the presence of alkaloids, carbohydrates, glycosides, flavonoids, phenols, tannins, lignin, saponins, terpenoids and amino acids in all the three solvents used. The present investigation reported the crucial fact that phytochemicals present in different extracts of selected plant parts are highly responsible for their ethnomedicinal and antimicrobial potency.

Keywords: Antimicrobial, Phytochemical, Caesalpiniaceae, *Bauhinia variegata*

1. INTRODUCTION

India is a varietal emporium of medicinal plants and is one of the richest countries in the world in regard to genetic resources of medicinal plants. It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition.

Moreover, the agro-climatic conditions are conducive for introducing and domesticating new exotic plant varieties .[1] The presence of antimicrobial substances in the higher plants is well established .[2] The current study was initiated because of the increasing resistance to antibiotics including bacteria and fungi. Plant extracts and compounds are proved to be of new interest as antiseptics and antimicrobial agent. Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers use primarily water as the solvent but we found in this study the plant extracts by ethanol and methanol provided more consistent antimicrobial activity compared to those extracted by water. The results of antibacterial activity of *Bauhinia variegata* of Caesalpiniaceae against the investigated bacterial and fungal strains are shown in different tables.

Correspondence

Nidhi Patearia Mishra
Department of Botany, Govt. (Auto.) Girls P.G. College of Excellence, Sagar (M.P.)

E mail: durva_2684@rediffmail.com

2. MATERIALS AND METHODS

2.1 Collection of Plant material

The fresh plant materials have been collected from the local areas of Sagar district of M.P from October 2008-September 2009. The plant specimen was botanically identified and authenticated by comparing the herbarium specimen available in the Department of Botany, Dr. H.S. Gour University, Sagar. The plant parts were shade dried, powdered in a kitchen blender and were stored in air tight plastic bags.

2.2 Extraction

It was performed by hot extraction method using Soxhlet apparatus.[3]For extraction, powdered plant material (~25g) was packed in Whatsmann filter paper No.1 and introduced into the Soxhlet apparatus. Successive extraction was performed with different solvents on basis of their polarity (methanol, ethanol, water). After each extraction the drug packet was air dried and then reintroduced for extraction with the successive solvent, while the respective extracts were concentrated and dried at 65-70˚C. Dried extracts were transferred to air tight bottles and stored at 4˚C.

2.3 Test Organisms

The bacterial and fungal culture were obtained from National Collection of Industrial Micro-organism (NCIM) Pune.The tested bacterial strains are *Eschherichia coli*(NCIM-2065), *Bacillus subtilis*(NCIM-2063), *Pseudomonas aeruginosa*(NCIM-2036), *Sarcina lutea*(NCIM-2103), *Staphylococcus aureus*(NCIM-2602).The tested fungal strains are *Aspergillus niger*(NCIM-920), *Aspergillus flavus*(NCIM-540), *Alternaria alternata* (NCIM-718), *Fusarium monilifer*(NCIM-1099), *Candida albicans*(NCIM-3102).

2.4 Preparation of media

There are so many methods for studying anti-bacterial and anti-fungal activities. In the present research work filter paper disc diffusion method would be followed. [5,6] The media used are given below.

2.4.1 Nutrient culture media used for anti-bacterial activity

Oxide nutrient agar medium having following composition was used for preparing the slants and plates-

Beef extract - 3g
Peptone - 10g
Glucose - 25g
Agar agar powder - 20g
Distilled water to make the solution 1liter.

2.4.2 Nutrient culture media used for anti fungal activity

Potato dextrose agar medium would be used for making the inoculums and the medium was prepared by taking the following composition of substances:

Potato slices - 200g
Dextrose - 25g
Agar agar powder - 20g
Distilled water to make the solution 1litre.

All the components were dissolved in freshly prepared distilled water. The flask were plugged very well with cotton and sterilized in an autoclave at 151bs pressure for 30 minutes.

2.5 Procedure for performing the Disc Diffusion test. [7]

The required amount of Petri plates were prepared and autoclaved at 121°C for 15 minutes. They were allowed to cool under Laminar air flow. Aseptically transfer about 20 ml of media into each sterile Petri dishes and allowed to solidify. 1 ml inoculum suspension was spread uniformly over the agar medium using sterile glass rod to get uniform distribution of bacteria. The readily prepared sterile discs were loaded with different plant extract. The paper diffuse discs were placed on the medium suitably apart and the plate were incubated at 5°C for 1 hour to permit good diffusion and then transferred to an incubator at 37°C and 28°C for 72hours and 24 hours for antifungal and antibacterial activity respectively. The antimicrobial activity was recorded by measuring the width of the clear inhibition zone around the disc using zone reader (mm).

2.6 Phytochemical analysis

The dried plant parts were ground into uniform powder and stored in containers. The dried plant parts were subjected to qualitative chemical screening for the identification of various classes of active chemical constituents using standards prescribed methods .[8-12]

3. RESULT AND DISCUSSION

Table 1-3 showed the antibacterial activity of *Bauhinia variegata* plant parts, viz.. root, stem ,leaf, seed in water, ethanol and methanol extracts against five bacterial strains were compared to Streptomycin (standard) by measuring the diameter of inhibition zone. All the extracts have exhibited different degrees of antibacterial activity against the tested bacteria; among them leaf and stem extracts showed broad spectrum activity against all the

test pathogenic bacteria. The ethanol leaf extract of *B. variegata* showed strong inhibitory activity against *S. aureus*(19mm). The study made on methanol leaf extract recorded maximum inhibition zone against *S. aureus*(18mm).

The zone of inhibition developed by extract of *B. variegata* stem in ethanol showed the maximum inhibition against *P. aeruginosa* (19mm). Similarly, methanolic extract showed the highest activity against *P. aeruginosa* (18.25mm).All the other samples of root and seed had either shown very negligible activity or showed no activity against all the five bacterial strains.

Table 4-6 presented the results of antifungal activity of *Bauhinia variegata* parts viz.. root, stem ,leaf, seed in water,ethanol and methanol extracts against five fungal strains. The ethanol stem extract of *B. variegata* showed strong inhibitory activity against *C.albicans* (17.25) and *A.niger* (17mm) followed by *A. flavus* (15mm), *F. monilifer* (14.5mm) and *A. alternata*(13.75mm). In methanol extract of stem the maximum inhibition zone was recorded in *A. niger*(18mm) followed by *A. flavus* (16.25mm), *C. albicans* (15.5mm) and *F. monilifer* (13.75mm) while *A. alternata* was least affected. In aqueous extract it showed minimum inhibition zone aganst all the fungal strains.

The ethanolic leaf extract of *B. variegata* (Table 5)showed strong inhibitory activity against *C. albicans* (16mm) and *A. alternata* (15.25mm) followed by *A. niger* (14.5mm), *A. flavus* (13mm) and *F. monilifer* (12.5mm). The study made on methanol leaf extract recorded maximum inhibition zone for *C. albicans*(17mm) and *A. alternata* (16.5mm) followed by *A. niger* (15.5mm), *A. flavus* (14.25mm) and *F. monilifer* (12.5mm). In aqueous extract it showed minimum inhibition zone against all the fungal strains. Hence ethanolic extract of leaves of *B. variegata* could be a potential source of new antibiotics against pathogenic fungi. All the other samples of root and seed had either shown very negligible activity or showed no activity against all the five fungal strains.

Investigations on phytochemical screening of leaves of *B. variegata* revealed the presence of alkaloids, carbohydrates, glycosides, tannin, phenols and amino acids in all the three solvents, methanol, ethanol and water (Table 7,8). While, saponins showed presence in aqueous extract, anthraquinones were present in methanol and aqueous extracts and steroids showed presence in methanol and ethanol extracts only. The results of phytochemical screening of methanol and ethanol extracts of *Bauhinia variegata* stem shows the presence of alkaloids, carbohydrates, saponins, phenols, tannins, flavonoids, amino acids, terpenoids and steroids, whereas, anthraquinones were present only in methanol and aqueous extract, carbohydrates, saponins, phenols, tannins, flavonoids and amino acids were present in aqueous extract also.

Table 1: Antibacterial activity of aqueous extract of *Bauhinia variegata*

Plant part used	*E. coli*	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*S. lutea*
Root	_	6.5mm	6mm	_	_
Stem	12mm	10mm	11.5mm	13.5mm	10.5mm
Leaf	12.25mm	11.5mm	10.25mm	13mm	13.5mm
seed	_	7.5mm	6mm	6.25mm	10mm
Streptomycin (standard)	28mm	24mm	20mm	30mm	32mm

(_) shows no inhibitory activity

Zone of inhibition (in mm) including the diameter of 6 mm of filter paper disc

Table 2: Antibacterial activity of ethanolic extract of *Bauhinia variegate*

Plant part used	*E.coli*	*B.subtilis*	*S.aureus*	*P.aeruginosa*	*S.lutea*
Root	8mm	8.5mm	8mm	_	_
Stem	16mm	14.5mm	18.5mm	19mm	15.25mm
Leaf	15mm	13.5mm	19mm	16mm	17mm
seed	_	_	_	_	9.25mm
Streptomycin (standard)	28mm	24mm	20mm	30mm	32mm

(_) shows no inhibitory activity

Note: Zone of inhibition (in mm) including the diameter of 6 mm of filter paper disc.

Table 3: Antibacterial activity of methanolic extract of *Bauhinia variegata*

Plant part used	*E.coli*	*B.subtilis*	*S.aureus*	*P.aeruginosa*	*S.lutea*
Root	_	_	6mm	7.5mm	_
Stem	16.5mm	14mm	17.75mm	18.25mm	17mm
Leaf	16.25mm	13.75mm	18mm	15mm	16.75mm
seed	_	8mm	10mm	_	12mm
Streptomycin (standard)	28mm	24mm	20mm	30mm	32mm

(_) shows no inhibitory activity

Note: Zone of inhibition (in mm) including the diameter of 6 mm of filter paper disc.

Table 4 Antifungal activity of aqueous extract of *Bauhinia variegate*

Plant part used	*A.niger*	*Fusarium*	*C.albicans*	*A.flavus*	*A.alternata*
Root	_	_	6.5mm	_	_
Stem	6.5mm	_	10mm	_	8.5mm
Leaf	_	13mm	7.5mm	13.25mm	_
Seed	_	_	_	_	_
Grassioflavin (standard)	21mm	22.5mm	20mm	20mm	24mm

(_) shows no inhibitory activity

Note: Zone of inhibition (in mm) including the diameter of 6 mm of filter paper disc.

Table 5: Antifungal activity of ethanolic extract of *Bauhinia variegata*

Plant part used	*A.niger*	*Fusarium*	*C.albicans*	*A.flavus*	*A.alternata*
Root	_	_	6.75mm	_	_
Stem	17mm	14.5mm	17.25mm	15mm	13.75mm
Leaf	14.5mm	12.5mm	16mm	13mm	15.25mm
seed	_	_	___	_	_
Grassioflavin (standard)	21mm	22.5mm	20mm	20mm	24mm

(_) shows no inhibitory activity

Note: Zone of inhibition (in mm) including the diameter of 6 mm of filter paper disc.

Table 6: Antifungal activity of methanolic extract of *Bauhinia variegata*

Plant part used	A.niger	Fusarium	C.albicans	A.flavus	A.alternata
Root	_	_	8mm	_	_
Stem	8mm	_	5.5mm	_	_
Leaf	15.5mm	12mm	17mm	14.25mm	16.5mm
Seed	_	_	_	_	_
Grassioflavin (standard)	21mm	22.5mm	20mm	20mm	24mm

(_) shows no inhibitory activity

Note: Zone of inhibition (in mm) including the diameter of 6 mm of filter paper disc

Table 7: Phytochemical analysis of *Bauhinia variegata* leaves

Tests for Extracts	Methanol	Ethanol	Water
Alkaloids Dragendorff's & Mayer's reagent	+	+	+
Carbohydrates Molish's test & Fehling's test	+	+	+
Glycosides Fehling's test	+	+	+
Saponins Froth test	-	-	+
Phenols	-	-	-
Tannin	+	+	+
Flavonoids Shinoda test	-	-	-
Amino acids			

Ninhydrin test	+	+	+
Terpenoids	-	-	-
Anthraquinone Borntrager's test	+	-	+
Steroids	+	+	+

+:present -:absent

Table 8: Phytochemical analysis of *Bauhinia variegata* stem

Tests for Extracts	Methanol	Ethanol	Water
Alkaloids Dragendorff's & Mayer's reagent	+	+	-
Carbohydrates Molish's test & Fehling's test	+	+	+
Glycosides Fehling's test	-	-	-
Saponins Froth test	+	+	+
Phenols	+	+	+
Tannin	+	+	+
Flavonoids Shinoda test	+	+	+
Amino acids Ninhydrin test	+	+	+
Terpenoids	+	+	+
Anthraquinone Borntrager's test	+	-	+
Steroids	+	+	-

+:present -:absent

4. CONCLUSION

On the basis of above studies it can be concluded that the presence of the antimicrobial activity and identified phytochemicals makes the plant pharmacologically active. Efforts to identify the constituent compounds responsible for this antioxidant activity are also in progress. The traditional medicine practice is recommended strongly for this plants as well as it is suggested that further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity. The current study was initiated because of the increasing resistance of pathogenic microbes against antibiotics. Plant extracts and compounds are proved to be of new interest as antiseptics and antimicrobial agent. In present study it was observed that *B. variegata* showed good activity against all the tested bacterial and fungal strains. This is inferred because more than one part of these plants are useful and gave good results. Apart from this, we also recognized that leaves are most active among all the plants parts studied.

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