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Preliminary phytochemistry and antimicrobial activity of bark of *Bauhinia racemosa* Lamk.

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Abstract
The bark of *Bauhinia racemosa* are reported to have great medicinal value. Phytochemical screening of the plant bark reveals the presence of carbohydrates, alkaloids, steroids and tannins. the methanol, ethanol, aqueous, acetone and petroleum ether extracts of bark of *B. racemosa* Lamk. prepared and antimicrobial activity were studied by agar well diffusion method against enteric bacterial pathogens such as *E. coli, S. aureus, B. subtilis, P. aeruginosa* and fungi *A. niger* and *C.albicans.* The methanol extracts had wide range of antimicrobial activity against enteric microbes than ethanol extracts, where as ethanol extract were slightly higher antibacterial activity than aqueous extract. Antimicrobial activity of various extracts of bark of *B. racemosa* was carried in attempt to develop a new pharmaceutical drug from natural origin for prevention of enteric infection.

Keywords: *Bauhinia racemosa*, antimicrobial activity, bark.

INTRODUCTION

The use of plant compounds for pharmaceutical purposes has gradually increased in India 80% of individuals from developed countries use traditional medicine which involves compounds derived from medicinal plants [1] . The use of plant extracts and phytochemical both with known antimicrobial properties can be of great significance in therapeutic treatments [2]. Hence studies involving the use plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes. The plant *B.racemosa* Lamk. belonging to the Caesalpiniaceae family. It occurs frequently in India, Ceylon, China and Timor. The stem bark of the plant is an astringent and is used in the treatment of headache, fever, skin diseases, tumors, blood diseases, dysentery and diarrhea. The fresh flower buds of the plant showed antiulcer activity [3 and 4].

Therefore the review revealed that the bark of *B. racemosa* Lamk. were used in various metabolic disorders but for their antimicrobial properties were not demonstrated. Hence attempt was made to find out the antibacterial properties of bark of *B.racemosa* Lamk. against enteric bacterial pathogens.

MATERIALS AND METHODS

Plant material

Fresh bark of B.racemosa Lamk. were collected from the natural population growing in different areas of Parbhani district of Maharashtra. Plant was identified and authenticated from the herbarium of Deptt. of Botany Dr. B.A.M. University, Aurangabad, flora of Marathawada [5] and encyclopida of Indian medicinal plants.

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Preparation of extracts

The fresh bark was dried under shed at room temperature and then powdered with a grinder and stored in air tight container. A soxhlet apparatus was used for extraction of antimicrobial active compounds from the powder. The dried powder of the bark of the plant (20 gm) was successively extracted using methanol, ethanol, aqueous, acetone and petroleum ether solvents respectively. The collected extracts were concentrated by evaporation under room temperature and used for the study.

Phytochemical screening

Different extracts were screened for the presence of alkaloids, glycosides, flavonoids, saponins, tannins, sterioids, resins, phytobatanine, oil and fats by using standard protocol [6 and 7].

Test microorganisms

Bacterial and fungal strain used for testing included authentic pure cultures of human pathogenic bacteria like. *Staphylococcus aereus* (SRTCC1073), *Bacillus subtilis (*SRTCC1091) and two are gram negative viz. *Pseudomonas aeruginosa* (SRTCC708), *Escherichia coli* (SRTCC3260). Two species of fungi viz. *Aspergilus niger (*SRTCC1073), *Candida albicans* (SRTCC3971).These were obtained from the school of life sciences, S.R.T.M. university, Nanded (M.S.).

Preparation of test organisms suspension

The test organisms were maintained on slants of medium containing nutrient agar (2.5 gm/ 10ml) and sub cultured once a week. The slants incubated at 37^0C for 24 hrs and stored under refrigeration. The inoculums was 1x 10^8 cells/ml.

Antimicrobial activity

The in vitro antimicrobial activity of different bark extracts of B. racemosa Lamk. was determined by agar well diffusion method [8] .

The plant extracts were dissolved in distilled water at concentrated 50 µg/ ml streptomycin was used as reference antibiotic. Each plate was inoculated with 20µl microbial suspension having concentration 1x 10^8 cells/ml. The 0.1 ml extract was added to each well. The plates containing bacteria were incubated at 37^0C for 24 hrs and those containing fungi were incubated at 25^0C for 7 days. Positive antimicrobial activity was based on growth inhibition zone and compared with standard drug [9]. The dimeter of zone of inhibition surroundings each of the well was recorded. All the experiments were performed in triplicate.

Statistical analysis

Results were expressed as mean ± S.D. statistical significance was determined using analysis of students t- test.

RESULTS AND DISCUSSION

Indian systems of medicine such as ayurveda and siddha uses majority of the crude drugs that are of plant origin. It is necessary that standards have to be laid down to control and check the identity of the plant an ascertain its quality before use. A detailed pharmacognostic evaluation there fore is highly essential perquisite [10].

The phytochemical investigation of the various solvent extract of bark of *B. racemosa* Lamk. Presented in table 1. the results revealed that the bark of *B. racemosa* Lamk. Show the presence of alkaloids,glycosides, carbohydrates, saponins, flavonoids, triterpenoids, anthroqunonine, phytobatalanine are present in methanol, ethanol and aqueous extracts. The result of the antimicrobial activity of the different extracts of bark of *B. racemosa* Lamk. are presented in table 2. In the present investigation methanol and aqueous extracts of bark showed higher antibacterial activity against the test organisms which was greater than the standard reference antibiotic streptomycin. The methanolic extract of bark also found more inhibitory activity on gram negative and gram positive bacteria *E. coli, P.aeruginosa, S. aureus and B.subtilis*. The various worker have already shown that gram positive bacteria are more susceptible towards plants extracts as compared to gram negative [11]. The difference in the activity may be due to the different secondary metabolites present in extracts. The gram negative activity bacteria *P. aeruginosa* shows the higher activity in both ethanol and water extracts the antimicrobial activity of the ethanol (23mm) was higher than that of water extract (19mm). Kumar (1999) [12] reported that methanol extract of *B. racemosa* Lamk. root bark possess significant inhibition effect against a broad range of microbes in *dysenteriae, vibrio cholera, S. aureus, S.pnemonia, M. luteus, C. albicans and A. niger.*

Table 1.Phytochemical analysis of bark of *Bauhinia racemosa* Lamk.

Sr. no.	Phyto Constituents	Chemical tests	*B. racemosa bark* extract				
			P.E	Met	Eth	Aq	Ac
1	Alkaloids	1. Mayer's test	-	-	+	-	-
		2. Dragendroff's test	-	+	-	+	-
		3. Wagner's test	-	-	+	-	-
		4. Hagers test	-	-	-	-	-
2	Carbohydrates	1. Molisch's test	-	+	+	+	-
		2. Benedicts test	-	-	-	-	-
		3. Fehling's test	-	-	-	-	-
3.	Glycosides	1. Modified Borntragers	-	+	+	+	
		2. Legal test	-	+	-	+	
4	Saponins	1. Foam test	-	+	-	+	-
		2. Froth test	-	-	-	-	-
5	Triterpenes	1. Salkowski test	-	-	+	+	-
		2. Libermann Burchard	-	-	-	-	+
		3. Tschugajew test	-	-	-	-	-
6.	Fats & Oil	1. Stain test	-	-	-	-	-
7	Tannins	1. Alkaline Reagent	-	+	-	+	-
8	Flavanoids	1. Gelatin test	-	+	+	+	-
		2. Lead acetate test	-	+	+	+	
		3. Shinoda test	-	+	+	+	+
		4. Zn-Hcl reduction	-	+	+	+	
9	Photobatalin		-	+	+	-	+
10	Anthraqunonines		-	+	+	-	+

Table 2. *In vitro* antimicrobial activity of bark of *Bauhinia racemosa* Lamk.

Microorganisms	Diameter of Zone of inhibition in mm of different extracts of bark (2mg/ml) Mean± S.D.					Standards reference antibiotic (Streptomycine)
Bacteria	Methanol	Ethanol	Aqueous	Pet. ether	Acetone	
Escherichia coli	15±1.23	14±1.23	14.3±1.11	12±2.12	11±2.11	14±1.11
Staphylococcus aureus	14.8±1.11	14±2.12	15±1.11	11±1.12	12±1.21	14.3±1.11
Bacillius subtilis	15±1.15	13±1.21	15±1.43	13±1.23	13±1.32	13±1.12
P.aeruginosa	15±2.23	14±2.31	14±1.11	14±1.21	14±1.23	14±1.14
Fungi						
Aspergillus niger	13±1.13	12±1.23	12±1.11	12±2.12	12±2.31	15±1.15
Candida albicans	12±0.12	12±3.33	13±1.24	11±1.11	12±1.11	14±1.13

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