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Research Article

Evaluation of antimicrobial activity of various bark extracts of *bombax malabaricum*

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ABSTRACT

This study was carried out with an objective to investigate the antibacterial and antifungal potential of Bark of *Bombax malabaricum.* Antibacterial activity of successive extracts (petroleum ether, chloroform, acetone, alcohol, water) of bark were carried out against two Gram positive bacteria – *Bacillus Subtilis* and *Staphylococcus aureus* and two Gram negative bacteria – *Escherichia coli*, *Pseudomonas aeruginosa.* The antifungal activity of the extracts was evaluated on two common pathogenic fungi – *Aspergillus niger* and *Candida albicans*. The testing was done by the agar diffusion method. Zones of inhibition of extracts were compared with that of standard Amoxycillin for antibacterial activity and Ketoconazole for antifungal activity. The extracts showed antibacterial and antifungal activities comparable with that of standard against the organisms tested. The results showed that the Petroleum ether and chloroform extracts showed no activity while the alcoholic extract showed more activity than the acetone and aqueous extracts. The highest inhibitory activity was determined for alcoholic extract against *E.coli* (19.50 ± 0.5000 mm, inhibition zone diameter). On the other hand, the weakest inhibitory activity was determined against *P. aeruginosa* for aqueous extract (7.00 ± 0.5774 mm, inhibition zone diameter).

Keywords: Antibacterial; antifungal; *Bombax malabaricum*; Amoxycillin; Ketoconazole.

1. INTRODUCTION

Medicinal plants have been used for years in daily life to treat disease all over the world. It is well known that some plants containing active compounds are able to inhibit the microbial growth. The potential of antimicrobial properties of plants are related to their ability to synthesize compounds by the secondary metabolism. Secondary metabolites proved to be the most important group of compounds that showed wide range of antibacterial and antifungal activity. These plant compounds have different structures and actions when compared with conventional fungicides used to control the microbial growth and survival.

Bombax malabaricum (Bombacacaea) is a tall and deciduous tree at a height of 20-25m, with smooth or butressed trunk with pyramidal spreading branches, gray or brown bark covered with hard, black, sharp, conical spines (Prajapati et al., 2003). It is found throughout the warmer parts of India and Srilanka. Mainly in the forest regions but cultivated in gardens and lining avenues for its beautiful flowers. (Singh et al., 2005). In Indian system of medicine 'Ayurveda', the plant is popularly known as Rakta shalmali (Sanskrit). This drug is a rasayana. It is a component of dashamulkwatha. Generally the plant exudates gum, light brown to opaque called as 'mochras' or 'semul gum' is used in vata diseases (Singh et al., 2005). Bark is astringent, diuretic, demulcent, diuretic, healing of abscesses, wounds and other skin eruptions, leaves are anti-inflammatory, roots are aphrodisiac, anti diarrheal, flowers are diuretic and laxative, gum is used in hemoptysis, seeds are used in gonorrhea (Deshpandey et al., 2008).

In recent years, multiple drug/chemical resistance in both human and plant pathogenic microorganisms have been developed due to indiscriminate use of commercial antimicrobial drugs/chemical commonly used in the treatment of infectious diseases. This situation forced the scientists to the searching of new antimicrobial substances from various sources like medicinal plants. Traditionally the decoction of the bark is used externally in inflammations, in fomenting, sealing of secondary infection, healing of wounds and skin eruptions in the form of paste and leaves of this plant are ground and mixed with milk are given for strangury and inflammations. Despite the traditional use of this plant, no scientific report is focused on the biological activity of *Bombax malabaricum*. We therefore investigated this study to evaluate the antimicrobial potential of the bark extracts of *Bombax malabaricum.*

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Table 1: Antimicrobial activity of *Bombax malabaricum* bark extracts on various bacterial strains by agar diffusion method

GROUP	TREATMENT	mg/ml	CONC.	AVERAGE DIAMETER OF ZONE OF INHIBITION OF DIFFERENT BACTERIA[a*] (mm)			
				S.aureus Gm(+ve)	*B. subtilis* Gm(+ve)	*E. coli* Gm(-ve)	*P. aureginosa* Gm(-ve)
I	Vehicle Control (DMSO)		50	NA	NA	NA	NA
II	Pet. ether	250	50	NA	NA	NA	NA
		250	50	NA	NA	NA	NA
		500	50	NA	NA	NA	NA
III	Chloroform	150	50	NA	NA	NA	NA
		250	50	NA	NA	NA	NA
		500	50	NA	NA	NA	NA
IV	Acetone	150	50	9.033 ± 0.7172	7.33 ± 0.666	10.00 ± 0.5774	7.667 ± 0.8819
		250	50	9.00 ± 0.5774	11.00 ± 0.000	12.00 ± 0.5774	11.00 ± 0.5774
		500	50	10.17 ± 0.7265	12.00 ± 0.0000	15.43 ± 0.3480	11.67 ± 0.333
V	Alcohol	150	50	16 ± 0.0000	13.00 ± 0.5774	14.00 ± 2.309	7.00 ± 1.155
		250	50	16.17 ± 0.7265	18.00 ± 0.5774	19.00 ± 1.732	17.50 ± 0.2887
		500	50	17 ± 0.2887	18.00 ± 1.155	19.50 ± 0.5000	18.67 ± 0.8819
VI	Aqueous	150	50	10 ± 0.2887	5.00 ± 1.155	9.00 ± 0.5774	6.00 ± 1.155
		250	50	12.00 ± 0.2887	9.667 ± 0.8819	10.00 ± 0.000	7.00 ± 0.5774
		500	50	14 ± 0.5774	12.83 ± 0.4410	17.00 ± 0.000	9.00 ± 0.5774
VII	Amoxycillin[b]	150µg/ml	50	22.33 ± 0.8819	20.83 ± 0.8819	22 ± 0.5774	22.00 ± 0.000

Values are mean±S.E.M. of three replicate experiments by one way ANNOVA
[*]All determinations were done in triplicate.
[a] Zone of Inhibition measured excluding cup diameter.
[b] Reference standard.,NA – No activity

2. MATERIALS AND METHODS

2.1. Collection of plant material

The bark of *Bombax malabaricum* were collected and authenticated from GKVK, Agricultural University, Bangalore in June, 2009. The voucher specimen (BM-10-01) has been kept in herbarium in Department of Pharmacognosy, PES College of pharmacy, Bangalore, Karnataka, India.

2.2. Extraction procedure and phytochemical screening

Air-dried and powdered bark of *Bombax malabaricum* were taken and subjected for successive solvent extraction. The extraction was carried out for 18 hrs with the following solvents, in the increasing order of the polarity i.e. Petroleum ether, chloroform, acetone, alcohol and chloroform water. The extracts were decanted, filtered with Whatman No. 1 filter paper and concentrated at reduced pressure below 40°C through rota vapour to obtain dry extract. These extracts were screened for antimicrobial activity. An attempt was also made to observe the presence and absence of different phytochemical constituents, viz. glycosides, carbohydrates, proteins, phenolic compounds, tannins, saponins, gums and mucilages.

2.3 Microorganisms

The test microorganisms used for the antimicrobial activity screening were 4 bacteria (2 Gram positive and 2 Gram negative) *Staphylococcus aureus, Bacillus subtilis*, *Escherichia coli Pseudomonas aeruginosa* and two fungi *Candida albicans and Aspergillus niger.* The strains are maintained and tested on Nutrient Agar (NA) for bacteria and Sabourand dextrose agar (SDA) for fungi.

Table 2: Antimicrobial activity of Bombax malabaricum bark extracts on various fungal strains by agar diffusion method

GROUP	TREATMENT	mg/ml	CONC.	AVERAGE DIAMETER OF ZONE OF INHIBITION OF DIFFERENT FUNGI[a*] (mm)	
				C. albicans	*A. niger*
I	Vehicle Control (DMSO)	NA	50	NA	NA
II	Pet.ether	150	50	NA	NA
		250	50	NA	NA
		500	50	NA	NA
III	Chloroform	150	50	NA	NA
		250	50	NA	NA
		500	50	NA	NA
III	Acetone	150	50	8.33 ± 0.333	12.67 ± 1.528
		250	50	11.33 ± 0.8819	10.67 ± 1.155
		500	50	12.33 ± 0.333	14.00 ± 0.5774
IV	Alcohol	150	50	9.667 ± 0.333	15.00 ± 0.0000
		250	50	14.00 ± 1.155	18.00 ± 0.5774
		500	50	16.00 ± 0.000	20.33 ± 0.333
V	Aqueous	150	50	9.333 ± 0.333	8.00 ± 1.000
		250	50	10.333 ± 0.6667	10.83 ± 0.7625
		500	50	13.17 ± 0.4410	12.33 ± 1.202
VI	Ketoconozole[b]	150µg/ml	50	22.00 ± 0.5774	23.67 ± 0.8819

Values are mean±S.E.M. of three replicate experiments by one way ANNOVA
[*]All determinations were done in triplicate.
[a] Zone of Inhibition measured excluding cup diameter.
[b] Reference standard.,NA – No activity

Table 3: The MIC values (µg/disc) of Bombax malabaricum bark extracts against the microorganisms

GROUP	Treatment	MINIMUM INHIBITORY CONCENTRATION[*] (µg/disc)					
		S.aureus Gm(+ve)	*B.subtilis* Gm(+ve)	*P.aureginosa* Gm (-ve)	*E. coli* Gm (-ve)	*Candida albicans*	*A. niger*
I	Vehicle Control (DMSO)	NA	NA	NA	NA	NA	NA
II	Pet. Ether	NA	NA	NA	NA	NA	NA
III	Chloroform	NA	NA	NA	NA	NA	NA
IV	Acetone	250	250	500	500	250	500
V	Alcohol	250	125	250	500	250	250

2.4 Antimicrobial activity

The dried plant extracts (Petroleum ether, Chloroform, acetone, alcohol and aqueous extracts of *Bombax malabaricum* bark was dissolved in DMSO to get a concentration of 150 mg/ml, 250 mg/ml, 500 mg/ml and sterilized by filtration by 0.45 µm Millipore filters. Amoxycillin was dissolved in DMSO to get a concentration of 150µg/ml. this concentration was used for testing antibacterial activity. Ketoconazole was dissolved in DMSO to get a concentration of 150µg/ml. this concentration was used for testing antifungal activity. Antimicrobial tests were then carried out by agar diffusion method (Murray et al., 1995) and modified by Olurinola (Olurinola et al., 1996) using 100µl of suspension containing 108 CFU/ml of bacteria, 106 CFU/ml of yeast and 104 spore/ml of fungi spread on nutrient agar (NA), sabourand dextrose agar (SDA), respectively(Singh M et al., 2007). Bacteria were cultured overnight at 37°C and fungi at 28°C for 72 h used as inoculum. 20ml of nutrient agar was dispensed into sterile universal bottles these were then inoculated, mixed gently and poured into sterile petri dishes. After setting a number 3-cup borer (6mm) diameter was properly sterilized by flaming and used to make three to five uniform cups/wells in each Petri dish. A drop of molten nutrient agar was used to seal the base of each cup. The cups/wells were filled with 50 µℓ of the extract concentration of 150 mg/ml, 250 mg/ml, 500 mg/ml and allow diffusing for 45 minutes. The plates were incubated at 37°c for 24 hours for bacteria. The above procedure is followed for fungal assays, the media used was sabourand dextrose incubated at 25°c for 48 hours. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in triplicate. The extracts and the phytochemicals that showed antimi-

crobial activity were later tested to determine the Minimal Inhibitory Concentration (MIC) for each bacterial and fungal sample. (Vadlapudi V et al., 2009)

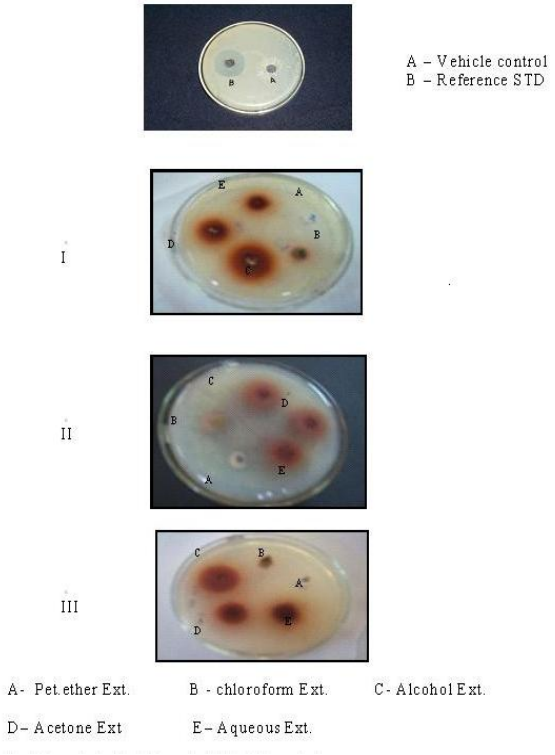

Figure 1: Antimicrobial activity – *S. aureus*

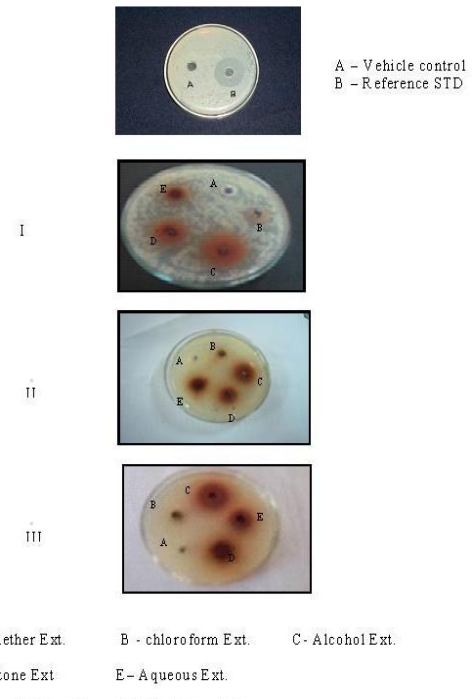

Figure 2: Antimicrobial activity – *B. subtilis*

2.5 Determination of minimum inhibitory concentration

MIC values were also studied for microorganisms, which were determined as sensitive to the extract in disc diffusion assay. Sterile filter paper discs (6mm in diameter) containing 2.5–1000 µg/disc of plant extracts were placed on the surface of a medium. MIC was defined as the lowest concentration of extract that inhibited visible growth on agar.

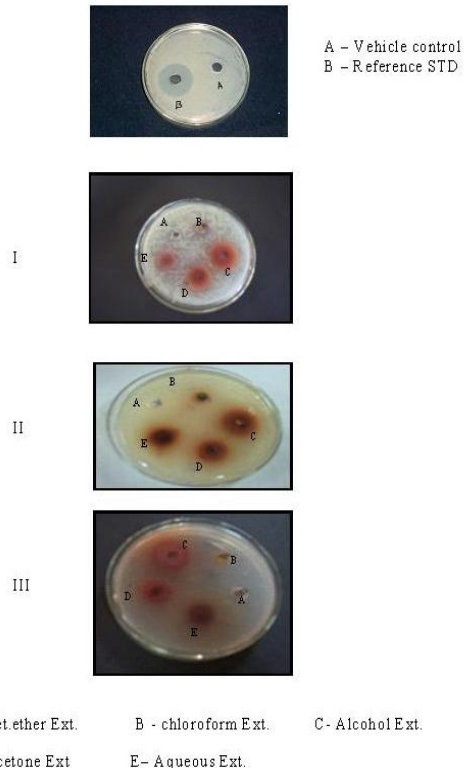

Figure 3: Antimicrobial activity- *E. coli*

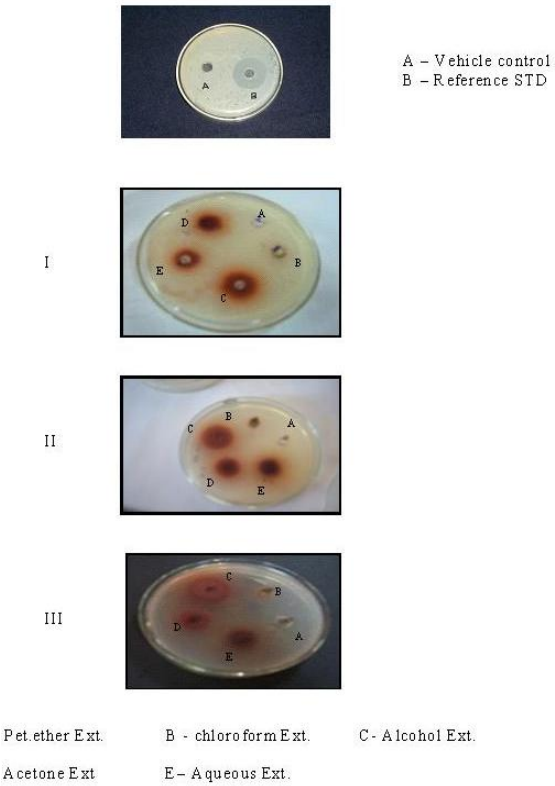

Figure 4: Antimicrobial activity- *P.aureginosa*

3. RESULTS

The results of investigation of antibacterial and antifungal activities of *Bombax malabar*icum are summarized in tables 1 and 2 Fig.1-6 respectively. The inhibition zones of *Bombax malabaricum* which were obtained against all test bacteria were in the range of 7–19 mm. Petroleum ether and chloroform extracts showed no activity while the alcoholic extract showed more activity than the acetone and aqueous extracts. The highest inhibitory activity was determined for alcoholic extract against *E.coli* (19.50 ± 0.5000 mm, inhibition zone diameter). On the other hand, the weakest inhibitory activity was determined against *P. aeruginosa* for aqueous extract (7.00 ± 0.5774 mm, inhibition zone diameter). Preliminary phytochemical analysis showed that the bark extracts of *Bombax malabar*icum possess phenolic compounds, saponins, tannins and glycosides. Phytoconstituents such as saponins, tannins, phenolic compounds and glycosides have been reported to inhibit bacterial growth and to be protective to plants against bacterial and fungal infections. MIC values for all the test organisms were indicated in table 3.

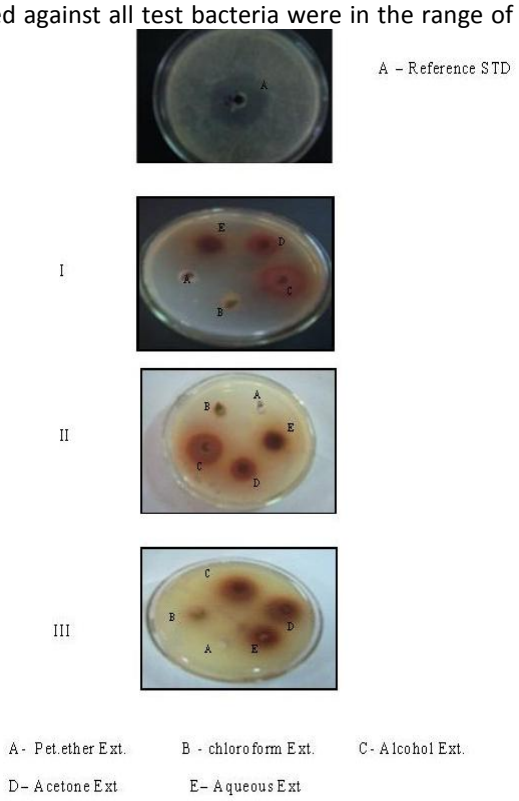

Figure 5: Antimicrobial activity- *C. albicans*

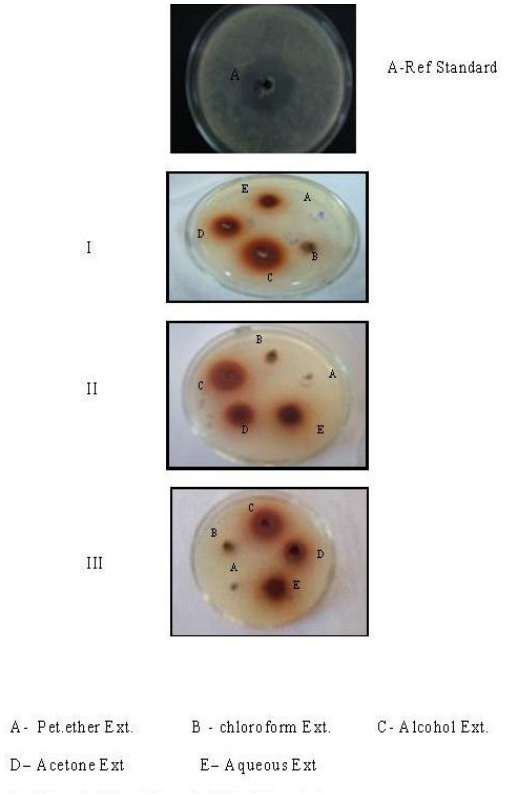

Figure 6: Antimicrobial activity- *A.niger*

4. DISCUSSION AND CONCLUSION

Phytoconstituents such as saponins, phenolic compounds and glycosides have been reported to inhibit bacterial growth and to be protective to plants against bacterial and fungal infections. (Okwute et al., 1992; Mather et al., 1982). Various extracts of *Bombax malabaricum* were subjected to phytochemical investigation and revealed presence of glycosides, saponins, flavonoids, tannins phenolic compounds, proteins, phytosterols, carbohydrates, gums and mucilages. From the above results it can be concluded that plant extracts have great potential as antimicrobial compounds against microorganisms and that they can be used in the treatment of infectious diseases caused by resistant microorganisms. The *Bombax malabaricum* showed maximum antibacterial activity and so this plant can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals that address hither to unmet therapeutic needs. Such screening of various natural organic compounds and identifying active agents is the need of the hour, because successful prediction of important lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development. Further studies need to be carried out to isolate the various classes of phytoconstituents and determine their antimicrobial potential.

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