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Research Article

In vitro Antimicrobial Activity of Ethyl Acetate Extract of *Ficus bengalensis* Lam.

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ABSTRACT

Ficus bengalensis belongs to the family Moraceae. This plant is used in Ayurveda for the treatment of diarrhoea, dysentery and piles, teeth disorders, Rheumatism, skin disorders like sores, to boost the immune system and as a hypoglycemic agent. The present study was designed to investigate antibacterial and antifungal activity of Ethyl acetate extracts from the leaves of Ficus benghalensis. The extract was screened for antibacterial and antifungal activity by the disc diffusion method. The highest zone of inhibition was found in the concentration of 200µg/ disc for Klebsiella species (18mm) and Alternaria Sp (20mm). The results confirm that Ethyl acetate extracts possessed moderate antibacterial activity against all tested bacterial and fungal strains. Ficus benghalensis showed strong activity against all the tested bacterial and fungal strains. Hence, this plant can be used to discover new antimicrobial products.

Key Words: *Ficus benghalensis*, Antibacterial, Antifungal and Disc diffusion assay.

INTRODUCTION

Many *Ficus* species have long been used in folk medicine as astringents, carminatives, stomachics, vermicides, hypotensives, anthelmintics and anti-dysentery drugs.[1] It is believed that some *Ficus* species can be used as a remedy for visceral obstructive disorders, diabetes, leprosy, respiratory disorders and certain skin diseases, [2] and as an absorbent for inflammatory swellings and burns.[3]

Ficus bengalensis belongs to the family Moraceae, which is commonly known as Banyan tree. Earlier glucoside, 20-tetratriaconthene-2-one, 6-heptatriacontene-10-one, pentatriacontan-5-one, beta sitostirolalpha- D-glucose and meso-inositol have been isolated from the bark of *Ficus benghalensis* [4] Leaves contain crude protein 9.63%, crude fibres-26.84%, CaO-2.53%, and Phosphorus-0.4 %. It yields latex containing Caoytchoue (2.4%), Resin, Albumin, Cerin, Sugar and Malic acid. It is used in Ayurveda for the treatment of diarrhoea, dysentery and piles ,[5, 6] teeth disorders ,[7] Rheumatism, skin disorders like sores ,[8] to boost immune system ,[9] as a hypoglycemic.[10, 11, 12, 13] The extracts of *Ficus benghalensis* were also reported to inhibit insulinase activity from liver and kidney.[14]

Fruit extracts exhibited anti-tumor activity in the potato disc bioassay.[15] Two Flavanoids compounds, viz. 5, 7-dimethyl ether of leucopelargonidin 3-0-alpha-L rhamnoside and 5, 3'-dimethyl ether of leucocyanidin 3-0-alpha-D galactosyl cellobioside were obtained from the bark of *F. benghalensis* and were evaluated for anti oxidant activity in hyperlipidemic rats.[16] It was also found to inhibit the lipid peroxidation.[17] Various extracts of *Ficus benghalensis* was screened for its antiallergic and anti stress potential in asthma by milk induced leucocytosis and milk induced eosinophilia.[18] Other species of Ficus viz. *Ficus septica* ,[19] *Ficus sycomorus, Ficus benjamina, Ficus religiosa* ,[20] *Ficus racemosa*,[21] *Ficus pumila* ,[22] *Ficus vasta* ,[23] *Ficus thonningii* [24] and *Ficus capensis* [25] was found to be reported to have antimicrobial activity. The present study was aimed to screen in-vitro antibacterial and antifungal activity against ten potentially pathogenic microorganisms and four pathogenic fungi at different concentrations.

MATERIALS AND METHODS

Plant materials: Leaves of *Ficus benghalensis* were collected during March 2010 from the local market in Bahadderhat Bazar of Chittagong city, Chandanish pourashava, and Bagichar hat, Chandanish, Chittagong.

Extraction: The leaves of *Ficus benghalensis* were dried in air and finally in mechanical drier at 60-70ºC. The dried samples were ground to course powder with a mechanical grinder and extracted with ethyl acetate for 7 days with occasional shaking in a beaker. The extracts were filtered. The filtrate was dried at 50 to 60 o. The extracts were dried and percentage yield was calculated.

Bacterial Media (Nutrient Agar Media): 36g of Nutrient Agar Media was mixed with distilled water and then sterilized in autoclave at 15lb pressure for 15 minutes. The sterilized media were poured into petri dishes. The solidified plates were bored with 5mm diameter cork bearer. The plates with wells were used for the antibacterial studies.

Fungal Media (Potato dextrose sugar): 200g of potato slices were boiled with distilled water. The potato infusion was used as water source of media preparation. 20g of dextrose was mixed with potato infusion. 20g of agar was added as a solidifying agent. These constituents were

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Table1. In vitro Antimicrobial activity of the ethyl acetate extract of *Ficus benghalensis Lam.* Leaves.

Name of the Bacteria	Zone of inhibition in mm			
	Kanamycin disc (standard)	*Ficus benghalensis* (Ethyl acetate extract)		
	30µg/disc	100µg/disc	150µg/disc	200µg/disc
Staphylococcus aureus	30 mm	11	12	15
Bacillus subtilis	29 mm	12	14	16
Vibrio cholera	25 mm	11	14	16
Bacillus cereus	29 mm	13	15	17
Salmonella typhi	30 mm	12	14	17
Shigella dysenteriae	30 mm	11	13	16
Pseudomonas aeruginosa	27 mm	12	14	16
Eschericia coli	30 mm	0	0	8
Klebsiella species	30 mm	12	15	18
Proteus species	24 mm	11	13	16

Table 2. In vitro Antifungal activity of the ethyl acetate extract of *Ficus benghalensis Lam.* leaves.

Name of the Fungi	Zone of the inhibition in mm			
	Nystatin (Standard)	*Ficus benghalensis* (Ethyl acetate extract)		
	30µg/disc	100µg/disc	150µg/disc	200µg/disc
Alternaria spp.	30mm	13	15	20
Colletotrichum spp.	27mm	8	9	10
Curvularia spp.	26mm	9	9	9
Fusarium spp.	30mm	14	15	17

mixed and autoclaved. The solidified plates were bored with 6mm diameter cork borer. The plates with wells were used for antifungal studies.

Antimicrobial and Antifungal screening (in *vitro*): The antimicrobial activity of the compounds *Ficus benghalensis,* were measured by disk diffusion method (Beur et al. 1966 and Barry et al. 1980).[26, 27] The prepared culture plates were inoculated with different selected strains of bacteria and fungi using streak plate method. Wells were made on the agar surface with 6mm cork borer. The extracts were poured into the well using sterile syringe. The plates were incubated at 37ºC±2ºC for 24 hours for bacterial and 25±2ºC for 48 hours for fungal activity. The plates were observed for the zone clearance around the wells.

The ethyl acetate extract was dissolved in sterile distilled water to form dilution such as 100µg, 150µg and 200µg. Each concentration of the plant extract was tested against different bacterial pathogens and fungal species. It was demonstrated by well diffusion assay (Bauer *et al*., 1996). The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter.

RESULTS

The present study showed that Leaves extract of *Ficus benghalensis* (ethyl acetate extract) exhibited both antimicrobial activity and antifungal activity against the tested microorganisms and fungi at the concentrations of 100, 150 and 200µg/disc. The potential sensitivity of the extract was obtained and the zone of inhibition was recorded and presented below in the tabulation drawn in Table 1 (antimicrobial activity) and Table 2 (antifungal activity).

DISCUSSION

In the present investigation, the antimicrobial and antifungal activity of leaves extract of *Ficus benghalensis Lam.* was assayed against ten potentially pathogenic microorganisms *Staphylococccus aureus, Bacillus subtilis, Vibrio cholera, Bacillus cereus, Salmonella typhi, Shigella dysenteriae, Pseudomonas aeruginosa, Klebsiella species, Eschericia coli* and *Proteus species* and antifungal activity against four pathogenic fungi- *Alternaria Sp., Colletotrichum Sp, Curvularia Sp and Fusarium Sp* at different concentrations of the extract to understand the most effective activity. The leaves extract of *Ficus benghalensis Lam.* showed a broad-spectrum antibacterial activity with a zone of inhibition of 8 to 18 mm and antifungal activity with a zone of inhibition of 8 to 20 mm. For ethyl acetate extract, the maximum zone of inhibition was obtained for *Pseudomonas aeruginosa* and *Bacillus cereus* at a concentration of 200µg/disc. While the maximum zone of inhibition was found for antifungal activity at a concentration of 200µg/disc for *Colletotrichum Sp.* and *Alternaria Sp.* All the tested microorganisms and fungi exhibited good sensitivity against above three concentrations except *Eschericia coli, Eschericia coli* showed no sensitivity against *Ficus benghalensis* at the concentrations of 100 and 150 µg/disc.

CONCLUSION

These findings suggest a new pathway in elucidating a potent antimicrobial agent from *Ficus benghalensis.* Present study indicates that the plant contains antimicrobial compound which can be further developed as phytomedicine for the therapy of infection. Such screening of various natural organic compounds and identification of active agents is the need of the hour because successful prediction of lead molecule at the onset

of drug discovery will pay off later in drug development. Lastly, to conclude the extracts were found to inhibit the growth of Gram-positive bacteria as well as the Gram-negative bacteria and the fungal species.

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