

ANTIMICROBIAL ACTIVITY OF *FICUS BENGHALENSIS*

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Summary

The various solvent extracts of underground roots of *Ficus benghalensis*, an important medicinal plant of Indian System of Medicine, were investigated for presence of various chemical groups. Preliminary phytochemical investigation showed presence of Carbohydrates, Flavanoids, Amino Acids/Proteins, Steroids, Saponins and Tannins in Aqueous and Methanolic extract while Flavanoids and Steroids were present in Chloroform extract. All the three extracts were screened for antimicrobial activity against six bacterial species (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosae*, *E.coli*, *E.coli mutant*, *Klebsiella pneumoniae*) and one fungal species (*Aspergillus niger*), by using the cup plate method. Amongst all the extracts tested, methanolic extract was comparably more effective to inhibit the growth of microbes than Aqueous and Chloroform extracts. A survey of literature showed that antimicrobial studies were conducted only on Bark and Fruit extracts of *Ficus benghalensis* so proposed study is to investigate whether the extracts of underground roots posses antimicrobial activity or not. This plant can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals that address unmet therapeutic needs.

Keywords: Antimicrobial activity, *Ficus benghalensis*, cup plate method, *Staphylococcus aureus*

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Introduction

Biologically active compound from natural source has always been of great interest to scientist working on infectious diseases. In recent years there has been a growing interest to evaluate plants possessing antimicrobial activity. Plant derived drugs serves as a prototype to develop more effective and less toxic medicines. *Ficus benghalensis* (Moraceae, Mulberry family) is commonly known as Banyan tree or Vata or Vada tree in Ayurveda. There are more than 800 species and 2000 varieties of *Ficus* species, most of which are native to the old World tropics. *Ficus benghalensis* a remarkable tree of India sends down its branches and great number of shoots, which take root and become new trunk. This tree is considered to be sacred in many places in India. Earlier glucoside, 20-tetratriacontene-2-one, 6-heptatriacontene-10-one, pentatriacontan-5-one, beta sitostiril-alpha-D-glucose and meso-inositol have been isolated from the bark of *Ficus benghalensis* (1) 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 (2,3), teeth disorders (4), Rheumatism, skin disorders like sores (5), to boost immune system (6), as a hypoglycemic (7,8,9,10). The extracts of *Ficus benghalensis* were also reported to inhibit insulinase activity from liver and kidney (11). Fruit extracts exhibited anti-tumor activity in the potato disc bioassay (12). Two Flavanoids compounds, viz. 5, 7-dimethyl ether of leucopelargonidin 3-O-alpha-L rhamnoside and 5, 3'-dimethyl ether of leucocyanidin 3-O-alpha-D galactosyl cellobioside were obtained from the bark of *F. benghalensis* and were evaluated for anti-

oxidant activity in hyperlipidemic rats (13). It was also found to inhibit the lipid peroxidation (14). Various extracts of *Ficus benghalensis* was screened for its anti-allergic and anti stress potential in asthma by milk induced leucocytosis and milk induced eosinophilia (15). Other species of Ficus viz. *Ficus septica* (16), *Ficus sycomorus*, *Ficus benjamina*, *Ficus religiosa* (17), *Ficus racemosa* (18), *Ficus pumila* (19), *Ficus vasta* (20), *Ficus thonningii* (21) and *Ficus capensis* (22) was found to be reported to have antimicrobial activity. Based on this, an attempt has been made to evaluate the antimicrobial potency of *Ficus benghalensis*.

Materials and Methods

1. Plant materials

Underground roots of *Ficus benghalensis* were collected from Satara District, Maharashtra, India in the month of October 2007. This plant material was authenticated at the Dept. of Botany, Sinhgad College of Science, STES Campus, Pune and the Voucher herbarium specimen is deposited in the Dept of Pharmacognosy of Sinhgad Institute of Pharmacy, Narhe, Pune. Around 3 kg of fresh root tubers was collected and washed under running tap water, dried and were cut into small pieces of 2-3 cms. These roots were then shade dried (30 °C, 45 % relative humidity) for 15 days and then homogenized to get a coarse powder. This powder was stored in an air tight container and used for further successive extraction.

2. Preparation of Extracts

i. Aqueous Extract (By Decoction Method)

160 g of coarse powder of underground roots of *Ficus benghalensis* was boiled with 1300 ml of double distilled water for 1 hour. Then it was kept at room temperature for 24 hours and then filtered through muslin cloth. The filtrate obtained was then concentrated to thick slurry and the residue left behind on muslin cloth was again boiled with double distilled water for 1 hour and filtered. The filtrate thus obtained was added to the thick slurry of first step. The resultant solution was boiled again to get a thick concentrated extract. It is then dried and used as a powder. The yield was found to be 9.99 g (22.22%).

ii. Solvent Extraction

The methanolic and Chloroform extracts of the roots of *Ficus benghalensis* were prepared by using soxhlet apparatus. In this extraction process, 45 g of dried powder was extracted with 500 ml of methanol and chloroform separately at 30 °C. A total of 50 cycles were runned to obtain thick slurry. This slurry was then evaporated to yield solid extracts of methanol and chloroform. The yield of methanol and chloroform was found to be 1.908g (04.24%) and 0.558g (01.24%) respectively.

3. Test Organisms

Bacillus subtilis, *Staphylococcus aureus*, *Pseudomonas aeruginosae* were the Gram positive bacterial species used and *E.coli*, *E.coli mutant*, *Klebsiella pneumonia* were the Gram negative bacterial species used for the antimicrobial study. The fungal species used was *Aspergillus niger*. These were obtained from Dept. of Microbiology of Sinhgad Institute of Pharmacy, Narhe, Pune.

4. Drugs and Chemicals

Streptomycin (Alembic Ltd.), Methanol A.R (Thomas Baker Chemicals Pvt Ltd.). Chloroform A.R (Loba Chemicals Pvt Ltd.), Dimethyl sulphoxide (Thomas Baker chemicals Pvt. Ltd), Nutrient agar – Meat beef extract, peptone, sodium chloride, agar and Potato dextrose agar- Potatoes, dextrose, agar were used for the experiment.

5. Antimicrobial Assay

The antimicrobial activity was evaluated by cup plate method by using Nutrient agar as growth media for bacterial species and Potato dextrose agar for the fungal species. The microorganism was activated by inoculating a loopful of the species in the nutrient broth and incubated for 24 hrs at 37°C and in case of fungi, the isolated colonies of *Aspergillus niger* were obtained from the stock and sub cultured using Potato dextrose agar at 37°C for 48 hrs. The sub culture in broth was used as the inoculum for seeding the agar plate. The growth media's were prepared and sterilized as directed by the manufacturer of the media and poured into presterilized Petri dishes on a flat horizontal surface to a depth of 4 mm and allowed to solidify. The prepared plates were inoculated with 0.1ml inoculum. With the help of the sterile spreader the inoculum was spread uniformly to obtain a nearly confluent lawn of growth covering the whole surface of the plate. The wells were bored into the inoculated Petri plates with the help of sterile borer depending upon the number of concentrations of the extracts. The extracts were freshly reconstituted with suitable solvents (Water/Dimethyl sulphoxide) Different dilutions of the extracts were added in the wells. The wells were uniformly and completely filled. The plates were then placed

in an incubator. They were incubated overnight at 37 ° C in the incubator. In case of fungi, the test plates were incubated for 48 hrs. Each experiment was carried out in triplicate and the mean diameter of the zone of inhibition (Z.O.I) was measured by the use of Antibiotic Zone Reader (Phathak electrical works) by placing the plate against a ruler or ruled screen. Normal control without any antibiotic or test compound was maintained to check experimental error. All the results were expressed as a mean \pm SEM

Preliminary phytochemical investigation

All the three extracts were subjected to qualitative chemical investigations using various reagents for the presence of the various phytochemical groups like tannins, flavonoids, steroids, proteins etc (23).

Results and discussions

Preliminary Phytochemical analysis showed the presence of Carbohydrates, Flavanoids, Amino acids, Steroids, Saponins and Tannins like phytoconstituents in the extracts of *Ficus benghalensis*. The antimicrobial potency of the plant may be attributed to single or combined effect of the mentioned chemical groups. The role of these constituents in eliciting antimicrobial properties requires further detailed phytochemical investigation.

From the observations made, all the extracts of roots of *Ficus benghalensis* were found to show antimicrobial activity when compared to the standard drug. Results of

antimicrobial assay showed that aqueous extract of the plant possess inhibitory activity at 0.4 mg/well concentration tested against all bacterial strains and the fungal strain used for the experiment, however no inhibition was observed at 0.04 and 0.004 mg/well concentration against any of the bacterial cultures or the fungal culture tested. The methanolic extract possess inhibitory activity at 0.4 mg/well concentration against all bacterial and the fungal strain used, however at 0.04 mg/well concentration shows inhibitory activity against all bacterial strain but no activity against fungal strain. At the least concentration used (0.004 mg/well) the extract shows activity against all except *E.coli*, *Bacillus subtilis* and *Aspergillus* species. The chloroform extract showed potential inhibitory activity at 0.4 and 0.04 mg/well concentration against all bacterial and fungal strains, however at 0.004 mg/well concentration no inhibitory activity against the fungal strain and the bacterial strains except *E.coli* mutants and *Staphylococcus aureus* were noted (Table:1).

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 also the fungal species and the methanolic extract was comparably more effective to inhibit the growth of microbes than Aqueous and Chloroform extracts.

Table no. 1: Comparative Zone of Inhibition of various extracts at various conc.

Test samples	Conc (mg/well)	Zone of inhibition (in mm)						
		<i>E.c.</i>	<i>Ecm</i>	<i>S.a</i>	<i>Bs</i>	<i>K.p.</i>	<i>P.a.</i>	<i>A.n.</i>
Standard	0.4	34.33± 0.56	37.5 ±0.76	38.66± 0.87	38.83± 0.60	35.00 ±0.58	38.83± 0.83	
Water extract.	0.4	7.16±0.26	7.27±0.01	7.82±0.03	7.62±0.01	6.7±0.09	6.65±0.05	6.35±0.07
	0.04	--	--	--	--	--	--	--
	0.004	--	--	--	--	--	--	--
Methanol extract.	0.4	10.35±0.15	11.38±0.1 3	10.26±0.18	9.43±0.11	8.3±0.11	11.7±0.13	6.35±0.16
	0.04	8.61±0.10	8.86±0.09	7.93± 0.09	7.73± 0.08	6.55 ±0.07	8.9 ±0.08	--
	0.004	--	6.61±0.11	6.56± 0.12	--	6.65± 0.11	6.65± 0.13	--
Chloroform extract.	0.4	9.68±0.10	10.55±0.1 2	10.19±0.11	8.55±0.07	9.91±0.13	9.92±0.14	8.61±0.09
	0.04	7.41± 0.11	8.22± 0.10	9.45± 0.05	6.6 ±0.11	6.46± 0.13	7.98± 0.08	6.58± 0.13
	0.004	--	6.45±0.11	6.55 ±0.12	--	--	--	--

Ec – *Escherichia coli* , *Ecm* – *E.coli mutants* , *Sa* – *Staphylococcus aureus* , *Bs* – *Bacillus subtilis*,
Kp – *Klebsiella pneumoniae*, *Pa* – *Pseudomonas aeruginosae* , *An* – *Aspergillus niger*.

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