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Research Article

A Research Paper On Phytochemical Profiling And In-Vitro Antibacterial Evaluation Of Ethanolic Extract Of Aerial Roots Of *FICUS BENGHALENSIS*

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ABSTRACT

Ficus benghalensis L. is a medicinally significant plant that has been widely used in traditional healthcare practices. The present investigation was designed to explore the phytochemical constituents and antibacterial potential of its aerial roots. The collected aerial roots were processed and extracted using an appropriate solvent system, followed by preliminary phytochemical evaluation to identify the major classes of secondary metabolites. The analysis indicated the occurrence of several phytoconstituents, including phenolic compounds, flavonoids, tannins, alkaloids, terpenoids, saponins, steroids, glycosides, and other metabolites, depending on the extract. The antibacterial efficacy of the prepared extracts was assessed against selected bacterial pathogens under in vitro conditions using a suitable antimicrobial assay. Different extracts demonstrated varying levels of inhibitory activity against the tested organisms, suggesting that the aerial roots contain compounds capable of suppressing bacterial growth. The antibacterial effect may be associated with the combined action of phenolic and flavonoid compounds along with other secondary metabolites present in the plant material. Overall, the study highlights the aerial roots of Ficus benghalensis as a potential natural source of antibacterial compounds. Further isolation, structural characterization, and pharmacological evaluation of the active constituents are required to determine their therapeutic relevance.

INTRODUCTION

Medicinal plants have been an important source of therapeutic agents since ancient times and continue to attract scientific interest because of their diverse secondary metabolites and biological activities. The genus *Ficus*, belonging to the family Moraceae, comprises numerous species that are traditionally used in different systems of

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medicine. Among them, *Ficus benghalensis* Linn., commonly known as the Indian banyan or banyan tree, is a well-known medicinal tree widely distributed in the Indian subcontinent. Different parts of the plant, including leaves, bark, roots, aerial roots, fruits and latex, have been traditionally used for various health-related conditions. Modern investigations have supported several pharmacological properties of *F. benghalensis*, including antioxidant, antidiabetic, anti-inflammatory, antimicrobial, wound-healing and hepatoprotective activities. These properties have been associated with the presence of chemically diverse phytoconstituents, making the plant an important candidate for pharmacological and natural-product research. [1–3]

The aerial roots of *F. benghalensis* are particularly interesting because they represent a specialized plant part that has received comparatively less systematic investigation than leaves and stem bark. Previous phytochemical studies have demonstrated the presence of several biologically relevant compounds in the aerial roots. Reported constituents include phenolic compounds such as 4-hydroxybenzoic acid, 4-hydroxymellein and *p*-coumaric acid, together with flavonoid-related compounds, triterpenoids and sterols. Compounds such as bengalensinone, benganoic acid, alpinumisoflavone, lupanyl acetate, stigmasterol and α-amyrin acetate have also been reported from the aerial roots. Preliminary phytochemical investigations further indicate the occurrence of flavonoids, phenolics and saponins. Such chemical diversity provides a reasonable scientific basis for investigating the biological activities of aerial-root extracts. [2,4,5]

Antibacterial activity is an important area of medicinal-plant research because pathogenic bacteria continue to cause a wide range of infections, while antimicrobial resistance has increased the need for alternative or complementary sources of antibacterial compounds. Plant-derived phenolics, flavonoids, tannins, terpenoids and other secondary metabolites may interfere with bacterial growth through several mechanisms, including disruption of cell membranes, alteration of cellular proteins and enzymes, and interference with essential metabolic processes. Studies on *F. benghalensis* have reported antibacterial or antimicrobial effects from different plant parts, and specific investigations of aerial-root extracts have demonstrated activity against several bacterial organisms. Recent research has also reported antibacterial activity of aerial-root extracts against *Escherichia coli* and *Staphylococcus aureus*, together with inhibition of bacterial biofilm formation. [5–7]

Ethanol is a useful extraction solvent for phytochemical investigations because it can extract a broad range of polar and moderately polar plant constituents, including many phenolic and flavonoid compounds. Therefore, systematic phytochemical profiling of an ethanolic extract of *F. benghalensis* aerial roots, followed by evaluation of its antibacterial activity against selected pathogenic bacteria, may provide useful evidence linking the chemical composition of the extract with its biological potential. The proposed study is consequently designed to characterize the major classes of phytoconstituents present in the ethanolic aerial-root extract and determine its in vitro antibacterial activity. Such findings may contribute to the scientific validation of the medicinal potential of *F. benghalensis* aerial roots and provide a basis for further isolation, characterization and development of plant-derived antibacterial agents. [1,3,6,7]

Therefore, the present study entitled “Phytochemical Profiling and In Vitro

Antibacterial Evaluation of Ethanolic Extract of *Ficus benghalensis* Aerial Roots Against Selected Pathogenic Bacteria" is designed to investigate the phytochemical constituents present in the ethanolic extract of *F. benghalensis* aerial roots and to evaluate its antibacterial potential against selected pathogenic bacterial strains. The study may provide useful preliminary scientific evidence regarding the medicinal value of the aerial roots and may contribute to the identification of plant-derived antibacterial resources for further pharmacological and phytochemical investigations.

1. MATERIALS AND METHODS

Collection and Authentication of Plant Material

Fresh aerial roots of *Ficus benghalensis* Linn., belonging to the family Moraceae, were collected from the Chail Chowk area of Mandi district, Himachal Pradesh, India, during the month of December. The plant material was collected from healthy and mature trees to ensure the quality and suitability of the sample for further investigation. Immediately after collection, the aerial roots were carefully examined and selected to remove any damaged or diseased portions. The collected material was thoroughly washed first with running tap water and subsequently with distilled water to remove adhering soil, dust, and other extraneous matter. The plant specimen was authenticated by experts at HP CDP JICA ODA, Mandi, Himachal Pradesh, confirming its botanical identity as *Ficus benghalensis* Linn. Following authentication, the aerial roots were processed appropriately and subsequently subjected to phytochemical profiling and in vitro antibacterial evaluation against selected bacterial strains.

Preparation of Plant Material

After collection and washing, the aerial roots will be shade-dried at room temperature under well-ventilated conditions, protected from direct sunlight to minimize degradation of heat- and light-sensitive phytoconstituents. The dried aerial roots will be cut into small pieces and pulverized into a coarse-to-fine powder using a clean mechanical grinder. The powdered material will be passed through an appropriate sieve to obtain relatively uniform particle size and stored in a clean, dry, airtight container until extraction. Similar drying and powdering procedures have previously been used for the preparation of *F. benghalensis* aerial-root extracts.

Preparation of Ethanolic Extract

The powdered aerial-root material will be extracted with ethanol because ethanol can extract a broad range of polar and moderately polar phytoconstituents. A predetermined quantity of powdered material will be mixed with ethanol in an appropriate solid-to-solvent ratio and subjected to maceration with intermittent shaking for sufficient extraction time. Alternatively, Soxhlet extraction may be employed depending upon the facilities available in the laboratory. After completion of extraction, the mixture will be filtered through Whatman filter paper to separate the plant residue from the extract. The filtrate will then be concentrated under reduced pressure using a rotary evaporator at a controlled temperature to obtain a semisolid or dry ethanolic extract. The percentage extraction yield will be calculated, and the prepared extract will be stored in a properly labelled airtight container under refrigerated conditions until further investigation. Published work on *F. benghalensis* aerial roots has used ethanol extraction followed by filtration and rotary evaporation, supporting this approach.

Preliminary Phytochemical Screening

The ethanolic aerial-root extract will be subjected to preliminary qualitative phytochemical screening to identify the major classes of secondary metabolites. Standard chemical tests will be performed for alkaloids, flavonoids, phenolic compounds, tannins, saponins, steroids, terpenoids, glycosides and other relevant phytoconstituents. Each test will be conducted using suitable reagents, and the appearance of characteristic colour changes, precipitates or foam formation will be recorded as evidence of the respective phytochemical class. The results will be expressed qualitatively as absent or present, with the intensity of the observed reaction recorded where appropriate. Similar phytochemical screening has been reported for aerial-root extracts of *F. benghalensis*.

Selection and Preparation of Bacterial Cultures

Selected pathogenic bacterial strains representing both Gram-positive and Gram-negative groups will be used to assess the antibacterial potential of the ethanolic aerial-root extract. The bacterial strains will be obtained from a recognized microbial culture collection or an appropriately authorized microbiology laboratory. The cultures will be maintained on suitable growth media and subcultured before experimentation to obtain fresh, actively growing bacterial cultures. The bacterial inoculum will be standardized before the antibacterial assay to ensure comparable microbial loading among different experimental plates. Published antibacterial studies commonly include organisms such as *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* when evaluating medicinal plant extracts.

In Vitro Antibacterial Evaluation

The antibacterial activity of the ethanolic extract will be evaluated using the agar-well diffusion method. Suitable Mueller–Hinton agar plates will be prepared and inoculated uniformly with the standardized bacterial suspension. Sterile wells of appropriate diameter will be prepared in the inoculated agar using a sterile cork borer. Different concentrations of the ethanolic aerial-root extract will then be introduced into the respective wells. A suitable standard antibacterial drug will be used as the positive control, while the solvent used for dissolving the plant extract will serve as the negative control. The plates will be incubated under appropriate conditions for bacterial growth, after which the diameter of the clear zone surrounding each well will be measured in millimetres. The antibacterial activity will be assessed by comparing the inhibition zones produced by the plant extract with those of the controls. Agar-well diffusion is a widely used method for evaluating antibacterial activity of medicinal plant extracts.

Determination of Minimum Inhibitory Concentration

If sufficient laboratory facilities are available, the minimum inhibitory concentration (MIC) of the ethanolic extract will also be determined using a broth dilution or microdilution procedure. Serial concentrations of the extract will be prepared in a suitable sterile broth medium and inoculated with standardized bacterial cultures. After incubation under appropriate conditions, bacterial growth will be assessed visually or by measuring optical density. The MIC will be considered the lowest concentration of the extract that prevents visible bacterial growth compared with the appropriate growth control. MIC determination provides additional quantitative information about the antibacterial potency of the extract beyond the inhibition-zone measurement.

Replication and Statistical Analysis

All experiments will be performed in appropriate replicates to improve reliability and reproducibility of the observations. The antibacterial results will be expressed as mean ± standard deviation wherever applicable. Differences between experimental groups will be statistically evaluated using an appropriate statistical test, depending on the experimental design and number of groups. A probability value of $p < 0.05$ will generally be considered statistically significant. The results will be presented in suitable tables and figures showing the phytochemical profile and antibacterial activity of the ethanolic extract.

2. RESULT

Test materials

In the present study, etahnolic extract of areal roots of tree Ficus benghalensis (FB) was prepared using Soxhlet apparatus and was evaluated for its antimicrobial activity against a gram positive and a gram-negative bacteria. A standard concentration of (1000 mg/ml) was prepared from crude plant extract in dimethyl sulfoxide (DMSO).

Microorganism used for antibacterial assay.

Standard strains of Staphylococcus aureus (MTCC-96) and E. coli (MTCC- 443) were used to evaluate the antibacterial potential of ethanolic extract of Ficus benghalensis (FB).

Antibacterial assay

Preparation of inoculum

The bacterial strains Staphylococcus aureus (MTCC-96) and E. coli (MTCC- 443) were freshly cultured on nutrient broth media. Inoculum of selected bacterial strains was prepared by transferring 2-3 loop-full of culture growth into 5 mL of sterile normal saline. The suspension was vortexed thoroughly to achieve a homogenous mixture. The turbidity of the suspension was then adjusted to match the 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL. This standardized inoculum was subsequently used for the antibacterial assay.

Agar well diffusion method

The antibacterial potential of extract FB was investigated using agar well diffusion assay, based on previously reported method (Sharma et al., 2012) with a few modifications. Muller Hinton Agar (MHA) plates were prepared and spread plated with prepared bacterial inoculum using sterile cotton swab. Three equidistant wells (8 mm in diameter) were aseptically punched into each plate using a sterile cork borer. Subsequently, 100 µL of test material (FB- 1000 mg/ml of DMSO) was dispensed into respective wells in two different plates. The second well of each plate was filled with 100 µL of Dimethyl sulfoxide (DMSO), serving as the negative control. The third well received standard antibiotic (AB) Piperacillin/Tazobactam (PIT 100/10 µg), as the positive control. Plates were incubated at 37 °C for 24 hours and observed for the formation of zones of inhibition around the wells. Post-incubation, zones of inhibition were measured in millimetres and recorded comparative analysis.

Results and discussion

In the present study in-vitro evaluation of antibacterial potential of ethanolic extract of *Ficus benghalensis* (FB) was evaluated against Staphylococcus aureus and E. coli.

Table 1. Details of test material along with controls used for antibacterial assay.

Sr. No.	Test material	Concentration used	Volume used for each assay
1.	*Ficus benghalensis* **(FB)**	1000 mg/ml of DMSO	100 microliters
2.	Dimethyl Sulfoxide **(DMSO) Negative control**	100 % (v/v)	100 microliters
3.	**Antibiotic (AB)** Piperacillin/Tazobactam **(Positive control)**	(PIT 100/10 µg)	100 microliters

Antibacterial activity of the ethanolic extract of Ficus benghalensis (FB) against Staphylococcus aureus after 24 hrs of incubation. The formation of distinct zones of inhibition surrounding the wells indicates the antibacterial activity of the extract. Dimethyl sulfoxide (DMSO) served as the negative control, while AB served as the positive control on Mueller Hinton agar (MHA) plates.

Antibacterial activity of the ethanolic extract of Ficus benghalensis (FB) against E. coli after 24 hrs of incubation. The formation of distinct zones of inhibition surrounding the wells indicates the antibacterial activity of the extract. Dimethyl sulfoxide (DMSO) served as the negative control, while AB served as the positive control on Mueller–Hinton agar (MHA) plates.

Table 2. Antibacterial assay of ethanolic extract of Ficus benghalensis (FB).

Test Samples	Dimeter of Zone of inhibition (in mm) *	
	Staphylococcus aureus	***E. coli***
Ethanolic extract of *Ficus benghalensis* (FB)	27	31
Antibiotic (AB) Piperacillin/Tazobactam (Positive control)	30	29
Negative control (DMSO)	Nil	Nil

The antibacterial activity of the ethanolic extract of Ficus benghalensis (FB) against Staphylococcus aureus and Escherichia coli is presented in Table 2. The extract demonstrated measurable antibacterial activity against both bacterial test organisms, as evidenced by the

formation of clear zones of inhibition around the treatment wells. The diameter of the inhibition zone was 27 mm against S. aureus and 31 mm against E. coli, indicating that the ethanolic extract exhibited appreciable inhibitory activity against both Gram-positive and Gram-negative bacteria.

The larger inhibition zone observed against E. coli (31 mm) than S. aureus (27 mm) suggests that the extract may have greater inhibitory efficacy against the tested E. coli isolate under the experimental conditions. However, differences in susceptibility between Gram-positive and Gram-negative bacteria can arise from variations in cell-envelope architecture, permeability, membrane composition, and the ability of individual bacterial strains to withstand plant-derived bioactive compounds. Therefore, the difference observed in the present study should be interpreted specifically for the tested isolates and should not be generalized to all strains of these species.

The activity of FB was also comparable with that of the positive control, piperacillin/tazobactam. Against S. aureus, the extract produced a zone of inhibition of 27 mm, compared with 30 mm for the antibiotic control. Against E. coli, the extract produced a zone of 31 mm, slightly exceeding the 29 mm zone observed with piperacillin/tazobactam. This finding demonstrates that, under the conditions of the agar diffusion assay, the extract exhibited substantial antibacterial activity. Nevertheless, direct comparison of inhibition-zone diameters between a crude plant extract and an antibiotic should be made cautiously because their active constituents, concentrations, diffusion characteristics, and mechanisms of action differ considerably.

No zone of inhibition was observed with DMSO against either S. aureus or E. coli, confirming that the solvent itself did not contribute to the observed antibacterial activity. Thus, the inhibition observed with FB can reasonably be attributed to constituents present in the ethanolic extract rather than to the extraction solvent. The results support the potential of F. benghalensis as a source of antibacterial phytochemicals.

The antibacterial activity may be associated with secondary metabolites present in F. benghalensis, including phenolic compounds, flavonoids, tannins, terpenoids, alkaloids, and other bioactive constituents reported from the genus Ficus. These compounds may exert antibacterial effects through multiple mechanisms, such as disruption of bacterial cell membranes, alteration of membrane permeability, inhibition of essential enzymes, interference with nucleic-acid synthesis, and oxidative stress. However, the specific compounds responsible for the activity observed in the present study cannot be established from the inhibition-zone assay alone and require further phytochemical and mechanistic investigation.

3. CONCLUSION

Overall, the results demonstrate that the ethanolic extract of Ficus benghalensis possesses appreciable antibacterial activity against both S. aureus and E. coli. The maximum zone of inhibition was recorded against E. coli (31 mm), followed by S. aureus (27 mm). The absence of inhibition with DMSO confirms the suitability of the solvent as a negative control. The observed activity, together with the comparable inhibition zones obtained with the positive antibiotic control, indicates that F. benghalensis may serve as a promising source of naturally occurring antibacterial compounds. However, these findings represent preliminary evidence of antibacterial potential and should not be interpreted as demonstrating equivalence to conventional antibiotics.

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