



Phytochemical Screening and Antibacterial Efficacy of *Ficus benghalensis* Using *in vitro* Models

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ABSTRACT

F. benghalensis is known to be an important medicinal herb since ancient times in India. It has been reported to possess several bioactive compounds of prime pharmacological importance. The present work was aimed to undergo the phytochemical screening and to investigate the antibacterial efficacy of its leaves in aqueous, methanol and chloroform extract by agar well diffusion assay. The phytochemical screening of the methanolic leaf extract revealed the presence of carbohydrates, reducing sugars, deoxysugars, sterols, glycosides, phenolic compounds, tannins, saponins and flavonoids. The *in vitro* antibacterial efficacy of methanolic extract was found to be the most effective followed by chloroform and aqueous extracts. The extract was more effective against gram positive bacteria as compared to gram negative bacteria. The study further concludes that *S. typhi* was completely resistant against all the extracts.

Keywords: *Ficus benghalensis*, medicinal herb, bioactive compounds, phytochemical screening, *in vitro* antibacterial efficacy.

INTRODUCTION

In recent years, multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases.¹ The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Medicinal herbs are a rich source of antimicrobial agents. A wide range of medicinal plant parts are used in the form of extracts as raw drugs and possess varied medicinal properties.² In comparison with modern medicine, herbal medicines cost less, are more often used to treat chronic diseases and the occurrence of undesirable side effects seems to be less frequent.³ Several factors have contributed to the growth of the use of traditional herbs worldwide, among which are: preference of consumers for natural therapies, concern regarding undesirable side effects of modern medicines and the belief that herbal drugs are free from side effects, great interest in alternative medicines; preference of populations for preventive medicine due to increasing population age, the belief that herbal medicines might be of effective benefit in the treatment of certain diseases where conventional therapies and medicines have proven to be inadequate; tendency towards self-medication, improvement in quality, proof of efficacy and safety of herbal medicines and high cost of synthetic medicines. Over the past twenty years, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents.⁴ Different extracts from traditional medicinal plants have been tested. Many reports have shown the effectiveness of traditional herbs against different micro-organisms, as a result, plants are

one of the bedrocks for modern medicine to attain new principles.⁵ Medicinal plants are finding their way into pharmaceuticals, nutraceuticals, cosmetics and food supplements. In this regard, plants have given western pharmacopoeia about biomolecules of plant origin appeared to be one of the alternatives for the control of such antibiotic resistant human pathogens.⁶ Keeping these points in view, the present study was aimed to investigate the antibacterial efficacy and phytochemical analysis of *Ficus benghalensis* (Banyan tree).

MATERIALS AND METHODS

Collection of plant material

The leaves of the plant *Ficus benghalensis* (Banyan tree) were collected for the study. Leaves suitable for extraction were plucked and were washed under running tap water followed by sterilized distilled water. Leaves were air-dried, powdered and were subjected to the following extraction protocols.

Aqueous Extraction

Air-dried powders of leaves (10 g) were boiled in 400 ml distilled water till one fourth of the extract, initially taken, was left behind after evaporation. The solution was then filtered using muslin cloth. Filtrate was then centrifuged at 5000 rpm for 15 minutes. The supernatant was again filtered using Whattmann filter paper no. 1 under strict aseptic conditions and the filtrate was collected in fresh sterilized bottles and stored at 4°C until further use.

Organic solvent Extraction

Air-dried powder of leaves (10 g) was thoroughly mixed with 100 ml of methanol and chloroform. The mixture



was placed at room temperature for 24 h on shaker with 150 rpm. Solution was then filtered through muslin cloth and then re-filtered through Whatman filter paper no. 1. The filtrate thus obtained was concentrated by complete evaporation of solvent at room temperature to yield the pure extract. Stock solutions of organic crude extracts were prepared by mixing well the appropriate amount of dried extract and suitable solvent to give rise to a final concentration of 100 mg/ml. The solution was stored at 4°C in sterilized bottles until further use.

Collection of bacterial strain

The bacterial strains, *S. aureus* (MTCC-737), *P. aeruginosa* (MTCC-429), *E. coli* (MTCC-1303), *B. subtilis* (MTCC-121), *K. pneumonia* (MTCC-109), *Streptococcus thermophilus* (MTCC-5461), *Proteus vulgaris* (MTCC-1771), *Shigella flexneri* (MTCC-1457) and *Salmonella typhi* (MTCC-734) were obtained from MTCC, IMTECH, Chandigarh, India. These strains were maintained in nutrient agar slants at 37°C. Each of the bacteria was reactivated prior to susceptibility testing by transferring them into a separate test tube containing nutrient broth and incubated overnight at 37°C.

Bacterial Susceptibility Assay

In vitro antibacterial activities of all extracts were determined by standard agar well diffusion assay.⁷ Petridishes (size 100 mm diameter) containing 18 ml of cool and molten Mueller Hinton Agar (MHA) (at 40°C) were seeded with 100 µl inoculum of bacterial strain (inoculum size was adjusted so as to deliver a final inoculum of approximately 1.0×10^8 CFU/ml). Wells of 6 mm diameter were cut into solidified agar media with the help of sterilized cork borer. An aliquot of 100 µl of each extract was poured in the respective well and the plates were incubated at 37°C overnight. Organic solvents, in which extracts were prepared, were used as negative control while ciprofloxacin (10 µg/ml) was used as positive control. The experiment was performed in triplicate under strict aseptic conditions. The antibacterial activity for each of the extract evaluated was expressed in terms of the average of the diameter of zone of inhibition (in mm) produced by the respective extract at the end of incubation period.

Minimum inhibitory concentrations (MIC)

MIC was determined using a microdilution assay according to the Clinical and Laboratory Standards Institute standard (NCCLS 2000).⁸ The bacterial strains were cultured in MH broth at 37°C in an incubator for 24 h and added to a 96-well plate to a final concentration of 1×10^6 CFU/ml. The methanolic extract of peel and leaves were added to each well to final concentrations of 1, 2, 4, 8, 16, 32, 64, 128 and 256 µg/ml. These were dissolved in dimethyl sulfoxide (DMSO, Sigma). The final DMSO concentration in each well was 1%. The 1% DMSO in the medium well was used as the negative control. Ciprofloxacin (Sigma, India) was used as a positive control.

Phytochemical Screening

The phytochemical screening of methanol and aqueous extracts of *Ocimum sanctum* was performed using standard procedures.^{9, 10} The qualitative analysis like test for alkaloids, tannins, saponin, steroid, glycosides, flavanoids, terpenoids, amino acids and carbohydrates were carried out for both the extracts.

RESULTS

Phytochemical screening

The phytochemical screening of the methanolic leaf extract was carried out, since the better antimicrobial properties have been found to be associated with the methanolic extract. Our study revealed the presence of carbohydrates, reducing sugars, deoxysugars, sterols, glycosides, phenolic compounds, tannins, saponins and flavonoids, as outlined in table 1.

Table 1: Analysis of phytochemical contents of the methanolic extract of *F. benghalensis* Leaves

Carbohydrates	+
Reducing sugar (Benedict's test)	+
Reducing sugar (Fehling's test)	+
Alkaloids	+
Sterols	+
Deoxy sugars	+
Glycosides	+
Saponins	+
Phenolic compounds	+
Tannins	+
Flavanoids	+
Amino acids	+

In vitro antibacterial activity assay

Agar Well Diffusion

The antibacterial efficacy of the aqueous, methanolic and chloroform extract of leaves of *Ficus benghalensis* were analyzed. The methanolic extract was found to be most effective against the selected bacterial strains. However, the extracts were completely ineffective against *S. typhi* (Table 2).

Minimum inhibitory concentration

MIC measurements were performed to determine the anti-bacterial effect of the methanolic extract of leaves of the plant on the selected bacterial strains. Table 3 summarizes the MIC values of these two extracts against different bacteria. Low MIC values were obtained against *S. aureus*, *S. thermophilus*, and *B. cereus*, suggesting its good antibacterial efficacy. However, these were not so effective against gram negative bacteria.

Table 2: Antibacterial efficacy of aqueous extract of different plant parts. The results are the means of the independent triplicates:

Name of the bacteria	Zone of Inhibition (in mm)		
	Aqueous Extract	Methanolic Extract	Chloroform Extract
<i>S. aureus</i>	11±0.34	13±0.20	11±0.80
<i>S. themophilus</i>	10±0.45	12±0.75	11±0.50
<i>B. cereus</i>	8±0.50	9±0.65	9±0.32
<i>P. vulgaris</i>	9±0.74	12±0.80	10±0.45
<i>P. aeuroginosa</i>	7±0.25	8±0.50	7±0.58
<i>E. coli</i>	10±0.86	12±0.50	11±0.45
<i>S. flexneri</i>	7±0.36	9±0.36	8±0.46
<i>S. typhi</i>	R	R	R
<i>K. pneumoniae</i>	R	7±0.30	8±0.50

R: Resistant

Table 3: MIC of methanolic extract of *Ficus benghalensis*

Name of the micro-organism	MIC (µg/ ml) of methanolic extract
<i>S. aureus</i>	32
<i>S. themophilus</i>	32
<i>B. cereus</i>	64
<i>P. vulgaris</i>	128
<i>P. aeuroginosa</i>	>128
<i>E. coli</i>	128
<i>S. flexneri</i>	>128
<i>S. typhi</i>	>256
<i>K. pneumonia</i>	>256

DISCUSSION

Nearly 80% of the world population is dependent on the traditional medicine for primary health care, mainly including the use of natural products.¹¹ Researchers have extensively studied the biological properties of *Ficus benghalensis* and their results showed that this plant is ethno medically valuable. The present study was designed to investigate the phytochemical properties and to analyze the antibacterial efficacy of the plant leaves.

The phytochemical analysis revealed the presence of carbohydrates, reducing sugars, deoxysugars, sterols, glycosides, phenolic compounds, tannins, saponins and flavonoids, thereby indicating its biological importance. The aqueous, methanolic and chloroform extract of leaves of the plant was prepared in order to investigate its antibacterial efficacy. The results indicated that the methanolic extract was most effective against bacteria. The results were in accordance with Aswar *et al.*, who also reported the potent antimicrobial activity of the methanolic extract of the plant leaves. The extracts were more effective against gram positive organisms as compared to gram negative organisms. The antibacterial

activity carried out by agar well diffusion method showed that the methanolic extract was most effective against *S. aureus*, *S. themophilus*, *P. vulgaris* and *E. coli*. Thus, observing the antibacterial efficacy of the methanolic extract, MIC of methanolic extract was carried out. MIC results confirmed that the methanolic extract of the plant leaves was most effective against *S. aureus*, *S. themophilus*, *P. vulgaris* and *E. coli*.

CONCLUSION

In the present study, an attempt has been made to decipher the phytochemical constituents and antibacterial activity of *Ficus benghalensis* leaves. The phytochemical analysis of the leaves revealed that the plant is a rich source of bioactive components. The antibacterial efficacy of the methanolic extract of the plant leaves was also quite considerable. Further purification and characterization of the active metabolites present in *Ficus benghalensis* followed by a detailed study of toxicity and pharmacological effects of the compounds present in it, *Ficus* may be used as a remedy against various diseases without any side effects.

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