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Preliminary phytochemical analysis of bark of *Ficus bengalensis* Linn and Antibacterial activity of its bark oil.

Neena Unnikrishnan^{1*}, Rinku Mathappan², Manu Somanath³, D Sheela⁴

¹ Department of Botany, St.Teresa's College, Ernakulam, Kerala, India 682035

² Department of Pharmacognosy, Gautham College of Pharmacy, Bangalore, Karnataka, India. 560032

³ Research and Development, Kerala Ayurveda Limited, Aluva, Kerala, India.

⁴ Department of Botany, St.Teresa's College, Ernakulam, Kerala, India 682035

ABSTRACT

The bark, aerial roots and leaf buds of *Ficus bengalensis* Linn, are used in the ayurveda for the treatment of dysentery, skin diseases, diabetes, piles, ulcers and inflammatory swellings. Phytochemical screening of the bark showed positive tests for sterols, flavanoids, glycosides, saponins and tannins. Agar well diffusion method proved that methanolic extracts of the oil prepared from the bark possess good antibacterial activity against the tested Gram negative strain *Salmonella typhimurium* and has shown intermediate action against tested few Gram positive and Gram negative strains.

Keywords: *Ficus bengalensis* Linn, Anti-bacterial activity, Agar well diffusion assay, phytochemical analysis

*Corresponding author



INTRODUCTION

Medicinal plants have been used as an exemplary source for centuries as an alternative remedy for treating human diseases because they contain numerous active constituents of therapeutic value [1]. Many plants derived from nature possess antimicrobial and insecticidal activities. The interest in these plants is increasing because of finding safer microbicides in combination with the need of preventing environmental degradation. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties [2].

Ficus bengalensis Linn of family Moraceae is used in ayurveda for diarrhoea, dysentery, piles, as a hypoglycemic, diuretic, tonic, astringent, in rheumatism, applied to gums to lessen inflammation [3]. The bark is astringent and tonic and used in diabetes and leucorrhoea, lumbago, sores, ulcers, pains and bruises [4]. The curative properties of medicinal plants are due to the presence of different phytochemicals having a wide range of activities, which help in protection against chronic diseases. The bark extract of *Ficus bengalensis* Linn exhibited anti-inflammatory activity [5].

The development of antimicrobial agents for clinical use has brought unquestionable benefit to individuals and society. However, mankind is now confronted with new re-emerging infections for which no effective treatments are available. The development of microbial resistance to antibiotics has led the researchers to investigate the alternative sources for the treatment of resistant strains [6].

MATERIALS AND METHODS

Plant collection and Extraction

The stem barks of the plant *Ficus bengalensis* Linn. (Moraceae) were collected from Herbal Garden Division of Kerala Ayurveda Limited (KAL), Aluva and authenticated at the Department of Botany, KAL and voucher specimen of the plant were deposited at KAL herbarium for future references.

The bark was dried separately under shade. The standardized coarse powder of bark was subjected to soxhlet extraction using methanol and water as solvent. Preliminary phytochemical analysis was carried out in aqueous and methanolic extract using standard analytical procedure [7]. Medicated oil of the powdered bark of *Ficus* was prepared in the laboratory of Kerala Ayurveda Limited. Crude methanolic extract of the oil was used for the antibacterial studies, obtained by using soxhlet extraction method using methanol as the solvent.

Test microorganisms

Bacillus subtilis (MTCC 3053), *E.Coli* (MTCC 727), *Staphylococcus aureus* (MTCC 3160), *Salmonella typhimurium* (MTCC 98), *Klebsiella pneumonia* (MTCC 3384) were procured from IMTECH Chandigarh. They were confirmed by Gram staining and biochemical tests and maintained in nutrient broth at 4⁰C.

Medium used

Medium used was Muller Hinton Agar and Nutrient Broth. All media was supplied by HiMedia.

Antibiotic disc

Antibiotic disc used was Gentamycin (10µg/disc) and supplied by HiMedia.

Preparation of extract for Antibacterial studies:

The extract was prepared by dissolving 10g of the Ficus oil in 40ml of methanol and extracted for 30min under reflux. The supernatant was filtered and the filtrate was freeze-dried for 2 days. The top methanol layer was filtered through Whatman No.40 filter paper from the solidified oil. This methanol extract was concentrated.

EVALUATION OF ANTIBACTERIAL ACTIVITY

Evaluation of antibacterial activity of the methanolic extract of the Ficus oil was performed by agar well diffusion method [8]. The plates were prepared by using Muller Hinton Agar. 18hrs culture of test organisms *Salmonella typhimurium*, *E.coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Bacillus subtilis* in Nutrient broth were taken for the study. Sterile cotton swabs were dipped into the culture broth and pressed around the side walls of the tubes in order to remove excess inoculum and swabbed it evenly on the Muller Hinton Agar plates. The wells of 5.0mm diameter were made in the agar plate by using sterile cork borer. Aliquots of 200µl of the methanolic extract of the oil of *Ficus bengalensis* bark were applied aseptically to the wells using the micropipette and sterile tips. Methanol was used as negative control. The antibiotic disc Gentamycin (10mcg/disc), used as positive control was placed aseptically over the medium at a sufficient distance between the wells containing the sample using sterile forceps. The plates were kept undisturbed at room temperature for 2hrs for the proper diffusion of test-drug into agar. Then the plates were incubated at 37⁰C for 24 hrs and the zone of inhibition was observed. The zone of clearance around the well was measured in millimeters (mm) using Himedia zone size checking scale. The experiment was repeated and took the average zone size.

RESULTS

In the present work, the preliminary phytochemical analysis on *Ficus bengalensis* bark extract reveals the presence of sterols, saponins, glycosides, flavanoids and tannins which was shown in Table 1. The data relating to antibacterial activity of Ficus oil is depicted in Table 2. The presence of ketosterols in the bark of *Ficus bengalensis* agrees with the findings of the Subramanian and Misra [9] who have isolated three ketones and two other compounds beta-sitosterol-alpha-D-glucose and meso-inositol. Tannins have been reported to be bacteriostatic against *Staphylococcus aureus* [10]. The methanolic extract of the oil of *Ficus bengalensis* bark showed strong antibacterial activity against *Salmonella typhimurium* and *Bacillus subtilis*. *Staphylococcus aureus* and *Klebsiella pneumoniae* were found to have intermediate action to the methanolic extract of the oil of *Ficus bengalensis* bark. The methanolic extract had no activity against *E.coli*. Studies have shown that glycosides, saponins, flavonoids, tannins and alkaloids have hypoglycemic activities and anti-inflammatory activities [11]. No antibacterial activity was observed in negative control. Antibiotic disc Gentamycin (10µg/disc) was used as positive control.

Table 1: Preliminary Phytochemical screening of Ficus bengalensis Linn bark extract

Secondary metabolites	Methanol	Aqueous
Alkaloids	-	-
Tannins	+	+
Flavanoids	+	-
Sterols	+	-
Glycosides	+	-
Saponins	-	+

(+) Presence and (-) Absence

Table 2: Antibacterial activity of methanolic extract of the oil prepared from bark of Ficus bengalensis Linn.

Organisms	Gentamycin	Methanol	Methanolic extract of oil of Ficus bengalensis	
	+ve control	-ve control	Zone size	Inference
<i>Staphylococcus aureus</i>	32mm	Nil	15mm	S
<i>Bacillus subtilis</i>	32 mm	Nil	16 mm	S
<i>Salmonella typhimurium</i>	24 mm	Nil	20 mm	S
<i>Escherichia coli</i>	24mm	Nil	Nil	R
<i>Klebsiella pneumoniae</i>	22mm	Nil	12 mm	S

S – Sensitive, R - Resistant

DISCUSSION

The phytochemical screening on qualitative analysis showed that the bark of Ficus is rich in popular phytochemical substances like flavanoids, sterols, glycosides, saponins and tannins. From the results obtained from the antibacterial activity it can be concluded that the methanolic extract of the oil of *Ficus bengalensis* bark have good antibacterial activity against the tested Gram-positive and Gram-negative strains except *E.coli*. The phytochemical

compounds present in the bark have great potential as compounds against microorganisms and is responsible for the antibacterial activity. Thus it can be summarized that, the oil of *Ficus bengalensis* bark can be used in the treatment of infectious diseases. Data from the literatures reveal that the bark of the plant has good anti-inflammatory activity. Therefore more studies can be conducted to isolate bioactive compounds from oil.

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