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ANTIMICROBIAL ACTIVITY OF LEAVES OF *FICUS BENGHALENSIS* AGAINST ISOLATED DENTAL PATHOGENS

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Keywords:

Ficus benghalensis, Leaf extract, Antimicrobial Activity, Antifungal Activity, and Dental Pathogens.

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ABSTRACT: Dental caries is considered one of the major oral health issues throughout the world. Synthetic drugs and antibiotics have major side effects and can develop resistance. *Ficus benghalensis F. benghalensis* is a plant with enormous established pharmaceutical properties. The present study was designed to evaluate the antimicrobial activity of water, and ethanolic extracts of *F. Benghalensis* leaves against human dental pathogens. The isolated dental pathogens are *Streptococcus mutans*, *Lactobacillus*, *Klebsiella pneumonia*, and *Candida albicans*. The cup plate method was used to evaluate the antimicrobial activity of water, and ethanolic extracts of *F. benghalensis* leaves against the isolated microbes. Data were analyzed in one-way variance analysis. The water extract of *F. benghalensis* leaves was active against *Klebsiella pneumonia* and *Candida albicans*. The ethanolic extract of *F. benghalensis* leaves was active against all the *Streptococcus mutans*, *Lactobacillus*, *Klebsiella pneumonia* and *Candida albicans*. Since both the water and ethanolic extracts of *F. benghalensis* leaves shows maximum antimicrobial activity against *Klebsiella pneumonia* with a diameter of inhibition zones 20 mm and 23 mm respectively, it was used to obtain MIC (minimum inhibitory concentration) values. Five different concentration (100, 50, 25, 12.5, 6.25 mg/ml was tested against the water and ethanolic extracts of *F. benghalensis* for its MIC (minimum inhibitory concentration) values. The antimicrobial activity of water and ethanolic extracts of *F. benghalensis* shows it is highly significant against *Klebsiella pneumonia*. Further antimicrobial and pharmaceutics studies are required to evaluate the safety, and therapeutic efficacy of *F. benghalensis* leaves in combating dental caries.

INTRODUCTION: Compounds isolated from nature were known to possess biological. Profiles and pharmaceutical potential far greater than anything that is synthetically produced. Natural products have, until recently, been the primary source of commercial medicines and drug leads. Pharmacognosy is the study of a chemical's physical, chemical, biochemical and biological properties and the discovery and development of a drug from natural sources.

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Natural products are obtained from plant sources, marine sources, animal sources, and microbial sources. The major source of natural product plants because they are abundant and easily obtained. Correct identification of the species and geographical locations is vital whenever plants are used as a source of natural products because

different growing conditions and different species may yield different metabolites or ratios of metabolites. Selecting the plant parts should be before the pharmacological activity as the Phytochemicals vary within the whole plant. Methods of extraction of plant material can affect the chemical composition and alter the activity of the plant [1]. *F. benghalensis* is chosen since it is abundantly available in Asia and its ability to survive harsh weather and drought [2]. In the olden days, there was a proverb in Tamil "Aalum velum palluku uruthi naalum rendum solluku uruthi" says that the twigs of the banyan tree and neem tree gives strength to teeth; therefore, the *F. benghalensis* was chosen to prove this Tamil proverb scientifically. *F. benghalensis* leaves extract possesses good antioxidant and radical scavenging properties, which is due to the presence of kaempferol [3]. *F. benghalensis* leaves, roots, bark, and seeds have antimicrobial activities against bacteria, yeast, dermatophytes, and helminths.

The leaves and flower extracts are capable of controlling parasitic worms. The leaves of *F. benghalensis* contain polyphenols, including quercitin-3-glycoside, kaempferol glycosides, rutin and other polyphenols which results in antidiabetic activity. Furthermore, mustard oil glycosides, thiocarbamate glycosides, and nitrile are compounds leading to the blood pressure-lowering effect in leaves. The seed leaves and pods are effective against eye problems and helpful in preventing night blindness.

Vitamin A presence will improve and delay cataract development [3]. Traditional medicines have been used since ancient times to treat dental infections. Few studies have been published involving tests with medicinal plants. In view of these aspects, the present work is carried out to evaluate the antimicrobial activity of *F. benghalensis* against dental pathogens. The objective of this study is to evaluate the antimicrobial activity of different extracts of *F. benghalensis* leaves against dental pathogens.

MATERIALS AND METHODS:

Plant Material: 3 kg of plant material of *F. benghalensis* was collected from Thanjavur in the month of September 2017. The seed was authenticated by Dr. Nagarajan, the botanist from Tamil University Thanjavur (voucher specimen no: S/H/M-021/2017). The fresh leaf of the plant was initially rinsed with distilled water and dried in an oven in a laboratory at 37 ± 1 °C for 24 h. After drying, the plant materials were homogenized in an electric blender to a fine powder and stored in an airtight bottle [4].

Preparation of Extracts: The powdered plant materials *F. benghalensis* were taken in an aspirator bottle separately and extracted successively by cold maceration technique with solvents like ethanol and water, respectively for 6 days. At the end of each extraction, there were filtered through filter paper. Then the filtered extracts were evaporated under vacuum in a rotatory evaporator. The colour and percentage yield of the extracts were recorded. The extracts were transferred to sterile bottles and stored in the refrigerator [5].

Collection of Samples: Five dental swab samples were collected from patients with dental caries from Dental Clinic, Thanjavur District, Tamil Nadu, and India.

Isolation: The collected samples were inoculated in Nutrient Agar Media to isolate bacteria and Sabouraud Dextrose Agar Media for the isolation of fungi by quadrant streaking technique. The Nutrient Agar culture plates were then incubated for 24 h at 37 °C, and the Sabouraud Dextrose Agar culture plates were incubated for 72 h at room temperature. After incubation, the isolated colonies were sub-cultured in Nutrient Agar slants for further use.

Preliminary Identification of Bacteria: Gram staining technique was used to separates bacteria into gram-positive bacteria and gram-negative bacteria. Crystal violet (the primary dye), Gram's Iodine (the mordant), an alcohol rinse (decolourizer), and a contrasting counterstain, safranin, are used in this procedure. A sample of a bacterial colony was mixed with water on a microbial slide to evenly spread out the bacterial sample. Before staining, the sample was heat fixed [6]. The bacterial smear was dried then flooded with crystal violet, Lugol's iodine, decolourizing agent

(alcohol 90%) and safranin. The microscope slides were then focused at × 100 magnification.

Biochemical Tests For Bacterial Species Identification:

Methyl Red (MR) Test: The MRVP medium was prepared and autoclaved 121 °C for 15 min. The medium was transferred to the sterilized test tube. After the medium solidified, organisms from pure culture were inoculated in the MRVP medium and incubated at 37 °C for 48 h. After 48 h of incubation, 2.5 ml of the broth was aliquoted to a sterilised test tube. Five drops of methyl red indicator were added, and a red color's immediate development shows a positive MR test. The yellow colour on the surface of the medium shows a negative MR test [7].

Voges-Proskauer (VP) Test: For Voges-Proskauer (VP) Test, 1ml of the broth was aliquoted to a clean test tube and re-incubated for an additional 24 hours. To the aliquoted broth, 2 drops of 5% alpha-naphthol were be added. Then 1 drop of 40% potassium hydroxide was added. The tube was shaken and left undisturbed for about 10-15 minutes. A pink-red colour on the surface of the medium shows a positive VP test, whereas a yellow colour shows a negative VP test 7.

Citrate Test: The Simmons Citrate Agar medium was prepared and autoclaved 121 °C for 15 min. The medium was transferred to the sterilized test tube. After the Simmons Citrate Agar medium solidified, colony of pathogens was inoculated and incubated at 37 °C for 24 to 48 h. The growth was visible on the slant surface, and the medium will be an intense Prussian blue show positive citrate test absence of growth and colour change shows negative citrate test 7.

Urease Test: The Urease medium was prepared and autoclaved 121 °C for 15 min. The medium was transferred to the sterilized test tube. After the medium solidified, the isolated colony was inoculated and incubated at 37 °C for 24 to 48 h. The colour of the slant changes from light orange to magenta shows positive. If organisms do not produce urease, the agar slant remains light orange 7.

SIM (Sulphide, Indole, Motility) Test: The SIM (Sulphide, Indole, Motility) medium was prepared and autoclaved 121 °C for 15 min. The medium was transferred to sterilised test tube. After the SIM (Sulphide, Indole, Motility) medium solidified, the isolated colony was inoculated and incubated at 37 °C for 24 to 48 h. Indole was detected upon the addition of Kovacs Reagent, **f**ollowed by incubation of the inoculated medium. A red band at the top of the medium indicates the presence of Indole. A negative indole test produces no colour change upon the addition of Kovacs Reagent 7.

TSI (Triple Sugar Iron) Test: The TSI (Triple Sugar Iron) medium was prepared and autoclaved 121 °C for 15 min. The medium was transferred to sterilised test tube. After the TSI (Triple Sugar Iron) medium solidified, the isolated colony was inoculated and incubated at 37 °C for 24 to 48 h. The formation of red colour on the slants shows the bacteria does not ferment either lactose or sucrose. The formation of yellow colour on the slants shows the bacteria ferment either lactose or sucrose [8].

***In-vitro* Antibacterial Screening for Extracts by Cup Plate Method:** The term antimicrobial assay designates a type of biological assay performed with microorganisms such as bacteria, yeasts, and moulds. This involves the measurement of the relative potency of the activity of compounds by determining the amount required to produce a stipulated effect on a suitable organism under standard conditions. Muller Hinton agar media (Hi-media) was used for the preparation of a medium for antibacterial screening. Subculturing of test organisms has been carried out every week using nutrient agar medium. The medium (15 ml in each test tube) was sterilized by autoclaving at 121 °C for 15 min.

The tubes were inoculated with bacterial strains and incubated at 37 °C for 24 to 48 h and stored at 5 °C. The inoculum was prepared by transferring a loopful of stock to a 150 ml Erlenmeyer flask containing 80 ml of nutrient broth. The composition of inoculum broth was the same as that of stock culture with the exception of agar. The inoculum flasks were incubated at 37 °C for 18 h and used for this study. Amoxicillin (125 mg/ml) was used as a standard antibiotic as it was a broad-spectrum antibiotic that is effective against Gram-positive bacteria and Gram-negative bacteria for dental pathogens.

The drug was diluted in distilled water. Fluconazole, a broad-spectrum antifungal drug, was used as a standard antifungal as it was effective against most of the fungi, including Candida albicans. Fluconazole (125 mg/ml) was diluted in distilled water. The Muller Hinton agar medium was sterilized by autoclaving at 121 °C (15 psi) for 15 min the petri dishes and pipettes were sterilized in an oven at 150 °C for an hour. About 25 ml of Muller Hinton agar medium (40-50 °C) was poured in each sterilized petri dish and approximately 0.5 ml of inoculums broth of bacterium was added to the respective petri dishes.

The contents of petri dishes were mixed thoroughly by rotary motion. The medium containing inoculums was allowed to solidify at room temperature. After solidification of the medium, three cups (diameter-9 mm) were made at equal distance with metallic borer. The uniform volumes of different extracts and standard solution were added to the cups in the petri dish and the solutions were allowed to diffuse by leaving the plates undisturbed for 40 min at room temperature. The petri plates were incubated at 37 °C for 24 h and the zone of inhibition were recorded in mm. The experiment was performed in triplicate and the average reading was recorded.

Determination of Minimum Inhibitory Concentration (MIC) of Water and Ethanolic Extracts of *F. benghalensis* Leaves: The MIC of the extracts was determined by diluting the various concentrations (100, 50, 25, 12.5, 6.25 mg/ml). Equal volume of the extracts (1 ml) and nutrient broth (1 ml) were mixed in the sterilized test tube. Specifically, 0.1 ml of standardized inoculums was added to each tube. The antibiotic amoxicillin added as standard control and inoculum (culture) as organism control. The tubes were incubated at 37 °C for 24 to 48 h. MIC was determined as the lowest concentration of the extracts permitting no visible growth when compared with the control tubes [9]. After 24 to 48 h, absorbance values were recorded at 600 nm.

Statistical Analysis: The assessment was performed in triplicate, and the values obtained from MIC study were subjected to ANOVA using a T-test. The data were expressed as mean ± S.E.M. and compared using one-way analysis of variance (ANOVA), and p values < 0.05 were considered as significant [10].

RESULTS AND DISCUSSION:

Macro Morphological Distribution of Plant: The Plant name was *F. benghalensis* belongs to the Family Moraceae. Its Vernacular name: Tamil: Allai, commonly known as Banyan tree. It is very commonly cultivated in Sub-Himalayan, Assam, Bengal, peninsular India, north-eastern Pakistan, north-eastern Bangladesh, Sri Lanka, West Asia, the Arabian Peninsula, East, and West Africa. The botanical Characters of the plant are mentioned in **Table 1** [11].

TABLE 1: THE BOTANICAL CHARACTERS

Part of the plant	characteristics
Bark	The bark of *F. benghalensis* is brownish grey when dried. The inner surface of the bark is usually yellowish-brown, the outer will be reddish-brown. the thickness of the will be 0.8 - 0.25 varies with age
Leaves	The leaves are green in colour, bitter in taste, a stimulant in odour, oval in shape, and thickly coriaceous.4-6 inches long
Flowers	They have both male and female flowers separately, Present near the receptacle.
Fruit	The fruits are globose, fleshly pericarp dark red in colour,1.5-2.0 cm in diameter.
Seeds	The seeds are very small in size and dispersed through wind

The morphological characters' colour, odour, taste, shape, and size of the *F. benghalensis* leaf were studied and recorded in **Table 2.**

Nature and Yield of Extract: The Ethanolic extract of *F. benghalensis* was found to be brownish-yellow in colour, oily, sticky mass in consistency. The %yield of the exact obtained was 9.6. The aqueous extract of *F. benghalensis* was found to be dark brown in colour, sticky mass in consistency. The %yield of the extract obtained was 13.63.

Isolation and Identification of Microorganisms From the Patients With Dental Caries: The collected dental swabs samples were brought to the

Microbiology Lab, Tamil University, Thanjavur. The samples were inoculated to Nutrient Agar Media and Sabouraud Dextrose Agar Media and incubated for 24 h at 37 °C and 72 h at room temperature, respectively. The isolated colonies and the colony characteristics of bacteria and fungi observations were recorded in **Tables 3** and **4.**

TABLE 2: THE MORPHOLOGICAL CHARACTERS OF THE *F. BENGHALENSIS* LEAF.

S. no	Characters	*F. benghalensis*
1.	Color	Green
2.	Odor	Stimulant
3.	Taste	Bitter
4.	Shape	Oval
5.	Size	4-6 inches long

TABLE 3: THE COLONY CHARACTERISTICS OF SAMPLES ON NUTRIENT AGAR MEDIA

Samples	Form	Elevation	Margin	Surface	Opacity	colour
S1	Circular	Raised	Entire	Smooth	Opaque	White
S2	Irregular	Raised	Undulate	Rough	Opaque	White
S3	Circular	Raised	Entire	Smooth	Opaque	White
S4	Irregular	Raised	Undulate	Rough and wrinkled	Opaque	White
S5	Irregular	Raised	Undulate	Wrinkled	Opaque	white

TABLE 4: THE COLONY CHARACTERISTICS OF SAMPLE ON SABOURAUD DEXTROSE AGAR MEDIA

Sample	Form	Elevation	Margin	Opacity	Colour
S6	Circular	Raised	Entire	Opaque	Creamy white

The isolated bacterial samples were identified using a biochemical test by gram staining technique. The microscope slides were then focused at × 100 magnification.

The observations were recorded in **Table 5.** The bacterial species were identified using the biochemical tests. The observations were recorded in **Table 6.**

TABLE 5: GRAM STAINING RESULTS ON ISOLATED BACTERIA

Samples	Gram-positive organisms	Gram-negative organisms
S1	+	-
S2	+	-
S3	+	-
S4	-	+
S5	-	+

+ indicates Positive; - indicates Negative

TABLE 6: BIOCHEMICAL TESTS FOR BACTERIAL SPECIES IDENTIFICATION

Samples	MR	VP	Citrate	Urease	SIM	TSI	Probably identified
S1	-	-	-	-	+	AG	*Streptococcus mutans*
S2	+	-	-	-	-	A	*Lactobacillus*
S3	-	-	-	-	+	AG	*Streptococcus mutans*
S4	-	+	+	+	-	A	*Klebsiella pneumoniae*
S5	-	+	+	+	-	A	*Klebsiella pneumoniae*
S6	-	-	-	-	-	-	*Candida albicans*

+ indicates Positive; - indicates Negative A indicates the production of acid AG indicates the presence of acid and gas

Antibacterial Activity of Water and Ethanolic Extracts of *F. Benghalensis* Leaves: The antibacterial activity of water and ethanolic extracts of *F. benghalensis* leaves against the isolated pathogens was performed. The zone of inhibition was measured in mm. The results were recorded in **Table 7.**

TABLE 7: ZONE OF INHIBITION OF WATER AND ETHANOLIC EXTRACTS OF F. BENGHALENSIS LEAVES

Organisms	Water extract (mm)	Ethanol extract (mm)	Standard (mm)
Streptococcus mutans	-	+	++
Lactobacillus	+	++	+++
Klebsiella pneumoniae	+++	+++	+++
Candida albicans	+	++	+++

-Indicates less than 5 mm + indicates 5 mm to 9 mm ++ indicates 10mm to 19 mm +++ indicates more than 20 mm

Minimum Inhibitory Concentration (MIC) of Water and Ethanolic Extracts of *F. benghalensis* Leaves: *Klebsiella pneumoniae* has the highest zone of inhibition in ethanol extract and water extract. The Minimum Inhibitory Concentration (MIC) was recorded in **Table 8.** The statistical analysis of water and ethanolic extracts of *F. benghalensis* leaves was recorded in **Table 9.**

TABLE 8: THE OPTICAL DENSITY VALUE OF DIFFERENT EXTRACTS AGAINST DIFFERENT CONCENTRATIONS OF *KLEBSIELLA PNEUMONIA*

Concentration (mg/ml)	Water extract (mm)	Ethanol extract (mm)	Standard (mm)
100	1.842	0.321	0.298
50	3.886	0.521	0.298
25	3.561	0.996	0.298
12.5	3.943	1.220	0.298
6.25	3.963	1.436	0.298

TABLE 9: STATISTICAL ANALYSIS OF WATER AND ETHANOLIC EXTRACTS OF *F. BENGHALENSIS* LEAVES

Treatment	Absorbance
Standard	0.39 ± 0.00
Water Extract	3.18 ± 0.17***
Ethanol Extract	1.21 ± 0.21***

*P<0.05, **P<0.01, ***P < 0.001 extract VS standard; Values are mean ± S.E.M., n = 5(different concentration).

CONCLUSION: The present study revealed that the *Ficus benghalensis* leaves possess potent antibacterial activity against dental pathogens. Since both the water and ethanolic extracts of *F. benghalensis* leaves show maximum antibacterial activity against Klebsiella pneumonia with the zone of inhibition at 20 mm and 23 mm respectively, it is highly significant (P < 0.001) against *Klebsiella pneumonia*.

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