



**ANTIMICROBIAL EFFECT OF *FICUS BENGHALENSIS* & *FICUS RELIGIOSA*
AGAINST ORAL MICROFLORA**

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ABSTRACT

The present study shows the Antimicrobial effect of *Ficus benghalensis* & *Ficus religiosa* against oral pathogens. Two types of extracts were prepared from tree leaf powder to check their antimicrobial activity on oral pathogenic bacterial culture. The effects of 60% aqueous & methanolic extracts of *Ficus benghalensis* & *Ficus religiosa* showed that both the tree extracts were highly effective on *Staphylococcus capitis*, *Staphylococcus* but showed very less or no effect on *Bacillus licheniformis*, *Paenibacillus macerans*. The aqueous extract of plants was more effective than methanolic extract. *Ficus benghalensis* tree extract showed better and more effective antibacterial effects. The effect of Soxhlet extracts of *Ficus benghalensis* & *Ficus religiosa* were studied that, the both plant extracts were effective on all bacterial culture and formation of zone inhibition was 1.20-2.20cm on agar plates. Thus, throughout the study it is reinforced that the gram +ve rod shaped bacteria are highly resistant to the *Ficus* tree extracts.

KEYWORDS: Oral microflora, *Ficus religiosa*, *Ficus benghalensis*, soxhlet extraction, antimicrobial activity.

1. INTRODUCTION

Oral diseases are the fourth most expensive diseases to treat in certain countries (Sheiham et al. 2005). Dental caries, also known as tooth decay which is the most important problem for dental health in developing countries (Patro, B. K. & B. R., Goswami (2008). Dental caries is a breakdown of teeth due to activities of bacteria. Dental plaque, a biofilm of microorganisms which are presents on tooth surface. Microorganisms plays an important role in the development of caries and periodontal disease (Marsh PD et al (1992). Dental plaque is produced by bacterial culture and their products accumulating on the surface of the teeth (Eley BM, 1999).

The oral cavity of humans is a house for Gram-positive and Gram-negative bacteria culture, as well as certain yeasts and fungi, due to the favorable microbial habitat at 37°C with a acidic pH (Madigan, M.T, et al 2003). *Streptococcus mitis*, *Streptococcus oralis*, *Actinomyces naeslundii*, *Fusobacterium nucleatum*, *Haemophilus parainfluenzae*, *Eikenella corrodens* and certain *Prevotella* species are present in the oral cavity. *Streptococcus* and *Lactobacillus acidophilus* are the principal microorganisms involved in the causation of dental caries (Anita P, 2014).

Plant parts such as fresh or dried roots, stems, leaves and bark are mostly used in remedies to treat toothache,

gingivitis, loose teeth, dental abscesses and general mouth sores (Tapsoba, H. and Deschamps, J. et al., 2005). Plants stems are also used in chewing sticks to form basic toothbrushes. Over time, people discovered invitro condition that the leaves of certain plants are effected on mouth sores, infections and toothache (Van Wyk, B. and Gericke N et al 2000).

The present study was undertaken to study the antimicrobial effect of *Ficus* tree *Ficus benghalensis* & *Ficus religiosa* leave extracts against oral microflora.

2. MATERIAL AND METHODS

2.1 Collection of Plant Material

The plant leaves were collected from local region of Noida. The leaves were washed with alcohol to remove contamination and then washed properly with sterile water. The leaves were dried at room temperature, grounded to powder and finally stored in air tight containers until further use.

2.2 EXTRACT PREPARATION

2.2.1 Aqueous and alcoholic extraction of Plant Leaves

The air dried plant leaves was crushed and added in sterile water and methanol to make 60 % aqueous and 60% alcoholic extracts respectively. The mixture was incubated at 50°C for overnight for complete extraction.

The mixture was centrifuged for 30 mins. Supernatant was collected and stored at 4°C in sterile tubes.

2.2.2 Soxhlet Extraction for alkaloid extraction

Dried plant leaves were crushed. Methanol was added to a round bottom flask, which was attached to a Soxhlet extractor and condenser on a heating mantle. The crushed plant material was loaded into the Soxhlet extractor. The solvent was heated using the heating mantle and began to evaporate, moving through the apparatus to the condenser. The condensate then drips into the dried leaves. Once the level of solvent reaches the siphon it pours back into the flask and the cycle begins again. The process should run for a total of 5 hours. Once the process was finished, the methanol was evaporated and leaving a small yield of extracted plant material. The extract thus purified was used for antimicrobial testing.

2.3 Oral microflora isolation and characterization

The oral bacteria cultures were isolated from oral sample. The oral samples were inoculated onto Nutrient Agar (NA) plates and then incubated at 37°C for 24 hrs. After incubation, different colonies were developed on agar plates. The characterization of bacteria culture was done by biochemical tests. Biochemical tests were used for the identification of isolates bacteria culture. Different Biochemical tests were performed to prove the identity of isolated bacteria culture. Urease, carbohydrate, nitrate, SIM medium, imvic test, starch,

casein hydrolysis test, catalase test, Acetate, high concentration of NaCl test, etc were used for identification. The bacteria strains were finally identified using 16S rRNA gene sequence analysis and BLAST comparison online.

2.5 Anti microbial activity by agar Well Diffusion Method

Antibacterial activity was carried out by agar well diffusion method described by Perez et al (1990). In each well 200µl of 20%, 40% and 60% extract was added and sterile water & methanol were used as control. The antibacterial activity of each extract was expressed in terms of diameter of zone of inhibition produced by respective extract. All the bacterial cultures used were grown on nutrient agar medium at 37°C. The diameter of the inhibitory zone was measured in cm.

3. RESULTS

The present study involved the analysis of the antimicrobial effect of Peepal (*Ficus religiosa*) & Banyan (*Ficus benghalensis*) extracts. For the study different types of leaf extracts were made and then tested against the oral microflora which were both gram negative and gram positive. Seven different bacterial strains were selected based on morphological by gram staining and purified by sub culturing on agar plates & slants. The bacteria cultures were identification by biochemical test and 16s rRNA sequencing analysis. The results are shown in table no 1.

Table No. 1 Biochemical test of isolated bacterial culture

S.No	Type of test	strain 1	strain 2	strain 3	strain 4	Strain 5	Strain 6	Strain 7
1.	Carbohydrate test	Lactose	-	-	-	-	+	+
2.		Fructose	-	-	-	+	-	+
3.		Glucose	-	+	+	-	-	+
4.		Sucrose	-	-	+	+	+	--
5.		Maltose	-	-	-	-	-	-
6.		Mannitol	-	-	-	-	-	-
7.		Glycerol	-	-	-	-	-	-
8.		Starch	-	-	-	-	-	-
9.		Sorbitol	+	-	+	-	-	-
10.		Arabinose	-	-	+	-	+	+
11.		Xylose	-	-	+	-	-	-
12.		Ribose	-	-	-	-	-	-
13.	Biochemical test	Citrate test	+	+	-	-	+	-
14.		Acetate test	-	+	-	-	+	-
15.		MR test	-	-	+	+	-	-
16.		VP test	-	-	-	-	-	+
17.		Indole test	+	+	-	-	-	-
18.		7% NaCl test	-	-	+	-	-	-
19.		Casein hydrolysis test	-	-	-	-	-	-
20.		Starch hydrolysis test	-	-	-	-	-	-
21.		Nitrate test	+	-	-	+	+	+
22.		Urease test	+	+	-	-	-	-
23.		Acetate test	-	-	-	-	-	-
24.		Sim media test	+	-	+	-	-	-

									+
25.		Catalase test	+	-	+	-	-	-	+
26.		Growth at 45°C	-	+	+	+	-	-	-

Depending upon the biochemical tests, sugar utilization results and sequence analysis of the 16S rRNA gene, identification of the unknown bacterial strains was done on basis of similarity index after comparison on BLAST. The results showed that:

S1 – *Bacillus licheniformis* (97%)

S2 – *Paenibacillus macerans* (91%)

S3 – *Pseudomonas aeruginosa* (99%)

S4 – *Staphylococcus capitis* (98%)

S5 – *Staphylococcus aureus* (99%)

S 6 – *Staphylococcus gallinarum* (98%)

S 7 - *Enterobacter gergovie* (97%)

To study the antimicrobial activity of *F. religiosa* & *F. benghalensis* tree leaf extracts, three types of extracts were prepared: aqueous extract, methanolic extract & soxhlet extract. The screening for antibacterial activity was carried out by agar well diffusion method. In each well, extract was added and sterile water & pure methanol were used as control for aqueous extract and methanolic extract, respectively. When the effect of 60% aqueous extracts of *Ficus benghalensis* & *Ficus religiosa* were studied against *Bacillus licheniformis*, *Paenibacillus macerans*, *Pseudomonas aeruginosa* and

Staphylococcus capitis, *Staphylococcus aureus*, *Staphylococcus gallinarum* and *Enterobacter gergovie*: the results (Table 2, fig 1) showed that, both plant extracts were comparatively more effective on *Staphylococcus capitis*, *Staphylococcus aureus*, *Staphylococcus gallinarum* and *Enterobacter gergovie* as compared to *Bacillus licheniformis*, *Paenibacillus macerans* bacteria culture. The formation of zone inhibition was 1.40 – 2.70 cm on agar plates in all the *Staphylococcus* strains as seen in table no 2 & fig no 1.

Table: 2 Zone of inhibition of 60% aqueous extract of *Ficus benghalensis* & *Ficus religiosa*.

Plant species	Zone of Inhibition (cm)	
	<i>Ficus religiosa</i>	<i>Ficus benghalensis</i>
<i>Bacillus licheniformis</i>	----	---
<i>Paenibacillus macerans</i>	--	--
<i>Pseudomonas aeruginosa</i>	1.5	1.4
<i>Staphylococcus capitis</i>	2.7	2.10
<i>Staphylococcus aureus</i>	2.6	2.3
<i>Staphylococcus gallinarum</i>	2.1	1.9
<i>Enterobacter gergovie</i>	2.5	2.3

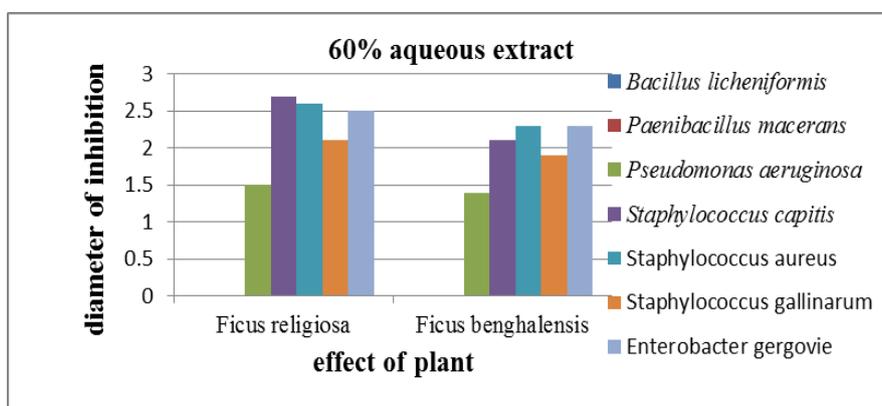


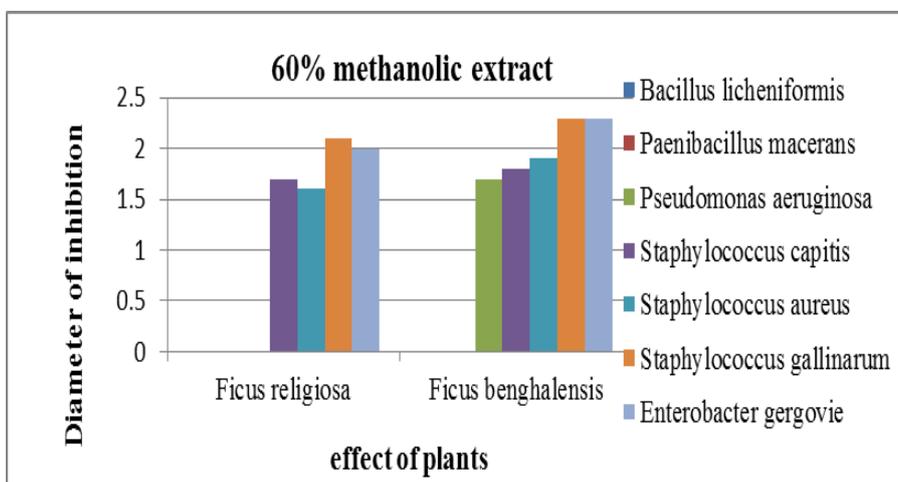
Fig 1: Graphical representation of antimicrobial activity of 60% aqueous extract of *Ficus benghalensis* & *Ficus religiosa*.

When the effect of 60% methanolic extracts of *Ficus benghalensis* & *Ficus religiosa* were studied against *Bacillus licheniformis*, *Paenibacillus macerans*, *Pseudomonas aeruginosa* and *Staphylococcus capitis*, *Staphylococcus aureus*, *Staphylococcus gallinarum* and *Enterobacter gergovie*. It was noted that, both plant

extracts were only effective on all the *Staphylococcus* strains and *Enterobacter gergovie*. The formation of zone inhibition was 1.30 & 2.3 cm on agar plates. *Ficus benghalensis* was also effective on *Pseudomonas aeruginosa* and formation of zone inhibition was 1.70cm. It is shown in table no 3 & fig no 2.

Table 3: Zone of inhibition by 60% methanolic extracts of *Ficus benghalensis* & *Ficus religiosa*.

Plant species	Zone of Inhibition (cm)	
	<i>Ficus religiosa</i>	<i>Ficus benghalensis</i>
<i>Bacillus licheniformis</i>	-----	---
<i>Paenibacillus macerans</i>	--	--
<i>Pseudomonas aeruginosa</i>	-	1.7
<i>Staphylococcus capitis</i>	1.7	1.3
<i>Staphylococcus aureus</i>	1.6	1.3
<i>Staphylococcus gallinarum</i>	1.9	0.9
<i>Enterobacter gergovie</i>	2.0	2.3

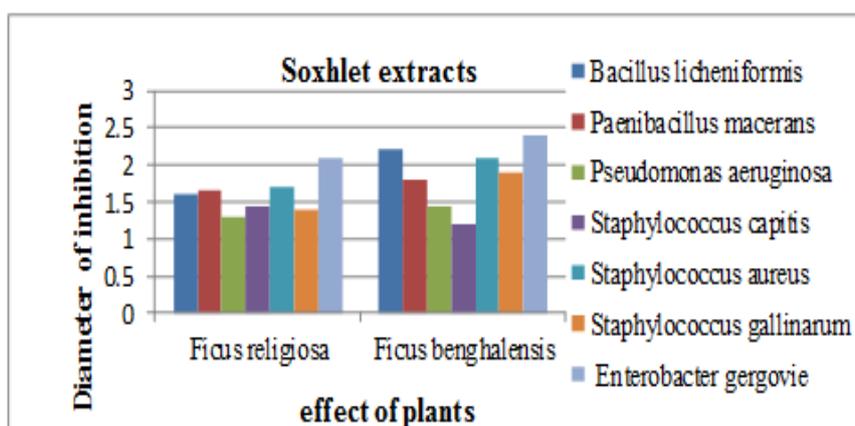
**Fig 2: Graphical representation of antimicrobial activity of 60% methanolic extracts**

Both the soxhlet plant extracts were effective on all bacterial culture and formation of zone inhibition was 1.20-2.20cm on agar plates. *Ficus benghalensis* plant

extract was more effective than *Ficus religiosa* as shown in table. 4 & fig no3.

Table 4: Zone of inhibition of soxhlet extracts of *F. religiosa* & *F. benghalensis*.

Plant species	Zone of Inhibition (cm)	
	<i>Ficus religiosa</i>	<i>Ficus benghalensis</i>
<i>Bacillus licheniformis</i>	1.6	2.2
<i>Paenibacillus macerans</i>	1.65	1.8
<i>Pseudomonas aeruginosa</i>	1.3	1.45
<i>Staphylococcus capitis</i>	1.45	1.2
<i>Staphylococcus aureus</i>	1.8	1.5
<i>Staphylococcus gallinarum</i>	2.2	1.9
<i>Enterobacter gergovie</i>	2.3	2.3

**Fig 3: Graphical representation of antimicrobial activity of soxhlet extracts.**

4. DISCUSSION

The present study shows the Antimicrobial effect of *Ficus* species against oral pathogens. Two types of extracts were prepared from plant leaf powder to check their antimicrobial activity on oral pathogenic bacterial culture. The oral pathogenic bacterial cultures were isolated from oral sample. The oral samples were inoculated onto Nutrient Agar (NA) plates. After incubation, different colonies were developed on agar plates. Totally 7 different bacterial strains were selected based on morphological by gram staining and purified by sub culturing on agar plates & slants.

Different Biochemical tests were performed to prove the identity of isolated bacteria culture. Depending upon the biochemical tests, sugar utilization results and sequence analysis of the 16S rRNA gene. The results showed that: (1) *Bacillus licheniformis* (2) *Paenibacillus macerans*, (3) *Pseudomonas aeruginosa*, (4) *Staphylococcus capitis* (5) *Staphylococcus aureus* (6) *Staphylococcus gallinarum* (7) *Enterobacter gergovie*

The antimicrobial activity of different common *Ficus* plant leaf extracts. Two types of extracts were prepared from plant leaf powder to check their antimicrobial activity such as aqueous extraction, methanolic extraction & soxhlet extraction. The different plant extract concentration which was used against oral pathogens microorganism were 60%. *F. religiosa* & *F. benghalensis* plants have used for the present studies.

The effects of 60% aqueous & methanolic extracts of *Ficus benghalensis* & *Ficus religiosa* were studied against *Bacillus licheniformis*, *Paenibacillus macerans*, *Pseudomonas aeruginosa* and *Staphylococcus capitis*, *Staphylococcus aureus*, *Staphylococcus gallinarum*, *Enterobacter gergovie*. It was noted that, both plant extracts were more effective on *Staphylococcus spp.* than *Pseudomonas aeruginosa* but not effected on *Bacillus licheniformis*, *Paenibacillus macerans* bacteria culture. The aqueous extract of plants was more effective than methanolic extract. *Ficus benghalensis* showed to be a plant which had a better antibacterial effect.

The effect of Soxhlet extraction of *Ficus benghalensis* & *Ficus religiosa* leading to better extraction of alkaloids and flavanoids etc were studied against *Bacillus licheniformis*, *Paenibacillus macerans*, *Pseudomonas aeruginosa* and *Staphylococcus capitis*, *Staphylococcus aureus*, *Staphylococcus gallinarum*, *Enterobacter gergovie*. It was noted that, both tree extracts were effective on all bacterial culture and formation of zone inhibition was 1.20-2.3cm on agar plates. *Ficus benghalensis* plant extract showed higher and better level of antibacterial activity against the cocci and gram –ve bacteria.

Another related study by Valsaraj et al. in 1997, demonstrated that antimicrobial activity of ethanolic extracts of *F. religiosa* (leaves) using the agar well

diffusion method is effective against *Bacillus species*, *Staphylococcus species* and *Pseudomonas aeruginosa*. The results showed that the extract was active against these bacteria. This is very similar to our results, with slight variation probably caused by change in the structure of alcoholic solvent and the reaction against gram +ve rod shaped bacteria namely *Bacillus licheniformis* and *Paenibacillus macerans*.

In this study, the zone of inhibition was for 60% methanolic extracts of *F. benghalensis* was 2.2 cm. Another related study by Chaudhary et al. showed in their experiment conducted this year, 2016, that 50% ethanolic extracts of *F. benghalensis* forms a 1.2cm zone of inhibition against *Bacillus sp.* This minor deviation may be caused by environmental factors or change in solvents. Even though both solvents are alcoholic, they have different structure.

Bacteremia by *Pseudomonas aeruginosa* causes very low blood pressure, known as hemodynamic shock, which can lead to failure of other organs including the heart, kidneys, and liver. It also causes infection of the lungs (called pneumonia) and folliculitis. It is reported to cause eye and ear infections (Cafasso, 2016). Extracts of *Ficus benghalensis* and *Ficus religiosa* at various concentrations showed formation of zone of inhibitions. This may indicate clinical importance of these two trees against *P. aeruginosa* & *S. capitis* and other *Streptococcus* strains.

5. CONCLUSIONS

Our present study focuses on, antimicrobial activity of *Ficus benghalensis* & *Ficus religiosa* against *Bacillus licheniformis*, *Paenibacillus macerans*, *Pseudomonas aeruginosa*, and *Staphylococcus capitis* *Staphylococcus aureus*, *Staphylococcus gallinarum* and *Enterobacter gergovie*. It was found that Soxhlet extracts of *Ficus benghalensis* & *Ficus religiosa* showed best results overall. Soxhlet extracts showed noteworthy zones of inhibition in all the leave extracts. This indicates that the lipids present in these leaves play a significant role in inhibition of these pathogenic microorganisms. It was able to inhibit the growth of all the four bacteria. *F. religiosa* and *F. benghalensis*, showed equally significant results to each other.

Today according to the World Health Organization (WHO), as many as 80% of the world's people depend on medicine for their primary healthcare needs. In the developed countries, 25 per cent of the medical drugs are based on plants and their derivatives (Principe 1991). Plant extracts are been used since ancient times in India. They had been involved in various medicinal purposes. This traditional knowledge can be now exploited as various scientific tools are now available. Thus, *F. benghalensis* and *F. religiosa* can be used to prevent such infections.

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