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Original Article

PHYTOCHEMICAL COMPOSITION AND INHIBITION OF ORAL PATHOGENS BY *FICUS BENGHALENSIS* (LINN.) ROOT EXTRACTS

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ABSTRACT

Objectives: Bactericidal effect of ethnomedicinal important plant species *Ficus benghalensis* (Family-Moraceae) was screened against six Gram +ve and one Gram –ve bacterial species associated with oral infections.

Methods: Antibacterial activity of aerial roots was assessed by using disc diffusion and minimum antibacterial inhibitory concentration method. Phytochemical analysis was also carried out.

Results: Phytochemical evaluation showed the presence of Alkaloids, Flavonoids, Simple Phenolics, Steroids and Saponins. Result of the disc diffusion study revealed that petroleum ether extract have the resistance activity against all the tested bacterial strain. *Ficus benghalensis roots* showed maximum antibacterial activity for *E. coli* and *L. rhamnosus* bacteria i.e 19.4% and 15.33% zone of inhibition. The minimum inhibitory antibacterial concentration of pet. Ether extracts of *F. benghalensis* is found to be 12.5mg/ml for *E. coli*, 25mg/ml for *S. mutans*, 50mg/ml for *L. rhamnosus* and *S. epidermidis* while for *B. subtilis* and *S. aureus* it is found to be 100mg/ml.

Conclusion: These findings suggest the excellent medicinal bioactivity of *Ficus benghalensis* and explain the popularity of this plant in the folk medicine as a remedy for oral disorders,thus supporting its folklore application as preventive remedy against oral microbial diseases.

Keywords: Ethnomedicinal plant, *Ficus benghalensis* roots, Oral infection, antibacterial activity, Disc diffusion method.

INTRODUCTION

A vast knowledge and venerable history of use of plants against different health problems has been known since antiquity. Alike different health illness, dental and oral diseases has becoming an alarming problems of the century. Poor hygiene, poor nutrition and smoking contribute to dental and oral problems. Due to colonization and accumulation of microorganism, oral diseases are included into a category of global infectious diseases. The prevalence of dental caries in industrialized countries like India is on resurgence today.

Prevalence and severity of periodontal diseases and dental carries varies according to age, sex, race, geographic areas, socioeconomic factors, local and systemic factors and methods of oral cleansing. Effective antimicrobial agents against these oral pathogens could play an important part in the prevention of dental caries. Natural products have been used for thousands of years in folk medicine for several purposes including oral health care.

As most of the oral diseases are due to bacterial infections and it has been well documented that medicinal plants confer considerable antibacterial activity against various microorganisms including bacteria responsible for dental caries [1]. In the present era, a vast interest has developed among the people regarding the potential of natural tooth brushing in preventing and treating the common diseases of oral cavity such as bad odors, tooth decay and plaque. The developed cavity can be mirror image of the body and many systemic illnesses which are manifested in the soft tissue of mucosa of the mouth [2]. The World Health Organization has recommended and encouraged the use of chewing sticks[3]. Chewing sticks are at least as effective as toothpaste in maintaining oral hygiene [4,5,6]. Africans that use chewing sticks have fever carious lesions than those that use toothbrushes [7]. In a related development, Enwowu reported that Chewing sticks, in addition to providing mechanical stimulation of the gums, also destroy microbes[8], advantages of the chewing sticks over the conventional toothpaste and brushes have been attributed to the strong teeth of Africans[9].

Survey of traditional ethnomedicinal plants used for oral health care by tribals of Melghat region.Dist. Amravati (M.S.) India. has been carried out and found that *F. Benghalensis* species is most predominantly used for oral care by tribal population [10].Hence in the present study, an attempt has been made to screen potential of ethnomedicinaly important plants species *Ficus benghalensis* (Family-Moraceae) from Melghat and Amravati region on oral bacterial flora in *in vitro* condition. *Ficus benghalensis* is a huge tree with high ethnomedicinal value. Old Indian medicinal systems like Ayurveda are using plants for many symptoms such as for snake bites the ground root is given with water until the patient vomits and regains consciousness, fresh piece of root is used as tooth brush, on gastric disorders and diarrhoe

The present investigation of medicinal plant and its efficacy on oral health care will open up new avenues; to scrutinize such rich, effective natural resources for further analysis in order to develop the potential of herbal medicines. Such screening may provide the basis for developing a novel mouth care agent without any possible side effects.

MATERIAL AND METHODS

Plant collection and identification

Areal roots of *Ficus benghalensis* of were collected from Amravati and Melghat region. Authentication and identification was performed at Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. Specimen sample was deposited at herbarium of BAMU, Department of Botany Aurangabad with voucher accession number 0568.

Phytochemical analysis[11]

Areal roots of *Ficus benghalensis* were screened for phytochemical analysis.

Preparation of extracts [11]

Shade dried powdered extract of roots was subjected to successive Sox let extraction using solvent of varying polarity such as water, petroleum ether, chloroform and acetone. After extraction solvent was removed under reduced pressure. Extracted material was stored in airtight container till use.

Test organism / Microbial flora

Seven lyophilized bacterial strains were procured from Institute of Microbial Technology (IMTECH), Microbial Type Culture Collection (MTCC) Chandigarh.

Table 1: List of Bacterial Strains

S. No.	Bacterial Strain	MTCC Code	Growth Medium (Agar and Broth)	Incubation time in hours
1.	*Lactobacillus rhamnosus**	1408	MRS agar	24
2.	*Streptococcus mutans*	890	Brain Heart Infusion(BHI)	48
3.	*Staphylococcus aureus*	3408	Soyabene Casein Digest	24
4.	*Actinomyces viscoscus*	7345	Pikoskaya's agar	24
5.	*Staphylococcus epidermidis*	3639	Nutrient agar and broth	24
6.	*Escherichia coli*	732	Nutrient agar and broth	24
7.	*Bacillus subtilis*	3160	Nutrient agar and broth	24

All the agars and broth medium are of HI-MEDIA.

Antibacterial activity by disc diffusion assay [12]

Antibacterial activity of 4 extracts i.e. aqueous, petroleum ether; chloroform and acetone were determined by paper disc diffusion method **[12, 13]**. Sterilized Whatman filter paper no. 1 discs of 5 mm diameter were soaked in respective 200 mg/ml extract solution.0.2 ml inoculums of test organism was spread on surface of respective bacterial agar plates. Previously soaked discs were placed on surface of inoculated plates. Ciprofloxacin was used as positive control and water, DMSO was used as negative control. Bacterial plates were initially transferred to refrigerator for 40-45 min to allow diffusion and then transferred to incubator set at 37^0 c. and incubated for given incubation period. All the tests were performed in triplicates and under the sterile condition. Zone of inhibition in mm were measured from edge of disc after incubation.

Analysis of data

% Zone of inhibition

% Zone of inhibition of 4 extracts of plant *Ficus benghalensis* against seven bacterial strains were calculated by formula-

$$\% \text{ Zone of inhibition in mm} = \frac{\text{Zone of inhibition of experimental plant extract in mm}}{\text{Zone of inhibition of positive control (standard drug) in mm}} \times 100$$

Minimum Inhibitory antibacterial concentration of extracts

The extract which showed highest inhibitory action in disc diffusion assay was selected for determination of its minimum antibacterial concentration. Nutrient growth agar media plates were prepared same as that of disc diffusion assay. Autoclaved petriplates containing 15 ml of agar media was then seeded with 0.2 ml of inoculums which contain test microorganism by pour plate technique and left for about 30min.Test extract solutions were prepared by dissolving extract powder at following concentration with 1 ml of 20% DMSO.

200mg/ml -Test solution 1

100mg/ml-Test solution 2

50mg/ml-Test solution 3

25mg/ml -Test solution 4

12.5mg/ml -Test solution 5

Sterile discs of Whatmann filter paper no.1 of 5mm diameter were deeped into the prepared extract solution for 15 min. The disc were allowed to air dry for 5-7 min. and loaded on petriplates at a distance of 24mm between two test sample discs. The petriplates were shifted to refrigerator for 30 min to allow diffusion and later shifted to incubator at 37^o c for incubation for given time period.

Entire work was carried out in aseptic condition using laminar air flow in between the two gas burners. Glass surface of laminar air flow was sterilized by Lysol and UV radiation before experimentation.

Statistical analysis [14]

Data obtained was subjected to two way ANOVA test.

RESULTS

Quantitative phytochemical analysis

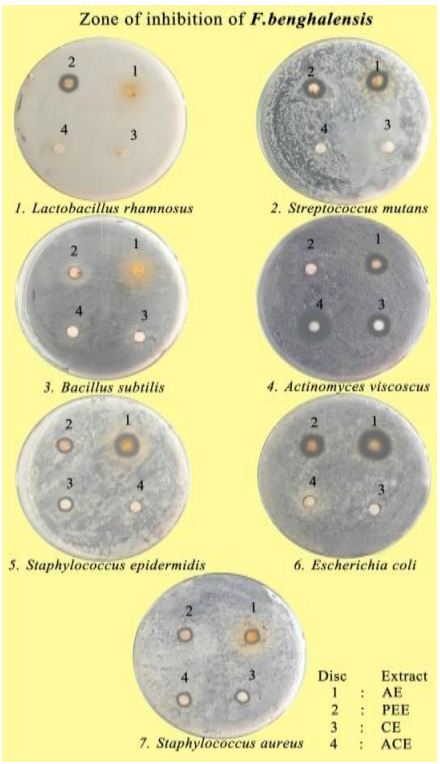
Quantitative phytochemical analysis of areal roots of *Ficus benghalensis* revealed the presence of Alkaloids, Flavonoids, Simple Phenolics, Steroids and Saponins **(Table no.2).**

Table 2: Phytochemical analysis of areal roots of *Ficus benghalensis*

	Test Inference
1.	Alkaloid++
2.	Anthraquinones-
3.	Flavonoids++
4.	Simple Phenolecs+++
5.	Steroid+
6.	Tanine--
7.	Saponins+++

Antibacterial activity against oral micro-organisms

Table no. 3 depicts the result of zone of inhibition of *F. benghalensis*. It is found that petroleum ether showed the resistance activity against all the tested bacterial strain. While aqueous extract showed activity against five strains of bacteria and chloroform, acetone extract inhibits growth of three and four bacterial strains respectively **Fig no.2**



Fig, 2: Zone of Inhibition of *F. benghalensis*.AE=aqueous extract, PEE= Pet. ether extract, CE= chloroform extract, ACE= acetone extract

Table 3: Zone of Inhibition of *F. benghalensis* in mm

Bacterial Strain		Aqueous	Pet. ether	Chloroform	Acetone	Standard
1.	*Lactobacillus rhamnosus*	00	1.3±0.11	00	00	25±00
2.	*Streptococcus mutans*	0.7±0.02	2.3±0.15	00	00	18±00
3.	*Bacillus subtilis*	0.1±0.05	0.8±0.13	00	00	14±00
4.	*Actinomyces viscoscus*	2.3±0.15	00	2.4±0.08	4±0.05	15±00
5.	*Staphylococcus epidermidis*	2.6±0.05	1.5±0.23	1.2±0.11	00	25±00
6.	*Escherichia coli*	4.1±0.05	4.5±0.2	0.5±0.02	1.0±0.06	28±00
7.	*Staphylococcus aureus*	1.6±0.05	1.7±0.14	1.1±0.08	1.1±0.09	30±00

The % Zone of inhibition of *F. benghalensi i*s shown in **Table no. 4.** It is found that extract showed maximum % zone of inhibition i.e. 5.97% against *L. rhamnosus,* 15.33% against *S. mutans*, 9.30% against *B. subtilis,* 7.38% against *S. epidermidis,* 19.14% against *E. coli,* and 6.61% against *S. aureus* while extract showed 0% activity against *A. viscoscus.*

Table 4: % Zone of Inhibition of *F. benghalensis*

Bacterial Strain		Aqueous	Pet. ether	Chloroform	Acetone	Standard
1.	*Lactobacillus rhamnosus*	00	5.96	00	00	100
2.	*Streptococcus mutans*	4.66	15.33	00	00	100
3.	*Bacillus subtilis*	1.16	9.30	00	00	100
4.	*Actinomyces viscoscus*	25.27	00	26.37	43.95	100
5.	*Staphylococcus epidermidis*	10.34	7.38	5.91	00	100
6.	*Escherichia coli*	17.44	19.14	2.12	4.25	100
7.	*Staphylococcus aureus*	6.22	6.61	4.28	4.28	100

Statistical Analysis

ANOVA two way analysis revealed that all bacterial strains of *F. benghalensis* effective against all bacterial strains tested at 5% level of significance. (Table no.5)

Table 5: Anova: Two-Factor without Replication

ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Rows	18.63357	6	3.105595	2.281063	0.081826	2.661305
Columns	5.408571	3	1.802857	1.324201	0.297494	3.159908
Error	24.50643	18	1.361468			
Total	48.54857	27				

***H0₁*:** There is no significant difference between inhibitions of seven bacterial species.

***H0₂*:** There is no significant difference between activity of four extract.

Solution-

***H0₁*:** Accepted.

***H0₂*:** Accepted.

Minimum Inhibitory antibacterial concentration

Table no.6 showed minimum inhibitory antibacterial concentration of petroleum ether extracts of *F. benghalensis* in mm. It is found that 12.5mg/ml concentration is minimum inhibitory antibacterial concentration for *E. coli*, 25mg/ml for *S. mutans*, 50mg/ml for *L. rhamnosus* and *S. epidermidis* while for *B. subtilis* and *S. aureus* it is found to be 100mg/ml. **Fig no.2**

Table 6: Minimum Inhibitory antibacterial concentration of pet. Ether extracts of *F. beghalensis* in mm

Bacterial Strain		200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml
1.	*Lactobacillus rhamnosus*	1.5	1	0.5	00	00
2.	*Streptococcus mutans*	2.3	1.6	1.1	0.8	00
3.	*Bacillus subtilis*	1.4	0.5	00	00	00
4.	*Actinomyces viscoscus*	00	00	00	00	00
5.	*Staphylococcus epidermidis*	2	2	1.6	00	00
6.	*Escherichia coli*	5.1	4.3	4	1	0.6
7.	*Staphylococcus aureus*	2.3	2	00	00	00

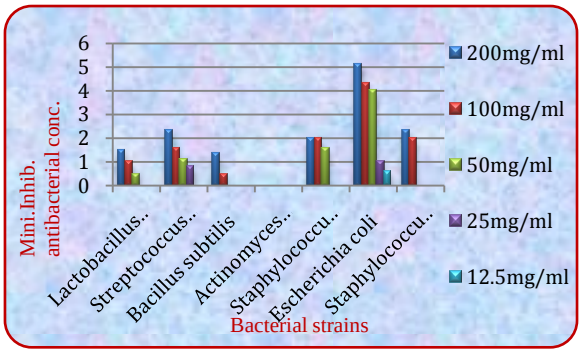


Fig. 2: Minimum Inhibitory antibacterial concentration of pet. Ether extracts of *F. beghalensis* in mm

DISCUSSION

It is generally accepted that oral hygiene maintenance through regular removal of dental plaque and food deposits is an essential factor in the prevention of dental caries and periodontal disease. Despite the widespread use of toothbrushes and toothpastes, natural methods of tooth cleaning using chewing sticks selected and prepared from the twigs, stems or roots from a variety of plant species have been practiced for thousands of years in Asia, Africa, the Middle East and the Americas [15]

Natural products have been used for thousands of years in folk medicine for several purposes. As most of the oral diseases are due to bacterial infections and it has been well documented that medicinal plants confer considerable antibacterial activity against various microorganisms including bacteria responsible for dental caries[1]. The curative properties of medicinal plants are perhaps due to the several aromatic compounds or secondary metabolites of plants which are serve as defense mechanism against predation of many microorganisms, insects and herbivores etc. Specifically phenolic compounds are known for antimicrobial activity[16].

In the present investigation four extract of areal roots of *Ficus benghalensis* species has been screened for its antibacterial potential against seven strains of oral bacteria i.e *Lactobacillus rhamnosus Streptococcus mutans*, *Staphylococcus aureus*, *Actinomyces viscoscus*, *Bacillus subtilis Escherichia coli* and *Staphylococcus epidermidis* by disc diffusion method. *Ficus benghalensis* showed maximum inhibition for bacterial species *L. rhamnosus, S. mutans*, *B. subtilis, S. epidermidis, E. coli, S. aureus* while extract showed 0% activity against *A. viscoscus*m respectively.

Similar type of study on varying concentration of extract of four species of seaweeds was screened against oral bacterial stains causing dental carries. The finding revealed that out of three oral pathogenic bacteria i.e. *A. viscosus, S. mitis* and *S.mutans*. Seaweeds showed more inhibition against *A. viscosus*[17]. Four extracts of *Ficus benghalensis* was effective and showed maximum inhibition of bacterial species *L. rhamnosus. Invitro* antibacterial activity of traditional plants like *J. curcas* and *F. benghalensis* against oral microorganisms and found that latex of *J. curcus* and aqueous arial root extract *of F. benghalensis* shows more inhibitory action against six oral bacterial species [18].Also in the present investigation, other extract such as aceton, chloroform and pet ether of *F. benghalensis* arial root showed effective inhibitory action. An Assessment of phytochemical composition and antibacterial activity of different extracts of *Merremia emargenata* leaves and *Barleria prionitis* against oral micrflora to improve dental hygiene has been evaluated and they got a resistant activity.[19,20]

CONCLUSION

This study has confirmed antimicrobial potential of *F. benghalensis* plant, thus supporting its folklore application as preventive remedy against oral microbial diseases. The present investigation is an attempt to give herbal products against the drugs used today.

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