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A Study on In *Vitro* Antibacterial Activity of *Ficus Bengalensis* Linn. on Dental Caries Pathogens *streptococcus Mutans* and *Actinomyces Viscosus*

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Abstract

The aerial roots of Ficus bengalensis Linn. were collected in and around Chennai, Tamilnadu, India They were washed, shade dried and were ground into powder. The powder was extracted with chloroform, petroleum ether, methanol and hexane. The antibacterial screening of the extracts was carried out by determining the zone of inhibition using disc diffusion method. The strains were grown to logarithmic phase in BHI broth and the inoculum was prepared by adjusting the turbidity of bacterial suspension to 0.5 McFarland's tube. The dried extracts were dissolved in 10% Dimethyl sulphoxide (DMSO) in required concentration. The sterile discs were impregnated with 20 µl of extract. The extract discs were placed on BHI agar plates, which were previously inoculated with test strains and incubated at 37°C for 24 hours. Ampicillin disc (10µg) and 10% DMSO impregnated discs were used as positive and negative controls respectively and the zones of inhibition were recorded. The Minimum inhibitory concentration was determined by agar dilution method. The results of the present study showed that the methanol and chloroform extracts of Ficus bengalensis Linn. have activity against both Streptococcus mutans and Actinomyces viscosus. From the present study it is concluded that Ficus bengalensis Linn. extracts can be used as an effective antibacterial agent against dental caries.

Keywords: *Antibacterial activity, Ficus bengalensis Linn., Streptococcus mutans, Actinomyces viscosus, Dental caries*

1. Introduction

Dental caries is the main oral health problem in both the developed and developing countries and it affects 60-90% of school aged children and adults [1]. In India, children comprise 40% of a rapidly growing population. The prevalence of dental caries varies from 33.7%-90% in children population and is increasing at an alarming rate [2].

Streptococcus mutans is considered to be an important cariogenic bacterium. It has the ability to adhere and colonise the tooth surface to form dental plaque. Further they can metabolise the simple sugars to develop dental caries [3]. They colonize the host after the first tooth erupts, and their preferential colonization site is the teeth [4].

Actinomyces viscosus, is a filamentous gram-positive bacterium and is the primary coloniser on the tooth surface [5]. It is considered to be involved in root caries.

A. viscosus of root caries origin synthesize large quantities of glycogen and subsequently degrade this stored polymer slowly with acid production, at acidic pH levels, may play an important role in the root caries process [6].

Medicinal plants are still the source of maintaining the oral hygiene in villages. Many plants have been reported to be used traditionally for oral care [7]. *Ficus bengalensis* Linn (Family-Moraceae), commonly known as Banyan tree is known for its branches and aerial roots. It is a large evergreen tree distributed all over India from sub-Himalayan region and in the deciduous forest of Deccan and south India [8]. It is used in Ayurveda for treatment of diarrhoea, piles, teeth and skin disorders [9], The twigs of tree is used as tooth brush traditionally in many parts of south India [10].

The present research work has been conducted to study the *in vitro* antibacterial activity of *Ficus bengalensis* Linn. on oral pathogens *Streptococcus mutans* and *Actinomyces viscosus*.

2. Materials and Methods

Collection of plant material: The aerial roots of *Ficus bengalensis* Linn. was collected in and around Chennai, India. The plant materials were authenticated by Dr. S. J. Kingsley, Professor and Head, Department of plant biology and Biotechnology, Loyola College. Chennai, India.

Preparation of extract: The plant material was washed and shade dried. The dried material was coarsely powdered and was stored in an air tight container for further use. 100 g of powder was extracted with chloroform, petroleum ether, methanol and hexane for 48 hours in a soxhlet apparatus. The extract was then concentrated with rotary evaporator under reduced pressure. The dried extract was weighed and the percentage yield of the extracts was calculated.

Bacterial strains: *Streptococcus mutans* (MTCC No. 890) and *Actinomyces viscosus* (MTCC NO. 7345) were obtained from Microbial Type Culture Collection and Gene Bank, Chandhigarh, India. They were maintained in Brain Heart Infusion Agar.

Antibacterial activity:

1. **Disc Diffusion method:** The dried extracts were dissolved in 10% Dimethyl sulphoxide (DMSO) in the concentration of 25mg/ml, 50 mg/ml and 75 mg/ml. The antibacterial activity was determined by standard disc diffusion method [11]. The strains were first grown to logarithmic phase in Brain

heart infusion broth and the inoculum were prepared by adjusting the turbidity of bacterial suspension to 0.5 McFarland's tube with brain heart infusion broth [12]. The sterile discs (6mm in diameter) were impregnated with 20 µl of extract. The extract discs were placed on BHI agar plates, which were previously inoculated with test strains and incubated at 37ºC for *S. mutans* and 30ºC for *A. viscosus for* 24 hours.

The disc impregnated with 20µl of 10% DMSO and ampicillin disc (10µg) were used as negative and positive control respectively. The agar plates were observed for zones of inhibition around the disc and the diameter of zone of inhibition is measured.

2. **Minimum Inhibitory concentration by Agar Dilution method:** The agar dilution method was used to determine the MIC of all the extracts. The extracts were prepared at the concentration of

100 mg/ml with the 10% DMSO. From this a dilution of 10 mg/ml of extracts were prepared by further dilution by 10% DMSO. Then a double fold dilution is performed in the concentration ranging from 10 mg/ml to 0.625 mg/ml. 1 ml each of the extracts were added to sterile molten brain heart infusion agar.

After the plates were prepared a loop full of the standardized bacterial cultures were inoculated and the plates were incubated at 37ºC and 30ºC for *S. mutans* and *A. viscosus* respectively for 24 hours. Growth of organisms on each concentration were checked to determine the minimum concentration that inhibits growth of test organism.

3. Results and Discussion

When percentage yield of extracts was compared for various solvents, it was found that it was better for methanol and chloroform than the other two solvents (**Table 1**).

Table 1: Percentage Yield of The Extracts For Various Solvents

S. No.	Extract	Percentage yield
1.	Methanol	5.07
2.	Chloroform	6.23
3.	Petroleum ether	2.46
4.	Hexane	0.72

The results of the present study showed that the methanol and chloroform extracts of *Ficus bengalensis* Linn. have activity against both *Streptococcus mutans* and *Actinomyces viscosus.*

Table 2 shows the zone of inhibition for various solvent extracts of *Ficus bengalensis* Linn. against *S. mutans*. From the table it is evident that only methanol and chloroform extracts have antibacterial activity. The petroleum ether and Hexane extracts did not show any antibacterial activity. Further from the table, it is clear that the antibacterial activity increased with increasing concentration.

Table 2: Zone Of Inhibition By Various Extracts Against *Streptococcus Mutans*

	Zone of inhibition ± SD in mm (N = 5)

***Ficus bengalensis* Linn. extract concentration**	**Methanol**	**Chloroform**	**Petroleum ether**	**Hexane**	**Negative control***	**Positive control****
25 mg/ml	10.58 ± 0.71	12.8 ± 1.01	Nil	Nil	Nil	24.1 ± 1.2
50 mg/ml	16.82 ± 0.99	18.12 ± 0.61	Nil	Nil		
75 mg/ml	19.92 ± 1.26	22.2 ± 0.95	Nil	Nil		

*Negative control – 10% DMSO, **Positive control – Ampicillin disc. Both the controls are common for all the concentrations

Figure 1 depicts the comparison of diameter of zone of inhibition of methanol and chloroform extracts. It is obvious that the chloroform extract has better antibacterial activity than the methanol extract.

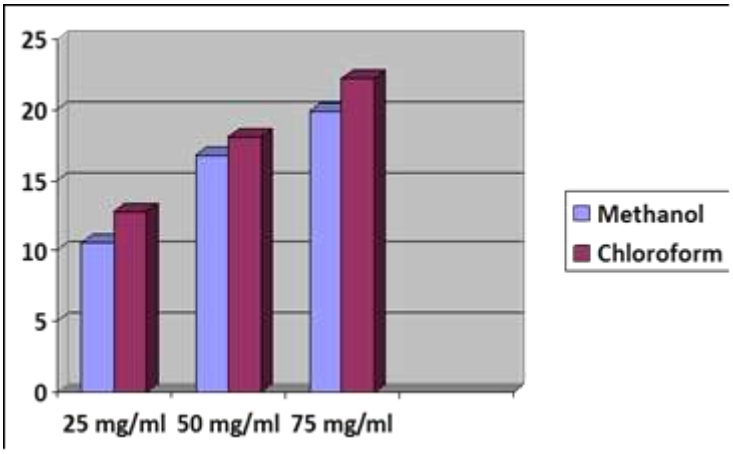

Figure 1: Comparison Of The Diameter Of Zone Of Inhibition For Methanol And Chloroform Extracts Against *Streptococcus Mutans*

Table 3 depicts the zone of inhibition for various solvent extracts of *Ficus bengalensis* Linn. against *A. viscosus*. From the date it is interpreted that only chloroform extract is having antibacterial activity against *A. viscosus*. However from the table it is evident that methanol extract showed antibacterial activity at high concentration of 75 mg/ml. The remaining extracts did not show any antibacterial activity against *A. viscosus.*

Table 3: Zone Of Inhibition By Various Extracts Against *Actinomyces Viscosus*

***Ficus bengalensis* Linn. extract concentration**	**Zone of inhibition ± SD in mm (N = 5)**					
	Methanol	**Chloroform**	**Petroleum ether**	**Hexane**	**Negative control***	**Positive control****
25 mg/ml	Nil	12.08 ±0.63	Nil	Nil	Nil	25.9 ± 0.9
50 mg/ml	Nil	18.52 ±1.03	Nil	Nil		
75 mg/ml	8.22 ±2.65	23.48 ± 0.72	Nil	Nil		

*Negative control – 10% DMSO, **Positive control – Ampicillin disc. Both the controls are common for all the concentrations

Figure 2 projects the comparison of diameter of zone inhibition of methanol and chloroform extracts. It is clear that the chloroform extract has better antibacterial activity than the methanol extract. The methanol and chloroform extracts were further analysed to find its minimum inhibitory concentration (MIC).

Table 4 shows the MIC for methanol and chloroform extracts for *S. mutans* and *A. viscosus*. From the table it is evident that the chloroform extract is superior compared to methanol extract with MIC of 1.25 mg/ml for both the bacteria. The present study has revealed that *Ficus bengalensis* Linn. exhibits antibacterial activity with special reference to *S. mutans* and *A. viscosus*.

Table 4: Minimum Inhibitory Concentration For Methanol And Chloroform Extracts

S. No.	**Extract**	***Streptococcus mutans***	***Actinomyces viscosus***
1.	Methanol	5 mg/ml	10 mg/ml
2.	Chloroform	1.25 mg/ml	1.25 mg/ml

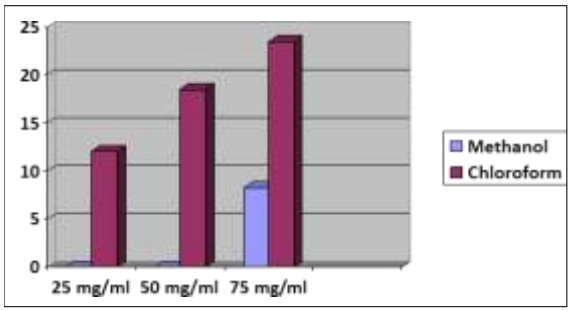

Figure 2: Comparison Of The Diameter Of Zone Of Inhibition For Methanol And Chloroform Extracts Against *Actinomyces Viscosus*

The methanol and chloroform extracts showed activity against both the bacteria. However, the activity is excellent for chloroform extract against both the bacteria. The antibacterial activity of *Ficus bengalensis* Linn. has been studied extensively [13]. It is has been established that it has antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* [14]. The antibacterial activity of the tree has been established against *A. viscosus* already with the methanol extract [15]. However in the present study it has been observed that the chloroform extract contains the antibacterial activity better than methanol extract. Further MIC of chloroform extract is 1.25 mg/ml and thus its antibacterial activity has been proved to be good.

The antibacterial activity is attributed to the various phytochemicals that are present in the extract. *Ficus bengalensis* Linn. is rich in sterols and flavanols [16]. These phytochemicals are attributed to the plant's antibacterial activity [17]. Further it has been revealed that the phytochemical constitution differs according to the solvent used for extraction [18]. Thus, it is not surprise that the results of the present study shows antibacterial activity for only chloroform and methanol extract.

4. Conclusion

The medicinal properties of various parts of *Ficus bengalensis* Linn. are well documented in the literature [19]. However, its antibacterial activity against the oral pathogens is less studied. The present study has revealed that *Ficus bengalensis* Linn. can be used as a source of antibacterial compounds against oral pathogens.

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