

Ficus Benghalensis and *Stevia* formulation based mouthwash and its Anti- cariogenic activity *in vitro* comparative study

R. Shruthi Devi¹, S. Rajeshkumar^{2*}, DR. Ezhilarasan³, T. Lakshmi⁴

¹Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-77, Tamilnadu, India.

²Associate professor, Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical sciences, Saveetha University, Chennai -77, Tamilnadu, India.

^{3,4}Department of Pharmacology, Saveetha Dental College and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-77, Tamilnadu, India.

ABSTRACT

Introduction: *Ficus benghalensis* commonly called alampattai is used to treat diabetes, piles, teeth disorders, rheumatism and to boost the immune system. *Stevia rebaudiana* commonly called berton leaves is a perennial shrub that contains 2 important glycosides, stevioside and rebaudioside that is responsible for producing sweetness intensity 300 times more than sucrose. It has various properties like anti oxidant, antibacterial etc. Mouthwashes are considered to act as potent delivery systems to prevent oral pathogens mainly the *S. Mutans*, the main organism that causes dental caries. Hence, the present study aims in evaluating the anticariogenic property of the aqueous mouthwash of *Ficus benghalensis* and stevia extract.

Mouthwash preparation - 0.3g of sucrose + 0.001 sodium Benzoate + 0.01g sodium Lauryl sulphate. Dissolved in 10ml distilled water. Then 600µl plant extract + 50µl peppermint oil added as flavouring agent.

Antibacterial activity - Studied against *S. aureus*, *E. faecalis* and *S. mutans*. MHA is the medium used to determine the zone of inhibition using 5 different concentrations of the mouthwash extract.

Antifungal activity- Studied against *C. albicans*. Sabouraud's dextrose agar is the medium used to determine the zone of inhibition using different concentrations. The graph shows that the zone of inhibition was greater in *S. mutans* than in *C. albicans*, *S. aureus* and *E. faecalis*. Hence the mouthwash can be considered to prevent bacteria present in oral cavities. The study concluded that the mouthwash preparation can be used to replace the pharmaceutical mouthwash. But further study and random clinical trials needs to be done to make it more efficient.

Corresponding Author: rajeshkumars.sdc@saveetha.com

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BACKGROUND

Ficus benghalensis commonly known as banyan tree belongs to the moraceae family. It is known as alampattai in Tamil. In Indian ayurveda it is called the vata tree and considered remarkable as its branches take up roots and form new trunks and hence are considered a sacred one. It produces a latex that contains resin, albumin, sugar and malic acid. In Indian Ayurveda it is used for treating Diarrhea, piles, teeth disorders, Rheumatism, to boost the immune system, as an anti- helminthic and as a hypoglycemic. Its extract inhibits insulinase activity from the liver and lipid peroxidation.^[1] It contains 20-tetratriacontane-2-one, 6-heptatriacontene-10-one, glucoside, pentatriacontan-5-one, beta-sitosterol alpha-D-glucose, and meso-inositol and crude protein, fibres, CaO, and phosphorus are present in its leaves. The fruit extract obtained from this showed anti-tumor activity in potato disc assay test.^[2] The milk obtained from stem and seeds is used for external application in treating rheumatism and inflamed sole and feet. The plant is also found to show immunomodulatory, antioxidant, anti stress and antiallergic properties based on the various scientific studies carried out.^[3]

Dental caries is considered as chronic, transmissible, biobehavioral and infectious disease that is present throughout the life of a person and caused by demineralisation of inorganic portion and destruction of organic substance by lactic acid production.^[4,5] *Streptococcus mutans* is considered as the main

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causative agent causing caries in the oral cavity as it ferments sugar present in the food in the form of sucrose to lactic acid. If more than 10^6 of this bacteria is present then it is an indicator for high risk of caries. Hence, it can be prevented by brushing teeth regularly, using antimicrobial agents and using mechanical plaque removal methods like flossing and chemical plaque control like mouthwashes or else it will result in loss of tooth structures.^[6] So for preventing this disease the focus should mainly be set on decreasing the cariogenicity of the bacteria, increasing the host's ability to respond to an insult. For this, various herbal extracts and mouthwash have been prepared.^[7] *Candida albicans* is a most frequent yeast group that is isolated from oral cavities and often found in association with *S. mutans* mainly in dental plaque of children.^[8]

Mouthwashes are considered to act as potent delivery systems for the antimicrobials.^[9] Wide range of chemical agents like triclosan, chlorhexidine (CHX), metal ions, and essential oils have been added to it to make it effective out of which chlorhexidine considered gold standard.^[10] But certain disadvantages like tooth staining, taste disturbance and rarely painful desquamation of the oral mucosa is seen. These act as carrier for daily exposure to these bacterial agents.^[11] Chemical agents present in mouthwashes are found to be less technically demanding adjuvant to mechanical control of plaque like toothbrush and flossing agents as the latter was found to be time consuming, does not remove the plaque completely from all tooth surfaces and most people lack motivation in doing such procedures.^[12]

Herbal medicines are considered to be both promotive and preventive in approach as it restores the health to normal in a least harmful way because it does not contain both alcohol or sugar, the two most common ingredients that is normally found in a chemical mouthwash. It prevents various oral diseases like halitosis, mouth ulcers, bleeding gums etc.^[13] *Stevia rebaudiana* commonly called bertonia leaves is a perennial shrub that belongs to the Asteraceae, the sunflower family mostly in Gujarat and available in powder form. Its leaf extract is used for sweetening foods due to the presence of glycosides like stevioside and rebaudioside-A thereby producing sweetness intensity 300 times more than that of sucrose.^[14] It is the only one among the stevia family that acts as a sweetening agent. Under critical environmental conditions it can exist either as an annual or a mixture of plant types. The phytochemical constituents present in stevia are tannins,

alkaloids, glycosides, saponins, sterols, triterpenes.^[15] Our team has extensive knowledge and research experience that has translate into high quality publications.^[16-50]

Previously, various epidemiological and *in vitro* studies have been done successfully for the betterment of communities.^[51,52] Though various studies have been made on the antibacterial activity less insight is made on anticariogenic activity. Hence the present study aims in evaluating the anticariogenic activity of *Ficus benghalensis* and stevia based mouthwash.

MATERIALS AND METHODS

The study was conducted at Saveetha dental college, Chennai. The plant extract was available in the local market in powder form, hence was easily obtained. The sample was collected by random sampling method in an unbiased manner. In the present study, anticariogenic activity of the plant extract was included and various other properties like anti-oxidant, antibacterial, anti-inflammatory activity etc were excluded from the study.

Preparation of the plant extract

1 g of the powder of plant extract was measured accurately. An aqueous extract of the plant was prepared and kept in a stirrer, followed by a shaker for 1 day after which it was boiled for 75 \square for 10min and then mouthwash was prepared.

Preparation of Mouthwash

0.3g of sucrose was added to 0.001g of sodium benzoate and 0.1g of sodium lauryl sulphate and mixture obtained was then made to dissolve in 10ml of distilled water. Then, 600 \square l of the plant extract along with 50 \square l of peppermint oil as a flavouring agent was added.

Anticariogenic activity

Antibacterial activity

Antibacterial activity of the plant extract against the strain *S.aureus*, *E.faecalis* and *S,mutans*. MHA (Muller Hinton agar) was utilized for this activity for determining the zone of inhibition. Muller Hinton agar were prepared and sterilised for 45min at 120lbs. Media poured into the sterilised plates and allowed them to solidify. The wells were then cut using a well cutter and the test organisms were swabbed. The mouthwash extract with different concentrations were loaded and the

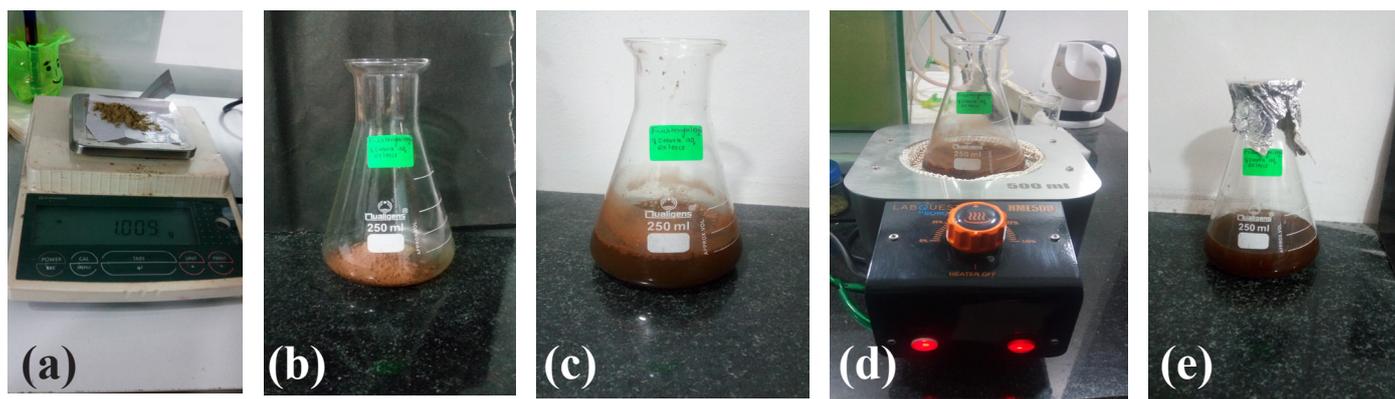


Fig 1: Preparation of plant extract a) The powder of *Ficus benghalensis* measured in weighing machine b) The extract taken in beaker c) aqueous extract of ficus and stevia prepared d) The extract was boiled e) Aqueous Extract after boiling

plates were incubated for 24hrs at 37° and after the incubation time, the zone of inhibition was measured.

Antifungal activity

Candida albicans was used as test pathogens by agar well diffusion method. Sabouraud’s dextrose agar is used to prepare the medium. The prepared and sterilised medium was swabbed with test organisms and the plant extract of different concentrations were added to the wells. The plate was incubated at 28μ for 48 - 72hrs. After incubation time, the zone of inhibition was measured.

RESULTS AND DISCUSSION

From the graph above, it is seen that *S.mutans* show higher anticariogenic activity than the antibiotic used. It also shows antifungal activity against *C.albicans*. The highest zone of inhibition for *S.mutans* was obtained at 28mm in 100μl concentration followed by *S.aureus* at 20mm in antibiotic, *E.faecalis* at 12mm in antibiotic and *C.albicans* at 15mm in 100μl concentration

Dental caries is a localised disease that is caused by the metabolic processes of the biomass when it comes in contact with the surface of the tooth. Recent investigations have shown stevia

as a non cariogenic sweetener as it inhibits *Streptococcus mutans*, *Streptococcus sobrinus* and *Lactobacillus acidophilus* that are involved in tooth decay by decreasing the production of bacterial insoluble polymers, producing anti plaque effect and by lowering the acidogenic potential of enamel [53]. Previous study has shown that antimicrobial effect of the plant extract obtained from leaves of stevia when dissolved in hexane, methanol, ethanol, ethyl acetate, and chloroform showed the best MIC was obtained in hexane extract for anticariogenic activity[54]. Another study by Tirantra et al has found 50% growth inhibition and decrease in acid production by bacteria.[55]

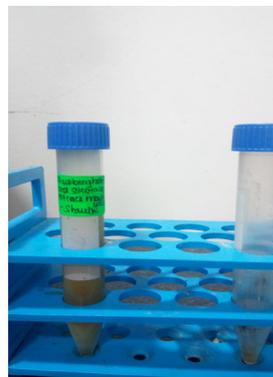


Fig 2: Mouthwash of *F. benghalensis* and *stevia* prepared

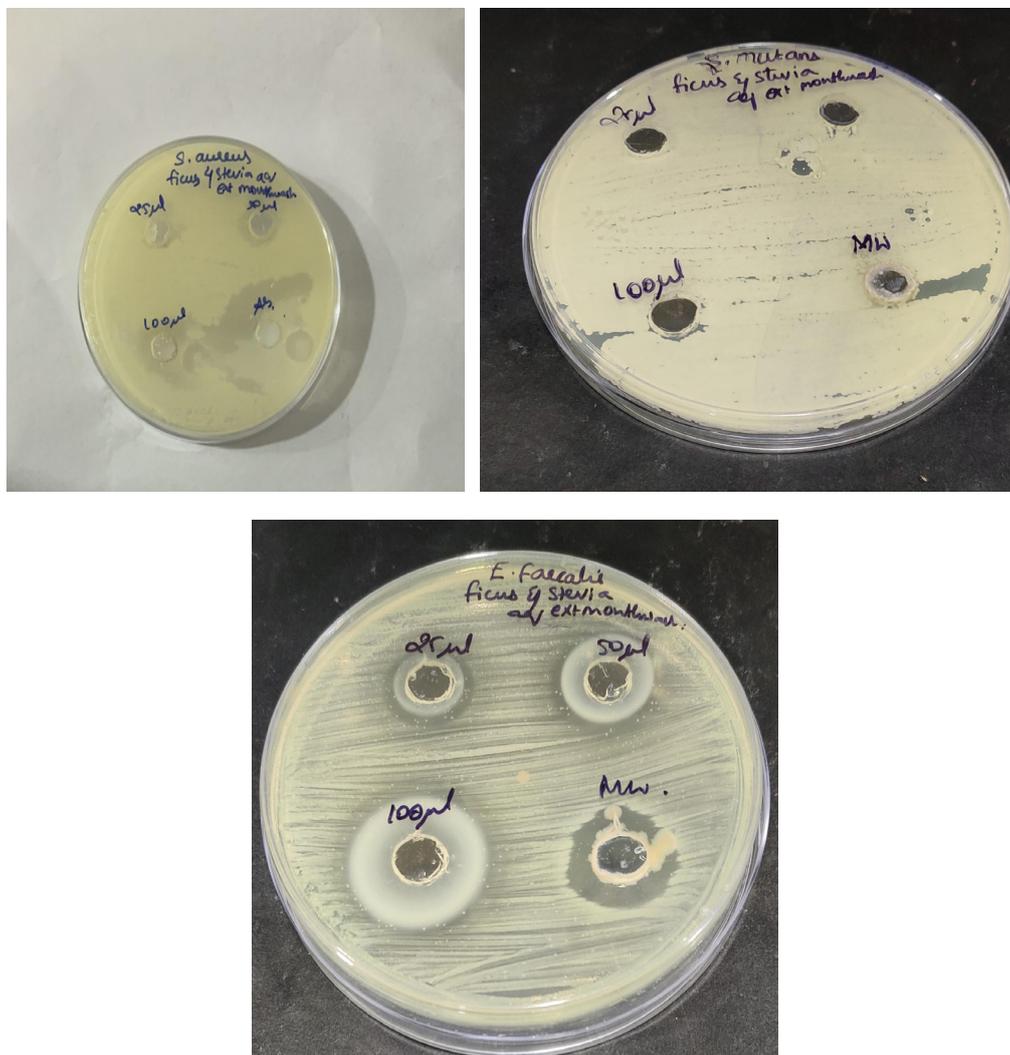


Fig 3: Antimicrobial plates of the mouthwash extract against a) *S. aureus* , b) *S. mutans* and c) *E. faecalis* from which the zone of inhibition was measured.



Fig 4: Antifungal activity of the mouthwash extract against *C.albicans* from which zone of inhibition was measured.

Our study showed that as the concentration increased in dose dependent manner, the zone of inhibition was found to be increased indicating a good anticariogenic property of the mouthwash.

The secondary metabolites are responsible for various physiological processes. The sweetening property of stevia comes from the presence of these secondary metabolites in it. Das et al 1992 performed an experiment on Sprague Dawley rats and found the glycosides present in stevia to be non cariogenic. Other studies with alcoholic and aqueous extract have shown reduction in plaque index.^[56] Our present study found that *Ficus benghalensis* showed good antimicrobial property against *S.aureus* and *S.mutans* in combination with stevia than with *Candida albicans*. A similar study showed that the ethanolic extract of both *F.benghalensis* and *F. religiosa* showed good antibacterial activity against *S.aureus* but that of *F. religiosa* was higher than *F.benghalensis*.^[57] Another similar study also showed that the ethanolic extract of *F. benghalensis* did not show antibacterial activity against *C.albicans* and other fungi when compared with test pathogens.^[58] Using plants that have antimicrobial activity as mouthwashes has various advantages than commercial mouthwashes like they are more economically viable, have less side effects but more *in vivo* studies need to be done.^[59]

In an attempt to prolong the efficiency of the antimicrobial activity against the oral pathogens nanoparticles are used as they have enhanced antimicrobial activities and in recent times antibiotic resistance against various chemical microbial agents. Hence, the use of natural products has started to gain importance^[60] It contributes to control disordered growth produced by the oral microbes due to the presence of flavonoids that disrupts the cell membrane of bacterial cells and inhibits the enzymatic activity. But change in period, region and season in the collection of plants for extract preparation may produce different outcomes.^[61] Since no previous study has been done on mouthwash based on a combination of *F.benghalensis* and *Stevia*, our study wanted to experiment with it and evaluate its anticariogenic activity so as to use it as a standard mouthwash instead of the gold standard chlorhexidine. Previous studies like^[62-75] helped the present study to gain best results

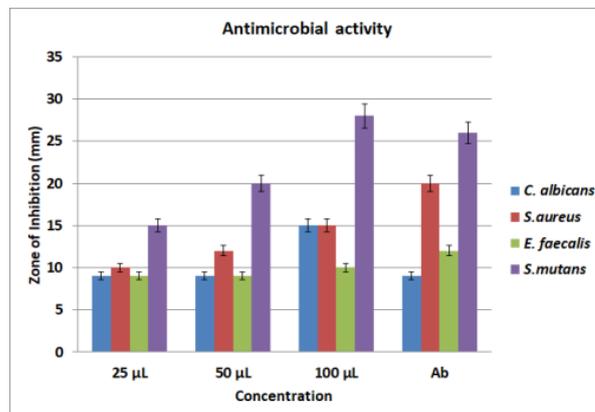


Fig 5: The graph shows the antimicrobial activity of the aqueous extract of mouthwash. X axis represents the concentration in microlitre and the Y axis represents the zone of inhibition in mm. The blue colour represents the *C.albicans*, red represents the *S.aureus*, green represents the *E.faecalis* and violet represents the *S.mutans*.

CONCLUSION

Thus it can be concluded that the mouthwash can be used to replace the pharmaceutical mouthwash to prevent the oral pathogens due to the higher zone of inhibition seen in *S.mutans* and *S.aureus*, hence exhibiting good anticariogenic potential. But further study and random clinical trials needs to be done to make it more efficient.

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