

Cytotoxic and Antimicrobial Effects of Herbal Formulation (*Ficus benghalensis*, *Azadirachta indica* and *Menthapiperita*) Based Mouthwash

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ABSTRACT

Background: *F.Benghalensis* is known to have medicinal effect and is beneficial in treating mouth ulcers. *A.Indicais* rich in antioxidants and helps to boost the immune response in gum and tissues of the mouth. Neem offers a good remedy for curing mouth ulcers, tooth decay and acts as a pain reliever. *M.Piperita* contains antimicrobial and anti-inflammatory properties that promote oral hygiene.

Aim: To prepare a mouth wash formulation containing *F.benghalensis*, *A.indica*, *M.piperita*. To determine the cytotoxic and antimicrobial effects of mouthwash formulation containing *F.benghalensis*, *A.indica*, *M.piperita*.

Materials and Methods: In the present study, preparation of herbal mouthwash formulation was carried out initially and then the cytotoxicity and antimicrobial activity of the mouthwash was assessed. The antimicrobial activity of the mouthwash formulation was carried out using agar well diffusion method. Different concentrations of mouthwash were tested against *Streptococcus mutans*, *E.faecalis*, *Lactobacillus* and *Candida albicans*. The cytotoxicity activity of the mouthwash was assessed using brine shrimp lethality assay method.

Results: The cytotoxic activity was found to be better at low concentration. 8 nauplii survived at 5 µL, 10 µL, 20 µL after 24 hours. The antimicrobial activity of the mouthwash formulation showed that the mouthwash formulation had the highest zone of inhibition seen in 100 µL concentration on *C.albicans* almost similar to the control antibiotic in comparison to the oral pathogens.

Discussion: *Ficus benghalensis*, *Azadirachta indica* & *Mentha piperita* extracts have many medicinal properties. Our study has evaluated the combination of these three extracts in a mouthwash formulation and has found positive results.

Conclusion: The mouth wash formulation had better antimicrobial activity and exhibited less cytotoxicity at lower concentrations.

Keywords: *F.benghalensis*, *A.indica*, *M.piperita*, cytotoxicity, antimicrobial

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INTRODUCTION

Active ingredients in commercial brands of mouthwash can include thymol, eucalyptol, hexetidine, methyl salicylate, menthol, chlorhexidine, gluconate, benzalkonium chloride, cetylpyridinium chloride, methylparaben, hydrogen peroxide, domiphen bromide, and sometimes fluoride, enzymes, and calcium.¹ Ingredients also include water and sweeteners such as sorbitol, sucralose, sodium saccharine, and xylitol (which doubles as a bacterial inhibitor).² Sometimes, a significant amount of alcohol is added, as a carrier for the flavor, to provide "bite" and to contribute an antibacterial effect.³ Due to the alcohol content, it is possible to fail a breathalyzer test after rinsing; in addition, alcohol is a drying agent and may worsen chronic bad breath.⁴ As such, it is possible for alcoholics to abuse mouthwash. Recently, some assumptions were made of a possible carcinogenic character of alcohol used in mouth rinses, but no clear evidence was found.⁵ Commercial mouthwashes usually contain a preservative such as sodium Benzoate to preserve freshness once the container has been opened.⁶ In the commercial market, many newer brands are of alcohol free and contain odor elimination agents such as oxidizers, as well as odor-preventing agents such as zinc ion technology to keep fu-

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ture bad breath from developing. In addition to being a fighter of unpleasant breath, the possible health benefits may include re-

relieving the symptoms of gingivitis, canker sores, swollen, sore or inflamed gums, sore mouth, inflamed or ulcerated throat, infections in the mouth, calculus, and bleeding gums.⁶ A general mouthwash for unpleasant breath can also be used as an aid for canker sores and other mouth or gum soreness or inflammation.⁷

Throughout India rural people use Neem (*A.indica*) twig, banyan (*F.benghalensis*) tree barks and leaves to brush their teeth, and keep their gums free of disease and infection, even though they have limited access to modern dental care. The ancient Ayurvedic practice of using Neem, banyan to heal and rejuvenate gum tissue and to prevent cavities and gum disease is verified in modern clinical studies.⁸ study shows that Neem and banyan extract is used to treat dental plaque and gingivitis.⁹ The medicinal utilities of neem, banyan have been described, especially for leaf, fruit and bark¹⁰. Neem, banyan bark and leaf extracts have been therapeutically used as folk medicine to control leprosy, intestinal helminthiasis, respiratory disorders, constipation and also as a general health promoter¹¹ Neem leaf aqueous extract effectively suppresses oral squamous cell carcinoma induced by 7,12-dimethylbenz[a] anthracene (DMBA), as revealed by reduced incidence of neoplasm.¹² The aqueous extract of neem leaf shows good antioxidant activity.¹³ The crude ethanolic extract of stem bark and root bark showed hypotensive, spasmolytic and diuretic activities.^{14,15} Peppermint leaves (*Menthapiperita*) is also known to have many medicinal properties as well as it is known to have beneficial aromatic effects.

So the aim of the present study to formulate a herbal based mouthwash formulation to provide more beneficial effects and reduce toxic effects of the commercially available chemical based mouthwashes.



Fig. 1: Various steps in the preparation of mouthwash and brine shrimp lethality assay testing.

MATERIALS AND METHODS

Mouthwash Formulation

The chemicals and media used in this study were purchased from Himedia, Mumbai, India. Q3 1g of each *F.benghalensis*, *A.indica*, *M.piperita* extract was taken in a beaker. The extract was then mixed with 100 ml of distilled water. The prepared mixture was then subjected to 15 minutes of constant boiling. An Extract was obtained which was then filtered to 50-60 ml to get the final extract.(Fig.1)

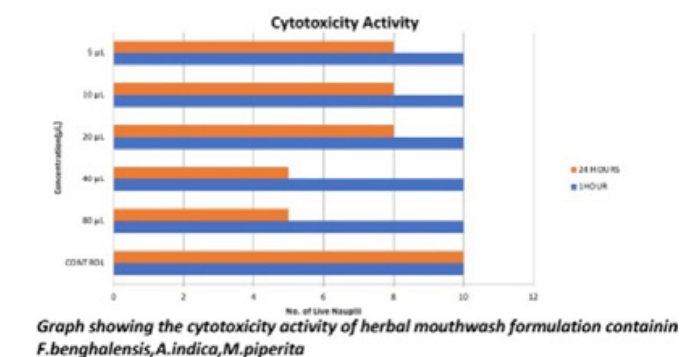
Cytotoxicity Activity Testing

Cytotoxicity activity of the prepared mouthwash formulation was done using Brine shrimp lethality assay.

Antimicrobial Effect Testing

Antibacterial Activity

The bacterial cultures used in this study was collected from Microbiology Lab, Saveetha Dental College and Hospitals, Chennai. The Antibacterial activity of the mouthwash against the strain *Staphylococcus aureus*, *Bacillus*, and *E.coli*. MHA agar was utilized for this activity to determine the zone of inhibition. Muller hinton



Graph showing the cytotoxicity activity of herbal mouthwash formulation containin *F.benghalensis*, *A.indica*, *M.piperita*

Fig. 2: Results of antimicrobial testing and cytotoxicity testing.

agar were prepared and sterilized for 45 minutes at 120 lbs. Media poured into the sterilized plates and let stable for solidification. The wells were cut using the well cutter and the test organisms were swabbed. The mouthwash with different concentration were loaded and the plates were incubated for 24 hours at 37°C. After the incubation time the zone of inhibition were measured.¹⁶⁻²¹

Antifungal activity

Aspergillus fumigatus, *Aspergillus flavus*, *Aspergillus niger* are used as test pathogens by agar well diffusion assay. Sabouraud's Dextrose Agar is used to prepare the medium. The prepared and sterilized medium was swabbed with test organisms and mouthwash with different concentration were added to the wells. The plates were incubated at 28°C for 48-72 hours. After the incubation time the zone of inhibition were measured.^{22,23}(Fig.2)

Brine Shrimp Lethality Assay:

Salt water preparation:

2g of iodine free salt was weighed and dissolved in 200ml of distilled water.

6 well ELISA plates were taken and 10-12 ml of saline water was filled. To that 10 nauplii were slowly added to each well (20µL, 40 µL, 60 µL, 80 µL, 100 µL). Then the mouth wash were added according to the concentration level. The plates were incubated for 24 hours.²⁴⁻²⁷

After 24 hours, the ELISA plates were observed and noted for number of live nauplii's present and calculated by using following formula,

number of dead nauplii/number of dead nauplii+number of live nauplii×100 (fig.2)

RESULTS & DISCUSSION

Antimicrobial activity

The antimicrobial activity of herbal mouthwash formulation containing *F.benghalensis*, *A.indica*, *M.piperita* is depicted in figure 2. The effects of herbal mouthwash formulation were tested for the antimicrobial activity against *Streptococcus mutans*, *Lactobacillus*, *E.faecalis* and *Candida albicans*. The mouthwash showed a very small zone of inhibition against *S. mutans*, *S.aureus* and *E.faecalis* showed 12mm, 14, 15 mm at 100µL concentration. The maximum zone of inhibition for *C.albicans* is 11 mm at 100 µL concentration and The antibacterial effect of mouthwash formulation on microbes have the ability to attach to the bacterial cell and eventually penetrate into it thus causing structural changes in the cell membrane like the permeability of the cell membrane and death of the cell.¹⁶⁻²⁰ The antimicrobial activity of the mouthwash formulation showed that the mouthwash formulation had the highest zone of inhibition seen in 100 µL concentration on *C.albicans* almost similar to the control antibiotic in comparison to the oral pathogens.(Fig.2)

Cytotoxicity Activity

The cytotoxic activity of herbal mouthwash formulation was assessed by using Brine shrimp Lethality test. The mouthwash formulation did not show cytotoxic activity on brine shrimp as all the shrimps in the 6 wells in which the extract were added survived on the first day. From the brine shrimp lethality test done, it was noted that on the first day all the nauplii survived, and on the second day 8 nauplii survived at lower concentrations. As far as the concentration of the herbal mouthwash formulation, the cytotoxicity was found to be better at lower concentrations where 8 nauplii survived. Hence from the current study, it was noted that the lower

concentrations can be used for biomedical applications. (Fig.1)

CONCLUSION

Ficus benghalensis, *Azadirachta indica* & *Mentha piperita* extracts have many medicinal properties. Our study has evaluated the combination of these three extracts in a mouthwash formulation and has found positive results. The cytotoxic activity was found to be better at low concentration. 8 nauplii survived at 5 µL, 10 µL, 20 µL after 24 hours. The antimicrobial activity of the mouthwash formulation showed that the mouthwash formulation had the highest zone of inhibition seen in 100 µL concentration on *C.albicans* almost similar to the control antibiotic in comparison to the oral pathogens. So the herbal mouthwash formulation can be used for oral hygiene practice with lower concentrations.

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