

Antimicrobial and Cytotoxic Effect of Herbal Mouthwash Prepared Using Ethanolic Extract of *Ficus benghalensis* and *stevia*

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ABSTRACT

Ficus benghalensis, commonly known as the banyan, banyan fig and they belong to the family moraceae. Around 750 species of plants occurring in tropical and subtropical forests belong to the genus *Ficus*. These have been found to possess medicinal properties. *Stevia* also known as *stevia rebaudiana* belongs to the family Chrysanthemum. This plant was called caá-êhê, meaning "sweet herb" by Guarani Indians of the Paraguayan highlands as they are nonnutritive sweeteners. The main aim is to study the antimicrobial and cytotoxic effect of herbal mouthwash prepared using ethanolic extract of *Ficus benghalensis* and *stevia*. 0.5g *stevia* and 0.5g *Ficus benghalensis* plant extract powder was measured and transferred into the conical flask. Mouthwash was prepared using ethanolic extract of *stevia* and *Ficus benghalensis* and the cytotoxicity and antimicrobial activity was determined. In order to determine whether the prepared bioactive compound is toxic to cells, evaluation of cytotoxicity is useful which is done using hatched nauplii. A clean ELISA plate was taken and the wells were marked with the concentration of 5µl, 10µl, 20µl, 40µl, 80µl, control respectively. Each well was filled with 3ml of salt water and 10 hatched nauplii or brine shrimp were collected and added to the wells. Mouthwash was pipetted with respective concentration into the wells with nauplii and kept for 24hrs. To analyse the toxicity of ethanol extract mouthwash, the count of nauplii was taken after 24 hrs excluding the dead nauplius. The mueller Hinton agar and nutrient broth were purchased from Hi-media, India. By agar well-diffusion method, the antimicrobial activity of prepared *stevia* and *Ficus benghalensis* ethanol extract based mouthwash was tested. Pathogenic isolates *S.mutans* and *Lactobacillus* were the culture used for bactericidal activity. Three different concentrations (25µl, 50µl, 100µl) of mouthwash solution were poured into each well on plates using sterile micropipette and were kept in an incubator at 37 degree Celsius for 24hrs. The bacterial zone of inhibition was observed and measured after incubation. Cytotoxicity and antimicrobial activity was determined. As the concentration increased the cytotoxicity of the herbal mouthwash prepared using ethanolic extract increased. The highest percentage of death of nauplii was at 80 µl concentration of ethanolic extract mouthwash. As the concentration of the mouthwash increased, the mean zone of inhibition (ZOI) was found to increase. Herbal mouth rinse showed antimicrobial activity which is as effective as antibiotics against *Lactobacillus* and has a superior effect on *S.mutans*. Findings from the study suggest that herbal mouthwash prepared using ethanolic extract of *Ficus benghalensis* and *stevia* have a potential cytotoxic and antimicrobial effect and can be used as an alternative to commercially available products.

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How to cite this article: Ranjana V, Rajeshkumar S, Ezhilarasan D, Lakshmi T. Antimicrobial and Cytotoxic Effect of Herbal Mouthwash Prepared Using Ethanolic Extract of *Ficus benghalensis* and *stevia*. Journal of Complementary Medicine Research, Vol. 13, No. 2, 2022 (pp. 102-108).

BACKGROUND

Ficus benghalensis, commonly known as the banyan, banyan fig and they belong to the family moraceae. Around 750 species of plants occurring in tropical and subtropical forests belong to the genus *Ficus*. These have been found to possess medicinal properties.^[1] Fruits and seeds of this plant have the property of coolant and tonic. Leaves of these plants are good for ulcers. For the treatment of inflammatory diseases such as rheumatism, the stem bark of *Ficus benghalensis* which contains milky juice (latex) is very effective.^[2] *Stevia* also known as *stevia rebaudiana* belongs to the family Chrysanthemum. This plant was called caá-êhê, meaning "sweet herb" by Guarani Indians of the Paraguayan highlands as they are nonnutritive sweeteners.^[3] *Stevia* contains sweet diterpene glycosides which have a defensive capacity acting as antimicrobial agents against specific herbivores, pathogens or pests.^[4]

KEYWORDS:

Anti microbial activity, Cytotoxic effect, Herbal mouthwash, Innovative technique, *Ficus benghalensis*, *Stevia*, Ethanolic extract,

ARTICLE HISTORY:

Received: Jan 30, 2022

Accepted: Mar 28, 2022

Published: May 12, 2022

DOI:

10.5455/jcmr.2022.13.02.20

Since this plant has very few calories, it is preferred as an alternative to added sugar in a variety of meals and beverages.^[5] Eight glycosides (stevioside, rebaudiosides A, rebaudiosides C, rebaudiosides D, rebaudiosides E, rebaudiosides F, steviolbioside, dulcoside A) are present in stevia which are the sweet components that are purified and isolated from the leaves of this plant. Stevia is more helpful in diabetic patients as they help in keeping the blood sugar level in check.^[6]

Mouthwashes, which is also known as oral rinse, is mainly being used to reduce the microbial load in the oral cavity and to control halitosis depending on the ingredients present in the particular mouthwash.^[7] Oral rinse comes in contact with the oral mucosa and with the underlying gingival connective tissue when the oral cavity is being flushed. Oral rinse contains aloe vera, anti-inflammatory glucocorticoids, coloring agent, flavouring agent, glycerin, antifungal nystatin, etc which helps in preventing or decreasing the severity of oral disease.^[8] Herbs like tulsi patra, pudina, triphala, green tea, neem, clove oil, aloe vera are used in oral rinse which is both promotive and preventive in its approach. All the herbal mouthwashes are alcohol/sugar free.^[9]

In order to control supragingival plaque and gingivitis, the antimicrobial activity is used and also it is utilized before oral and periodontal surgery which includes implant placement and tooth extraction.^[10] Ethanol shows antimicrobial activity and is effective against oral microorganisms, *streptococcus mutans*. Cetylpyridinium chloride, present in mouthwash, possess antimicrobial properties.^[11] Natural herbal oral rinse has beneficial properties like anticariogenic and antiplaque effects. Antibacterial action is because of the presence of flavonoids which inhibits the enzymatic activity and disrupts the cytoplasmic membrane on the cell. In general, compared to synthetic compounds, natural extracts are known to show a lower cytotoxicity.^[12]

There are very few validation studies that have been carried out previously on the Antimicrobial and cytotoxic effect of herbal mouthwash prepared using ethanolic extract of *Ficus benghalensis* and *stevia*. Although there is evidence that single ingredients in mouthwash are effective against bacteria and are cytotoxic,^[13] the impact of a complex cocktail of supplements

on oral cell cytotoxicity and antimicrobial activity are largely unknown.

The main aim is to study the Antimicrobial and cytotoxic effect of herbal mouthwash prepared using ethanolic extract of *Ficus benghalensis* and *stevia*.

MATERIALS AND METHOD

Study Design

This is an In-vitro study. Before scheduling of the In-vitro study, the official permission was obtained from the institutional ethical committee (Ethical approval number - IHEC/SDC/UG-1951/21/73).

Preparation of stevia and ficus benghalensis

Stevia and *Ficus benghalensis* plant extract powder were collected. 100 ml clean conical flask was taken and 0.5g *stevia* and 0.5g *Ficus benghalensis* was measured and transferred into it [Figure 1]. 50ml ethanol solution was added to the conical flask and it was placed in the stirrer for 24-48 hours. Later color change was observed where pale brown turned dark brown [Figure 2]. Then the mixture was boiled until the solution became 10ml which was then filtered in the conical flask with a filter paper. Color change of the extract from dark brown to yellowish brown was observed [Figure 3].

Preparation of mouthwash with ethanol extract

A clean centrifuge tube was taken and 0.3g of sucrose, 0.001g of sodium benzoate, 0.01g of SLS, 100µl of peppermint oil, 100ml of water (H₂O) were measured and added in it and 600µl prepared ethanol extract of *stevia* and *Ficus benghalensis* was measured and mixed well in the centrifuge [Figure 4].

Evaluation of cytotoxic activity in stevia and ficus benghalensis ethanol extract based mouthwash

In order to determine whether the prepared bioactive compound is toxic to cells, evaluation of cytotoxicity is useful which is done using hatched nauplii [14]. A clean ELISA plate was taken and the wells were marked with the concentration



Fig. 1: Depicts the measured stevia and Ficus benghalensis powder in a conical flask.

of 5µl, 10µl, 20µl, 40µl, 80µl, control respectively. Each well was filled with 3ml of salt water and 10 hatched nauplii or brine shrimp were collected and added to the wells. Mouthwash was pipetted with respective concentration into the wells with nauplii and kept for 24hrs. To analyse the toxicity of ethanol extract mouthwash, the count of nauplii was taken after 24 hrs excluding the dead nauplius [figure 5].

$$\% \text{ Death} = \frac{\text{Number of dead nauplii}}{\text{Number of dead nauplii} + \text{number of live nauplii}} \times 100$$

Antimicrobial activity of *stevia* and *Ficus benghalensis* ethanol extract and its mouthwash

Preparation of plates

The mueller Hinton agar and nutrient broth were purchased from Hi-media, India. By agar well-diffusion method, the antimicrobial activity of prepared *stevia* and *Ficus benghalensis*



Fig. 2: Depicts the mixture of ethanol and *stevia* and *Ficus benghalensis* powder

ethanol extract based mouthwash was tested. Pathogenic isolates *S.mutans* and *Lactobacillus* were the culture used for bactericidal activity.^[15] Three different concentrations (25µl, 50µl, 100µl) of mouthwash solution were poured into each well on plates using sterile micropipette and were kept in an incubator at 37 degree Celsius for 24hrs. The bacterial zone of inhibition was observed and measured after incubation.^[16] [figure 6].

RESULTS AND DISCUSSION

Graph 1 depicts Cytotoxicity: Assessing the number of live nauplii after 24 h where X axis represents the concentration and Y axis represents the live nauplii on day 1 (blue) and day 2 (red). From the graph, it is shown that 100% nauplii were present in the control group, 5µl and 10µl concentration there was a death of 10% of nauplii, at 20µl concentration there was a death of 20% of nauplii, at 40µl concentration there was a death of 50% of nauplii and at 80µl concentration there was a death of 90% of nauplii. Table 1 depicts Cytotoxicity: Assessing the number of live nauplii after 24 h. There were 10 nauplii present on day 1 in all concentrations of the mouthwash whereas there was a gradual decrease in the number of live nauplii as the concentration of the mouthwash increased. The highest percentage of death of nauplii was at 80 µl concentration of ethanolic extract mouthwash. Graph 2: Zone of inhibition by *Lactobacillus* (blue) and *S. Mutans* (red). From the graph, it is shown that the zone of inhibition by *lactobacillus* at 25µl, 50µl and 100µl concentration was 9mm and zone of inhibition by *S.mutans* at 25µl concentration was 10mm, at 50µl concentration was 18mm and at 100µl concentration was 26mm. Table 2 depicts the Zone of inhibition by *Lactobacillus* and *S. Mutants*. As the concentration of the mouthwash increased, the mean zone of inhibition (ZOI) was found to increase where the antibiotics were 21 mm and 30 mm for *lactobacillus* and *S. mutans* respectively. Figure 1 depicts the measured *stevia* and *Ficus benghalensis* powder in conical flasks. Figure 2 depicts the mixture of ethanol and *stevia* and *Ficus benghalensis* powder. Figure 3 depicts the preparation

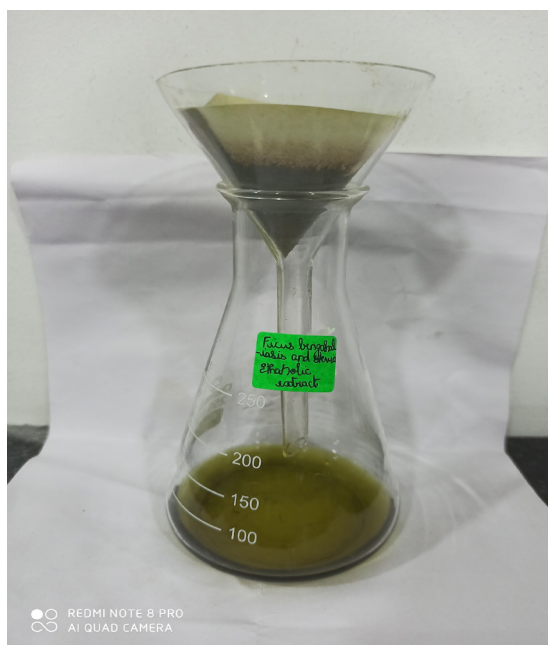
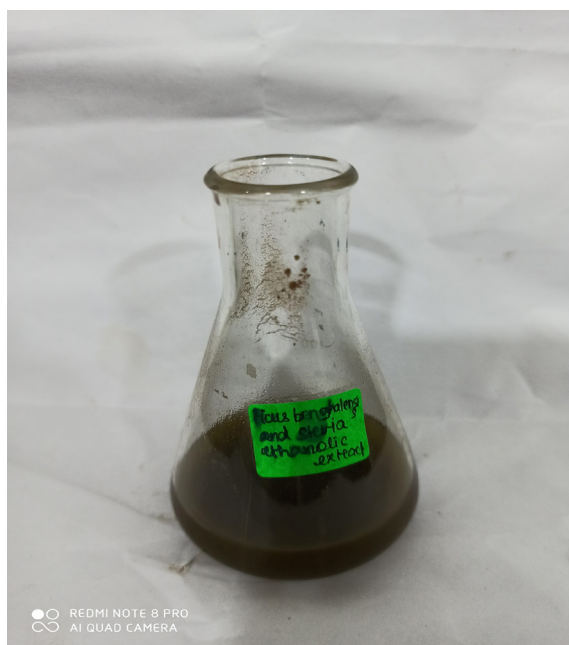


Fig. 3: Depicts the preparation of *stevia* and *Ficus benghalensis* of ethanol extract

of *stevia* and *Ficus benghalensis* of ethanol extract. Figure 4 depicts the preparation of mouthwash. Figure 5 depicts the cytotoxicity: assessing the number of live nauplii after 24hrs. Figure 6 depicts the zone of inhibition by *stevia* and *Ficus benghalensis* ethanol extract based mouthwash.

In this research, we investigated herbal mouthwash prepared using ethanolic extract of *Ficus benghalensis* and *stevia* with regard to their cytotoxic and antimicrobial effects on bacteria and oral cells. In the medical field, there is an increasing tendency to operate for therapeutic agents from natural sources which is also useful in management of dental caries .^[17] Herbal mouthwash is beneficial for unpleasant breath, gum soreness or inflammation. It also serves as an aid for canker sores.^[18] The properties of the hygiene products can be improved with antimicrobial activity, in order to preserve oral health.^[19] Oral rinse with natural ingredients helps in good and complete removal of the oral infections causative organisms with less after effects.^[20] Therefore Natural products can be a template to synthetic chemical substances. As a result herbal



Fig. 4: Depicts the preparation of mouthwash

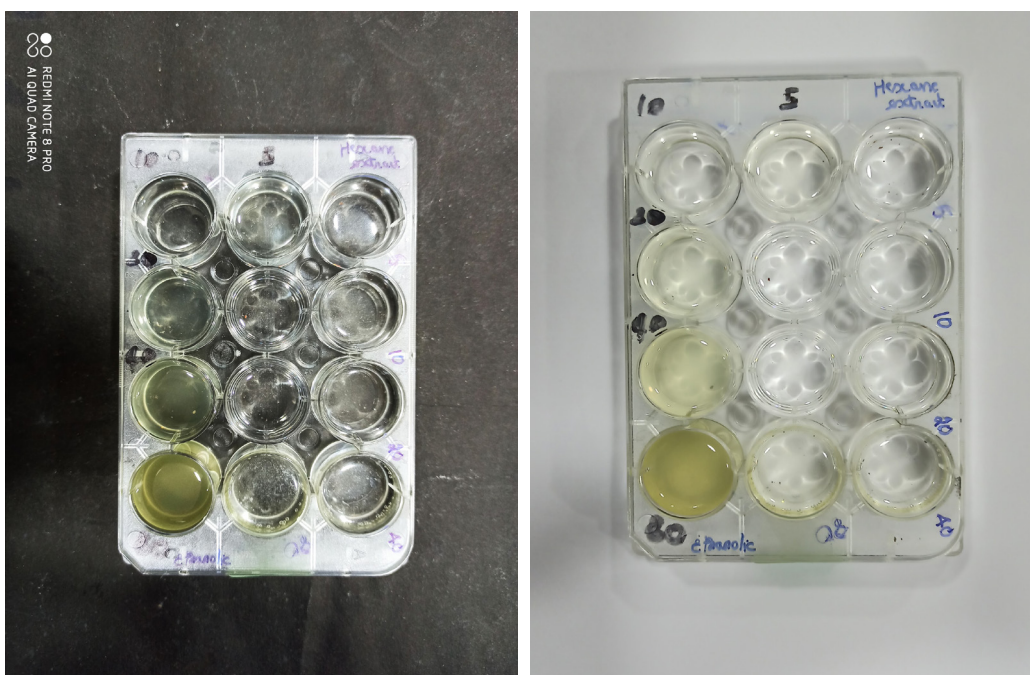
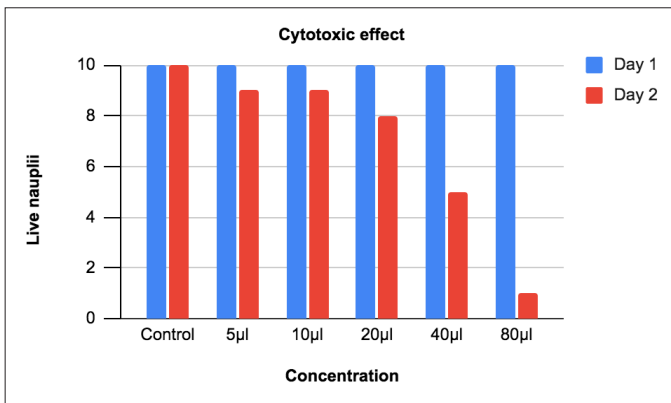


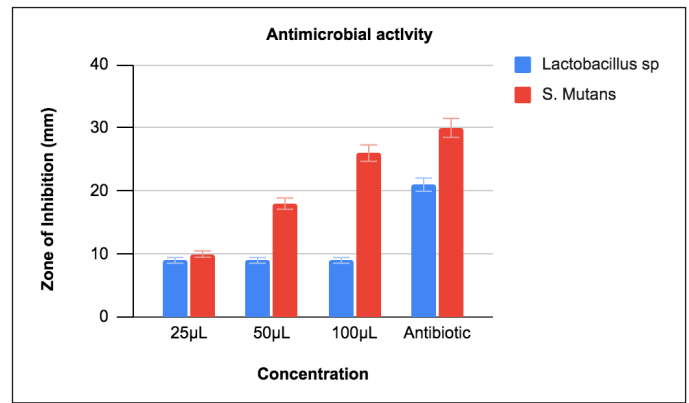
Fig. 5: Depicts the cytotoxicity: assessing the number live of nauplii after 24hrs



Fig. 6: Depicts the zone of inhibition by *stevia* and *Ficus benghalensis* ethanol extract based mouthwash.



Graph 1: Cytotoxicity: Assessing the number of live nauplii after 24 h where X axis represents the concentration and Y axis represents the live nauplii on day 1 (blue) and day 2 (red).



Graph 2: Zone of inhibition by Lactobacillus (blue) and *S. Mutans* (red).

Table 1: Cytotoxicity: Assessing the number of live nauplii after 24 h

	Control	5µl	10µl	20µl	40µl	80µl
Day 1	10	10	10	10	10	10
Day 2	10	9	9	8	5	1

Table 2: Zone of inhibition by Lactobacillus and *S. Mutans*

Concentration	Lactobacillus(mm)	S. Mutans(mm)
25µl	9	10
50µl	9	18
100µl	9	26
Antibiotic	21	30

products with antimicrobial activity may be used to get rid of infecting microorganisms in appropriate concentration.^[21]

In a study done by Satheesh et al, they revealed that the antimicrobial activity against both gram positive and negative bacteria was shown by the leaf extracts from *Diospyros blancoi*. The crude ethanolic extract of *D. blancoi* produced Zone of inhibition which was microorganism specific and ranged from 9.00-12.00 mm compared with standard erythromycin 7.50-32.50 mm at a concentration of 250 µg/disc and 500 µg/disc,^[22] Whereas in this study, it was seen that the zone of inhibition by *lactobacillus* at 25µl, 50µl and 100µl concentration was 9mm and zone of inhibition by *S.mutans* at 25µl concentration was 10mm, at 50µl concentration was 18mm and at 100µl concentration was 26mm. As the concentration of the mouthwash increased, the mean zone of inhibition (ZOI) was found to increase.^[23] Herbal mouth rinse showed antimicrobial activity which is as effective as antibiotics against *Lactobacillus* and has a superior effect on *S.mutans*.^[24]

In a study done by Howlader et al, it was revealed that the lethality was shown by the ethanolic extract indicating the biological activity of the compound present in the extract. Different mortality rates were shown by the test samples at different concentrations,^[25] Whereas in the present study, it was seen that at 5µl and 10µl concentration there was a death of 10% of nauplii, at 20µl concentration there was a death of 20% of nauplii, at 40µl concentration there was a death of 50%

of nauplii and at 80µl concentration there was a death of 90% of nauplii. As the concentration increased, the cytotoxicity of the herbal mouthwash prepared using ethanolic extract increased. The highest percentage of death of nauplii was at 80 µl concentration of ethanolic extract mouthwash.

Our team has extensive knowledge and research experience that has translated into high quality publications.^[26-30-60]

We therefore undertook this study to evaluate the cytotoxicity, antimicrobial effect of herbal mouthwash prepared using ethanolic extract of *Ficus benghalensis* and *stevia*.

Limitation

The study was conducted in vitro, so it cannot be assumed that the results of cytotoxicity, anti-microbial activity could be translated into clinical effectiveness.

Recommendation

In vivo studies are recommended with people' acceptance values in further studies. This product in the form of mouthwash can be given to the patients.

CONCLUSION

Findings from the study suggest that herbal mouthwash prepared using ethanolic extract of *Ficus benghalensis* and *stevia* have a potential cytotoxic and antimicrobial effect and can be used as an alternative to commercially available products.

Acknowledgement

The authors would like to thank Saveetha Institute of Medical and Technical Sciences for providing a platform to conduct this research.

Source of funding

The present study was supported by the following agencies:

Saveetha Dental College, SIMATS, Saveetha University, Edubridge learning private limited.

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