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RESEARCH ARTICLE
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Preparation of *Stevia* and *Ficus Benghalensis* Hexane Extract and Its Based Mouthwash

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ABSTRACT

Stevia is a non-caloric sweetener mostly used for medicinal and dental purposes as it is found in mouth rinse and chewing gums due to its decreased cariogenic and demineralizing potential. *Ficus benghalensis* (banyan tree) was used in a previous study to prepare an ethanolic extract to analyze its antimicrobial efficacy among gram negative and gram positive bacterium and it was found to be a potent antimicrobial and antiseptic against the infection caused by gram positive and gram negative bacteria. *Stevia* and *Ficus benghalensis* plants were collected and hexane extract was prepared, using the prepared extract mouthwash with which cytotoxicity and antimicrobial activity was checked. The antibacterial activity of the prepared mouthwash was confirmed by the zone of inhibition formed against *Lactobacillus sp., Streptococcus mutans* and cytotoxicity tested and the death and inhibition of growth of the organisms was observed only in higher concentrations of 40 and 80µl which were comparatively higher than citronella oil. *Stevia* and *Ficus benghalensis* hexane extract based mouthwash has potent antimicrobial and anti cariogenicity which can be patented and used for maintaining oral hygiene.

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BACKGROUND

Mouthwashes are aqueous solutions used world wide to maintain oral hygiene as it has various antimicrobial agents, antiseptic agents which helps in controlling plaque. It is also used as a deodorant which may contain various flavouring agents, colouring agents, synthetic sweeteners, glycerine and so on. There are many commercially available mouthwashes such as chlorhexidine, listerine etc. which have certain side effects such as dry mouth, taste disturbance. Considering the side effects of commercially available mouthwash. The current study has taken the herbal ingredients such as *Stevia* and *Ficus benghalensis* to prepare mouthwash and to determine its toxicity.[1-2]

Stevia originated from South America Specifically Brazil, Argentina and Paraguay. It is used as a substitute for sugar as it is a natural sweetening agent.[1] It is a non-caloric sweetener mostly used for medicinal and dental purposes as it is found in mouth rinse and chewing gums due to its decreased cariogenic and demineralizing potential.[2] *Ficus benghalensis* originated from the Indian subcontinent, commonly called banyan fig or banyan.[3] It is rich in flavonoids which are responsible for defensive action against microbial infections. It acts as a potent antibacterial and antifungal agent.[3-6]

Ficus benghalensis (banyan tree) was used in a previous study to prepare an ethanolic extract to analyze its antimicrobial efficacy among gram negative and gram positive bacterium and it was found to be a potent antimicrobial and antiseptic against the infection caused by gram positive and gram negative bacteria.[7] Similarly the *Ficus benghalensis* was used to synthesize green silver nanoparticles by Antariksh saxena et al (2012) to detect antimicrobial activity against *E.coli* which was interpreted to be a positive antimicrobial agent.[8] The property of being toxic to cells is called cytotoxicity which affects the growth by various factors or leads to death. The toxic compounds are even present in plants or other natural sources and cause cell death in varying proportions. Depending on concentration the toxicity of each compound differs. The *Stevia* and *Ficus benghalensis* are tested for its toxicity.[9]

The current study is done with an exclusive combination of *Stevia* and *Ficus benghalensis* to prepare hexane extract mediated mouthwash[9] due to there potent antimicrobial and antiseptic property against variety of gram positive and gram negative bacterium which may act effective against oral pathogens, no

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similar combination had been used to prepare hexane extract mediated mouthwash. Our team has extensive knowledge and research experience that has translated into high quality publications.[10, 14-16],[10],[17-44] Hence, It is a unique study done with the combination to prepare a potent mouthwash with antimicrobial activity.[45] So, the current study is done to prepare *Stevia* and *Ficus benghalensis* hexane extract and its based mouthwash.

MATERIALS AND METHODS

Preparation of Stevia and Ficus benghalensis hexane extract :

Stevia and *Ficus benghalensis* plant powders were collected 0.5g of *Stevia* and 0.5g of *Ficus benghalensis* was measured and poured into a 100ml clean conical flask (Figure 1). 50ml of cyclohexane chemical solution was measured and poured into a conical flask and the extract was placed in the stirrer for 24-48 hours, the color change was observed from pale brown to dark brown (Figure 2). Then the mixture was boiled till 10ml of solution was obtained, it was filtered into conical flasks with a Whatman No.1 filter paper and the extract was observed for the color change from dark brown to yellowish brown (Figure 3) .

Preparation of mouthwash with the hexane extract

A clean centrifuge tube was taken and 0.3g of sucrose, 0.001g of sodium benzoate, 0.01g of SLS, 100¤l of peppermint oil, 100ml of water (H2O) were measured and poured into the centrifuge tube and 600¤l prepared *Stevia* and *Ficus benghalensis* hexane extract was measured and mixed well (Figure 8).

Evaluation of Cytotoxicity in Stevia and Ficus benghalensis hexane extract based mouthwash

Cytotoxicity determines whether the prepared bioactive compound is toxic to cells [46]. The hatched nauplii is used to determine cytotoxicity. ELISA plate was taken and washed with running water and wells were marked with the concentration of 5µl, 10µl, 20µl, 40µl, 80µl respectively and one well was considered as a control. Each well was filled with 3ml of salt water and 10 hatched nauplii or brine shrimp were collected and added in wells and the mouthwash was pipetted with respective concentration and mixed to the wells with nauplii and kept for 24 hours. The nauplius were counted after 24 hours to analyze the toxicity of hexane based mouthwash (Figure 5).

Antimicrobial activity of Stevia and Ficus benghalensis hexane extract and its mouthwash:

Preparation of plates

From Hi-media, The Nutrient broth and Muller Hinton agar was purchased. The prepared *Stevia* and *Ficus benghalensis* hexane extract based mouthwash was tested for antibacterial activity by agar well-diffusion method. The cultures used for the

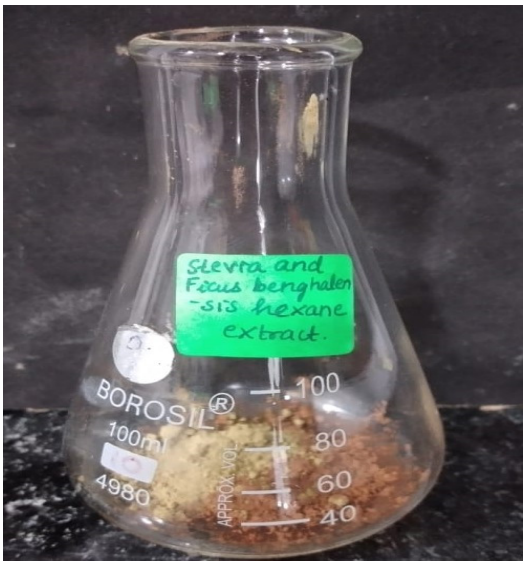


Fig. 1: Depicts measured Stevia and Ficus benghalensis powder in conical flask with digital balance

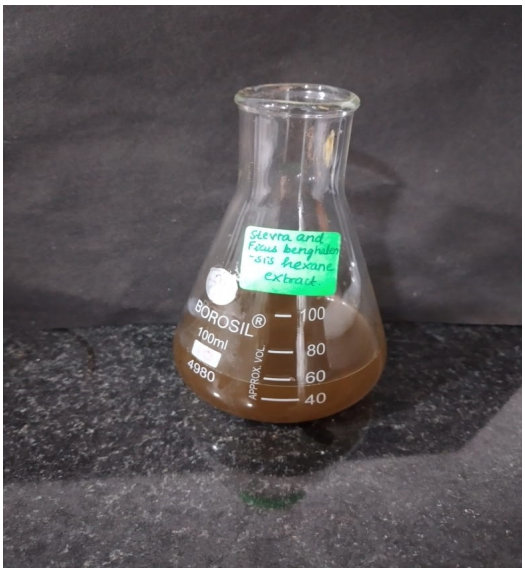


Fig.2: Depicts the mixture of hexane and Stevia and *Ficus benghalensis*

Fig. 3: Depicts the preparation of Stevia and Ficus benghalensis of hexane extract

bactericidal activity were pathogenic isolates *Streptococcus mutans* and *Lactobacilli* .The three different concentration of mouthwash samples (25µl,50µl,100µl) solution was poured onto each well on all plates using a sterile micropipette and kept in incubator for 24 hours at 37°C. After incubation, the different levels of the zone of inhibition of bacteria were observed and measured (Figure 6).

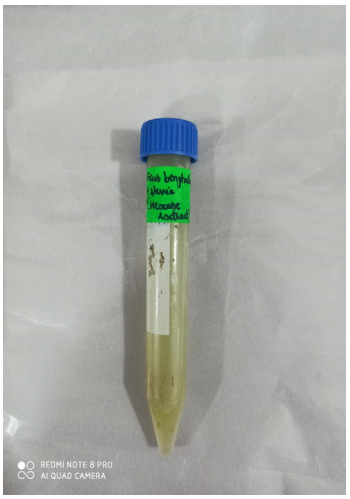


Fig. 4: Depicts the preparation of mouthwash

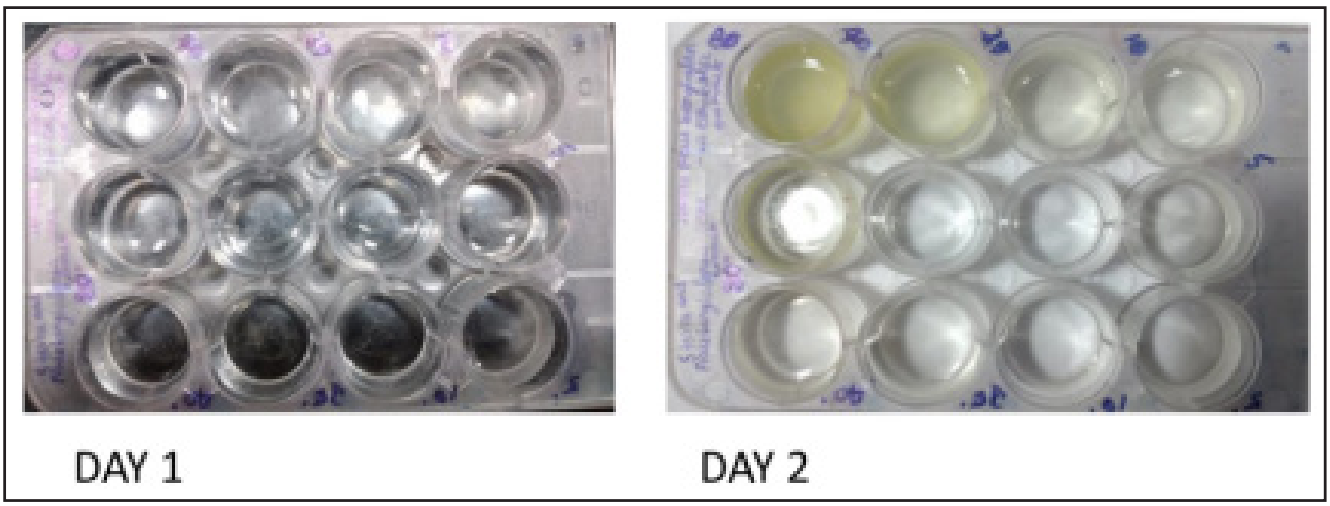
RESULT:

The antibacterial activity of the prepared mouthwash was confirmed by the zone of inhibition formed against *Lactobacillus sp.* (Figure 6), *Streptococcus mutans* (Figure 6) as shown in Table 1 and graph 1 . Potent antibacterial activity was observed against *Streptococcus mutans* in 100µl with zone of inhibition 30mm which was equal to an antibiotic whereas the zone of inhibition in 25µl and 50µl was 18mm and 25mm whereas the zone of inhibition (9mm) was less when observed for *Lactobacillus species* in all concentration (25µl, 50µl, 100µl) on comparison with antibiotic.

On testing the cytotoxicity of prepared mouthwash, the death of nauplii was observed only in higher concentrations 40µl and 80µl (Figure 3) (Table 3). There was no cytotoxic potential observed in lower concentrations as the majority of nauplii were alive (5µl, 10µl, 20µl).

DISCUSSION

As we all know that Tulsi is known for many of its medicinal properties and used by people in daily life but it may be new to hear about *Stevia* which is a type of tulsi and used as a potent sweetening agent which was found to have anticariogenic property.[47]



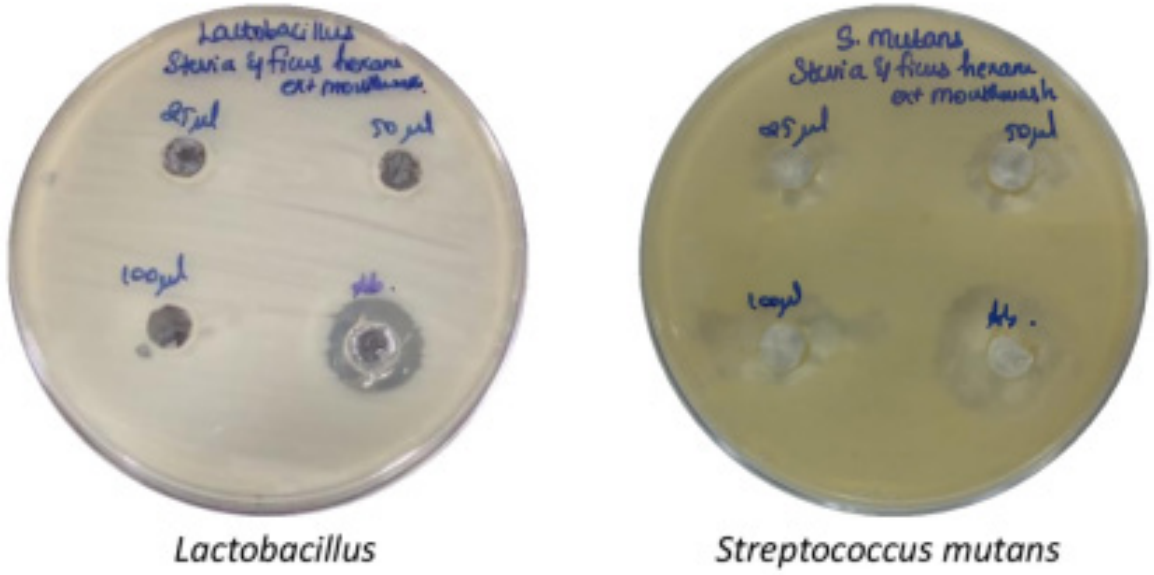
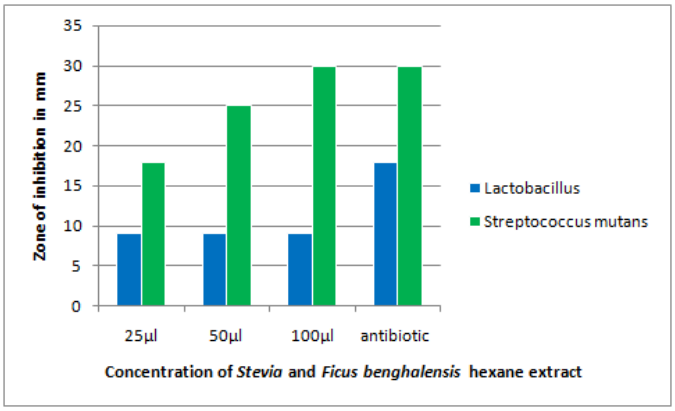


Figure 6 Depicts the Zone of inhibition by *Stevia* and *Ficus benghalensis* hexane extract based mouthwash

Table 1: The Zone of inhibition by *Stevia* and *Ficus benghalensis* hexane extract based mouthwash for two different oral pathogens (*Lactobacillus*, *Streptococcus mutans*)

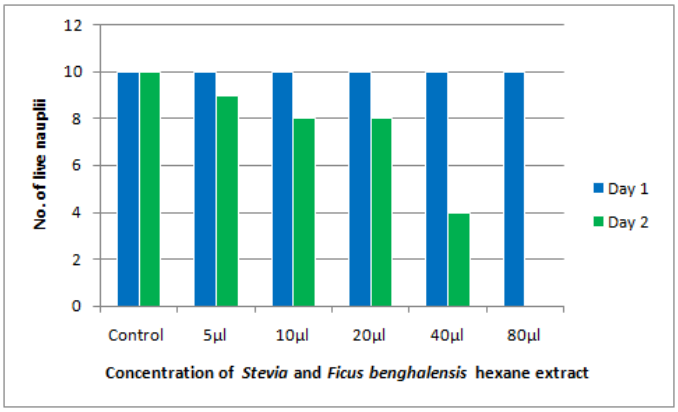
Concentration of Stevia and Ficus benghalensis hexane extract based mouthwash	*Lactobacillus (in mm)*	*Streptococcus mutans (in mm)*
25µl	9	18
50µl	9	25
100µl	9	30
Antibiotic	18	30



Graph 1: Bar chart depicts the Zone of inhibition by *Stevia* and *ficus benghalensis* hexane extract based mouthwash for two different oral pathogens. Lactobacillus is signified by blue color with maximum zone of inhibition (18mm) in antibiotic whereas Streptococcus mutans signified by green color with maximum zone of inhibition (30mm) at 100µl concentration. The X- axis represents the concentration of Stevia and Ficus benghalensis hexane extract (25µl, 50µl, 100µl) and the Y-axis represents the zone of inhibition in mm against Lactobacillus and Streptococcus mutans.

Table 2: The Cytotoxicity effect of prepared hexane based herbal mouthwash by assessing the number of live napulii after 24 h

Concentration of Stevia and Ficus benghalensis hexane extract based mouthwash (In microlitres)	*No. of live organism (Day 1)*	*No. of live organism (Day 2)*
Control	10	10
5µl	10	9
10µl	10	8
20µl	10	8
40µl	10	4
80µl	10	0



Graph 2: Bar chart depicts the Cytotoxicity effect of prepared hexane based herbal mouthwash by assessing the number of live napulii after 24 h. On day 1, 10 nauplii were alive in all concentration whereas on day 2 the maximum number of nauplii died at 80µl and maximum alive at 5µl. The blue color signifies day 1 and green signifies day 2. The X-axis represents the concentration of Stevia and Ficus benghalensis hexane extract (5µl, 10µl, 20µl, 40µl, 80µl) and the Y-axis represents number of live nauplii on day 1and day 2.

Therefore the current study has used *Stevia* in preparation of mouthwash along with cyclohexane[48] as the chemical has potent antimicrobial properties against gram positive and negative bacteria and antifungal activity.[49] *Ficus Benghalensis* is used for it's antimicrobial and antifungal properties against infection of bacteria.[7]

In 2018 Verma UP et al has tested the cytotoxicity effect[50] of chlorhexidine mouthwash and found the maximum cell death[51] in 10% chlorhexidine whereas in the current study hexane extract based mouthwash with *Stevia* and *Ficus benghalensis* was prepared and found its cytotoxicity (Figures 9 and 10) which showed maximum effect at 40µl and 80µl.[52] Similarly, another study done by Cunha BG et al in 2020 reports that Citronella oil showed maximum cell death in 50µl with 3 nauplius alive whereas in the current study the maximum cell death was seen only in 40µl and 80µl.

Based on the results of the current study we determine that the zone of inhibition for *Streptococcus mutans* was maximum at 100µl with 30mm inhibition whereas the study done by Manali Deb Barma et al shows 15 mm of zone of inhibition for *Streptococcus mutans* in 100µl with the mouthwash prepared from silica nanoparticles.[9,53,54] Similarly another study done by Subashree Mohapatra et al reports that the Zinc Oxide nanoparticle synthesized by clove and cinnamon formulation showed 15mm of inhibition against *Streptococcus mutans* in 100µl of concentration which is less compared to the present study.[55]

A previous study done in 2020 had reported the preparation of citronella extract based mouthwash and its antimicrobial activity was detected. The zone of inhibition was found to be maximum at 50µl measured as 12mm for *Staphylococcus aureus* and 40µl measured as 10 mm for *Candida albicans* whereas in current study the zone of inhibition was maximum only at 100µl measured as 9mm for *Lactobacillus* (Figure 11) and 30mm for *Streptococcus mutans* (Figure 12).[56] Hence the current study states that the mouthwash has potent inhibition with *Streptococcus mutans* it can be used to prevent dental caries.

The limitation of current study is that the preparation of mouthwash can be done with the plant extract alone to reduce the toxicity more and any other combination can be used to prepare mouthwash to see the potency of other plants and the powder of the plants can be prepared in laboratory instead of using a preserved commercial product and a toothpaste can be prepared with same combination instead of mouthwash to determine its potency.

The future scope of the current study will be that the prepared mouthwash has to get patent to make it a potent prophylactic agent which can be used worldwide to prevent dental caries risk in population and based on the results of current study more studies can be done to prepared many dental products with the combination as its antimicrobial activity is stronger against *Streptococcus mutans*.

CONCLUSION

Within the limitations of the study, the current study concludes that *Stevia* and *icus benghalensis* hexane extract based mouthwash has potent antimicrobial activity against *Streptococcus mutans an*d has less cytotoxicity. Hence it has to be patented in future to make it a prophylactic agent against dental caries and increase oral hygiene.

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