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RESEARCH ARTICLE

EVALUATION OF THE ANTI-CARIOGENIC PROPERTY OF *STEVIA REBAUDIANA* - BASED NOVEL LOZENGES

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ABSTRACT

Background & Aim: Dental caries is a widespread oral health issue predominantly caused by *Streptococcus mutans*, a bacterium known for its ability to form biofilms on tooth surfaces. The use of non-cariogenic sweeteners with potential therapeutic benefits offers a promising strategy for caries prevention. *Stevia rebaudiana*, a natural plant-based sweetener, has shown antimicrobial properties, but its application in child-friendly formulations such as lozenges remains underexplored. This study aimed to develop lozenges using *Stevia rebaudiana* and evaluate their antibacterial and antibiofilm efficacy against *Streptococcus mutans*, comparing their activity to commercial sugar-free alternatives.

Methods: Lozenges were prepared using *Stevia rebaudiana* extract, palm sugar, ginger, lemon, and water. Extracted molar teeth were inoculated with *S. mutans* prepared in Brain Heart Infusion (BHI) broth. After incubation, treatments included natural sugar-free (control), commercial sugar-free green, and Stevia lozenges. The biofilm biomass was quantified using crystal violet staining, and viable bacterial counts were assessed through colony-forming unit (CFU) analysis after sonication and plating on BHI agar.

Results: The Stevia lozenges demonstrated a significant reduction in *S. mutans* viability and biofilm formation. CFU analysis showed 20 CFU/ml for both Stevia lozenge and sugar-free green treatments, in contrast to 1000 CFU/ml for the natural sugar-free control. Biofilm quantification supported these findings, indicating lower biomass in treated samples.

Conclusion: Lozenges formulated with *Stevia rebaudiana* effectively inhibited *S. mutans* growth and biofilm formation, showing comparable efficacy to commercial sugar-free products. This study highlights the potential of Stevia lozenges as a natural, non-cariogenic, and child-friendly alternative with therapeutic applications in oral health care.

Keywords: Anti-cariogenic, antimicrobial, biofilm, dental caries, lozenges

INTRODUCTION

Stevia rebaudiana, a species native to tropical South America, is renowned for its medicinal properties and natural sweetness[1]. This plant contains sweet compounds like rebaudioside A and stevioside, making it a potential aid in managing conditions such as diabetes, obesity, and hypertension. With the rise in demand for natural sweeteners, *Stevia rebaudiana*, containing stevioside and other glycosides, has gained popularity as a low-calorie alternative to sugar, particularly beneficial for diabetic individuals[2].

Stevia rebaudiana Bertoni leaves extract has been found to possess significant anti-cariogenic properties. This is attributed to its ability to combat the growth of cariogenic bacteria such as Lactobacillus acidophilus and Streptococcus mutans, which are known to be responsible for the development and progression of dental caries by forming biofilms on the surfaces of teeth[3,4]. Stevia extract contains various bioactive compounds, including flavonoids, alkaloids, saponins, sterols, and tannins, that have been shown to inhibit the growth of these bacteria, thereby providing a natural alternative for preventing dental caries[5]. Moreover, Stevia extract's antimicrobial properties have been found to extend to reducing bacterial load, biofilm formation, and maintaining oral pH levels, further supporting its anti-cariogenic potential[6].

This implies that Stevia extract can prevent the colonization and growth of cariogenic bacteria on the surface of the teeth, thus reducing the risk of dental caries. Additionally, Stevia extract is a safe and effective alternative to synthetic antimicrobial agents, making it an ideal choice for those seeking natural alternatives to prevent dental caries. Furthermore, Stevia's ability to reduce bacterial load, biofilm formation, and act as an anti-inflammatory agent contributes to its anti-cariogenic potential[7]. In vitro studies have demonstrated that *Stevia rebaudiana* ethanolic extract inhibits the acidogenic potential and formation of insoluble polysaccharides in Streptococcus mutans, further supporting its anti-cariogenic effects[8]. Overall, *Stevia rebaudiana* emerges as a promising option for oral health, offering benefits beyond just its sweetening properties. Therefore, this study aims to evaluate the anti-cariogenic property of a novel lozenge formulation containing *Stevia rebaudiana* extract. The study will utilize in vitro assays to assess the antimicrobial activity of the lozenges against common cariogenic bacteria, including Streptococcus mutans.

MATERIALS AND METHODS

Preparation of Lozenges

Ingredients needed to make lozenges include water, lemon, ginger, palm sugar, and *Stevia rebaudiana* leaves or powder. Palm sugar and water are cooked over a low flame until the sugar melts and the mixture takes on a liquid consistency. For flavor and medicinal benefits, lemon and ginger paste are added. Stevia powder is then added while they are thoroughly mixed. Further heating produces a paste-like consistency that is dried out and molded into appealing forms for kids to consume.

Treatment of Molar teeth with S. mutans

Bacterial inoculum in Brain Heart Infusion broth was prepared with 0.5 McFarland standard. And distributed in bottles. The teeth were put in the bacterial suspension and allowed to adhere to the teeth for 4 hours with frequency supplementation of media. Centrifugation was used to separate the planktonic cultures, which were then floated in Brain Heart Infusion broth after being spun at 2700 relative centrifugal force (RCF), for 10 minutes at 25 °C.

Quantification of Biofilm Biomass

After being fixed with 200 L of methanol at 25 °C for 30 minutes, biofilms were allowed to dry. About 250 µL of a 0.01% crystal violet solution was used to stain the biofilms for 15 minutes at 25 °C. The control solution contained 0.01% crystal violet. The wells were twice washed in 250 µL of sterile distilled water and then allowed to air dry at 25 °C. After a 30-minute static incubation period at 25 °C, 200 µL of a 30% acetic acid solution was added to each well, and the absorbance (AU) was measured at 570 nm.

Antibiofilm Activity

Based on colony-forming units (CFU), viable microbial colony counts were calculated. To spread the bacteria in the biofilm, 200 µL of sterile PBS was poured into each well, and the wells were then sonicated for 30 min. 10µL of the serially diluted scattered biofilms were observed on Brain Heart Infusion agar.

RESULTS

In our study, treatment with the extract has significantly decreased the bacterial viable count indicating the inhibition of growth of *S.mutans* and biofilm synthesis. The colony count of *S. mutans* was assessed following treatment of tooth samples with different sweetening agents: sugar-free green (positive control), natural sugar-free (negative control), and plant-based lozenges containing *Stevia rebaudiana* extract. The composition of each sample is given in **Table 1**

Table 1 shows the exact composition of the samples used in our study

Treatment	Type	Key Components	Role in Study
Natural Sugar-Free (Aquazyl Isomalt Lozenges Sugar Free Mint)	Commercial product	Sorbitol, erythritol, natural flavorings	Negative control – does not contain known antimicrobial agents
Sugar-Free Green (Eugica Coff Herbal Lozenges Sugar Free)	Commercial product	Stevia, xylitol, herbal extracts	Positive control – marketed for oral health, known anti-cariogenic ingredients
Stevia Lozenges	Experimental product	*Stevia rebaudiana* extract, lemon, ginger, palm sugar	Test sample – combines natural sweeteners with plant-based antimicrobial agents

Upon exposure to *S. mutans* inoculum prepared in Brain Heart Infusion (BHI) broth, the treated teeth showed varying levels of bacterial adherence and biofilm formation. After incubation and quantification procedures, a significant reduction in viable colony-forming units (CFU/ml) was observed in samples treated with Stevia lozenges and sugar-free green, as compared to the control (natural sugar-free):

- **Natural sugar-free (Control):** 1000 CFU/ml
- **Sugar-free green:** 20 CFU/ml
- **Stevia lozenges:** 20 CFU/ml
- The antimicrobial efficacy of *Stevia rebaudiana* lozenges against *Streptococcus mutans* was evaluated using a broth microdilution method, and the Minimum Inhibitory Concentration (MIC) was determined by monitoring turbidity as an indicator of bacterial growth. The results are depicted in **Figure 1**, which shows a clear dose-dependent reduction in bacterial turbidity with increasing concentrations of *Stevia rebaudiana* lozenge extract.

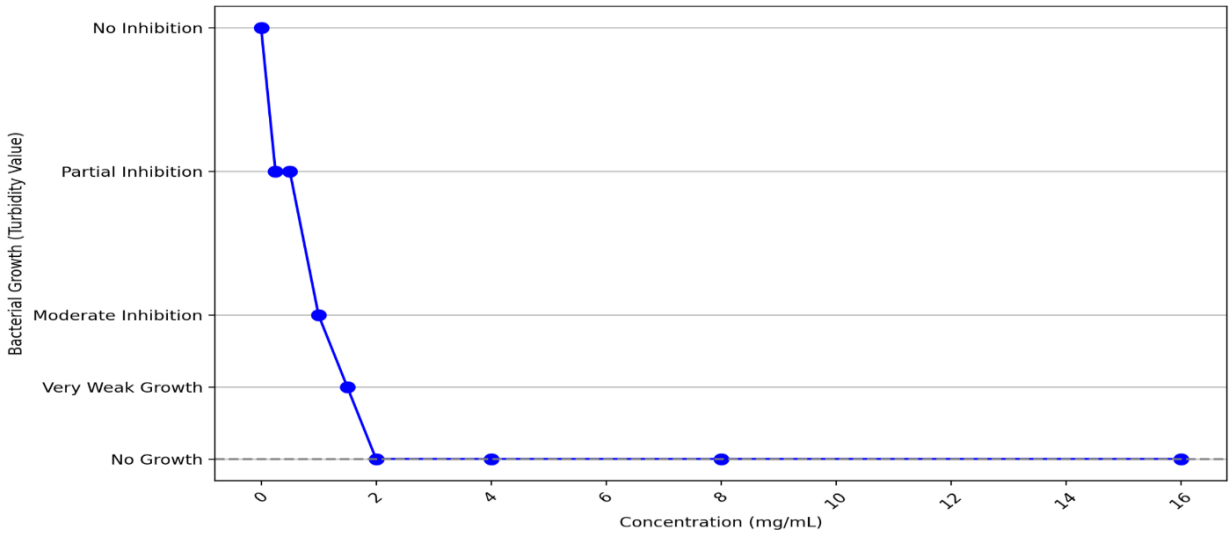

Figure 1 shows the bacterial growth in the teeth treated with prepared lozenges from *Stevia rebaudiana* against *S. mutans* at various concentrations

At 0 mg/mL (control), *S. mutans* exhibited uninhibited growth, corresponding to the highest turbidity values on the graph, indicating the absence of antibacterial activity in the absence of the lozenge extract. Partial inhibition of bacterial growth was observed at concentrations between 0.5 and 1 mg/mL, as seen by a modest reduction in turbidity. A further decrease in bacterial turbidity was recorded at 1.5 mg/mL, suggesting moderate inhibition. Notably, at 2 mg/mL, the bacterial growth was significantly reduced, with minimal turbidity values, indicating a near-complete inhibition of *S. mutans* growth.
Complete inhibition was observed at concentrations of 4 mg/mL and above (4, 8, and 16 mg/mL), where the turbidity values plateaued near zero, suggesting no observable bacterial growth. These concentrations are considered bacteriostatic or bactericidal under the test conditions. Thus, the Minimum Inhibitory Concentration (MIC) of *Stevia rebaudiana* lozenges against *S. mutans* was determined to be 2 mg/mL, with complete inhibition achieved at ≥4 mg/mL. These findings support the potent antibacterial properties of Stevia-based lozenges and their applicability in oral health care, particularly for the prevention of dental caries caused by *S. mutans*.
Quantification of biofilm biomass using crystal violet staining revealed a marked decrease in biofilm accumulation in Stevia lozenge-treated samples. Absorbance readings at 570 nm confirmed reduced staining intensity, suggesting lower biofilm density. The use of 30% acetic acid to solubilize the dye enabled accurate spectrophotometric measurement of biofilm mass. Additionally, viable counts following sonication and plating on BHI agar further supported the anti-adhesive and antibiofilm activity of the Stevia-based formulation. The reduction in CFU/ml in Stevia-treated samples was consistent across all replicates, demonstrating reproducibility and reliability.
The data indicate that Stevia rebaudiana lozenges significantly inhibit the growth and biofilm formation of *S. mutans*, with results comparable to the commercially available sugar-free green product. The natural sugar-free control, lacking antimicrobial components, showed the highest CFU count and densest biofilm, reinforcing the importance of incorporating bioactive plant extracts in oral formulations. These findings illustrate the potential of Stevia lozenges as a functional, non-cariogenic sweetener with significant anti-*S. mutans* activity, suggesting a promising role in the prevention of dental biofilm-associated diseases.

DISCUSSION

Dental caries remains one of the most prevalent chronic diseases worldwide, largely due to the activity of *Streptococcus mutans*, a bacterium well known for its capacity to adhere to tooth surfaces, form biofilms, and ferment dietary sugars into acids that demineralize enamel. This biofilm-mediated pathogenesis is strongly influenced by the type and frequency of carbohydrate intake, with sucrose being particularly cariogenic.[9] In this context, the need for non-cariogenic sweeteners with therapeutic properties is more pressing than ever. Our study demonstrates that lozenges made from *Stevia rebaudiana* extract significantly reduce both bacterial viability and biofilm formation by *S. mutans*, providing a potential dual-function oral health product—offering both sweetness and protection.

The lozenges formulated with *Stevia rebaudiana* showed a dramatic reduction in *S. mutans* colony-forming units (20 CFU/ml) compared to the control (natural sugar-free; 1000 CFU/ml). This represents a three-log reduction in bacterial load, highlighting the potent antibacterial activity of the Stevia-based lozenges. Interestingly, this efficacy was on par with a leading commercially available sugar-free green product, positioning Stevia lozenges as a competitive and potentially superior natural alternative. The observed reduction in biofilm biomass, as indicated by reduced crystal violet absorbance at 570 nm, supports the antibacterial findings and suggests that Stevia lozenges not only inhibit bacterial growth but also impair biofilm development. This is consistent with previous studies where Stevia reduces biofilm formation in *S.mutans* and *C.albicans* by decreasing the expression of genes such as gtfB and gbpB, which are crucial for EPS synthesis and thus demonstrating superior suppression compared to sucrose.[10,11] This is a critical feature, as the biofilm matrix plays a central role in protecting bacteria from host immune responses and antimicrobial agents. By disrupting biofilm formation, Stevia-based lozenges can directly influence caries development at an early stage.

Previous research has explored the antimicrobial properties of *Stevia rebaudiana* in various forms. and reported that aqueous Stevia extracts could inhibit *S. mutans* growth and reduce acidogenic potential. However, most of these studies relied on extract solutions or powders rather than edible formulations.[12-15] The current study extends these findings by incorporating the Stevia extract into a lozenge form that is suitable for pediatric and general use, improving both palatability and compliance. Moreover, prior work has primarily focused on in vitro inhibition zones or metabolic assays, while our study utilized a quantitative biofilm model involving real tooth surfaces, mimicking in vivo conditions more closely.[16-18]

This methodological approach enhances the translational value of the findings and offers a practical framework for future product development.

The mechanism by which *Stevia rebaudiana* exerts its antibiofilm effects is not yet fully elucidated, but it is hypothesized that compounds such as stevioside and rebaudioside A possess antimicrobial properties that interfere with bacterial adhesion, quorum sensing, and metabolic pathways.[19,20]

These glycosides may alter the surface properties of enamel or interact with bacterial cell membranes, leading to reduced colonization and impaired biofilm integrity.[21-23] In addition to Stevia, the lozenge formulation in this study included lemon and ginger—both of which possess known antibacterial and antioxidant properties. Lemon provides citric acid, which can modulate pH and disrupt bacterial metabolism, while ginger contains gingerol, a compound with proven antimicrobial action. These components likely exert a synergistic effect, amplifying the antibacterial activity of the formulation without compromising taste or safety. The novelty of this study lies in the development of a natural, Stevia-based lozenge formulation specifically designed to be both therapeutic and child-friendly. Unlike synthetic sweeteners, which have raised concerns over metabolic effects and long-term safety, Stevia offers a natural and safer alternative that not only replaces sugar but actively contributes to oral health. The fact that the lozenges performed as well as a top-tier commercial sugar-free product underscores their potential value.

Furthermore, the study's reproducibility across replicates enhances the credibility of the results and suggests potential for scalability in commercial production. As dental caries prevention moves toward integrative and preventive care, functional formulations like Stevia lozenges could play an important role in public health strategies, especially in pediatric populations and individuals with high caries risk.

Limitations and Future Directions

While the results of this study are promising, several limitations must be acknowledged. As the experiments were conducted under in vitro conditions, the findings may not fully reflect the complex dynamics of the oral environment in vivo. Therefore, future studies should include clinical or in vivo evaluations to confirm the real-world efficacy of the Stevia lozenges. Additionally, long-term assessments are necessary to understand the effects of regular lozenge use on the overall oral microbiome, ensuring that beneficial bacteria are not adversely impacted. Consumer acceptability, especially among children, should also be explored through sensory evaluation and taste preference studies to optimize palatability. Further research into the formulation's stability, storage conditions, and shelf life will be essential for commercial development. Finally, isolating and characterizing the specific bioactive compounds in *Stevia rebaudiana* responsible for the observed antibiofilm activity could enable the refinement and enhancement of the formulation for greater therapeutic benefit.

CONCLUSION

This study provides compelling evidence that *Stevia rebaudiana*-based lozenges significantly reduce *Streptococcus mutans* viability and biofilm formation, with efficacy comparable to established sugar-free products. The combination of natural ingredients with proven health benefits highlights the potential for such formulations to serve as effective, safe, and consumer-friendly alternatives in oral care, especially in the pediatric segment. This positions Stevia lozenges not only as a non-cariogenic sweetening solution but also as a novel, bioactive preventive strategy against dental caries.

DECLARATIONS

Ethical approval and consent to participate

Not Applicable

Availability of data and material

All data generated or analyzed during this study are included in the published article

Competing interest

The authors declare that there are no competing interest.

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Ethical approval

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