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Effect of Sugar-Free Chewing Gum Containing Tulsi Extract on Salivary Streptococcus Mutans in a Group of Children

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ORIGINAL STUDY

Effect of Sugar-free Chewing Gum Containing Tulsi Extract on Salivary *Streptococcus mutans* in a Group of Children

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Abstract

Purpose: This study aimed to evaluate the antimicrobial effect of Tulsi extract incorporated into chewing gum on salivary *Streptococcus mutans* count in a group of children. **Patients and methods:** A total of 33 samples of systemically healthy children with ages ranged from 4 to 11 years old, without any removable prosthetics or orthodontic appliances, and without any recent history of antibiotic use in the previous 2 weeks were selected in the current study. Participants were instructed to masticate gum containing tulsi extract for duration of 5 min and subsequently discard the gum. Two salivary samples were collected from each participant: one before beginning the 5 min gum chewing session, and another 30 min after the cessation of gum chewing. All samples were streaked onto mitis salivarius agar plates and incubated at 37 °C for 24 h, and then *Streptococcus mutans* colonies were counted. **Results:** The statistical analysis of this study revealed that there was a significant decrease in the mean value of the number of *Streptococcus mutans* colonies in the salivary samples collected 30 min after the cessation of gum chewing (Mean ± SD 8655.69 ± 4306.52 × 10^4 for the initial sample; Mean ± SD 0.91 ± 0.47 × 10^4 for nonstimulated saliva after 30 min; $P = 0.000$). **Conclusion:** This study concluded that Tulsi chewing gum had the potential to reduce salivary *Streptococcus mutans* count in children.

Keywords: Caries prevention, Chewing gum, Pedodontics, *Streptococcus mutans*, Tulsi extract

1. Introduction

Dental caries poses significant challenges to children's oral health and overall well-being [1]. Untreated carious lesions can lead to severe consequences, including infections that extend into the bone through the tooth root, resulting in pain, difficult eating, and malnutrition [2]. Moreover, dental caries can have profound social and psychological impacts, affecting children's self-image, self-esteem, and social interactions. It may also hinder speech and cognitive development, potentially affecting academic performance and attendance [3].

Globally, dental caries, including early childhood caries, affect a considerable proportion of children, with prevalence rates ranging from 30 to 60% in preschoolers and approximately 66% in children aged 6–15 years [1–4]. Furthermore, special needs populations are particularly vulnerable to dental caries [5]. In Egypt, dental caries prevalence is alarmingly high, impacting around 78.2% of the population across all age groups, with a notably elevated prevalence of 74% among children, highlighting the urgent need for effective preventive strategies [6,7].

Caries formation is primarily attributed to bacteria, particularly cariogenic strains that produce acids from carbohydrate metabolism, leading to the demineralization of tooth enamel [8]. Among these bacteria, *Streptococcus mutans* is a key initiator of this process because it can trigger the creation of biofilms

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and the onset of caries [9]. *Streptococcus mutans* use sucrose to create extracellular polysaccharides, encouraging dense bacterial growth and acid production, which, in turn, lowers oral pH, promoting the growth of other cariogenic bacteria and enamel demineralization [10]. Additionally, *Streptococcus mutans* produces adhesive extracellular polymers called glucans, facilitating cariogenic biofilm formation by promoting cellular adhesion to teeth and interactions with other oral microbes [11,12].

As the adage goes, prevention is always preferable to cure. Traditionally, caries prevention meant preventing caries from starting [13]. Dental caries is a localized condition; hence preventative measures work better than systemic measures [14]. Inhibiting cariogenic bacteria, reducing sugar consumption, and utilizing anti-biofilm substances are all effective prophylactic measures. The objective is to reduce the overall biofilm quantity or the presence of specific pathogens [15].

Regarding the negative consequences of chemical antimicrobial agents in children, A pressing demand exists for a novel antibacterial substance that is both efficient and safe to use regularly. Natural herbs have grown in popularity as alternatives since they have strong anticariogenic effects and are secure for long-term usage [16]. Several natural products demonstrated effectiveness against *Streptococcus mutans* over the past 20 years, including propolis curcumin, cranberry, and Tulsi extracts, among many others. The use of natural remedies has gained growing interest since they are natural, readily available in local communities, inexpensive, simple to use, and less problematic. Moreover, herbal therapy may be an effective alternate medication with limited adverse effects and drug resistance [16–18].

Tulsi, holy basil or *Ocimum sanctum* is an aromatic plant renowned for its therapeutic attributes, and utilized for centuries [19–21]. The leaves of *Ocimum sanctum* hold medicinally important compounds including volatile oil eugenol and methyl eugenol [22,23]. Tulsi is cost-effective, easy to obtain, and safe medicine that has good therapeutic outcomes with few to no adverse effects [24]. It also exhibits a variety of therapeutic activities including analgesic, anticancer, antidiabetic, anti-fertility-stress, antiemetic, hepatic protective, and cardio protective effects [25]. It is also recognized as being successful in preserving dental hygiene by its ability to combat both *Streptococcus mutans* and *Lactobacillus acidophilus*, demonstrating antimicrobial efficacy [26].

Several caries preventive agents have been developed including mouthwash, gels, and varnishes. Chewing gum also has been introduced as a dosage form for many applications including dental caries. For both adults and kids, chewing gum is a socially acceptable, pleasurable, and regular pastime. Hence, incorporating antibacterial agents in chewing gum can achieve effective delivery and maintenance of the incorporated drug in the oral cavity. This could be due to the longer retention of chewing gum in mouth time than toothpaste and rinses [27–35]. Various studies have incorporated xylitol into chewing gums and have found its efficacy against *Streptococcus mutans* [36,37]. Chewing gums containing xylitol, a blend of xylitol and sorbitol, sorbitol alone, and a blend of sorbitol and mannitol were linked to average caries prevention rates of 58, 52, 20, and 10.7%, respectively [38].

So far, there have been no documented clinical trials evaluating the effectiveness of chewing gum containing Tulsi extract against salivary *Streptococcus mutans*. This research holds profound importance as it not only addresses this substantial knowledge gap but also emphasizes the broader significance of early prevention strategies for dental caries in children. It has the potential to inspire additional research efforts and, ultimately, make a meaningful contribution to enhancing oral health outcomes for children across the globe.

2. Patients and methods

2.1. Materials

Tulsi plants were purchased from local markets, Health In Gum (Cafosa, Barcelona, Spain), and Glycerol (Al Kahira Co., Cairo, Egypt).

2.1.1. Preparation of tulsi leaves extract

Tulsi leaves were plucked from the plant stem and left to dry for four days in the shade. The dried leaves were then crushed finely. Total 125 g of finely crushed Tulsi leaves were mixed with 1 l of ethanol 96% and solicited for 30 min and allowed to macerate for 1 day before filtration using standard filter papers. There were two more repeats of this procedure. Following collection, the extract was vacuum-dried at 50 °C [39].

2.1.2. Ingredients and preparation of chewing gums

Chewing gums with directly compressible gum base (Health-In Gum) were prepared by direct compression method. The chewing gum used in the study was composed of 80% gum base (Health-In Gum), 3% glycerol as a plasticizer, 10% Tulsi extract, and 7% filler by weight [32] no sweeteners or flavors were added to the used chewing gum. The prepared blends were compressed using flat-faced punches of

a tablet compression machine to form 800 mg tablet-shaped chewing gums. We then used heart-shaped and butterfly-shaped plunger cutter molds with dimensions of ~1 cm × 1 cm to transform the tablet-shaped gums to heart-shaped and butterfly-shaped ones to gain children acceptance (Fig. 1).

2.1.3. Clinical registry information

Effect of sugar-free chewing gum containing tulsi extract on salivary *Streptococcus mutans* in a group of children clinical Trials.gov ID: NCT06174194 URL: https://clinicaltrials.gov/study/NCT06174194 Year: 2023.

2.1.4. Evaluation of antimicrobial effect

Sample size calculation: Based on a previous study [40], sample size calculation was estimated using CDC Epi Info program version 7.2.0.1 (Atlanta, USA), assuming a power of 80%, alpha = 0.05 to detect the significant antimicrobial effect of Tulsi leaves extract chewing gum on *Streptococcus mutans* count in saliva. A total sample of 33 children was needed based on mean ± SD logs of the colony of *Streptococcus mutans* count of 3.37 ± 0.67 in Sample 2 (30 min after chewing the gum) compared with 5 ± 0.0 in Sample 1 (Unstimulated baseline whole saliva).

Case selection: A total of 33 children of both sexes (15 boys and 18 girls) participated in this study. All participants were selected from attending the outpatient Pedodontics clinic. Ethical Approval was granted by the Research Ethics Committee of the Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt. The code for approval was [REC-PE-23-14]. Faculty of Dental Medicine, Al-Azhar University according to the following criteria.

Inclusion criteria [40]:

(a) Children aged between 4 and 12 years old.
(b) No fixed or removable orthodontic appliances or removable prostheses.
(c) Systemically healthy patients.
(d) No history of recent antibiotic administration (previous 2 weeks).

Exclusion criteria [40]:

(a) History of using antimicrobial mouthwash (previous 12 h).
(b) History of fluoride treatment (previous 2 weeks).

Participation in the study was entirely voluntary. To ensure comprehensive and systematic data collection, our study utilized standardized data sheets tailored for two key purposes: gathering demographic information (age, sex), medical history, and past treatments and documenting the results of collected samples. These data sheets were meticulously designed to capture relevant variables essential for our research objectives while maintaining consistency and clarity in data recording.

2.1.5. Sample collection [40]

Samples were taken between 9 and 10 AM. Before saliva collection, participants were instructed to refrain from eating, drinking (except water), or performing oral hygiene procedures for at least 1 h to minimize potential contamination and ensure the collection of unstimulated saliva. Children were asked to spit into a sterile container to collect samples. To prevent contamination, participants were instructed not to touch the inside of the collection container.

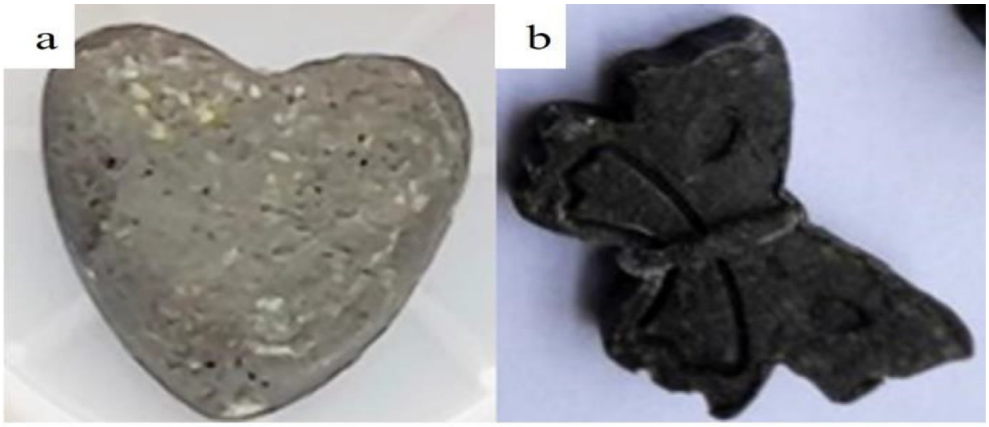

Fig. 1. Prepared chewing gums (a) heart shape (b) butterfly shape.

(a) Sample 1: unstimulated baseline whole saliva was collected from each child by asking them to spit in a labeled, sterile, plastic container until a suitable amount of unstimulated saliva was collected. Children were asked to chew the chewing gums, which contain Tulsi extract, for 5 min, after which they were asked to through down the chewing gum.
(b) Sample 2: unstimulated whole saliva: After 30 min of through down the chewing gums, participants were asked to spit in labeled, sterile plastic containers.

After saliva collection, participants were instructed to carefully cap the collection container without touching the inside of the caps to avoid contamination. Within an hour of collection, the samples were transferred in a cooler bag to the Regional Center for Mycology and Biotechnology laboratory at Al-Azhar University. Saliva samples were examined as soon as they arrived at the laboratory. Mitis Salivarius bacitracin agar is a microbiological growth medium used to quantify the overall bacterial growth of samples using the spread technique to form single colonies. After the samples were plated onto the agar, the Petri dishes were incubated aerobically at 37 °C for 48 h. Colony counting was performed manually. The count of *Streptococcus mutans* was measured in colony-forming units (CFU)/ml.

2.2. Statistical analysis

Statistical Package for Social Sciences (SPSS) version 20 was used to analyze the data. Utilizing mean, standard deviation, median, and range, numerical data were summarized. By examining the data distribution and performing the Kolmogorov–Smirnov and Shapiro–Wilk tests, data were examined for normality. *Streptococcus mutans* count showed an on parametric distribution and was compared between observations using the Friedman test, and Wilcoxon signed Rank test. Percentage reduction of *Streptococcus mutans* count showed a parametric (normal) distribution and was compared between intervals using repeated measures analysis of variance test and paired *t*-test. All *P* values are two-sided. *P* values less than or equal to 0.05 considered significant.

3. Results

3.1. Demographic data

Age: the study participants had a mean age of 7.42 years, with a SD of 1.82, and a range from 4 to 11 years. Sex: The study participants were 18 females and 15 males.

3.2. Comparison of Streptococcus mutans colonies count between first and second observations (Figs. 2 and 3, Table 1)

A higher value was recorded in initial non-stimulated saliva [mean: 8655.69 ± 4306.52; median 4600] × 10^4 (CFU)/ml. A significantly lower value was recorded in unstimulated saliva after 30 min of gum spitting out [mean .91 ± 0.47; median 0.07] × 10^4 (CFU)/ml. The difference between the two observations was statistically significant ($P = 0.000$). The percentage of reduction noted in the interval from initial unstimulated saliva to unstimulated saliva after 30 min [mean −99.98 ± 0.07; median −99.99] %.

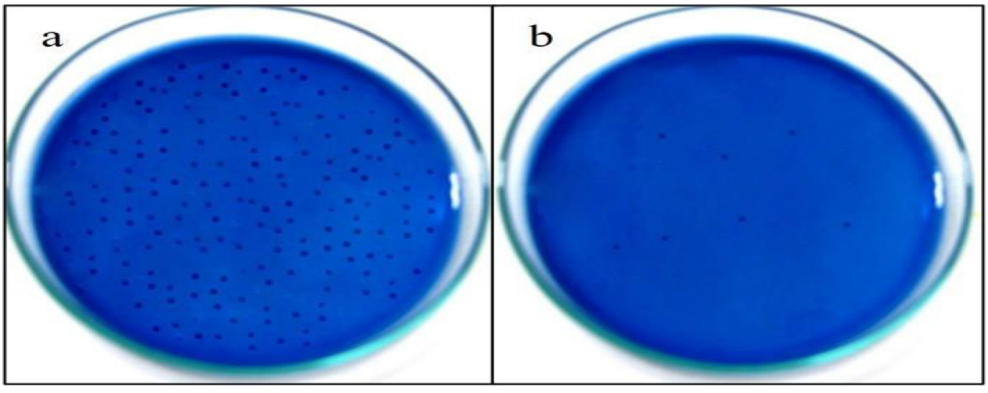

Fig. 2. Streptococcus mutans *colonies count between (a) first and (b) second observations.*

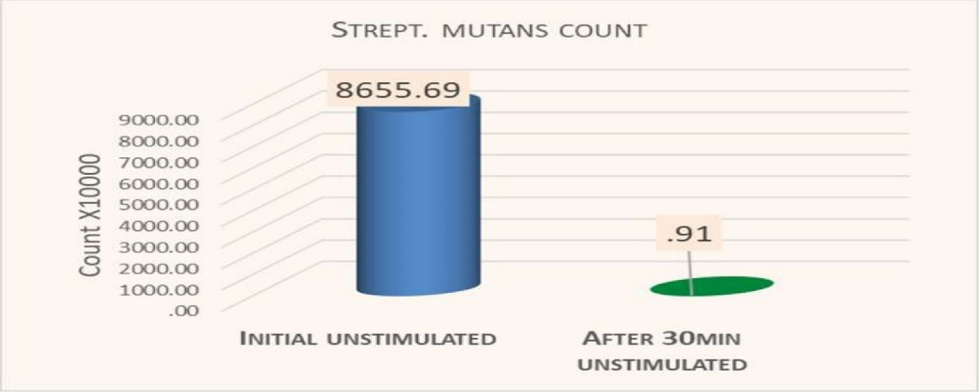

Fig. 3. Bar chart Compare the count of Streptococcus mutans *colonies between first and second observation percent.*

Table 1. Mean and standard deviation values of Streptococcus mutans *count (× 10^4) in initial unstimulated saliva and after 30 min of gum spitting out unstimulated saliva (CFU)/ml.*

	Mean ± SD	Median	Minimum	Maximum	*P* value
Initial unstimulated	8655.69 ± 4306.52	4600	21	68,000	0.000*
After 30 min Unstimulated	0.91±−0.47	0.07	0.01	9.3	

Significance level *P* less than or equal to 0.05, * significant.

3.3. Comparison of Streptococcus mutans *counts in males and females*

In initial nonstimulated saliva (S1), there was no significant difference in the colonies count ($P = 0.94$). After 30 min of gum chewing unstimulated saliva (S2), there was no significant difference between sexes ($P = 0.194$).

4. Discussion

Plants have been used medicinally by humans for millennia; Tulsi is one of these plants. It is an aromatic herb from the Lamiaceae family. Tulsi can be advised for long-term usage because it is widely accessible, affordable, culturally acceptable, and has potentially few negative effects when compared with herbal remedies [23,25].

Chewing gum is a widely accepted and enjoyable activity for both adults and children. It serves as a convenient vehicle for delivering various medicines, including Tulsi extracts, which offer positive effects on oral health. Chewing gum acts as a mechanical plaque control mechanism, reducing plaque index scores when used alongside regular brushing. Additionally, it stimulates saliva production and taste buds, promoting teeth cleaning and neutralizing acids in the mouth [28–36].

In the current study, chewing gum has been chosen as a carrier for Tulsi extracts due to its favorable drug release profile and numerous advantages over mouthwash formulations. Chewing gum is more attractive, easier to use, and more accessible than mouthwash. It also allows for increased consumption capacity, enhances safety with lower risk of overdose, and provides prolonged and controlled drug release [41].

Chewing gums containing xylitol have been extensively studied for their caries prevention benefits. Xylitol chewing gums have demonstrated great efficacy in reducing caries risk by inhibiting bacterial growth, stimulating saliva production, and promoting remineralization of tooth enamel [13–15]. This was in accordance with a previous studies have demonstrated the antibacterial activity of Tulsi extract in mouthwash [39] and gel [24] formulation against cariogenic bacteria such as *Streptococcus mutans*, but due to there have been no reported clinical trials evaluating the efficacy of incorporating Tulsi extract into chewing gum formulations for this purpose. Thus, the present study was undertaken to assess the potential benefits of Tulsi-infused chewing gums in inhibiting salivary *Streptococcus mutans*.

Chewing gum containing Tulsi extract was employed in this investigation. Chewing gum has been examined extensively as a delivery mechanism for numerous topical dental preventive and therapeutic drugs. However, our study is the first to use

Tulsi extract in chewing gum. The chewing gums were compacted into tablet shaped forms weighing 800 mg using flat-faced punches in a tablet compression machine. We then used heart-shaped and butterfly-shaped molds to transform the tablet-shaped gums into heart-shaped and butterfly-shaped ones as we thought that children would prefer them to tablet-shaped chewing gums.

Streptococcus mutans was chosen in the current study due to it stability to endure acidic conditions, produce acids, and adhere to tooth surfaces when exposed to sucrose, that plays a significant role in causing dental caries. Lowering the levels of *Streptococcus mutans* in saliva and plaque is a highly effective strategy in preventing tooth decay [9,15]. Hence; this study aimed to investigate the efficacy of chewing gum containing Tulsi extracts as a novel delivery method for reducing the salivary *Streptococcus mutans*.

Children between the ages of 4–11 were enrolled in the current study to encompass both primary and mixed dentition age groups [11]. Children selected for this study did not have fixed or removable orthodontic appliances or removable prostheses, as these devices can potentially modify the oral microbe and result in elevated *Streptococcus mutans* bacteria counts [42,43]. All participants were systemically healthy children who had not received any antibiotics or fluoride treatments during the two weeks leading up to the study and had refrained from using antimicrobial mouthwash for 12 h before the study as all these factors have the potential to modify the oral microbiota [44,45].

The present study utilized a before-and-after design to evaluate the effectiveness of chewing gum containing Tulsi extracts in reducing salivary *Streptococcus mutans* in children. While the before-and-after design offers insights into the effects of the intervention over time, it is susceptible to certain limitations, including potential biases associated with participant expectations and variations in individual responses. Additionally, the lack of a control group limits the ability to attribute observed changes solely to the intervention, as other factors may contribute to the outcomes.

In the current study, ethanol was chosen as the solvent for Tulsi extraction due to its superior solvency compared with distilled water, and because the ethanol extract of Tulsi (*Ocimum sanctum*) exhibits a potent antibacterial effect [46].

Saliva samples were selected in this study because they offer a noninvasive and convenient collection method. Salivary levels of cariogenic bacterial species have been explored as potential markers for assessing the risk of caries. Furthermore, salivary fluid consistently comes into contact with every tooth, rendering it a precise indicator of *Streptococcus mutans* presence throughout the complete set of teeth and a suitable medium for the isolation of oral bacteria [11,47–49]. In the current investigation, two nonstimulated saliva samples were collected at different time points. The first sample (Sample 1) was obtained before 5 min of gum chewing, and the second sample (Sample 2) was collected 30 min after the cessation of gum chewing. We used non-stimulated saliva at baseline and 30 min after chewing gum cessation due to its lower concentration of bicarbonate ions which can buffer the effects of saliva [50].

Morning saliva samples were obtained to eliminate potential circadian rhythm-related variances in saliva concentration. Participants were instructed to observe a 1-h fasting period (except water consumption) before saliva collection to minimize the influence of food remnants and saliva stimulation [51]. It was selected to isolate *Streptococcus mutans* for this investigation since it is the main pathogen and a key promoter of tooth caries.

In this study, the isolation, identification, and quantification of *Streptococcus mutans* were conducted using the selective medium, Mitis Salivarius bacitracin agar, as it is considered the most specific test for this organism [50]. The findings of this study demonstrated that there was a significant decrease in the number of *Streptococcus mutans* bacteria in saliva after participants chewed gum containing Tulsi, 30 min after finishing the gum. This reduction supports the idea that Tulsi could be viewed as an agent that prevents tooth decay. These results of the current study was in accordance with a previous study by Lolayekar and Kadkhodayan Mohapatra *et al*. [40,52] who provide further confirmation of Tulsa's antibacterial effect against *Streptococcus mutans*. In addition to the results of the current study support the findings Ahmed *et al*. study that investigated the effect of Tulsi extract mouth rinse on Salivary *Streptococcus mutans* Count [39].

However, the finding of the present study was found to be in disagreement with another study by Gadiyar *et al*. that assessed the in vitro antimicrobial activity of *Ocimum sanctum* against *Streptococcus mutans* [26].

The anti-bacterial components of Tulsi, including eugenol, urosolic acid, carvacrol, linalool, limatrol, and methyl carvicol, might be the cause of this effect. Moreover, the abundance of secondary metabolites found in Tulsi extract, including flavonoids, terpenoids, alkaloids, and tannins with antimicrobial activities, might further contribute to its effectiveness. These photochemicals enhance

bacteriolysis on tooth surfaces and in saliva, produce high molecular weight compounds with soluble proteins in saliva, and disrupt bacterial adhesion mechanisms on tooth surfaces [52].

In the recent study, the results indicated that the count of *Streptococcus mutans* colonies in non-stimulated saliva after 30 min of discontinuing gum chewing showed a significant decrease. Furthermore, a significant percentage reduction from the initial nonstimulated saliva to nonstimulated saliva after 30 min was observed. This outcome was similar to that of Lolayekar and Kadkhodayan [40] study, which observed that the least number of *Streptococcus mutans* in the salivary samples collected after 30 min of chewing Tulsi, leaves.

This study results revealed that chewing gum infused with Tulsi extracts was significantly reduced the *Streptococcus mutans* colony counts in saliva samples compared with baseline measurements. This observation suggests that Tulsi extracts may possess antimicrobial properties that inhibit the growth of cariogenic bacteria in the oral cavity, thereby contributing to the prevention of dental caries [53,54]. Moreover, These results offer fresh perspectives on caries prevention in children, shedding light on innovative approaches and strategies to combat dental caries. This research may pave the way for novel prevention methods and addressing a long-standing challenge in pediatric oral health.

4.1. Conclusion

Within the limitation of the current study: The efficiency potential of Tulsi extract in fused chewing gum as an antibacterial agent against *Streptococcus mutans* in children. This highlights the promising potential of Tulsi-infused gum as a novel approach to prevent dental caries in this demographic.

Ethics approval and consent to participate

Approval was granted by the Research Ethics Committee of the Faculty of Dental Medicine, Al-Azhar University, and Cairo, Egypt [REC-PE-23-14]. The ethics committee of the Faculty of Dental Medicine for Girls, Al-Azhar University is constituted, and operates according to ICH GCP guidelines and applicable local and Institutional regulations and guidelines which govern IRB operation.

The study objective and the protocol were explained in detail to parents in their local language. Participation in the study was completely voluntary. Before starting the study, detailed procedures were explained to the children's parents/guardians.

Written patient informed consent, comprehensively outlining the entire study procedure, was assigned by the parents, signifying their consent to include their children in this research. In addition verbal consent was also obtained directly from the children themselves, further affirming their willingness to participate.

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Biographical information

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Conflict of interest

There are no conflicts of interest.

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