
Potential Application of Bioactive Gambier Gum as a Next-Gen Oral Care Product: An In Vitro Study on *Streptococcus mutans* Reduction and Salivary pH Modulation

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Abstract

Dental caries is the most prevalent oral health problem in Indonesia. Caries occurs due to bacterial colonization, primarily by *Streptococcus mutans*. Prevention of dental caries can be achieved by utilizing gambier (*Uncaria gambier*, Roxb) as an antimicrobial agent in the form of chewing gum combined with xylitol. This study aimed to develop an innovative bioactive formulation of gambier gum and to determine its effectiveness in reducing *Streptococcus mutans* colony counts and increasing salivary pH, both of which play roles in improving oral health and hygiene. This experimental study was conducted at the Biomedical Laboratory, Faculty of Medicine, Universitas Negeri Padang, from July to October 2025. Three formulations of gambier gum containing catechin concentrations of 0%, 70%, and 95% were tested for effectiveness by mixing them with participants' saliva samples. The results showed a reduction in *Streptococcus mutans* colony counts compared to the control. Culture results of gambier gum with 0%, 70%, and 95% catechin demonstrated reductions of 40%, 60%, and 100%, respectively, relative to the control colonies. pH measurements indicated that gambier gum with 95% catechin prevented the saliva from becoming acidic. The gambier gum formulation containing 95% catechin exhibited the greatest antimicrobial effectiveness.

Keywords: Antimicrobial, Catechin, Dental Caries, Gambier, Gambier Gum

INTRODUCTION

Oral and dental health are a crucial aspect of human capital and overall well-being. Globally, oral diseases affect approximately 3.5 billion people (WHO, 2022). Based on the Indonesian Health Survey (SKI) 2023, an average of 57% of the population aged ≥ 3 years re-ported having oral and dental problems in the past year. In children, the DMF-t index reached 6.7, indicating a very high caries experience (Kementerian Kesehatan Republik Indonesia, 2024). Oral health problems can lead to pain, difficulty eating, impaired concentration, sleep disturbances, and can negatively affect nutritional status, learning processes, and child development (Nugraheni, Sadimin and Sukini, 2019; Carsita *et al.*, 2023).

The most prevalent oral health problem is dental caries. Caries occurs due to the colonization of *Streptococcus mutans*, which is the primary etiological agent. This bacterium forms plaque, which gradually erodes the tooth enamel and its underlying structures. According to Lemos *et al.* (2019) *Streptococcus mutans* can even enter the bloodstream (bacteremia), triggering an increase in systemic inflammatory mediators (Lemos *et al.*, 2019). This can elevate the risk of heart disease, including subacute bacterial endocarditis, and stroke (Sen *et al.*, 2024; Ramadhani *et al.*, 2025).

Current preventative approaches, referencing the study by Walsh *et al.* (2019) show that fluoride toothpaste use demonstrates a caries prevented fraction of approximately 24% compared to non-fluoride toothpaste. This exhibits a clear dose-response gradient, where concentrations above 1,000 ppm provide greater protection than lower concentrations (estimated effect range ~ 20 –40% depending on concentration and baseline risk) (Walsh *et al.*, 2019). A 5% fluoride varnish provides a greater protective effect; Iheozor-Ejiofor *et al.* (2015) reported a prevented fraction of about 40% in permanent teeth and $\approx 35\%$ in primary teeth when applied periodically. At the population level, water fluoridation is associated with an average caries reduction of about 26% in permanent teeth and $\approx 35\%$ in the primary teeth of children, with variations according to baseline caries and program coverage

(Iheozor-Ejiofor *et al.*, 2015).

For occlusal interventions, Ahovuo-Saloranta *et al.* (2017) explained that resin-based pit and fissure sealants significantly reduce the formation of occlusal caries. A pooled analysis indicated that the caries risk in sealed molars was $\approx 50\text{--}70\%$ lower at 2–4 years of follow-up compared to no sealing. For non-cavitated initial lesions, resin infiltration reduces the progression of proximal lesion (Ahovuo-Saloranta *et al.*, 2017). Meta-analyses report that the risk of progression is reduced by approximately 45–65% relative to control/monitoring alone over 12–36 months. For 38% silver diamine fluoride (SDF), clinical trial evidence shows high lesion arrest rates (Dorri *et al.*, 2015).

Given its significant potential, a local Indonesian commodity, the gambier plant (*Uncaria gambier*, Roxb), shows great promise in preventing dental caries (Saad *et al.*, 2020). The catechin content in gambier possesses antimicrobial, antioxidant, and anti-inflammatory properties (Taufiq, 2017). Gambier, often used in the tradition of menyirih (betel-chewing), is utilized for its antimicrobial, antioxidant, anti-inflammatory, antifungal, and antiseptic properties (Saad *et al.*, 2020). One of the beneficial components of gambier for caries prevention is catechin. Gambier catechin can prevent plaque formation by inhibiting the activity of the glucosyltransferase (GTF) enzyme, which plays a role in forming glucan for *Streptococcus mutans* adhesion to the tooth Surface (Dharsono *et al.*, 2022). Meanwhile, the tannins in gambier are astringent and can accelerate wound healing in the oral mucosal tissue (Kováč *et al.*, 2022).

Gambier can be utilized in a chewing gum product to prevent dental caries. This innovation of gambier and chewing gum offers both mechanical benefits and active substances that can prevent plaque formation. Several studies have attempted to solve oral health problems by developing various chewing gum products, but they are still sucrose-based. This, however, can increase the risk of plaque formation due to bacterial fermentation of sucrose, which causes salivary pH to become acidic. Xylitol, a non-fermentable sweetener, can be used as a sucrose substitute, thus preventing the drop in oral pH that triggers caries (Mayasari, 2020).

This bioactive innovation, combining gambier extract, gum base, and xylitol, is expected to provide a synergistic effect in preventing dental caries. This research aims to formulate a "gambier gum" as a next-gen oral care product and to assess its effectiveness in reducing the colony count of *Streptococcus mutans* and increasing salivary pH, which are crucial for enhancing dental and oral hygiene. This study has significant potential for developing a local natural resource as an anti-caries agent that provides dual protection by increasing salivary pH and reducing cariogenic bacteria. The results could encourage the downstream processing of gambier -derived products as a flagship commodity for West Sumatra, as well as provide a basis for further research, including broader clinical trials, formulation refinement, and the development of other gambier extract-based preparations such as toothpaste or mouthwash.

RESEARCH METHODS

This study was conducted during the period of July–October at the Biomedical Laboratory, Faculty of Medicine, Universitas Negeri Padang (UNP). Ethical approval was obtained from the UNP Research Ethics Committee (KEPK-UNP) under approval number No.037/KEPK-UNP/8/2025. The study employed a Posttest-only control group design. A total of 12 subjects participated in the research. Inclusion criteria required subjects to be free of dental caries and not be using fixed orthodontic appliances. All subjects provided written informed consent prior to participation.

The sample used in this study was saliva, which was divided into four groups: (1) control saliva, (2) saliva mixed with gambier gum containing 0% catechin, (3) saliva mixed with gambier gum containing 70% catechin, and (4) saliva mixed with gambier gum containing 95% catechin. The gambier catechin was procured from PT. Salimbado Jaya Indonesia, Lima Puluh Kota Regency, West Sumatra. The gambier catechin material underwent material analysis and certification by the UPTD (Regional Technical Implementation Unit) for Quality Testing and Certification of Goods, under the

Department of Industry and Trade of the West Sumatra Provincial Government. The analysis results indicated that the material was a pale-yellow powder with the following composition: 95.50% catechin content, 0% moisture content, 0% ash content, 0% water-insoluble substances, and 0% alcohol-insoluble substances. The gambier catechin was stored in an airtight container and protected from direct sunlight exposure.

The equipment used in this study included beaker glasses, stirring spatulas, a hot plate, plastic containers, an autoclave, Erlenmeyer flasks, dropper pipettes, test tubes, graduated cylinders, petri dishes, toothbrushes, toothpaste, saliva collection pots, distilled water (aquades), inoculating loops, masks, gloves, a colony counter, a digital scale, a pH meter, a microbiology incubator, micropipettes, a magnetic stirrer, microscope slides, aluminum foil, and plastic wrap.

The materials used comprised gambier extract, gum base, xylitol, saliva, 0.9% NaCl saline solution, TYCSB (Trypticase-Yeast-Cysteine-Sucrose Bacitracin) agar medium, gentian violet, carbol fuchsin, and Lugol's solution.

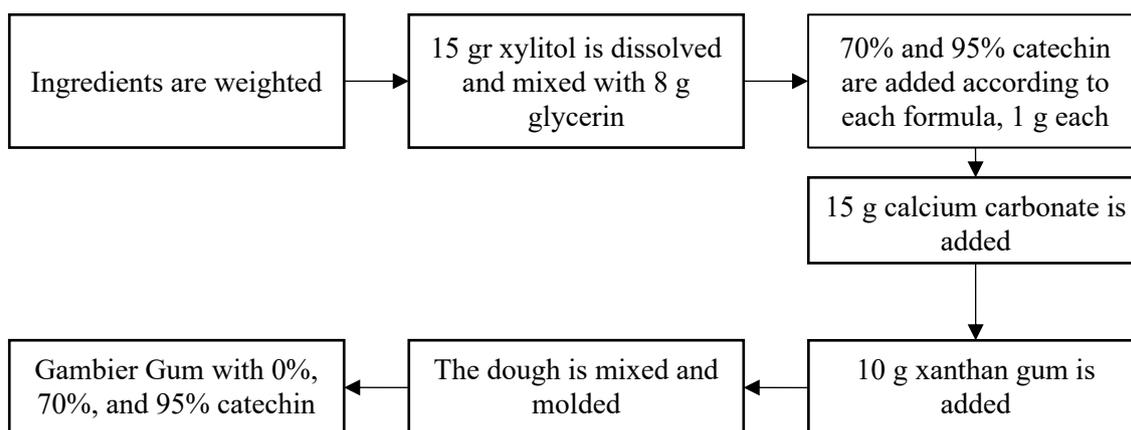
The research was divided into two phases: the product formulation phase and the efficacy testing phase.

Formulation of Gambier Gum

This phase involved experimental laboratory research to formulate the gambier gum product as a safe and stable chewing gum preparation. Three gum formulations were created: gum with 95% catechin, 70% catechin, and 0% catechin (control).

The gambier gum was prepared in three formulations: with 95% gambier catechin extract, 70% gambier catechin extract, and without catechin extract as a control. The 70% concentration was prepared by diluting 2.8 grams of the 95% gambier catechin extract with 1 ml of distilled water (aquadest).

- Fifteen grams (15 g) of xylitol were dissolved in water using a hot plate and magnetic stirrer.
- The xylitol solution was mixed with 8 grams of glycerin.
- One gram (1 g) of the 95% catechin extract and 70% catechin extract, respectively, was added to its corresponding formulation and stirred until homogeneous.
- Fifteen grams (15 g) of calcium carbonate were then added to the mixture.
- Ten grams (10 g) of xanthan gum were added.
- The dough was kneaded until it reached a smooth and pliable, non-sticky consistency and was then formed into chewing gum.



Gambier gum formulation flow diagram

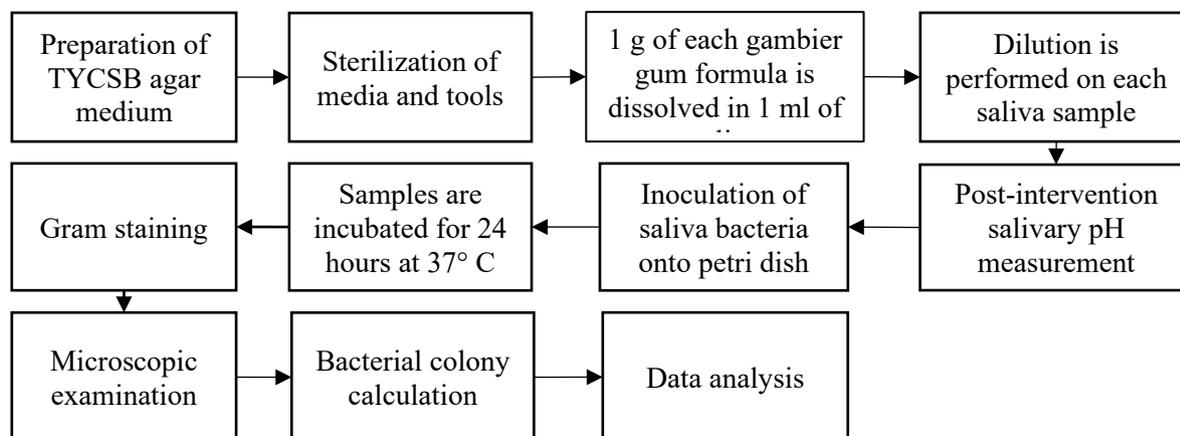
Saliva Sample Collection

- Participants were instructed to first brush their teeth using the Bass technique.
- Participants were required to refrain from eating and drinking for one hour prior to collection.
- Participants expectorated (spat) saliva into the provided sterile collection pots.

Efficacy Testing

The effectiveness of each formula obtained was tested to see the decrease in the number of Streptococcus mutans colonies and the increase in saliva pH compared to the control.

- Preparation of TYCSB agar medium.
- Saliva samples were divided into four groups (Control, 0%, 70%, and 95%).
- For each respective group, 0.1 g of each gambier gum formulation was dissolved in 1 ml of the sample saliva.
- Each solution was then diluted with 4.5 ml of NaCl solution, achieving a 10^{-1} dilution.
- Post-intervention salivary pH was measured.
- Bacterial culture from the saliva was performed on petri dishes using the spread plate and pour plate methods.
- All samples were incubated at 37°C for 24 hours.
- Gram staining was performed for morphological confirmation.
- Calculation of Streptococcus mutans colonies. The Streptococcus mutans colonies were counted using a colony counter and expressed in Colony Forming Units (CFU)/ml using the formula: $\text{CFU/ml} = (\text{Number of colonies formed} \times \text{dilution factor}) \times 4.5$ (Monica, Susiana and Widura, 2018).



Gambier gum effectiveness test Flow diagram

RESULTS AND DISCUSSION

Formulation

The result of this research is the formulation of gambier gum with catechin contents of 0%, 70%, and 95% (Figure1). The resulting gambier gum has a wood-brown color, an aroma of catechin, and a chewy texture typical of chewing gum. The formulated products subsequently underwent efficacy testing to determine their effect on the colony count of Streptococcus mutans in the saliva samples.

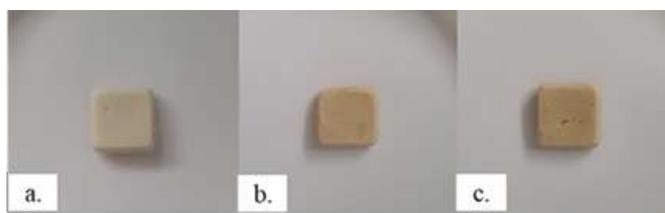


Figure1. Gambier gum with catechins (a) 0%, (b) 70%, and (c) 95%

Culture Results

The culture results showed the growth of Streptococcus mutans colonies (Figure 3). The efficacy test of the formulated products against Streptococcus mutans colonies in the saliva samples is shown in Figure 5.

The culture indicated the growth of Streptococcus mutans colonies, which was confirmed by the morphology of Gram-positive cocci observed during Gram staining. The bacteria appeared in clusters and short chains, as is common for this species.

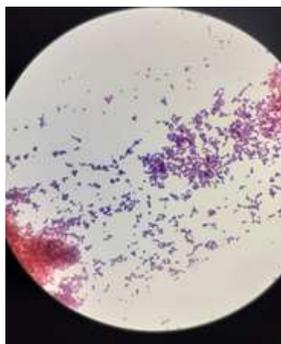


Figure2. Streptococcus mutans bacteria in gram staining

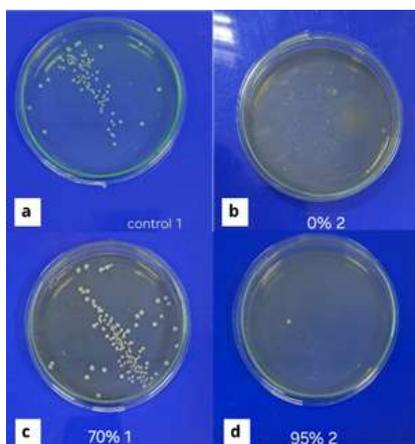


Figure3. Streptococcus mutans bacterial colonies, a) control, b) 0% catechin, c) 70% catechin, d) 95% catechin

Salivary pH

An increase in pH to an alkaline state (pH=8) was observed only with the use of the 95% catechin formulation (Figure 3). The salivary pH remained unchanged in the control group, the catechin-free formulation (0%) group, and the 70% catechin formulation group.



Figure4. Results of pH test, a) control, b) 0% catechin, c) 70% catechin, d) 95% catechin

Colony Calculation

A reduction in the Streptococcus mutans colony count was observed corresponding to the increase in catechin concentration in the formulations used. Reductions of 40%, 60%, and 100% were found for the 0%, 70%, and 95% catechin formulations, respectively, relative to the control (Figure

5). This indicates that the higher the catechin concentration used in the gambier gum, the more effective it is in reducing the bacterial colony count of Streptococcus mutans.

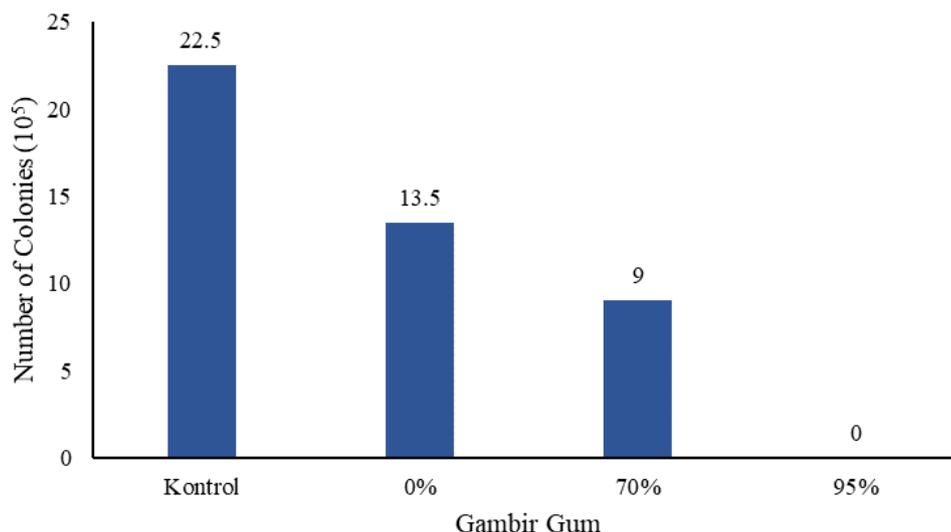


Figure5. Streptococcus mutans Colony Count

The data were subsequently analyzed using One-Way ANOVA. Figure 6 showed a downward trend in Streptococcus mutans colony counts as the catechin concentration increased, with the largest reduction occurring in the 95% catechin formulation ($p > 0.05$).

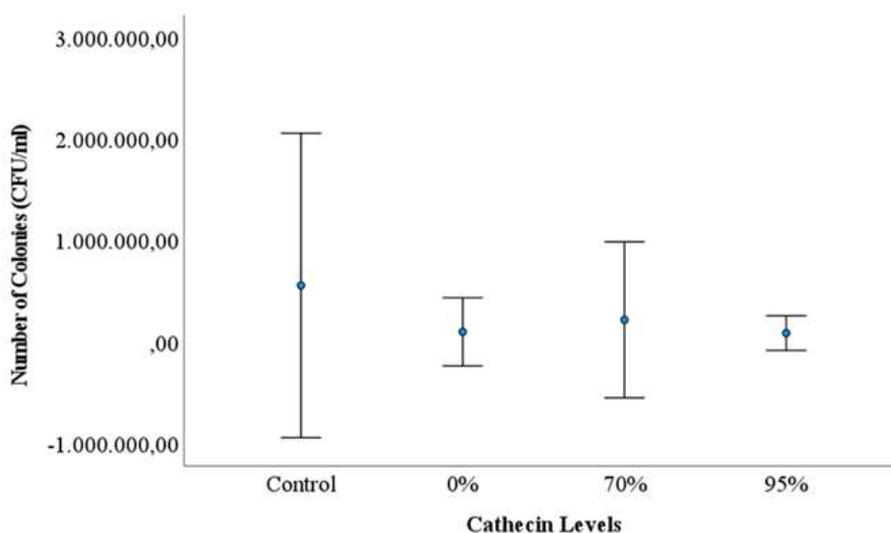


Figure6. Decreasing trend in the number of Streptococcus mutans colonies

The statistically non-significant trend in the reduction of Streptococcus mutans colonies in this study ($p > 0.05$) may be attributed to the study design, which only involved a post-intervention assessment of each sample compared to a control (a post-test only control group design). This design has a limitation as there is no known baseline to serve as a benchmark for assessing colony counts post-culture (Krishnan, 2019). Saliva samples from each individual possess varying colony counts depending on the time of day and the individual's oral conditions (Najafi *et al.*, 2022). Therefore, to more specifically observe the effect of the gambier gum, a comparison between pre-test and post-test samples against a control is necessary.

The efficacy test of the catechin-based gambier gum formulations against *S. mutans* in saliva samples demonstrated a trend: the higher the catechin concentration, the greater the inhibition of

colony growth. On the control and 0% catechin treatment plates, colonies still grew distinctly. The 70% concentration showed partial inhibition, whereas at 95%, the colony count was substantially reduced.

This finding is in line with recent evidence that catechins, particularly gallate fractions such as EGCG, possess potent antibacterial and antibiofilm activity against *Streptococcus mutans*. Recent studies indicate that EGCG can inhibit planktonic growth and biofilm formation, downregulating the expression of virulence genes (*gtfB*, *gtfC*, *ftf*), reducing EPS (Extracellular Polysaccharide) production, and disrupting bacterial membrane integrity (Schneider-Rayman *et al.*, 2021). Recent reviews also confirm that gallate catechins target the cell membrane, virulence factors, and glucan-forming enzymes involved in the colonization and cariogenicity of *S. mutans*. Additional evidence indicates that catechin gallate fractions can inhibit adhesion and biofilm formation, while catechins from *Uncaria gambier* have been shown to possess activity against bacterial enzymatic targets (Aragão *et al.*, 2024).

pH test showed that the 95% catechin gambier gum is effective in preventing a drop in salivary pH, thereby preventing the pH from becoming acidic and creating an oral environment that is unfavorable for *Streptococcus mutans*. This has the potential to inhibit the activity of *Streptococcus mutans* bacteria, which causes dental caries.

This finding aligns with results from other studies indicating that catechin can prevent the formation of extracellular glucan, which is essential for the adhesion of *Streptococcus mutans* to the tooth surface (Dharsono *et al.*, 2022). *Streptococcus mutans* forms an extra-cellular matrix from sucrose, which aids in its permanent colonization on teeth. *Streptococcus mutans* metabolizes carbohydrates into organic acids and can survive in acidic conditions (Lemos *et al.*, 2019). Dewi *et al.* (2023) described a study on the effectiveness of gambier extract contained in sucrose candy and showed that gambier extract can reduce the number of *Streptococcus mutans* colonies contained in saliva.

Glucosyltransferase (Gtf) is a crucial virulence factor for oral microbes, including *Streptococcus mutans*, in caries formation. *Streptococcus mutans* produces three Gtf enzymes, encoded by the *gtfB*, *gtfC*, and *gtfD* genes. *GtfB* synthesizes the majority of insoluble glucans that facilitate adhesion and accumulation on the tooth surface and promote cell clustering and microbial cohesion within the biofilm. *GtfC* produces a mixture of insoluble and soluble glucans. *GtfD* forms glucans that are predominantly soluble, which can be digested and used as a fermentable reserve energy source, contributing to the low pH in cariogenic (Zhang *et al.*, 2021).

Catechin inhibits the glycolytic process and competes with glucosyltransferase, which reduces saccharides, thereby preventing glucan formation. Catechin also interferes with the peptidoglycan in the bacterial wall, triggering the destruction of the cell wall (Dewi, Kamaluddin and Pambayun, 2016). The antimicrobial activity of catechin reduces the number of *Streptococcus mutans* bacteria, with higher catechin concentrations demonstrating better antimicrobial effectiveness.

The xylitol in the gambier gum serves as both a sweetener and a caries-prevention agent. Xylitol cannot be fermented by bacteria, thus preventing the plaque formation that initiates caries (Mayasari, 2020). Xylitol can penetrate the *Streptococcus mutans* bacterial cell, where it is converted into xylitol-5-phosphate and accumulated. This accumulation ultimately disrupts the glycolytic process and ATP (Adenosine Triphosphate) production. This phosphorylated sugar is toxic to the bacteria, which subsequently induces bacterial death (Monica, Susiana and Widura, 2018). Xylitol also plays a role in preventing a drop in pH, making it difficult for *Streptococcus mutans* to thrive in the oral cavity (Setyowati and Tiana, 2023).

CONCLUSION

Based on the research findings, there are three formulations of gambier gum that has an effect on reducing the colony count of *Streptococcus mutans*. The culture results demonstrate that the higher

the catechin concentration used in the gambier gum, the greater the reduction in formed colonies. Gambier gum with a 95% catechin concentration prevents the oral pH from shifting to an acidic state, showing potential for dental caries prevention.

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