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Evaluation of a Herbal Toothpaste Formulation with the Addition of *Nginang* Ingredients as an Antimicrobial Agent Against *Streptococcus mutans*

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Abstract: This study aimed to determine the antimicrobial activity of herbal toothpaste formulations containing betel leaf (*Piper betle* L.) extract against *Streptococcus mutans* and to evaluate the physical characteristics of the resulting preparations. The research employed a laboratory experimental design with variations in betel leaf extract concentrations of 5%, 10%, and 15%, each conducted in four replicates. The analyses included phytochemical screening, antimicrobial activity testing using the disc diffusion method, and evaluation of the toothpaste preparations, including pH measurement, organoleptic assessment, homogeneity testing, foam formation analysis, and viscosity determination. The results of the phytochemical screening indicated that the betel leaf extract positively contained triterpenoids, flavonoids, saponins, phenolic compounds, and alkaloids. The antimicrobial activity test demonstrated that the betel leaf extract produced average inhibition zones of 16.4 mm, 22.7 mm, and 25.6 mm at concentrations of 5%, 10%, and 15%, respectively. The evaluation of the toothpaste formulations showed pH values ranging from 8.04 to 8.42, which fall within the Indonesian National Standard (SNI) range of 4.5–10.5. The preparations exhibited a semi-solid and homogeneous texture, with the highest foam height of 20.6 mm observed at the 15% extract concentration, and viscosity values within the required acceptable range. In conclusion, the herbal toothpaste formulation containing betel leaf extract demonstrated strong antimicrobial activity against *Streptococcus mutans* and met the required physical parameters for toothpaste preparations. Therefore, it has potential to be developed as an alternative herbal toothpaste based on traditional natural ingredients suitable for oral health applications.
Keywords: Herbal toothpaste; *Piper betle*; *Uncaria gambir*; *Areca catechu* L.; *Streptococcus mutans*

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INTRODUCTION

Dental caries is one of the most prevalent non-communicable diseases worldwide and remains a significant public health problem. According to a global report by the World Health Organization (WHO), approximately 3.5 billion people worldwide experience oral health disorders, with caries of permanent teeth representing the most dominant condition (WHO, 2022). The Global Burden of Disease Study also identifies dental caries as one of the most common chronic diseases affecting the global population (GBD 2019 Oral Disorders Collaborators, 2020). This high prevalence indicates that caries prevention strategies still require effective, safe, and affordable innovations.

From a microbiological perspective, dental caries is closely associated with the activity of cariogenic bacteria, particularly *Streptococcus mutans*. This Gram-positive bacterium is capable of fermenting carbohydrates into lactic acid through glycolytic metabolism, which leads to a decrease in dental plaque pH below the critical threshold ($pH < 5.5$), thereby initiating the demineralization process of tooth enamel (Selwitz et al., 2007; Takahashi & Nyvad, 2011). If the buffering capacity of saliva fails to neutralize this acidic condition, damage to the hard tissues of the tooth may progress into carious lesions. Caries prevention is generally achieved through mechanical plaque control, such as regular tooth brushing, as well as through chemical approaches involving the use of antibacterial agents in toothpaste and mouthwash (Marsh, 2003; Yuliastri & Prasetyo, 2019).

In recent years, the utilization of natural products as alternative therapeutic agents has gained increasing attention, particularly in the field of oral health. Herbal materials are considered to possess antibacterial, anti-inflammatory, and antioxidant properties with relatively lower risks of adverse effects compared with certain synthetic compounds (Prabu et al., 2015; Palombo, 2011). Traditionally, Indonesian communities have long practiced *menginang* (betel chewing) as part of cultural practices aimed at maintaining oral and dental health. This tradition involves a mixture of betel leaf (*Piper betle*), areca nut (*Areca catechu*), gambier (*Uncaria gambir*), slaked lime, and occasionally tobacco (Novianti & Khusniati, 2022).

Several studies have demonstrated that betel leaf contains phenolic compounds, chavicol, and eugenol, which exhibit antibacterial activity against oral bacteria, including *Streptococcus mutans* (Chakraborty & Shah, 2011). Gambier is known to be rich in catechins and tannins that inhibit microbial growth through mechanisms involving protein denaturation and disruption of cell membrane permeability (Anggraini et al., 2019). Meanwhile, areca nut extract has been reported to exhibit significant antimicrobial activity against Gram-positive bacteria (Huang et al., 2010). Observational studies have also reported that some communities with habitual betel chewing practices tend to have relatively strong teeth and experience fewer caries until old age, although tooth discoloration may occur due to pigments from the chewing materials (Sutana et al., 2021).

Nevertheless, the utilization of betel chewing formulations in standardized modern preparations, such as herbal toothpaste, remains limited. On the other hand, synthetic antibacterial agents commonly used in oral health products, such as chlorhexidine, are known to be effective but may cause side effects including tooth staining and taste disturbances with long-term use (Jones, 1997). In addition, increasing concerns regarding microbial resistance further emphasize the importance of exploring safer and more sustainable natural antibacterial agents (Ventola, 2015).

Based on this background, the development of an herbal toothpaste formulation based on the traditional *menginang* mixture represents a relevant and strategic innovation. The integration of local traditional knowledge with modern scientific approaches has the potential to produce an effective preventive product capable of inhibiting the growth of *Streptococcus mutans*, while also supporting the development of Indonesian natural resources as bioactive sources in dental health. Therefore, this study aims to evaluate the antimicrobial activity of an herbal toothpaste formulation supplemented with a *menginang* mixture against the growth of *Streptococcus mutans* as a natural-based preventive approach to dental caries.

METHOD

This study employed a laboratory experimental design using a Completely Randomized Design (CRD) with different concentrations of *nginang* herbal extract as treatments, namely 5%, 10%, and 15%, along with positive and negative controls. The research was conducted from August to October 2025 at the Microbiology Laboratory of Universitas Islam Negeri Sumatera Utara and the Laboratory of the Polytechnic of Industrial Chemical Technology.

The equipment used in this study included a juicer, mortar and pestle, digital balance, autoclave, incubator, stirring rod, petri dishes, oven, pH meter, beaker glass, inoculating loop, Bunsen burner, and Erlenmeyer flask. The materials used consisted of betel leaves (*Piper betle*) (3 kg), gambir (*Uncaria gambir*), slaked lime, areca nut (*Areca catechu L.*), glycerin (15%), CMC-Na (1.0%), *Streptococcus mutans* bacteria, Mueller Hinton Agar (MHA), Nutrient Agar (NA), saccharin (0.1%), methyl paraben (0.1%), peppermint oil (20%), sodium lauryl sulfate (1.5%), distilled water (35.09%), and calcium carbonate.

Preparation of NA and MHA Media

Nutrient Agar (NA) was used as the initial growth medium for the rejuvenation and purification of *Streptococcus mutans* bacterial cultures prior to further testing. Mueller Hinton Agar (MHA) was used as the medium for antimicrobial activity testing using the disk diffusion (Kirby–Bauer) method. For the preparation of NA medium, 2 g of NA powder was weighed and dissolved in 100 mL of distilled water, then heated while stirring at 70°C until homogeneous. The medium was subsequently sterilized in an autoclave at 121°C for 15 minutes (Mayasari & Sapitri, 2020). For the preparation of MHA medium, 34 g of MHA powder was weighed and dissolved in 1 L of distilled water, then heated until homogeneous. The medium was sterilized in an autoclave at 121°C for 15 minutes. After sterilization, 20 mL of MHA medium was poured into sterile petri dishes (Prihandani, 2015).

Preparation of Nginang Extract

The preparation of *nginang* herbal extract for toothpaste formulation began with preparing the ingredients, namely betel leaves, slaked lime, gambir, and areca nuts. Fresh ingredients such as betel leaves and young areca nuts were first washed thoroughly. The cleaned materials were then processed using a juicer to obtain the extracts of betel leaves and areca nuts. This extraction process was conducted separately for each material. After the extracts were obtained, they were filtered to separate the pure extract from the residue. Filtration was performed twice using filter paper or gauze cloth to obtain a clear extract (Dewi et al., 2020).

Phytochemical Screening of Nginang Extract

Phytochemical screening of the *nginang* extract was conducted to identify the secondary metabolite compounds present in the extract. A total of 2 g of nginang extract was weighed and alkalized with NH_4OH. Subsequently, 10 mL of chloroform and 10 mL of distilled water (1:1) were added and shaken in a separatory funnel. The mixture was allowed to stand until two distinct layers formed. The aqueous layer was used for the detection of flavonoids, phenolics, and saponins, while the chloroform layer was used for the detection of triterpenoids, tannins, and alkaloids (Widyastuti et al., 2019).

Antimicrobial Activity Test of Nginang Extract

Sterile petri dishes were prepared for the antimicrobial test. A bacterial suspension was prepared by taking several pure colonies of bacteria using a sterile inoculating loop and suspending them in 0.9% NaCl solution. The turbidity of the suspension was adjusted to the 0.5 McFarland standard, equivalent to approximately 1.5×10^8 CFU/mL. A volume of 0.1 mL of the bacterial suspension was added into a petri dish. Subsequently, 15 mL of NA medium was added and mixed evenly by gently rotating the dish in a figure-eight motion, then allowed to solidify. *Streptococcus mutans* colonies grown on Muller Hinton Agar (MHA) plates were treated by placing Kirby–Bauer paper disks that had been previously soaked in *nginang* extract for 15 minutes. The plates were then incubated at 37°C for 24 hours. After incubation, the diameter of the inhibition zones formed at each concentration and replication was measured. The values were summed and divided by the number of replications to obtain the average inhibition zone diameter for concentrations of 5%, 10%, and 15%.

Procedure for Toothpaste Preparation

The active ingredient nginang extract was weighed according to concentrations of 5%, 10%, and 15%, along with additional ingredients including calcium carbonate, glycerin, sodium carboxymethyl cellulose (CMC-Na), sodium lauryl sulfate, saccharin, methyl paraben, peppermint oil, and distilled water. First, Na-CMC was dissolved in hot water and allowed to stand for 15 minutes, then stirred until homogeneous to form Mass 1. Calcium carbonate was ground and mixed with sodium lauryl sulfate until homogeneous, then added to Mass 1 while continuously grinding to form Mass 2. The ethanol extract of betel leaves was dissolved in glycerin, mixed thoroughly, and added to Mass 2 while grinding until homogeneous. Nipagin (methyl paraben) and saccharin were dissolved in the remaining water until completely dissolved and then added to Mass 2, followed by grinding until a paste mass was formed. Finally, peppermint oil was added to the paste and mixed until homogeneous. The prepared paste was then transferred into toothpaste containers (Nuzulia, 2019).

pH Test

The pH measurement was conducted using universal pH paper. A total of 1 g of toothpaste was diluted with distilled water to a final volume of 100 mL. The universal pH strip was then immersed into the prepared solution. The pH value of the preparation had to comply with the Indonesian National Standard (SNI), which ranges from 4.5 to 10.5. If the pH was above or below this standard range, the preparation was considered non-compliant (Rahmnah, 2019).

Organoleptic Test

Organoleptic observations of the toothpaste included color, odor, and texture, which were evaluated objectively. This test aimed to determine whether significant changes occurred in the prepared formulation (Rahmnah, 2019). The expected characteristics included a whitish-reddish or brownish color, a mint aroma combined with natural ingredients, and a semi-solid texture typical of toothpaste.

Foam Formation Test

A 1 g sample of toothpaste was weighed and placed into a graduated cylinder, then distilled water was added to reach a total volume of 10 mL. The cylinder opening was covered with aluminum foil. The cylinder was shaken for 20 minutes and then allowed to stand for 5 minutes. The foam height was measured using a ruler. The maximum acceptable foam height for toothpaste preparations is 15 mm (Rahmnah, 2019).

Homogeneity Test

The homogeneity test was conducted by applying a small amount of toothpaste onto a glass slide and observing its uniformity. If no coarse particles were observed on the glass surface, the toothpaste was considered homogeneous. Conversely, the presence of coarse particles indicated that the toothpaste formulation was not homogeneous (Rahmnah, 2019).

Viscosity Test

Viscosity determination was conducted using a Brookfield viscometer equipped with spindle number 6 at a rotation speed of 2 rpm. The spindle was attached to the spindle holder and lowered into the sample until fully immersed. The spindle was allowed to rotate, and the viscosity value indicated by the red needle was recorded.

The test was performed weekly for three weeks during storage (Afni et al., 2015).

Antimicrobial Test of Nginang Toothpaste

Five petri dishes were prepared and filled with approximately 15 mL of Mueller Hinton Agar (MHA). The medium was allowed to solidify for several minutes. *Streptococcus mutans* bacteria were collected using a sterile cotton swab and evenly spread across the surface of the solidified MHA medium. Paper disks were then soaked in toothpaste samples with concentrations of 5%, 10%, and 15%, along with a positive control (Shasha Sirih Siwak herbal toothpaste) and a negative control (toothpaste formulation without nginang extract). After 5–15 minutes, the disks were placed on the surface of the agar medium. The plates were then incubated for 24 hours at 35–37°C. Following incubation, the diameter of the inhibition zone (clear zone) was measured (Handayani et al., 2017). The inhibition zone was interpreted according to the following criteria: < 5 mm : weak activity, 5–10 mm : moderate activity, 10–20 mm : strong activity, > 20 mm : very strong activity against the test microorganism.

RESULTS AND DISCUSSION

Phytochemical Screening of the *Nginang* Herbal Mixture

Phytochemical screening is a qualitative test conducted to determine the presence of bioactive compounds in a material or to identify the classes of active compounds contained in an extract. The phytochemical screening performed in this study included tests for triterpenoids, flavonoids, saponins, tannins, phenolics, and alkaloids (Prayitno & Utami, 2024).

Based on the results of phytochemical screening conducted at the Research Laboratory of the Polytechnic of Industrial Chemical Technology, the findings are presented in Table 1.

Table 1. Phytochemical screening results of the *nginang* herbal mixture

No	Compound Test	Result
2	Flavonoids	Positive
3	Saponins	Positive
4	Tannins	Negative
5	Phenolics	Positive
6	Alkaloids	Positive

The triterpenoid compounds showed positive results in the phytochemical screening of the *nginang* herbal extract, as indicated by the formation of red, purple, or orange coloration after the addition of acetic anhydride and concentrated sulfuric acid. This color change indicated the presence of triterpenoid compounds in the sample. Triterpenoids are known to disrupt the structure of bacterial cell membranes, thereby inhibiting the growth of pathogenic microorganisms (Syafitri et al., 2020). Previous research on the formulation of herbal toothpaste based on traditional ingredients reported that the presence of triterpenoid compounds contributed to increased antibacterial activity against *Streptococcus mutans*, the primary bacterium responsible for dental caries (Suparno et al., 2021).

The phytochemical screening results also indicated that the *nginang* herbal mixture contained flavonoid compounds, which were characterized by the appearance of a pale yellow color following the addition of magnesium powder, concentrated hydrochloric acid, and amyl alcohol. This result confirmed the presence of flavonoids in the sample. Flavonoids are known to exhibit antibacterial activity by inhibiting bacterial cell wall synthesis and interfering with microbial enzyme activity. In the context of herbal toothpaste, flavonoids play an important role in reducing plaque-forming bacteria and maintaining oral health (Faizah et al., 2024).

The presence of saponins in the phytochemical screening of the *nginang* herbal mixture also showed positive results, which supports its potential use in herbal toothpaste formulations. Saponins possess natural surfactant properties that facilitate the tooth-cleaning process by enhancing foam formation. In addition, they exhibit antibacterial activity by increasing the permeability of bacterial cell membranes (Hayati et al., 2024).

The phytochemical screening results further demonstrated that the *nginang* herbal mixture contained phenolic compounds, as indicated by color changes (green, red, blue, purple, or black—positive result) after the addition of 1% $FeCl_3$ solution. Phenolic compounds are known to possess antibacterial and antioxidant activities by damaging bacterial cell walls and disrupting microbial metabolic processes. The presence of phenolic compounds in the *nginang* herbal mixture therefore strengthens its potential as a natural antimicrobial agent capable of inhibiting the growth of *Streptococcus mutans*.

Alkaloid compounds also showed positive results in the phytochemical screening of the *nginang* herbal mixture. Alkaloids are known to exhibit antibacterial activity that can inhibit the growth and development of pathogenic bacteria. The presence of alkaloids in the *nginang* herbal mixture therefore supports its potential use as an active ingredient in herbal toothpaste formulations (Saraswati et al., 2019).

Antimicrobial Activity of the *Nginang* Mixture in Herbal Toothpaste

Based on the results of antimicrobial activity testing of the *nginang* mixture conducted at the Microbiology Research Laboratory of the State Islamic University of North Sumatra, the results obtained are presented in Table 2.

Table 2. Antimicrobial activity of the nginang mixture at different concentrations

Concentration	Streptococcus mutans Inhibition Zone (mm)				Mean Inhibition Zone (mm)	Criteria
	U1	U2	U3	U4		
K+	24,3 mm	22,9 mm	22,4 mm	19,9 mm	22,3 mm	Sangat kuat
K-	0 mm	0 mm	0 mm	0 mm	0 mm	Tidak ada Aktivitas
5%	14,9 mm	16,5 mm	17,0 mm	17,2 mm	16,4 mm	Kuat
10%	22,7 mm	23,7 mm	21,9 mm	22,7 mm	22,7 mm	Sangat kuat
15%	23,8 mm	25,5 mm	25,5 mm	27,8 mm	25,6 mm	Sangat kuat

Note: K+ = Chloramphenicol, K− = DMSO

Based on the results of the antimicrobial activity test of the *nginang* mixture, differences in inhibition zone diameters were observed across each concentration variation, namely 5%, 10%, 15%, as well as the negative and positive controls. These differences indicate that the concentration of the *nginang* mixture influenced its ability to inhibit the growth of microorganisms.

At a concentration of 5%, the inhibition zones formed in each replicate showed varying values with an average diameter of 16.4 mm. These results indicate that the *nginang* mixture at a 5% concentration was able to inhibit microbial growth, although the inhibitory activity remained lower compared with higher concentrations. The variation in inhibition zone diameters among replicates may have been influenced by differences in microbial responses to the active compounds contained in the *nginang* mixture.

At a concentration of 10%, the inhibition zone diameter increased with an average value of 22.7 mm. This increase suggests that a higher concentration of the *nginang* mixture enhanced its antimicrobial activity. The inhibition zones observed at this concentration were larger and more uniform than those at 5%, indicating a more optimal inhibitory effect.

Meanwhile, the 15% concentration produced the largest inhibition zone diameter with an average value of 25.6 mm. These results demonstrate that the 15% concentration provided the strongest inhibitory effect on the growth of the test microorganisms. The large inhibition zone formed indicates that increasing the concentration of the *nginang* mixture increases the amount of active compounds involved in inhibiting microbial growth (Muchtaromah, 2016).

Overall, the results of this study demonstrate a relationship between increasing concentrations of the *nginang* mixture and the enlargement of inhibition zone diameters. The 5% concentration exhibited the lowest inhibitory activity, the 10% concentration showed moderate inhibitory activity, and the 15% concentration demonstrated the highest inhibitory activity. Therefore, the *nginang* mixture has potential to be used as an active ingredient in herbal toothpaste formulations due to its ability to effectively inhibit microbial growth.

The negative control (−) used DMSO solution and produced an inhibition zone of 0 mm, indicating no antibacterial activity. This result confirms that DMSO does not possess antibacterial properties. In contrast, the positive control (+) used the antibiotic chloramphenicol and produced an inhibition zone of 22.3 mm, indicating very strong antibacterial activity. This result demonstrates that chloramphenicol inhibits bacterial growth by interfering with protein synthesis during antibacterial activity.

pH Test of Herbal Toothpaste

The results of the pH measurement of the herbal toothpaste formulation conducted at the Microbiology Research Laboratory of Universitas Islam Negeri Sumatera Utara are presented in Table 3. Based on the test results, all formulations showed pH values within the slightly alkaline range.

Table 3. Results of pH test of herbal toothpaste

No	Sample Code	pH Value
1	K-	8,42
2	K+	8,40
3	F1	8,35
4	F2	8,20
5	F3	8,04

Note: K− = Toothpaste formulation without the addition of *nginang* herbal mixture
K+ = Shasha Herbal Toothpaste Betel & Siwak

Based on the results presented in Table 3, the pH values of the herbal toothpaste ranged from 8.04 to 8.42. The base formulation (K−) exhibited the highest pH value of 8.42, whereas the formulation containing the highest concentration of *nginang* herbal mixture (F3) showed the lowest pH value of 8.04. The decrease in pH observed from K− to F3 indicates that the addition of the *nginang* herbal mixture influenced the pH of the herbal toothpaste formulation, although the reduction remained relatively small.

Nevertheless, all formulations still exhibited pH values that met the acceptable requirements for toothpaste, which fall within the neutral to slightly alkaline range. According to the Indonesian National Standard (SNI 12-3524-1995), the safe pH range for toothpaste intended for daily use is 4.5–10.5. Therefore, the herbal toothpaste formulations developed in this study can be considered safe for use and are unlikely to damage tooth enamel or cause irritation to oral tissues.

Organoleptic Test of Herbal Toothpaste

The organoleptic test included evaluations of color, aroma, and texture, which were conducted with 20 panelists consisting of university students around the Microbiology Research Laboratory of Universitas Islam Negeri Sumatera Utara. After the questionnaire data from the panelists were analyzed, the organoleptic evaluation results were obtained as presented in Table 4.

Table 4. Organoleptic characteristics of herbal toothpaste (aroma, color, and texture)

Concentration	Aroma	Color	Texture
K-	Mint aroma	White	Semi-solid
F1 (5%)	Mint aroma & nginang herbal mixture	Slightly brownish	Semi-solid
F3 (15%)	Mint aroma & nginang herbal mixture	Dark brown	Semi-solid

Based on the organoleptic results presented in the table above, differences were observed among several herbal toothpaste formulations tested, namely those with 0% (K−), 5%, 10%, and 15% concentrations of the *nginang* herbal mixture. In the K− (0%) concentration or base formulation, the toothpaste exhibited a typical mint aroma commonly found in toothpaste products. This indicates that mint aroma is commonly added to toothpaste to provide a refreshing sensation. Meanwhile, formulations with 5%, 10%, and 15% concentrations displayed a mint aroma combined with the aroma of the *nginang* herbal mixture. The increasing intensity of the herbal aroma with increasing active ingredient concentration indicates that the extract of the *nginang* mixture possesses a distinctive characteristic aroma that becomes more prominent in the formulation (Polontalo et al., 2025).

The color of the toothpaste also changed with increasing concentrations of herbal ingredients. At 0% concentration (K−), the toothpaste appeared white, similar to typical commercial toothpaste. At 5% concentration, the color changed to slightly brownish, while at 10% and 15% concentrations, the color became progressively darker, ranging from brown to dark brown. This indicates that the coloration of the formulation originates from natural pigments present in betel leaves, areca nut, and gambir (Putri, 2023).

All formulations exhibited a semi-solid texture, indicating adequate physical stability during handling, pressing, and application on a toothbrush. The absence of overly soft or hard textures suggests a balanced composition between the herbal ingredients and texture-forming additives. This observation aligns with Nugroho et al. (2021), who reported that semi-solid herbal toothpaste is easier to apply and more acceptable to users.

Foam Formation Test of Herbal Toothpaste

Based on the results of the foam formation test of the *nginang* herbal toothpaste conducted at the Microbiology Research Laboratory of Universitas Islam Negeri Sumatera Utara, the results obtained are presented in Table 5.

Table 5. Foam formation test results of herbal toothpaste

Concentration	Foam Height
K-	16,5 mm
F1 (5%)	15,1 mm
F2 (10%)	15,5 mm
F3 (15%)	20,6 mm
K+	20,3 mm

Note: K− = Toothpaste formulation without the addition of *nginang* herbal mixture
K+ = Shasha Herbal Toothpaste Betel & Siwak

Based on Table 5, formulation F1 (5%) produced a foam height of 15.1 mm, which represents the lowest value among the tested formulations. The relatively low foam height in F1 may be attributed to the relatively small concentration of the *nginang* herbal active ingredients, which had not yet contributed optimally to foam stability. In formulation F2 (10%), the foam height increased to 15.5 mm, indicating that the increase in *nginang* herbal concentration began to influence foam formation ability. Furthermore, formulation F3 (15%) showed the highest foam height among the tested formulations, reaching 20.6 mm. This value was even higher than the negative control and approached the positive control. These results indicate that increasing the concentration of the *nginang* herbal mixture significantly enhanced foam formation.

The negative control (K−) produced a foam height of 16.5 mm, indicating adequate foam-forming ability but still lower than F3 and the positive control. This suggests that although the base formulation without the *nginang* mixture could still produce foam, the addition of herbal active ingredients contributed to increasing the foam volume. Meanwhile, the positive control (K+) showed a foam height of 20.3 mm, which was nearly equivalent to that of formulation F3. This similarity indicates that formulation F3 possessed foam-forming ability comparable to that of the commercial reference toothpaste. This finding suggests that herbal toothpaste containing 15% *nginang* mixture not only excels in natural ingredient content but also meets the physical parameters expected from modern toothpaste formulations.

Homogeneity Test of Herbal Toothpaste

Based on the results of the homogeneity test conducted at the Microbiology Research Laboratory of Universitas Islam Negeri Sumatera Utara, the results are presented in Table 6.

Table 6. Homogeneity test results of herbal toothpaste

No	Concentration	Category
1	K-	Homogen
2	F1	Homogen
3	F2	Homogen
4	F3	Homogen

Based on the homogeneity test results presented in Table 6, all formulations (K−, F1, F2, and F3) were classified as homogeneous. This observation was indicated by the absence of visible lumps, coarse particles, or color variations during visual inspection of the preparations. The uniform appearance suggests that all components in the formulation were evenly distributed throughout the toothpaste matrix.

These findings indicate that the mixing process between the active ingredients of the *nginang* herbal mixture—comprising betel leaves (*Piper betle*), areca nut (*Areca catechu*), gambir (*Uncaria gambir*), and slaked lime—and the toothpaste base was successfully performed. Effective mixing ensures that the active compounds are consistently dispersed within the formulation, which is essential for maintaining the stability, quality, and uniformity of the product. Furthermore, a homogeneous formulation helps ensure that each portion of the toothpaste contains relatively equal concentrations of active ingredients, thereby supporting consistent performance during use.

Viscosity Test of Herbal Toothpaste

The viscosity test of the herbal toothpaste was conducted to determine the thickness and flow characteristics of the herbal toothpaste formulations. The results obtained from testing at the Industrial Chemical Technology Polytechnic Research Laboratory are presented in Table 7.

Table 7. Viscosity test results of herbal toothpaste

Concentration	Kinematic Viscosity (cP)
K+	27.853,41
K-	19.917,27
F1 (5%)	24.601,76
F2 (10%)	26.613,29
F3 (15%)	30.459,05

Note: K− = Toothpaste formulation without the addition of *nginang* herbal mixture
K+ = Shasha Herbal Toothpaste Betel & Siwak

Based on the viscosity test results, each herbal toothpaste formulation exhibited different viscosity values. This indicates that differences in the composition of herbal active ingredients and formulation base concentrations influenced the flow properties of the preparations. Viscosity is an important parameter because it is associated with ease of application, spreadability, and the ability of the toothpaste to retain active ingredients on the tooth surface during brushing. In toothpaste formulations, the ideal semi-solid texture is generally achieved through a balance between thickening agents and natural active ingredients.

The K− formulation showed the lowest viscosity value of 19,917.27 cP, indicating a relatively thinner consistency compared with other formulations. The F1 formulation showed an increased viscosity value of 24,601.76 cP, suggesting that the addition or increased concentration of herbal ingredients such as betel leaves, areca nut, and gambir began to influence the internal structure of the preparation. Furthermore, F2 exhibited a viscosity value of 26,613.29 cP, indicating a thicker consistency than F1. This value suggests that the composition of active ingredients and base components in F2 approached the expected viscosity characteristics for toothpaste preparations. The F3 formulation exhibited the highest viscosity value of 30,459.05 cP, making it the thickest formulation among all samples. This high viscosity indicates strong interactions among formulation components, both from thickening agents and the herbal biomass of the *nginang* mixture.

Compared with the positive control (K+), which exhibited a viscosity value of 27,853.41 cP, formulation F3 demonstrated a slightly higher viscosity, indicating a thicker consistency, while F2 showed a viscosity value that was relatively close to that of the positive control. According to the Indonesian National Standard (SNI 12-3524-1995) for toothpaste, the recommended viscosity range is 20,000–50,000 cP (Wahyuni & Fitria, 2024). Therefore, all tested formulations that fall within this range can be considered physically acceptable in terms of viscosity.

The results indicate that the viscosity characteristics of the *nginang* herbal toothpaste formulations were comparable to those of the commercial reference product, with some formulations, particularly F3, even exhibiting higher viscosity values. This suggests that the incorporation of herbal ingredients did not compromise the rheological quality of the toothpaste preparation. Instead, the presence of natural components such as betel leaves, areca nut, gambir, and slaked lime may have contributed to strengthening the internal structure of the formulation. Consequently, these herbal materials not only serve as antibacterial agents but also play a role in enhancing the physical stability and consistency of the toothpaste formulation.

Antimicrobial Test of Herbal Toothpaste

Based on the results of antimicrobial activity testing of the *nginang* herbal toothpaste conducted at the Microbiology Research Laboratory of Universitas Islam Negeri Sumatera Utara, the results are presented in Table 8.

Table 8. Antimicrobial activity of nginang herbal toothpaste

Concentration	Inhibition Zone of *Streptococcus mutans* (mm)				Mean Inhibition Zone (mm)	Criteria
	U1	U2	U3	U4		
K+	19,9 mm	19,7 mm	20,9 mm	21,0 mm	20,3 mm	Sangat kuat
K-	18,1 mm	19,5 mm	18,3 mm	18,2 mm	18,5 mm	Kuat
F1	17,9 mm	18,8 mm	21,3 mm	21,8 mm	19,9 mm	Kuat
F2	19,7 mm	14,5 mm	20,0 mm	18,3 mm	18,1 mm	Kuat
F3	22,4 mm	19,3 mm	17,8 mm	19,5 mm	19,7 mm	Kuat

Note: K− = Toothpaste formulation without the addition of *nginang* herbal mixture
K+ = Shasha Herbal Toothpaste Betel & Siwak

Based on the antimicrobial test results, formulation F1 (5%) showed an average inhibition zone diameter of 19.9 mm, obtained from four repetitions with variations in horizontal and vertical measurements. These results indicate that at a concentration of 5%, the *nginang* herbal toothpaste already exhibited the ability to inhibit bacterial growth within the moderate to strong category.

Formulation F2 (10%) showed an average inhibition zone diameter of 18.1 mm, slightly lower than that of F1 despite having a higher concentration of the *nginang* mixture. This difference may be caused by interactions among active compounds at higher concentrations, such as alkaloids from areca nut, which at certain levels may form complexes that reduce the diffusivity of active compounds in the agar medium.

Formulation F3 (15%) showed an average inhibition zone diameter of 19.7 mm, indicating that increasing the concentration of the *nginang* mixture to 15% was able to maintain strong and relatively stable antimicrobial activity. The inhibition zone diameter in F3 was larger than that in F2 and close to that of F1, suggesting that at certain concentrations, the combination of active compounds from betel leaves, areca nut, gambir, and slaked lime works optimally to inhibit bacterial growth.

The negative control (K−) showed an average inhibition zone diameter of 18.5 mm, indicating that the toothpaste base without the *nginang* mixture still possessed antibacterial activity. This activity may originate from base components such as surfactants or other supporting ingredients in the formulation. Meanwhile, the positive control (K+) exhibited the highest average inhibition zone diameter of 20.3 mm among all tested samples, indicating very strong antimicrobial activity and serving as the effectiveness reference in this study. The inhibition zone diameters of F3 (19.7 mm) and F1 (19.9 mm) were close to the positive control value, indicating that the *nginang* herbal toothpaste possesses antibacterial potential comparable to the commercial reference product.

A statistical analysis was conducted to evaluate the effect of different treatments on the antibacterial activity of the herbal toothpaste formulations. The results of the One-Way ANOVA test showed a significance value of 0.318 ($p > 0.05$), indicating that there were no statistically significant differences in the inhibition zones of *Streptococcus mutans* among the tested treatments. These findings suggest that variations in the concentration of the *nginang* herbal mixture did not produce significantly different antibacterial effects under the experimental conditions. However, the presence of base-forming components in the toothpaste formulation may still contribute to antibacterial activity, as reflected by the observed inhibition zone diameters. Several formulation excipients used in toothpaste, such as surfactants and preservatives, are known to exhibit mild antimicrobial properties that may influence the overall inhibitory effect (Pratiwi, 2005; Marsh & Martin, 2016).

The fluctuations in the average inhibition zone diameters among the formulations may be influenced by several factors. One important factor is the diffusion ability of active compounds in the agar medium, which determines how effectively antibacterial compounds spread and inhibit bacterial growth. According to Balouiri et al. (2016), the size of inhibition zones in agar diffusion assays can be affected not only by antimicrobial potency but also by the molecular size, solubility, and diffusion capacity of the active substances. In addition, interactions between herbal compounds and formulation additives, such as CMC-Na and calcium carbonate, may alter the release and diffusion of active compounds within the medium. These interactions can influence the availability of antibacterial agents and consequently affect the measured inhibition zone diameter.

Furthermore, other ingredients present in the toothpaste formulation, including sodium lauryl sulfate, nipagin (methyl paraben), and peppermint oil, may also contribute to the overall antibacterial effect. Sodium lauryl sulfate is known to possess antimicrobial activity against oral bacteria due to its ability to disrupt bacterial cell membranes, while essential oils such as peppermint oil contain bioactive compounds with antibacterial properties (Marsh & Martin, 2016; Pratiwi, 2005). The combined activity of these components may explain why several formulations showed relatively similar inhibition zone values, such as between K− and F2 and between F1 and F3, even though their herbal concentrations differed.

Despite these similarities, the positive control still demonstrated the highest antibacterial activity among all tested samples. This finding indicates that while the herbal toothpaste formulations exhibited promising antibacterial potential, commercial formulations may contain optimized combinations of active ingredients and excipients that enhance antibacterial efficacy. Overall, these results suggest that increasing the concentration of active herbal ingredients does not necessarily lead to a proportional increase in inhibition zone diameter, as the antibacterial effect in a complex formulation is influenced by multiple factors, including compound diffusion, interactions among ingredients, and the contribution of supporting antimicrobial agents.

CONCLUSION

Based on the results of this study, it can be concluded that: (1) The nginang mixture consisting of betel leaves (*Piper betle*), gambir (*Uncaria gambir*), areca nut (*Areca catechu* L.), and slaked lime was found to contain secondary metabolites, including triterpenoids, flavonoids, saponins, phenolics, and alkaloids, which have potential antimicrobial properties. (2) The extract of the nginang mixture exhibited antimicrobial activity against *Streptococcus mutans*, with average inhibition zones of 16.4 mm (5%), 22.7 mm (10%), and 25.6 mm (15%), where the 15% concentration demonstrated the highest inhibitory effect. (3) The formulation of herbal toothpaste with the addition of the nginang mixture met the physical preparation parameters, including pH values ranging from 8.04–8.42 in accordance with the Indonesian National Standard (SNI), homogeneous consistency, semi-solid texture, foam height of up to 20.6 mm, and viscosity within the required range. (4) Antimicrobial testing of the herbal toothpaste showed that all formulations (5%, 10%, and 15%) were capable of inhibiting the growth of *Streptococcus mutans* in the strong to very strong category, with effectiveness approaching that of the positive control. Therefore, this formulation has the potential to be developed as an alternative herbal toothpaste based on traditional ingredients for maintaining oral and dental health.

RECOMMENDATION

Further research is required to conduct long-term stability testing and safety evaluation (oral mucosal irritation test) of the nginang-based herbal toothpaste formulation before it can be developed into a commercial product. Future studies may also examine its antibacterial activity against other oral bacteria involved in plaque formation and dental caries in order to determine a broader spectrum of antimicrobial activity.

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