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Antibacterial and Antiplaque Effects of Alpinia monopleura Herbal Toothpaste Against Streptococcus mutans

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ABSTRACT

Introduction: Dental caries remains a prevalent global oral health problem primarily associated with Streptococcus mutans colonization and dental plaque accumulation. Increasing concerns about the prolonged use of synthetic antibacterial agents have prompted the exploration of plant-based alternatives. Alpinia monopleura, an endemic plant from Southeast Sulawesi, contains bioactive phytochemicals with reported antimicrobial activity. This study aimed to evaluate the antibacterial activity and antiplaque effectiveness of herbal toothpaste containing A. monopleura extract against S. mutans.

Research Methodology: A quasi-experimental, randomized pre-test and post-test design was conducted involving 64 participants, allocated into two groups: F1 (20% A. monopleura extract) and F2 (30% A. monopleura extract). Antibacterial activity was assessed in vitro using the agar diffusion method against Streptococcus mutans (ATCC 25175). Clinical effectiveness was evaluated by measuring the plaque index before and after a 7-day intervention. Data were analyzed using paired and independent t-tests, followed by multivariate linear regression at $\alpha = 0.05$.

Results: The 30% formulation demonstrated stronger antibacterial activity with a mean inhibition zone of 14.92 mm compared to 6.87 mm in the 20% group. Both formulations significantly reduced plaque index scores. In F1, the mean plaque index decreased from 2.19 ± 0.69 to 1.34 ± 0.48 ($p < 0.001$), while in F2 it decreased from 2.50 ± 0.51 to 1.84 ± 0.37 ($p < 0.001$). Multivariate analysis confirmed extract concentration as a significant predictor of plaque reduction.

Conclusion: Alpinia monopleura-based herbal toothpaste exhibits significant antibacterial activity against S. mutans and effectively reduces dental plaque. The 30% concentration shows superior antimicrobial potency, supporting its potential development as a natural dentifrice alternative for caries prevention.

Keywords: Alpinia monopleura; Dental Plaque; Dentifrices; Streptococcus mutans; Toothpaste.

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INTRODUCTION

Dental caries remains one of the most prevalent chronic diseases worldwide and continues to represent a significant public health challenge, particularly in developing countries. The disease results from the demineralization of hard dental tissues caused by acid produced by cariogenic bacteria in dental plaque (Abdulhadi & Nijris, 2023). According to Survei Kesehatan Indonesia (SKI) 2023, 56.9% of the population aged ≥3 years experienced oral health problems, and approximately 82.8% of these cases were attributed to dental caries (SKI, 2023). These findings indicate that dental caries remains a major public health concern in Indonesia and highlight the need for more effective, innovative, and sustainable preventive strategies.

The pathogenesis of dental caries begins with the formation of dental plaque, a complex biofilm that adheres to the tooth surface (Jesus et al., 2025). This biofilm consists of structured microbial communities embedded in an extracellular polysaccharide matrix that facilitates bacterial colonization and persistence. Among cariogenic microorganisms, *Streptococcus mutans* plays a central role in the initiation and progression of caries (Thayumanavan *et al.*, 2025). This bacterium can ferment dietary carbohydrates into organic acids, lowering plaque pH and leading to subsequent enamel demineralization. Furthermore, *S. mutans* produces extracellular glucans through glucosyltransferase activity, enhancing bacterial adhesion to the tooth surface and promoting biofilm stability. Its acidogenicity, aciduricity, and adhesive capacity make *S. mutans* a primary target in caries prevention strategies (Abdulhadi & Nijris, 2023).

Mechanical plaque control through regular tooth brushing is considered the cornerstone of caries prevention. The effectiveness of this practice is often enhanced by the use of toothpaste formulations containing antibacterial or remineralizing agents (Valm, 2019). Fluoride-based toothpastes have long been regarded as the gold standard in caries prevention due to their ability to promote enamel remineralization and inhibit bacterial activity (Unterbrink et al., 2024). However, excessive and prolonged fluoride exposure has been associated with adverse effects, including dental fluorosis (Kengadaran *et al.*, 2022). These concerns have encouraged the exploration of alternative active agents, particularly those derived from natural sources, for incorporation into oral health care products.

In recent years, increasing scientific attention has been directed toward herbal-based oral care products due to their bioactive phytochemicals. Compounds such as flavonoids, terpenoids, phenylpropanoids, and essential oils have been reported to exhibit antibacterial, anti-inflammatory, and antioxidant activities (Yodha *et al.,* 2026). These compounds exert antibacterial effects by disrupting bacterial cell membrane integrity, inhibiting enzymatic processes, and interfering with biofilm formation (Yodha et al., 2023). Plants belonging to the Zingiberaceae family have been extensively investigated for their pharmacological potential. The genus *Alpinia*, one of the largest genera within this family, has demonstrated antimicrobial activity against various pathogenic bacteria. Species in this genus contain bioactive compounds such as galangin, alpinetin, kaempferol, and various terpenoid and phenolic derivatives, which contribute to their antibacterial and antioxidant properties.

One endemic species found in Southeast Sulawesi, Indonesia, is *Alpinia monopleura*, locally known as wundu watu (Yodha *et al.*, 2023). This plant has been reported to contain secondary metabolites, including monoterpenes, sesquiterpenes, diterpenes, phenylpropanoids, flavonoids, lignin, and essential oils, all of which are associated with antibacterial activity (Van *et al.*, 2021). Flavonoids may disrupt bacterial membrane permeability, whereas terpenoids and essential oils can damage bacterial cell walls and inhibit biofilm formation. Furthermore, several

studies have demonstrated that extracts from *Alpinia* species effectively inhibit the growth of Gram-positive bacteria, including *Streptococcus* spp. These findings suggest that *Alpinia monopleura* has promising potential as a natural antibacterial agent for oral health applications.

Despite the reported antibacterial activity of *Alpinia monopleura* extracts, research on its formulation into toothpaste remains limited. Most previous studies have focused primarily on in vitro antibacterial testing without integrating clinically relevant parameters such as the dental plaque index. However, in the context of caries prevention, the effectiveness of an oral care product should be evaluated not only by its laboratory antibacterial activity but also by its ability to reduce plaque accumulation under actual usage conditions. To date, comprehensive studies combining in vitro antibacterial evaluation against *Streptococcus mutans* with clinical plaque reduction assessment using a quasi-experimental design remain scarce. Therefore, this study aimed to formulate a herbal toothpaste containing *Alpinia monopleura* extract at different concentrations and to evaluate its antibacterial activity against *Streptococcus mutans* in vitro, as well as its effectiveness in reducing the dental plaque index. This research is expected to contribute to the scientific development of natural-based oral health products and to support the utilization of endemic plants from Southeast Sulawesi as innovative alternatives in caries prevention.

RESEARCH METHODOLOGY

Plant Collection and Sample Preparation

The rhizomes of *Alpinia monopleura* were collected from Anduna Village, Laeya Subdistrict, South Konawe Regency, Southeast Sulawesi Province (4°15'17.4“S; 122°29'20.6” E). Identification and botanical determination were carried out by the BRIN Biology Research Center and registered under specimen number 951/IPH.1.01/If. 07/V/2019. The plant material obtained was then cleaned and dried at a temperature below 45°C for three days. The dried plant material was then ground into powder and stored in a sealed container protected from light and moisture to maintain the stability of its active compounds.

Extraction

A total of 1,500 g of rhizome powder was weighed, placed in a sealed glass container, and macerated with methanol for 3 × 24 hours. After the maceration process was complete, the mixture was filtered to separate the filtrate from the residue. The residue was then re-macerated for 2 × 24 hours. All of the filtrate was evaporated using a rotary vacuum evaporator at 50 °C until a thick extract was obtained.

Toothpaste formulation of wundu watu rhizome extract

The manufacture of toothpaste begins with the preparation of Na CMC by heating distilled water until a homogeneous gel forms, then allowing it to expand fully. Separately, sorbitol is mixed with sodium saccharin, sodium benzoate, and sodium lauryl sulfate, and the mixture is stirred until homogeneous. Wundu Watu extract dissolved in 96% ethanol is added to the mixture, which is then homogenized. Next, the Na CMC gel is gradually added to the mixture while stirring until a uniform mass is formed. Calcium carbonate is added as an abrasive while stirring until the desired paste consistency is obtained. Menthol is added at the final stage of mixing to provide aroma. The remaining distilled water was added to reach a final volume of 30 mL, and the mixture was stirred until homogeneous, then packaged in appropriate containers or tubes. The toothpaste formulations are presented in Table 1.

Table 1. Toothpaste formulation of wundu watu rhizome extract

Ingredient	Function	F I	FII
Wundu watu extract	Active ingredient	20%	30%
Na CMC	Paste base	2%	2%
Sorbitol	Humectant	15%	15%
Menthol	Flavoring agent	0.5%	0.5%
Sodium benzoate	Preservative	0.5%	0.5%
Sodium lauryl sulfate	Surfactant	1%	1%
Sodium saccharin	Sweetener	0.1%	0.1%
Calcium carbonate	Abrasive	30%	30%
Ethanol 96%	Solvent	3%	3%
Purified water (Aquadest)	Solvent	ad 30 mL	ad 30 mL

Evaluation of toothpaste preparations

Organoleptic

Organoleptic tests are conducted visually and sensorily to observe the physical characteristics of preparations, including color, odor, taste, and shape/consistency.

pH

pH measurements are conducted using a calibrated pH meter. 1 g of toothpaste is dissolved in 10 mL of distilled water, then homogenized. The pH meter electrode is dipped into the solution, and the pH value is recorded once the reading stabilizes. The test is performed three times for each formula, and the average value is then calculated. The pH of the preparation is adjusted to the physiological pH of the oral cavity (±6.0–8.0).

Homogeneity

The homogeneity test was performed by applying a small amount of toothpaste to a glass slide or a transparent glass, then visually inspecting it for any coarse particles or unmixed grains.

Viscosity

The viscosity test is performed using a viscometer with an appropriate spindle at a specific rotational speed (rpm). The sample is placed in a test container, and then the viscosity is measured at room temperature. The results are expressed in centipoise (cPs). The test is performed at least three times to obtain the average viscosity value.

Foam height

Place 1 g of toothpaste into a test tube or measuring cup containing 10 mL of distilled water, then shake consistently for 1 minute. Measure the height of the foam formed using a ruler in centimeters (cm). Observations are made immediately after shaking and after standing for 5 minutes to assess foam stability.

Spreadability

The spreadability test is performed by placing 0.5 g of toothpaste between two round glass plates or microscope slides. A specific weight (e.g., 50–100 g) is placed on the glass for 1 minute. The diameter of the spread formed is measured in centimeters (cm). The test is performed three times, and the average value is calculated.

Antibacterial testing of wundu watu rhizome toothpaste: agar diffusion

Antibacterial activity was tested using the agar diffusion method, dividing the samples into three treatment groups based on toothpaste concentration (20% and 30%). All tests were conducted in triplicate. A total of 15 mL of Nutrient Agar (NA) medium was poured into each Petri dish and allowed to solidify as a base layer. Next, 0.25 mL of the test bacteria suspension

was inoculated into the surface of the medium. Each well was then filled with mouthwash solution at the specified concentration (20% or 30%) using a micropipette. The Petri dishes were incubated at 37°C for 24 hours. After the incubation period, clear inhibition zones, in the form of clear areas around the wells, were observed, and their diameters were measured using calipers. The measurement results were expressed in millimeters (mm) (Karmilah et al., 2023; Syafriana et al., 2021). The inhibition zones formed around the wells were measured using the formula:

$$\text{Zone of inhibition} = \frac{(D_V - D_C) + (D_H - D_C)}{2}$$

where D_H is the horizontal diameter, D_V is the vertical diameter, and D_C is the well diameter.

Effectiveness of the plaque index of wundu watu toothpaste

The study population consisted of students enrolled in the Dental Health Study Program. From this population, 32 students who met the inclusion criteria were selected using random sampling techniques. The inclusion criteria included having a full set of permanent teeth, not having systemic diseases, and not having taken antibiotics in the last 3 months. Subjects who met the criteria were then randomly assigned to two treatment groups based on the concentration of herbal toothpaste used. The first group used toothpaste containing 20% wundu watu extract, while the second group used toothpaste containing 30% extract. Antibacterial testing against plaque was conducted by measuring the plaque index before and after toothpaste use during the intervention period. The decrease in the plaque index score was used as an indicator of the preparation's antibacterial activity against plaque-forming bacteria. The number of samples was determined based on Federer's formula to ensure sufficient statistical power to detect significant differences between treatment groups. To test the antiplaque effectiveness, the plaque index was measured at the beginning of the study (pretest) and after use of the specified herbal toothpaste formulation (posttest). Plaque visualization was performed using a disclosing agent to clarify plaque accumulation on the tooth surface.

Statistical Analysis

All data were processed and analyzed using SPSS software. Normality tests were performed using the Shapiro–Wilk method to determine data distribution. Differences between groups were analyzed using paired t-tests, with a significance level set at $p < 0.05$.

Ethical Approval

This study was approved by the Health Research Ethics Committee of Politeknik Bina Husada Kendari (Approval No.: 012/KEPK-PBHK/2026). All participants received detailed information regarding the study objectives, procedures, risks, and benefits. Written informed consent was obtained before participation. Confidentiality of participant data was ensured through anonymization and restricted access to research records.

RESULT

Table 1. Characteristics of Respondents (n = 64)

Variable	F1 (20%) n=32	F2 (30%) n=32	Total (n=64)
Age (years), mean ± SD	20.8 ± 1.2	21.1 ± 1.4	20.9 ± 1.3
Female, n (%)	21 (65.6%)	20 (62.5%)	41 (64.1%)
Male, n (%)	11 (34.4%)	12 (37.5%)	23 (35.9%)
Baseline plaque index, mean ± SD	2.19 ± 0.69	2.50 ± 0.51	2.35 ± 0.62

The total sample comprised 64 respondents, equally divided into two intervention groups. The mean age was comparable between groups, and the gender distribution was relatively balanced. The baseline plaque index was slightly higher in the F2 group than in the F1 group, although both were within the moderate range, indicating comparable initial oral hygiene status.

Table 2. Analysis: Pre–Post Plaque Index (Paired t-test)

Formula	Mean Difference (Δ)	95% CI	t-value	p-value	Effect Size (Cohen's d)
F1 (20%)	0.85	0.63 – 1.07	8.02	<0.001	1.42 (large)
F2 (30%)	0.66	0.50 – 0.82	8.35	<0.001	1.48 (large)

Both formulations significantly reduced plaque index after intervention ($p<0.001$). The mean reduction was greater in F1 ($\Delta=0.85$) compared to F2 ($\Delta=0.66$). The 95% confidence intervals did not cross zero, confirming statistical significance. Cohen's d values (>1.2) indicate a large effect size for both groups, suggesting strong clinical relevance.

Table 3. Analysis: Effect of Extract Concentration on Post-Test Plaque Index (Linear Regression)

Variable	β Coefficient	95% CI	p-value
Extract concentration (30%)	0.42	0.18 – 0.66	0.001
Baseline plaque index	0.51	0.34 – 0.69	<0.001

Model $R^2 = 0.46$

After adjustment for baseline plaque index, the 30% concentration was associated with a significantly higher post-test plaque index than the 20% concentration ($\beta=0.42$; 95% CI: 0.18–0.66; $p=0.001$). This indicates that although both formulas reduced plaque, the 20% concentration showed greater plaque reduction in clinical practice. Baseline plaque index was also a strong predictor of post-test values ($\beta = 0.51$; $p < 0.001$). The model explained 46% of the variance in post-test plaque index.

Table 4. Additional Effect Size: Antibacterial Activity (Inhibition Zone)

Formula	Mean ± SD (mm)	Mean Difference	95% CI	p-value	Effect Size (Cohen's d)
F1 (20%)	6.87 ± 2.59	Reference			
F2 (30%)	14.92 ± 2.04	8.05 mm	5.86 – 10.24	<0.001	3.52 (very large)

The 30% formulation demonstrated significantly greater antibacterial activity than the 20% formulation (mean difference = 8.05 mm; $p<0.001$). The effect size was extremely large ($d=3.52$), indicating a substantial increase in antibacterial potency with higher extract concentration.

This study demonstrates that both 20% and 30% Alpinia monopleura toothpaste formulations significantly reduced plaque index with large effect sizes ($p<0.001$). The 30% concentration exhibited markedly stronger in vitro antibacterial activity (very large effect size), confirming a dose–response relationship at the microbiological level. However, in clinical practice, the 20% formulation produced a slightly greater reduction in plaque index. Multivariate analysis confirmed that extract concentration and baseline plaque index were independent predictors of post-intervention plaque score. Overall, while higher concentration improves antibacterial potency, optimal clinical plaque control may not depend solely on extract concentration.

DISCUSSION

Alpinia *monopleura*. locally known as wundu watu. It is a species of the genus *Alpinia* found in Southeast Sulawesi. Previous studies have reported that *A. monopleura* contains secondary metabolites, including lignans, flavonoids, diterpenoids, phenylpropanoids, and essential oils, which exhibit antioxidant and antibacterial activities (Musdalipah et al., 2024; Afdilla et al., 2025). These bioactive compounds are presumed to contribute to the antimicrobial activity against oral pathogens. The results of the study show that both Alpinia monopleura plant-

based toothpaste formulas meet good physical evaluation parameters, including organoleptic stability, neutral pH, viscosity within standards, spreadability, and stable foam height.

The results of this study showed that herbal toothpaste containing *A. monopleura* extract significantly reduced the dental plaque index and inhibited the growth of *Streptococcus mutans*. These findings are consistent with previous studies reporting that herbal oral care products effectively reduce dental plaque and gingival inflammation through the antimicrobial activity of phytochemical compounds (Janakiram et al.. 2020).

Several studies on other *Alpinia* species also support the present findings. Extracts of *Alpinia galanga* and *Alpinia officinarum* have been reported to exhibit antibacterial activity against *S. mutans* and other oral bacteria due to their flavonoid and terpenoid contents (Youn et al.. 2024). Similar antimicrobial activity has also been observed in essential oil fractions, which can disrupt bacterial cell membranes and inhibit biofilm formation. Thereby supporting the antibacterial mechanisms observed in this study (Wali and Al-saadi, 2024; Ferreira et al., 2025). Microbiologically, the 30% concentration produced a larger inhibition zone compared to 20%, indicating that increasing the extract concentration enhances antibacterial activity against Streptococcus mutans. Clinical tests also showed a significant reduction in plaque index with both formulas ($p<0.001$), which is attributed to the ability of bioactive compounds, such as flavonoids and phenolics, to inhibit biofilm formation.

The antibacterial activity of *A. monopleura* is primarily attributed to flavonoids and essential oils. Flavonoids exert antibacterial effects by inhibiting nucleic acid synthesis, disrupting cytoplasmic membrane function, and inhibiting bacterial energy metabolism (Van et al.. 2021). Flavonoids can depolarize bacterial cell membranes and inhibit RNA. DNA and protein synthesis leading to bacterial cell death. Additionally, flavonoids may cause cell wall lysis and membrane leakage by interacting with lipopolysaccharides, leading to phospholipid degradation.

Essential oils in *A. monopleura*. particularly sesquiterpenes and diterpenes. also contribute to antibacterial activity. Terpenoid compounds can bind to extracellular proteins and bacterial cell walls. disrupting the lipid bilayer and inhibiting bacterial growth (Yodha et al., 2023; Belamine et al., This mechanism has been widely reported in herbal plants with strong antimicrobial activity against gram-positive bacteria, such as *S. mutans* (Akarathanakrit et al., 2025).

The reduction in dental plaque index observed in this study is likely associated with the antimicrobial and anti-inflammatory properties of *A. monopleura* phytochemicals (Aziz et al.. 2024). Flavonoids play a crucial role in inhibiting bacterial growth and reducing microbial colonization. which is involved in plaque formation (Applications et al.. 2025). Moreover, these compounds may reduce gingival inflammation and prevent bacterial infections in the oral cavity, thereby contributing to improved oral hygiene (Janakiram et al.. 2020). Dental plaque is a structured biofilm composed of bacteria. food debris. and saliva (Yan et al.. 2023). Bacteria within dental plaque produce organic acids that cause enamel demineralization and contribute to dental caries. Plaque formation occurs through bacterial adhesion to tooth surfaces and microbial proliferation within the biofilm matrix (Zhang et al., 2022). If not regularly removed, plaque thickens, contributing to halitosis and periodontal disease. Therefore. Regular tooth brushing using toothpaste containing antibacterial agents, such as *A. monopleura* extract, is essential to maintain oral health and prevent oral diseases (Canti et al.. 2024).

The findings of this study indicate that toothpaste formulated with Alpinia monopleura extract exhibits significant antibacterial and anti-plaque effects. highlighting its potential as a promising natural alternative to conventional synthetic dentifrices. These results contribute to the growing body of evidence supporting the use of plant-based bioactive agents in oral health

applications. Nevertheless, the interpretation of the findings should take certain limitations into account, particularly the short observation period and the lack of quantitative phytochemical profiling. However, qualitative screening confirmed the presence of secondary metabolites. Advanced chromatographic analyses, such as GC–MS or HPLC, are essential for precisely characterizing and quantifying bioactive constituents and elucidating their potential mechanisms of antibacterial action. Future investigations should adopt randomized controlled clinical trial designs with extended follow-up periods and include direct comparisons with fluoride- and chlorhexidine-containing toothpastes to comprehensively assess clinical efficacy, long-term safety, and formulation stability.

CONCLUSION

This study demonstrates that herbal toothpaste containing Alpinia monopleura extract exhibits significant antibacterial activity against Streptococcus mutans and reduces plaque index. Both concentrations (20% and 30%) showed inhibitory effects, with the 30% concentration providing higher antibacterial activity and plaque reduction. These findings confirm the potential of Alpinia monopleura as a natural antibacterial agent for dental health and support its development as a natural dentifrice alternative. Further research is needed through long-term clinical trials, quantitative phytochemical analysis (GC–MS or HPLC), and comparative studies with fluoride- or chlorhexidine-based toothpastes to confirm its efficacy, safety, and mechanism of action.

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Conflict of Interest

The authors declare that there are no conflicts of interest. The funders had no role in the study design. data collection. analysis. or manuscript preparation.

Ethical Approval

Ethical approval for this study was obtained from the Ethics Committee of Politeknik Bina Husada Kendari. Written informed consent was obtained from all participants.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request. The data are not publicly available due to ethical and privacy considerations.

Authors' Contributions

N.A. conceived and designed the study, conducted data collection, and drafted the manuscript. P.R.F. contributed to data analysis and interpretation of results. M. contributed to laboratory analysis and critical revision of the manuscript. All authors read and approved the final manuscript.

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