JURNAL AR RO'IS MANDALIKA (ARMADA)
Journal website: https://ojs.cahayamandalika.com/index.php/armada

ISSN: 2774-8499 Vol. 6 No. 3 (2026)

Research Article

Effectiveness of Cocoa Pod Husk Extract Toothpaste against Staphylococcus aureus In Vitro

Thisa Sefblianda[1], Armia Syahputra[2], Rini Octavia Nasution[3], Martina Amalia[4]

1. Universitas Sumatera Utara, Indonesia
2. Universitas Sumatera Utara, Indonesia; armia.syahputra@usu.ac.id
3. Universitas Sumatera Utara, Indonesia
4. Universitas Sumatera Utara, Indonesia

Corresponding Author, Email: armia.syahputra@usu.ac.id (Thisa Sefblianda)

Abstract

This study investigates the antibacterial activity of toothpaste formulated with Theobroma cacao L. pod husk extract against Staphylococcus aureus ATCC®29213. The extract was incorporated into toothpaste formulations at concentrations of 2.5%, 5%, and 10%. The antibacterial activity was evaluated using the well diffusion method, and inhibition zones were measured after a 24-hour incubation period at 37°C. The results showed that all concentrations of the toothpaste exhibited significant antibacterial effects, with the 10% concentration demonstrating the highest efficacy. Phytochemical screening revealed the presence of bioactive compounds such as flavonoids, alkaloids, saponins, and terpenoids, which are known for their antimicrobial properties. The 10% concentration of the extract showed the largest inhibition zone, reaching 19.3 mm, significantly higher than the 2.5% and 5% concentrations, which yielded zones of 13.4 mm and 15.9 mm, respectively. While the positive control (Siwak-F) demonstrated the highest inhibition zone (20.9 mm), the cocoa pod husk extract at 10% concentration showed comparable efficacy. Physical evaluations of the toothpaste formulations indicated good stability, with acceptable levels of pH, spreadability, and viscosity. These findings suggest that Theobroma cacao pod husk extract has potential as a natural antibacterial agent in toothpaste formulations, offering an alternative to synthetic chemical agents.

Keywords: Theobroma cacao, antibacterial activity, Staphylococcus aureus, cocoa pod husk extract, toothpaste, phytochemical screening, inhibition zone.

INTRODUCTION

Periodontal disease remains one of the most prevalent oral health problems worldwide and constitutes a major public health concern, particularly in developing countries such as Indonesia. Periodontal disease is a chronic inflammatory condition affecting the supporting tissues of the teeth, including the gingiva, cementum, alveolar bone, and periodontal ligament, and is primarily caused by microbial infection (Susanto et al., 2020; Ulfah et al., 2023). Clinically, periodontal disease is classified into gingivitis and periodontitis, with gingivitis representing a reversible inflammatory stage that may progress to periodontitis if left untreated. Periodontitis is characterized by irreversible periodontal attachment loss and alveolar bone resorption, which can ultimately result in tooth loss and significant functional, aesthetic, and psychosocial consequences (Wahyuni et al., 2024; Yuniawati et al., 2023).

Epidemiological data indicate that periodontal disease is highly prevalent in Indonesia. The Indonesian Health Survey (SKI) in 2023 reported that 56.9% of the population experienced oral and dental problems, with periodontal disease identified as one of the most common conditions (Kemenkes, 2023). Furthermore, data from the Basic Health Research Survey (Riskesdas) in 2018 revealed that the prevalence of periodontal disease in Indonesia reached 74.1% (Wahyuni et al., 2024). These findings highlight the urgency of implementing effective preventive and therapeutic strategies to control periodontal disease and improve oral health outcomes.

Dental plaque is recognized as the primary local etiological factor in the development of periodontal disease. Plaque is a soft, structured biofilm composed of diverse microorganisms that adhere to tooth surfaces and gingival margins (Pratiwi et al., 2022). Based on its anatomical location, plaque can be classified into supragingival and subgingival plaque, both of which play critical roles in periodontal inflammation (Peeran & Ramalingam, 2021). The microbial composition of plaque is complex and dynamic, involving both commensal and opportunistic pathogenic bacteria that contribute to tissue destruction under favorable conditions.

Among the microorganisms associated with dental plaque, Staphylococcus aureus has attracted increasing attention due to its pathogenic potential and ability to persist within the oral cavity. Although S. aureus is considered part of the normal oral flora, it may act as an opportunistic pathogen in the presence of predisposing factors such as immune suppression, microbial imbalance, and poor oral hygiene (Nasution & Daulay, 2022). This bacterium has been isolated from various oral niches, including the oral mucosa, tongue dorsum, saliva, supragingival plaque, and periodontal pockets (Chmielewski et al., 2024). Colombo et al., (2023) reported that Staphylococcus species were present in subgingival biofilms of periodontitis patients, with S. aureus accounting for approximately 22% of the isolates. The pathogenicity of S. aureus is closely related to its ability to form biofilms, which are resistant to host immune responses and antimicrobial agents through the production of extracellular polysaccharides, autolysin enzymes, and quorum sensing systems (Schilcher & Horswill, 2020). These characteristics justify the selection of S. aureus as a relevant target organism in antibacterial studies related to plaque control.

Effective plaque control is a fundamental strategy for preventing periodontal disease. Plaque control can be achieved through mechanical, chemical, and natural approaches. Mechanical plaque control, primarily through regular tooth brushing, remains the most widely recommended method for disrupting biofilm accumulation on tooth surfaces (Penda & Kaligis, 2015). Chemical plaque control involves the use of antibacterial agents such as mouthwashes and dentifrices to suppress bacterial growth in areas inaccessible to mechanical cleaning (Adnyasari et al., 2023). The effectiveness of tooth brushing largely depends on the antibacterial properties of the toothpaste used, making dentifrice formulation a critical factor in plaque control (Wulandari et al., 2020).

Conventional toothpastes commonly contain synthetic antibacterial agents such as triclosan and fluoride, which have been proven effective in inhibiting plaque-forming bacteria and preventing dental caries (Ucuncu et al., 2024). Fluoride plays a crucial role in strengthening enamel and reducing demineralization; however, excessive fluoride exposure has been associated with adverse effects, including dental and skeletal fluorosis and potential systemic toxicity (Ucuncu et al., 2024). Consequently, there is growing interest in developing alternative toothpaste formulations based on natural or herbal ingredients that are safer for long-term use (Susi et al., 2015).

Cocoa (Theobroma cacao L.) is a widely cultivated plant in Indonesia with substantial potential as a source of natural bioactive compounds. While cocoa beans are extensively utilized in the food industry, cocoa pod husk represents a major agricultural by-product that remains underutilized. Cocoa pod husk constitutes approximately 73–75% of the total fruit weight, resulting in a large volume of agricultural waste (Habiburrahim, 2016). According to data from the Indonesian Central Bureau of Statistics, national cocoa production reached 632.12 thousand tons in 2023, positioning Indonesia as one of the world's leading cocoa producers (BPS, 2024). North Sumatra Province, particularly Deli Serdang Regency, is among the regions with significant cocoa production, indicating a high availability of cocoa pod husk for further utilization (BPS, 2023).

Previous studies have demonstrated that cocoa pod husk contains various bioactive compounds, including flavonoids, tannins, saponins, alkaloids, and triterpenoids, which exhibit antibacterial, antioxidant, and antiviral activities (Chusniasih et al., 2020; Rahayu et al., 2023). Several investigations have reported the antibacterial activity of cocoa pod husk extract against oral pathogens such as Streptococcus mutans and Streptococcus sanguinis (Habiburrahim, 2016; Lestari & Asri, 2021). Moreover, in vitro studies have shown that cocoa pod husk extract can inhibit the growth of S. aureus at various concentrations (Chusniasih et al., 2020; Lestari & Asri, 2021).

Despite the growing body of evidence supporting the antibacterial potential of cocoa pod husk extract, most existing studies have focused on its activity in crude extract form. Research specifically evaluating cocoa pod husk extract as an active ingredient in toothpaste formulations targeting Staphylococcus aureus remains limited. This gap highlights the need for further investigation into the effectiveness of cocoa pod husk extract incorporated into dentifrice formulations as a natural antibacterial agent for plaque control.

Therefore, this study aims to evaluate the effectiveness of cocoa pod husk extract toothpaste at concentrations of 2.5%, 5%, and 10% in inhibiting the growth of Staphylococcus aureus in vitro. The findings of this study are expected to contribute to the development of herbal-based toothpaste formulations and promote the sustainable utilization of cocoa agricultural waste for oral health applications.

METHODS

Study Design

This study employed a laboratory experimental design using a post-test only control group design, in which outcomes were measured after completion of all treatments. The primary outcome was the inhibition zone diameter (mm) formed around wells on Mueller–Hinton Agar (MHA) following exposure to experimental toothpaste formulations against Staphylococcus aureus ATCC®29213. In vitro antimicrobial evaluation was conducted using agar well diffusion principles commonly applied for antimicrobial screening and comparative assessment (Balouiri et al., 2016; Savana et al., 2024).

Setting, Location, and Study Period

The work was performed across three laboratories at Universitas Sumatera Utara (USU), Indonesia:

- Cosmeceutical Laboratory, Faculty of Pharmacy: preparation of cocoa pod husk extract, toothpaste formulation (2.5%, 5%, 10%), base toothpaste, and physical evaluations.
- Organic Chemistry and Natural Products Laboratory, FMIPA: phytochemical screening of the cocoa pod husk extract.
- Microbiology Laboratory, FMIPA: bacterial culture preparation and antibacterial activity testing against S. aureus ATCC®29213.

The study was conducted from September to November 2025.

Ethical Approval

Ethical approval was obtained from the Health Research Ethics Committee (KEPK), Faculty of Medicine, Universitas Sumatera Utara, approval number No.1138/KEPK/USU/2025.

Materials

1. Plant Material

Mature cocoa pod husks (Theobroma cacao L.) were collected purposively from Tanjung Morawa District, Deli Serdang Regency, North Sumatra, Indonesia. A total of 2.5 kg fresh cocoa pod husks were obtained. Inclusion criteria were mature, non-rotten husks in good condition; exclusion criteria were rotten and moldy husks.

2. Bacterial Strain

The test organism was Gram-positive Staphylococcus aureus ATCC®29213, subcultured and maintained on MHA. Inclusion criterion was successful growth of a pure culture upon subculture; exclusion criterion was contamination by other microorganisms. The strain was obtained from Microbiologics (USA) as stated in the thesis method description.

Sample Size and Experimental Groups

Sample size estimation followed the Federer formula:

$$(t-1)(r-1) \geq 15$$

With t = 5 groups, the minimum replication was r ≥ 5. Thus, each group was tested in five independent replicates (n=5), resulting in 25 experimental units in total.

Five groups were evaluated:

- F1: Toothpaste with cocoa pod husk extract 2.5%
- F2: Toothpaste with cocoa pod husk extract 5%
- F3: Toothpaste with cocoa pod husk extract 10%
- F4 (Positive control): Commercial herbal toothpaste Siwak-F
- F5 (Negative control): Toothpaste base (placebo), without active ingredient

Operational Definitions and Outcome Measure

The indepenent variable was toothpaste formulation 2.5%, 5%, 10%, positive control, negative control). The dependent variable was mean inhibition zone diameter (mm) against S. aureus ATCC®29213, measured after 24 h incubation. The inhibition zone was defined as the clear zone around the well indicating suppression of bacterial growth, and was measured using a caliper after incubation (Balouiri et al., 2016).

(

Preparation of Simplisia (Dried Plant Powder)

Fresh cocoa pod husks were washed under running water, drained, cut into smaller pieces to facilitate drying, and dried in a drying cabinet until completely dry. The dried material (simplisia) was then ground using a blender to produce a fine powder for extraction.

Figure 1. Preparation of cocoa pod husk simplisia (washing, slicing, drying, milling).

Extraction Procedure

Extraction was conducted by maceration using 70% ethanol:

- 200 g cocoa pod husk powder was placed in a closed container.
- 2 L of 70% ethanol was added and stirred periodically for the first 6 h.

- The mixture was macerated for an additional 18 h, with intermittent stirring.
- The mixture was filtered using cotton and filter paper to obtain macerate I.
- The residue was re-extracted using 1 L of 70% ethanol following the same procedure to obtain macerate II.
- Macerates I and II were combined and concentrated using a rotary evaporator at 40°C (or water bath) until a viscous extract was obtained.

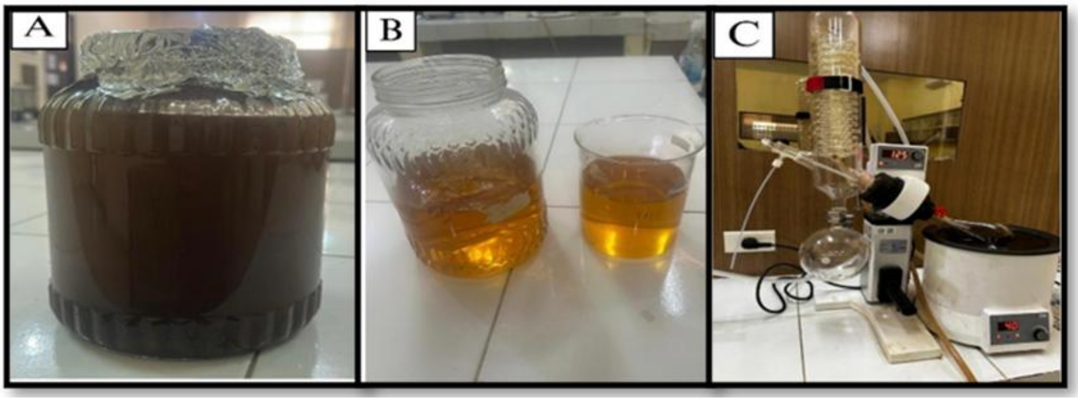


Figure 2. Extraction workflow (maceration, filtration, solvent evaporation)

Phytochemical Screening

Phytochemical screening was conducted at the Organic Chemistry and Natural Products Laboratory (FMIPA USU). The extract was reacted with appropriate reagents in test tubes for qualitative detection of secondary metabolites, and color changes were recorded as indicators of the presence of metabolite classes.

Toothpaste Formulation

Toothpaste formulations were prepared with cocoa pod husk extract concentrations of 2.5%, 5%, and 10%. A commercial herbal toothpaste (Siwak-F) served as positive control and the base toothpaste served as negative control. The role of excipients included abrasive (calcium carbonate), humectant (glycerin), binder (CMC-Na), surfactant (sodium lauryl sulfate), sweetener (saccharin), preservative (methyl paraben), and solvent (distilled water).

Table 1. Toothpaste formulation for each group (total weight 10 g)

Component	Function	F1 (2.5%)	F2 (5%)	F3 (10%)	F4 (+) Siwak-F	F5 (-) Base
Cocoa pod husk extract	Active ingredient	0.25 g	0.50 g	1.00 g	–	–
Siwak-F toothpaste	Active ingredient	–	–	–	10 g	–
Calcium carbonate	Abrasive	4.5 g	4.5 g	4.5 g	4.5 g	4.5 g
Glycerin	Humectant	1.5 g	1.5 g	1.5 g	1.5 g	1.5 g
CMC-Na	Binder	0.1 g	0.1 g	0.1 g	0.1 g	0.1 g
Sodium lauryl sulfate	Surfactant	0.15 g	0.15 g	0.15 g	0.15 g	0.15 g
Saccharin	Sweetener	0.01 g	0.01 g	0.01 g	0.01 g	0.01 g

Methyl paraben	Preservative	0.01 g	0.01 g	0.01 g	0.01 g	0.01 g
Distilled water	Solvent	q.s. to 10 g	q.s. to 10 g	q.s. to 10 g	q.s. to 10 g	q.s. to 10 g

Note. "q.s." indicates sufficient quantity added to reach total formulation weight.

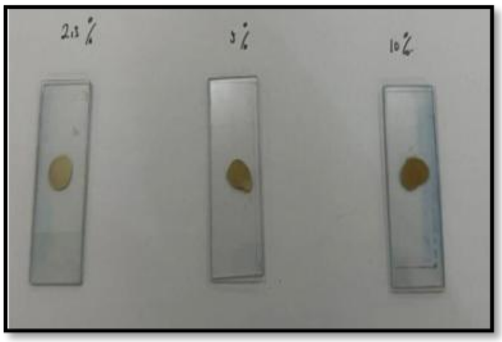
Toothpaste Preparation Procedure

CMC-Na was dispersed in hot water and allowed to hydrate for 15 min to form the first mass. Calcium carbonate and sodium lauryl sulfate were triturated to homogeneity and incorporated into the hydrated CMC-Na mass to form a second mass. Cocoa pod husk extract was mixed with glycerin until uniform and then incorporated into the second mass with continuous trituration. Methyl paraben and saccharin were dissolved in the remaining solvent portion and added to the mixture. The final mass was triturated until a homogeneous paste was obtained and then transferred into clean, tightly closed toothpaste containers.

Physical Evaluation of Toothpaste

Physical evaluation was performed to confirm basic quality characteristics:

- Organoleptic test: appearance/consistency, color, and odor, guided by SNI No. 12-3524-1995 criteria described in the thesis.
- Homogeneity test: spreading a thin layer on a glass slide to assess uniformity and absence of coarse particles.
- pH test: 1 g toothpaste dispersed in 10 mL distilled water; pH measured using a calibrated pH meter. The acceptable pH range referenced in the thesis was 4.5–10.5.
- Spreadability test: 0.5 g sample placed on a glass plate and loaded sequentially (50 g to 250 g) with diameter measured after stabilization; acceptable range was 2–5 cm (as per thesis criterion).
- Viscosity test: measured using a Brookfield viscometer with appropriate spindle and rotational speed settings. A viscosity of 20,000–50,000 cP was treated as an acceptable semi-solid range according to the thesis criterion.



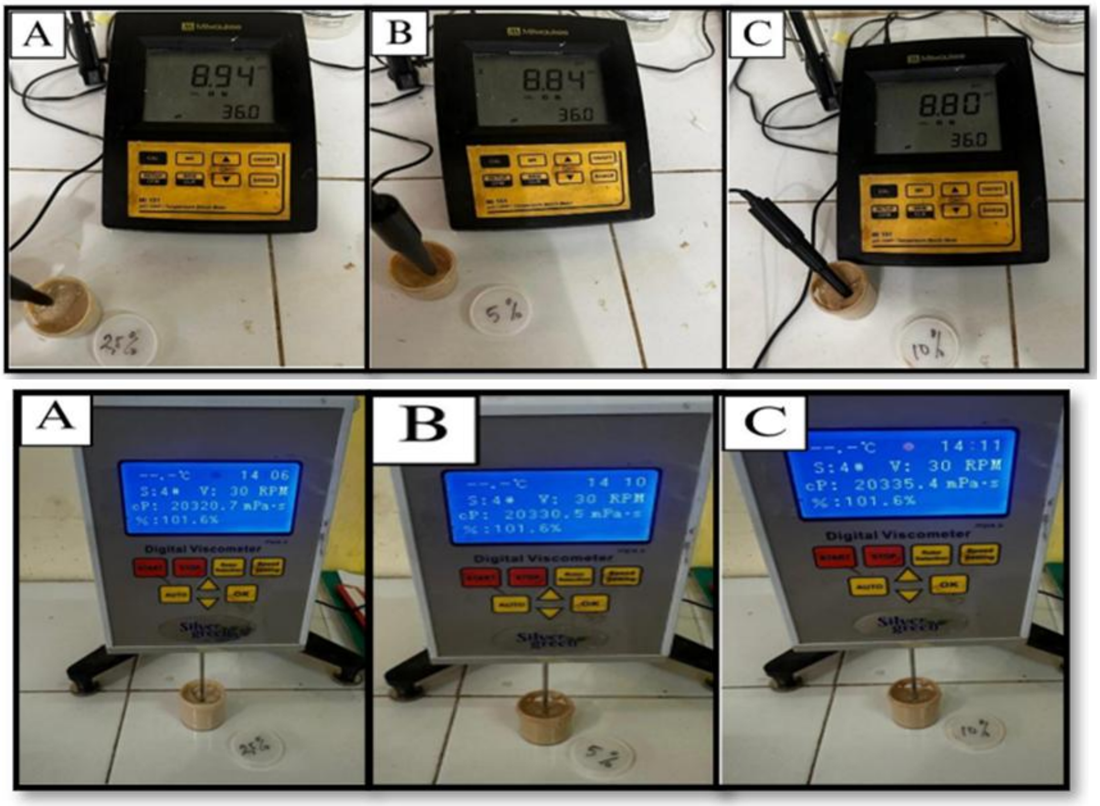


Figure 3. Physical evaluation (pH, viscosity, spreadability, homogeneity)

Antibacterial Activity Assay

1. Sterilization

All instruments and materials were sterilized prior to use. Sterilization was conducted in a biosafety cabinet for 30 min as described in the thesis.

2. Mueller–Hinton Agar (MHA) Preparation

MHA powder (3.8 g) was dissolved in 100 mL distilled water in a sterile 250 mL Erlenmeyer flask, heated to boiling, covered with gauze and cotton, and sterilized by autoclaving at 121°C for 15 min. The medium was cooled to approximately 50°C, poured into sterile Petri dishes, and allowed to solidify.

3. Preparation of Bacterial Stock and Inoculum

A colony of S. aureus was inoculated onto MHA and incubated at 37°C for 24 h. For inoculum preparation, bacterial colonies were suspended in sterile physiological NaCl and vortexed to achieve turbidity equivalent to 0.5 McFarland.

4. Agar Well Diffusion Procedure

Antibacterial testing used the agar well diffusion approach (Balouiri et al., 2016; Savana et al., 2024). After MHA solidification, the 0.5 McFarland bacterial suspension was swabbed evenly over the agar surface using a sterile cotton swab. Wells were created using a sterile metal borer. Each well received 0.1 g of the assigned toothpaste (F1, F2, F3, F4, or F5). Plates were incubated at 37°C for 24 h. Clear zones around wells were recorded as inhibition zones.

5. Measurement of Inhibition Zone Diameter

Inhibition zones were measured with a caliper by drawing three diameters (horizontal, vertical, diagonal). Each measured diameter was corrected by subtracting the well diameter, then the three corrected values were averaged to produce a single mean inhibition zone diameter (mm) per plate.

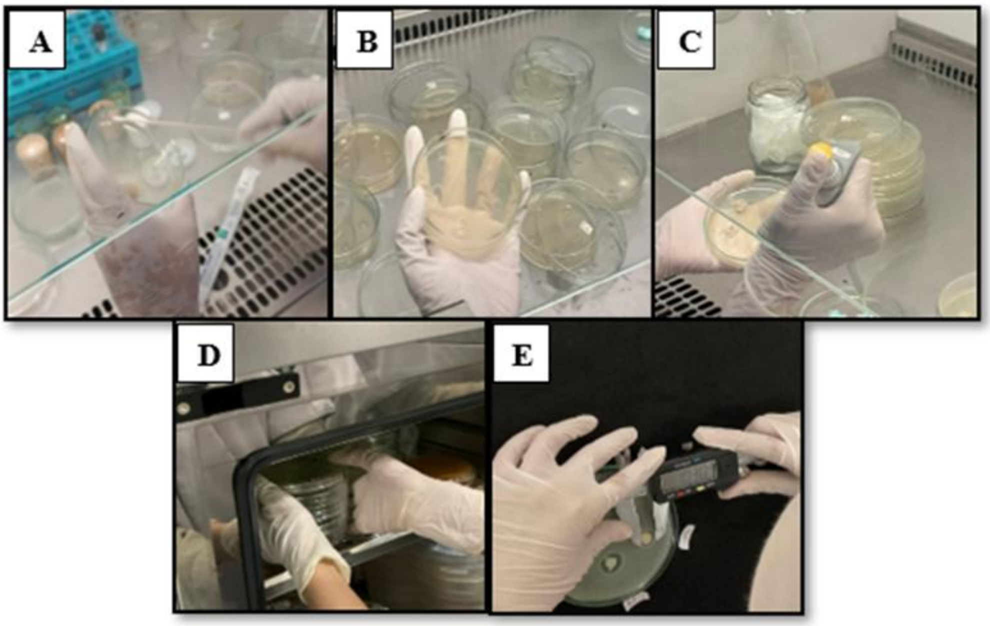


Figure 4. Antibacterial assay workflow (inoculation, well formation, sample placement, incubation, measurement)

Statistical Analysis

Data processing and statistical analyses were conducted using SPSS. Normality was assessed using the Shapiro–Wilk test ($p<0.05$ indicating non-normal distribution). If data were normally distributed ($p>0.05$), one-way ANOVA was used to test differences among groups, followed by LSD post hoc tests when ANOVA was significant. If data were not normally distributed ($p<0.05$), the Kruskal–Wallis test was applied, followed by Mann–Whitney pairwise comparisons for significant results.

RESULT AND DISSCUSSION

This study evaluated the antibacterial effectiveness of cocoa pod husk extract toothpaste (Theobroma cacao L.) against Staphylococcus aureus ATCC®29213. The experimental assessments included phytochemical screening of the extract, physical evaluation of toothpaste formulations, antibacterial activity testing using agar well diffusion, and statistical analysis of inhibition zone diameters.

Phytochemical Screening Results

Phytochemical screening was performed to identify the major secondary metabolites present in cocoa pod husk extract prior to antibacterial testing. The qualitative analysis demonstrated positive reactions for several classes of bioactive compounds.

Table 2. Phytochemical screening results of cocoa pod husk extract

Compound class	Reagent	Result
Flavonoids	Mg + HCl	Positive (+)
Alkaloids	Bouchardat	Positive (+)
	Mayer	Positive (+)
	Dragendorff	Positive (+)
Saponins	Distilled water + 96% ethanol + 2N HCl	Positive (+)
Terpenoids	Liebermann–Burchard	Positive (+)

The results confirmed the presence of flavonoids, alkaloids, saponins, and terpenoids in the cocoa pod husk extract, indicating that the extract contains multiple classes of secondary metabolites with potential bioactivity.

Physical Evaluation of Toothpaste Formulations

1. Organoleptic Properties

Organoleptic evaluation was conducted on day 0 and day 7 to assess physical stability during short-term storage. Parameters observed included consistency, color, aroma, and taste.

Table 3. Organoleptic characteristics of cocoa pod husk extract toothpaste

Formulation	Day	Consistency	Color	Aroma	Taste
F1 (2.5%)	Day 0	Semi-solid, homogeneous	Light brown	Mint/fresh	Sweet
	Day 7	Semi-solid, homogeneous	Light brown	Mint/fresh	Sweet
F2 (5%)	Day 0	Semi-solid, homogeneous	Brown	Mint/fresh	Sweet
	Day 7	Semi-solid, homogeneous	Brown	Mint/fresh	Sweet
F3 (10%)	Day 0	Semi-solid, homogeneous	Dark brown	Mint/fresh	Sweet
	Day 7	Semi-solid, homogeneous	Dark brown	Mint/fresh	Sweet

No changes in organoleptic properties were observed in any formulation during the 7-day observation period.

2. Homogeneity Test

All toothpaste formulations showed homogeneous characteristics without visible coarse particles throughout the evaluation period.

Table 4. Homogeneity test results

Formulation	Result
F1 (2.5%)	Homogeneous
F2 (5%)	Homogeneous
F3 (10%)	Homogeneous

3. pH Measurement

The pH values of all formulations were within the acceptable range for toothpaste preparations.

Table 4. pH values of toothpaste formulations

Formulation	pH	Assessment
F1 (2.5%)	8.94	Acceptable
F2 (5%)	8.84	Acceptable
F3 (10%)	8.80	Acceptable

4. Spreadability Test

Spreadability was measured under increasing loads (50–250 g). All formulations demonstrated spread diameters within the acceptable range.

Table 5. Spreadability of toothpaste formulations (cm)

Formulation	50 g	100 g	150 g	200 g	250 g
F1 (2.5%)	2.7	3.2	3.5	3.8	4.0
F2 (5%)	3.1	3.5	3.8	3.9	4.1
F3 (10%)	3.4	3.8	4.0	4.3	4.6

5. Viscosity Measurement

Viscosity testing using a Brookfield viscometer showed that all formulations met the standard viscosity range for semi-solid toothpaste preparations.

Table 6. Viscosity of toothpaste formulations

Formulation	Viscosity (mPa·s)
F1 (2.5%)	20,320
F2 (5%)	20,330
F3 (10%)	20,335

Antibacterial Activity

Antibacterial activity was assessed using the agar well diffusion method against Staphylococcus aureus ATCC®29213 with five replicates per group. Clear inhibition zones formed around wells containing active toothpaste formulations.

Table 7. Mean inhibition zone diameters against Staphylococcus aureus

Group	Mean inhibition zone (mm)	Activity category
Negative control (base toothpaste)	0	No activity
Positive control (Siwak-F)	20.9 ± 0.9	Very strong
F1 (2.5%)	13.4 ± 0.82	Strong
F2 (5%)	15.9 ± 1.2	Strong
F3 (10%)	19.3 ± 2.4	Strong

The negative control showed no inhibition zone, whereas all extract-containing formulations produced measurable antibacterial activity.

Normality and Homogeneity Tests

Shapiro–Wilk testing indicated that data from all treatment groups were normally distributed ($p > 0.05$). However, Levene's test showed that the data were not homogeneous; therefore, non-parametric analysis was applied.

Table 8. Normality and homogeneity test results

Group	Shapiro–Wilk (p)	Homogeneity (p)
Negative control	–	–
Positive control	0.70	0.00
F1 (2.5%)	0.52	
F2 (5%)	0.47	
F3 (10%)	0.09	

Kruskal–Wallis Test

The Kruskal–Wallis test demonstrated significant differences in antibacterial activity among all groups.

Table 9. Kruskal–Wallis test results

Group	Mean ± SD (mm)	p-value
Negative control	0	
Positive control	20.9 ± 0.9	
F1 (2.5%)	13.4 ± 0.82	0.000*
F2 (5%)	15.9 ± 1.2	
F3 (10%)	19.3 ± 2.4	

*Significant at $p < 0.05$.

Mann–Whitney Post Hoc Analysis

Pairwise comparisons were conducted to identify differences between groups.

Table 10. Mann–Whitney test results (p-values)

Group	Negative control	Positive control	2.5%	5%	10%
Negative control	–	0.005*	0.005*	0.005*	0.005*
Positive control		–	0.009*	0.009*	0.347
F1 (2.5%)			–	0.016*	0.009*
F2 (5%)				–	0.028*
F3 (10%)					–

*Significant at $p < 0.05$.

Discussion

The results of this study demonstrate that cocoa pod husk extract toothpaste (Theobroma cacao L.) exhibits significant antibacterial activity against Staphylococcus aureus ATCC®29213, as evidenced by the formation of inhibition zones in all concentration groups (2.5%, 5%, and 10%). The inhibition zones increased with higher concentrations of the extract, indicating that the active compounds present in the extract play a crucial role in inhibiting bacterial growth. This antibacterial effect is supported by the phytochemical screening, which identified the presence of secondary metabolites such as flavonoids, alkaloids, saponins, and terpenoids, all of which are known to have antimicrobial properties (Chmielewski et al., 2024; Susanto et al., 2020).

Flavonoids are known to interact with bacterial membrane proteins, disrupting cell permeability (Schilcher & Horswill, 2020). Alkaloids have been shown to inhibit peptidoglycan synthesis, weakening the bacterial cell wall (Wahyuni et al., 2024). Saponins exert their antimicrobial activity by lowering the surface tension of bacterial membranes, leading to cell lysis (Peeran & Ramalingam, 2021). Additionally, terpenoids can disrupt the outer membrane porins, which are crucial for nutrient uptake in bacterial cells (Rahayu et al., 2023). The synergistic action of these compounds likely contributes to the observed strong antibacterial activity of the cocoa pod husk extract, supporting its potential as a natural antimicrobial agent.

However, the screening did not detect tannins in the extract, which contrasts with the findings of Chusniasih et al. (2020), where tannins were identified in cocoa pod husk extract. This discrepancy could be due to differences in solvent type and concentration used for extraction. Tannins are large polyphenolic compounds that are more effectively extracted with polar solvents such as 96% ethanol or methanol (Lestari & Asri, 2021). The maceration method used in this study, which involves soaking without heating, may not be optimal for extracting tannins, as it typically extracts only about 50% of the active compounds. Therefore, the absence of tannins in our results may reflect suboptimal extraction conditions rather than the complete absence of these compounds in the cocoa pod husk.

The antibacterial activity observed in this study is consistent with previous research, such as the work of Chusniasih et al. (2020), where a pure cocoa pod husk extract (25%) produced an inhibition zone of 8.89 mm against Staphylococcus aureus. In contrast, the present study found a larger inhibition zone of 13.4 mm at a much lower concentration (2.5%). This difference could be attributed to the formulation used; Chusniasih et al. employed a pure extract, while this study used a toothpaste formulation that included a base of Na-CMC and glycerin. The base likely improved the stability and spreadability of the active compounds on the agar plate, resulting in a wider inhibition zone. Additionally, the well diffusion method used in this study may allow for more even distribution of the active compounds compared to a liquid extract, which can lead to a more pronounced antibacterial effect.

The results from the 5% concentration of the extract, which produced an inhibition zone of 15.9 mm, align with the findings of Habiburrahim (2016), where a 5% cocoa pod husk toothpaste demonstrated an inhibition zone of 16.63 mm against Streptococcus mutans. Although the bacteria tested were different (S. mutans vs. S. aureus), the findings support the strong antibacterial potential of cocoa pod husk extract, especially at higher concentrations. The difference in inhibition zone size between S. aureus and S. mutans may be due to the structural differences in their cell walls and their inherent resistance mechanisms. S. aureus, for example, possesses a thicker peptidoglycan layer and more complex defense mechanisms, such as hydrolytic enzymes that break down cell wall components (Wiegand et al., 2008), making it more resistant to some antimicrobial agents.

The positive control, Siwak-F toothpaste, showed the highest inhibition zone of 20.9 mm, categorizing it as "very strong." This result can be attributed to the presence of active compounds such as tannins, flavonoids, saponins, and sulfur in the siwak, which are known for their potent antimicrobial activity (Ucuncu et al., 2024). Although the cocoa pod husk toothpaste did not surpass the positive control in terms of inhibition zone size, it still showed significant antibacterial activity. This suggests that cocoa pod husk extract, particularly at 2.5%, 5%, and 10% concentrations, has potential as an alternative herbal toothpaste formulation with effective antimicrobial properties.

The physical evaluation of the toothpaste formulations demonstrated good stability and quality, with no significant changes observed in organoleptic properties, homogeneity, or pH over a 7-day storage period. The formulations remained semi-solid, homogeneous, with a fresh mint aroma and sweet taste. The color of the formulations deepened with increasing concentrations of the extract, which is consistent with the natural brown color of cocoa pod husk and its increasing concentration. The pH of all formulations fell within the acceptable range (8.80–8.94), which is safe for the oral mucosa and does not pose a risk of irritation (Peeran & Ramalingam, 2021). The spreadability of the toothpaste formulations (2–5 cm) and the viscosity (20,300 cP) also met the ideal standards for toothpaste formulations, ensuring ease of application and appropriate consistency.

In conclusion, while the Siwak-F toothpaste remains the most effective in terms of antibacterial activity, the cocoa pod husk extract toothpaste, even at lower concentrations, shows significant antibacterial potential. This study supports the use of cocoa pod husk extract as a promising natural alternative for toothpaste formulations. Furthermore, the results emphasize the importance of optimizing extraction methods to maximize the yield of active compounds like tannins and to enhance the overall efficacy of the toothpaste formulations.

CONCLUSION

This study demonstrates that toothpaste formulated with Theobroma cacao L. pod husk extract, at concentrations of 2.5%, 5%, and 10%, exhibits significant antibacterial activity against Staphylococcus aureus in vitro. The antibacterial efficacy was evident from the formation of inhibition zones at all concentrations tested, with the 10% concentration showing the highest antibacterial activity. These findings suggest that Theobroma cacao pod husk extract could be a promising natural alternative for incorporation into toothpaste formulations due to its ability to inhibit bacterial growth effectively.

Recommendations

Further research is necessary to fully explore the potential of Theobroma cacao pod husk extract as an antimicrobial agent. It would be beneficial to extend the evaluation of its antibacterial effectiveness to other bacterial species commonly associated with oral health, such as Streptococcus mutans and Porphyromonas gingivalis. These bacteria are significant contributors to oral diseases like tooth decay and periodontal disease, and testing against them could provide a broader understanding of the extract's application in oral hygiene.

In addition, future studies should focus on assessing the long-term safety and toxicity of Theobroma cacao pod husk extract when used in toothpaste formulations. Toxicological studies, particularly those investigating any potential side effects from prolonged use, will be crucial in ensuring that the product remains safe for consumers. This includes evaluating its effect on the oral and systemic health of individuals who use the toothpaste regularly.

Lastly, it is essential to conduct in vivo studies to test the real-world effectiveness and safety of this toothpaste formulation. While in vitro results are promising, in vivo testing would offer insights into the product's behavior in a living organism, including its impact on the oral microbiome, plaque formation, and its overall contribution to oral health. This would provide a more comprehensive understanding of its potential benefits and limitations in everyday use.

Bibliography

Adnyasari, N. L. P. S. M., Syahriel, D., & Haryani, I. G. A. D. (2023). PLAQUE CONTROL IN PERIODONTAL DISEASE: KONTROL PLAK PADA PENYAKIT PERIODONTAL. *Interdental Jurnal Kedokteran Gigi (IJKG)*, *19*(1), 55–61.

BPS. (2023). *Luas Tanaman dan Produksi Coklat Tanaman Perkebunan Rakyat menurut Kabupaten/Kota, 2021*. Badan Pusat Statistik Provinsi Sumatera Utara. https://sumut.bps.go.id/en/statistics-table/2/MjA5IzI=/luas-tanaman-dan-produksi-coklat-tanaman-perkebunan-rakyat-menurut-kabupaten-kota.html

BPS. (2024). *No Statistik Kakao Indonesia 2023*. Badan Pusat Statistik. https://www.bps.go.id/id/publication/2024/11/29/ed255af0c9059f288fb7e1de/statistik-kakao-indonesia-2023.html

Chmielewski, M., Załachowska, O., Komandera, D., Albert, A., Wierzbowska, M., Kwapisz, E., Katkowska, M., Gębska, A., & Garbacz, K. (2024). The oral cavity—another reservoir of antimicrobial-resistant Staphylococcus aureus? *Antibiotics*, *13*(7), 649.

Chusniasih, D., Tutik, T., & Syakira, N. (2020). Uji aktivitas ekstrak etanol kulit buah kakao (Theobroma cacao L.) Terhadap pertumbuhan jamur candida albicans dan bakteri Staphylococcus aureus. *JFM (Jurnal Farmasi Malahayati)*, *3*(2), 196–205.

Colombo, A. P. V., do Souto, R. M., Araújo, L. L., Espíndola, L. C. P., Hartenbach, F. A. R. R., Magalhães, C. B., da Silva Oliveira Alves, G., Lourenço, T. G. B., & da Silva-Boghossian, C. M. (2023). Antimicrobial resistance and virulence of subgingival staphylococci isolated from periodontal health and diseases. *Scientific Reports*, *13*(1), 11613.

Habiburrahim, B. (2016). Formulasi dan Uji Stabilitas Fisik Ekstrak Kulit Buah Kakao (Theobroma cacao L.) pada Sediaan Pasta Gigi sebagai Antibakteri terhadap Streptococcus mutans. *Jurnal Pharma and Med Sci*, *1*(2), 5–10.

Kemenkes. (2023). *Laporan Survei Kesehatan Indonesia (SKI) 2023*. Kementerian Kesehatan Republik Indonesia. https://layanandata.kemkes.go.id/katalog-data/ski/ketersediaan-data/ski-2023

Lestari, H. D., & Asri, M. T. (2021). Aktivitas antibakteri ekstrak kulit buah kakao (Theobroma Cacao L.) terhadap Staphylococcus Epidermidis. *LenteraBio: Berkala Ilmiah Biologi*, *10*(3), 302–308.

Nasution, L. W., & Daulay, A. S. (2022). Perbandingan Efektivitas Formulasi Pasta Gigi Ekstrak Daun Sirih Merah (Piper Ornatum NE Br) Dan Daun Sirih Hijau (Piper Betle L.) Terhadap Bakteri Staphylococcus Aureus. *Journal of Health and Medical Science*, 36–44.

Peeran, S. W., & Ramalingam, K. (2021). Essentials of periodontics & oral implantology. *Saranraj JPS Publication*.

Penda, P. A., & Kaligis, S. H. (2015). J. Perbedaan Indeks Plak Sebelum dan Sesudah Pengunyahan Buah Apel. *J. e-GIGI*.

Pratiwi, R., Nursyaputri, F., Indraswary, R., & Ratnawati, I. D. (2022). THE EFFECTIVENESS OF PHALERIA MACROCARPAâ€™ S LEAF NANOEMULSION GEL ON STAPHYLOCOCCUS AUREUS BIOFILM THICKNESS (IN VITRO). *Odonto: Dental Journal*, *9*, 69–79.

Rahayu, Y. C., Setiawatie, E. M., Rahayu, R. P., & Ramadan, D. E. (2023). Analysis of antioxidant and antibacterial activity of cocoa pod husk extract (Theobroma cacao L.). *Dent J*, *56*(4), 220–225.

Schilcher, K., & Horswill, A. R. (2020). Staphylococcal biofilm development: structure, regulation, and treatment strategies. *Microbiology and Molecular Biology Reviews*, *84*(3), 10–1128.

Susanto, A., Carolina, D. N., Amaliya, A., Pribadi, I. M. S., & Miranda, A. (2020). Periodontal health status and treatment needs of the community in Indonesia: A cross sectional study. *Journal of International Oral Health*, *12*(2), 114–119.

Susi, S., Bachtiar, H., & Sali, N. (2015). Perbedaan daya hambat pasta gigi berbahan herbal terhadap pertumbuhan Streptococcus mutans. *Majalah Kedokteran Andalas*, *38*(2), 116–123.

Ucuncu, M. K., Guven, K., & Yazicioglu, O. (2024). Investigation of the constituents of commercially available toothpastes. *International Journal of Dental Hygiene*, *22*(4), 913–932.

Ulfah, N., Yani, S., & Utami, N. D. (2023). Profil Penyakit Periodontal di Rumah Sakit Umum Daerah Abdoel Wahab Sjahranie Samarinda: Periodontal Diseases Profile in Abdoel Wahab Sjahranie Hospital. *Jurnal Sains Dan Kesehatan*, *5*(4), 458–465.

Wahyuni, P. S., Rahardjo, A., & Novrinda, H. (2024). Determinan Status Periodontal Pada Remaja Di Indonesia: Analisis Data Riskesdas Tahun 2018. *Cakradonya Dental Journal*, *16*(1), 7–16.

Wiegand, I., Hilpert, K., & Hancock, R. E. W. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols*, *3*(2), 163–175.

Wulandari, L. G. P. J., Kusumadewi, S., & Sudirman, P. L. (2020). Perbandingan efektivitas penggunaan pasta gigi herbal dan non herbal terhadap penurunan indeks plak. *Bali Dental Journal*, *4*(1), 49–53.

Yuniawati, F., Andriani, I., Hartanti, H., Cahyo, G. H., & Lestari, C. (2023). Edukasi Kesehatan Gigi dan Mulut pada Kegiatan Pengabdian Masyarakat Kelurahan Lubuk Minturun Kota Padang. *Jurnal Kreativitas Pengabdian Kepada Masyarakat (PKM)*, *6*(8), 3159–3167.