PHRAJ
Public Health Risk Assesment Journal
PHRAJ 2(2): 86-101
ISSN 3025-1109

Institute for Advanced Science, Social and Sustainable Future
MORALITY BEFORE KNOWLEDGE

Effectiveness of herbal toothpaste with ambon banana tree sap extract (*Musa Paradisiaca var. Sapientum*) on inhibiting the growth of Streptococcus Mutans Serotype-d (OMZ-176) bacteria

Yochidamai Akhsanitaqwim[1,3,*], Galang Wirasat[2,3], Fahim Rafa Nugraha[3]

[1] *Applied Natural Medicine, Graduate School of Pharma-Medical Sciences, Toyama University, Toyama City, Toyama Prefecture 930-0151, Japan;*
[2] *Civil Engineering, Faculty of Engineering, Sebelas Maret University;*
[3] *Science Hunter Indonesia, PT Riset Prestasi Indonesia.*
*Correspondence: yochidamai31@gmail.com

Received Date: December 8, 2024 Revised Date: January 8, 2025 Accepted Date: January 31, 2025

ABSTRACT

Background: This study aims to determine the antibacterial activity of Ambon banana stem sap extract against Streptococcus mutans serotype-d (OMZ-176) and to evaluate the physicochemical characteristics of toothpaste formulated with Ambon banana stem sap extract, along with its antibacterial activity against S. mutans serotype-d (OMZ-176). **Methods:** This study involved the extraction of Ambon banana stem sap, its phytochemical and antibacterial testing, and the formulation of herbal toothpaste. Quality tests included homogeneity, viscosity, pH, foaming, and spreadability. Data were analyzed using descriptive analysis and ANOVA to assess effectiveness and product quality. **Findings:** The research employed an experimental method on a laboratory scale. Ambon banana stem sap extract inhibited the growth of S. mutans at concentrations of 600 mg/mL and 700 mg/mL, with inhibition zone diameters of 9.405±0.0613 mm and 10.623±0.0044 mm, respectively. Toothpaste containing Ambon banana stem sap extract at a concentration of 600 mg/mL exhibited characteristics of a creamy color, thick consistency, homogeneity, a pH of 9.4, foam height of 0.8 cm, viscosity of 196.58 dPa·s, and a spreadability of 3.65 cm. Meanwhile, toothpaste with a 700 mg/mL extract concentration had a brownish color, thick consistency, homogeneity, a pH of 9.43, foam height of 0.5 cm, viscosity of 196.65 dPa·s, and a spreadability of 3.38 cm. Both toothpaste formulations at 600 mg/mL and 700 mg/mL were effective in inhibiting the growth of S. mutans, with inhibition zone diameters of 13.6233±0.8519 mm and 11.565±0.8674 mm, respectively, categorized as strong inhibition. **Conclusion:** Ambon banana stem sap extract effectively inhibits Streptococcus mutans growth and shows strong antibacterial activity in toothpaste formulations at 600 mg/mL and 700 mg/mL concentrations. This highlights its potential as a natural, safe alternative to fluoride for effective oral care. **Novelty/Originality of this Study:** This study presents an innovative approach to oral health by using Ambon banana stem sap as a fluoride alternative, showing strong antibacterial properties and offering a safer, natural option for effective oral care.

KEYWORDS: antibacterial, ambon banana stem, extract, toothpaste.

1. Introduction

Dental caries is a common dental and oral health issue with a high prevalence in the population. In Indonesia, its prevalence across all age groups is 96.58% (Siregar et al.,

Cite This Article:
Akhsanitaqwim, Y., Wirasat, G., & Nugraha, F. R. (2025). Effectiveness of herbal toothpaste with ambon banana tree sap extract (*Musa paradisiaca var. sapientum*) on inhibiting the growth of Streptococcus Mutans Serotype-d (OMZ-176) bacteria. *Public Health Risk Assesment Journal*, *2*(2), 86-101. https://doi.org/10.61511/phraj.v2i2.2025.1464

2016). Untreated dental caries can lead to other dental diseases such as cavities, gingival injuries (gingivitis), and periodontitis. According to the 2018 Riskesdas data, the prevalence of damaged, decayed, or painful teeth in Indonesia is 45.3%. Moreover, the majority of Indonesian residents suffer from gum problems, including swollen gums or abscesses, affecting approximately 14% of the population. This indicates a persistent high rate of dental and oral diseases due to poor dental and oral hygiene.

Although there have been many advancements in preventing dental caries, including identifying causes and providing treatments, plaque formation remains an unavoidable trigger. Dental plaque is a colorless, well-organized biofilm structure of bacteria that adheres to the tooth surface. If not regularly cleaned, it can cause dental cavities (caries) or other periodontal problems like gingivitis. Dental plaque mainly comprises biofilm-forming bacteria, particularly Streptococcus mutans (Lemos et al., 2019).

One common preventive measure taken by the community to maintain dental health is brushing teeth with fluoride toothpaste (Nilasary et al., 2020). Fluoride is highly effective in preventing dental caries as it inhibits enamel demineralization by neutralizing acidic conditions, suppressing bacterial acid production, and promoting remineralization of enamel surfaces. This mechanism helps to strengthen tooth enamel and protect against cavities. However, continuous and excessive use of fluoride can lead to unintended consequences. While fluoride makes tooth surfaces more resistant to demineralization caused by cariogenic bacteria (Nurjannah & Nugrahani, 2018), overexposure can result in dental fluorosis. Fluorosis occurs due to high fluoride intake during enamel formation and is characterized by tooth discoloration that progresses from white spots to yellow, brown, and even black stains as severity increases.

Children are particularly susceptible to fluorosis because they often lack proper spitting habits while brushing. A study by Angela (2013) estimated that 25–38% of individuals, particularly children, swallow toothpaste while brushing. This unintentional ingestion increases their risk of exceeding the recommended fluoride levels. Beyond cosmetic concerns, severe fluorosis can compromise the structural integrity of teeth, making them more prone to chipping and wear. Additionally, chronic fluoride overexposure has systemic health implications, including skeletal fluorosis, which affects bone structure and density. Long-term ingestion of high fluoride levels has also been associated with potential adverse effects on the nervous system, thyroid function, and kidney health, raising concerns about the safety of fluoride-containing products when used improperly or in excessive amounts.

The exploration of natural materials with antibacterial properties is promising, given Indonesia’s remarkable biodiversity. One material that has not been tested as an antibacterial agent in toothpaste is the stem of the Ambon banana (*Musa paradisiaca var. sapientum*). According to the official website of the Pekalongan City Central Bureau of Statistics, banana production in South Pekalongan District reached 256.60 tons in 2021. Despite high fruit productivity, the remaining banana stems are rarely utilized. This underutilization presents an opportunity to explore the potential of Ambon banana stems for antimicrobial applications, especially in dental hygiene products such as toothpaste, offering a sustainable and effective solution for oral health care.

Ambon banana stems contain sap rich in secondary metabolites such as flavonoids (8.18%), saponins (6.73%), tannins (4.38%) and occasionally alkaloids. These secondary metabolites possess well-documented antibacterial properties, as they disrupt bacterial cell walls, inhibit protein synthesis, and interfere with essential enzyme activities. Additionally, they function as antioxidants, reducing oxidative stress and enhancing oral health (Aini et al., 2024).

The Ambon banana (*Musa paradisiaca var. sapientum (L.) Kuntze*) is a tropical fruit plant known for its versatile uses, from the roots to the leaves (Mutmainnah et al., 2023). The distinctive characteristics of the Ambon banana include a greenish-black stem that grows approximately 2.5 to 3 meters tall, dark green, wide, and thick leaves, and fruit bunches that are 60 to 100 cm long, with each bunch containing 8 to 13 hands (each hand having about 12-22 individual bananas). The fruit ends are pointed and straight, with a moderately thick peel that turns from green-yellow to light yellow. The banana flesh is creamy-white with a sweet and slightly sticky taste.

Ambon bananas thrive easily in tropical regions like Indonesia. They are economically valuable, easy to care for, and grow quickly, often bearing fruit within a year. Despite the high productivity of bananas, the stems are often underutilized and regarded as agricultural waste, frequently discarded (Ariadi et al., 2024). Morphologically, the greenish-black stem of the Ambon banana is robust, cylindrical, and smooth, contributing to its strong structural integrity, which makes it suitable for further investigation into its potential applications beyond waste disposal.

The banana stem is composed of two parts: a pseudostem (a cluster of leaf sheaths) and the true stem, commonly known as the bulb. Banana stems contain several secondary metabolites, including flavonoids, saponins, tannins, and terpenoids (Salau et al., 2012). These natural compounds contribute to the antimicrobial properties of banana stems and play key roles in plant defense and medicinal applications. Flavonoids are phenolic compounds that are polar and thus dissolve easily in polar solvents like water, ethanol, and methanol. They exhibit antimicrobial, antioxidant, and anti-inflammatory properties. Flavonoids in banana sap, such as flavanones and flavonols, are derived from secondary metabolism via the malonyl and shikimic acid pathways. These compounds are known to disrupt bacterial cell membranes by interfering with nutrient exchange, causing loss of cellular integrity, and ultimately leading to bacterial cell death. Additionally, banana stem sap contains hydroxycinnamic acid, a derivative of phenylalanine synthesized through the shikimic acid pathway. Hydroxycinnamic acids have been shown to exhibit antimicrobial and antioxidant activities, further enhancing the medicinal potential of banana stems (Pothavorn et al., 2010).

Saponins, another prominent group of compounds in banana stems, are glycosides with surfactant properties. These compounds act as antibacterial agents by reducing the surface tension of bacterial cell walls, disrupting membrane permeability, and causing leakage of cytoplasmic content, which results in bacterial cell death (Rai et al., 2021). Tannins, complex polyphenolic compounds found in banana stems, also exhibit strong antimicrobial activity. They work by forming hydrogen bonds with proteins, leading to protein precipitation and denaturation. This process disrupts bacterial cell membrane integrity and physiological functions, preventing the uptake of essential nutrients required for energy production and ultimately causing bacterial death (Budi, 2020). Additionally, tannins have been noted for their ability to chelate metal ions, which can further inhibit bacterial enzymatic activity and growth.

The terpenoids in banana stems, though less studied, also contribute to antimicrobial effects by disrupting microbial membrane structure and interfering with signaling pathways essential for bacterial survival. These combined properties make banana stems a potential natural source of antimicrobial agents suitable for applications in food preservation, pharmaceuticals, and alternative medicine. Toothpaste is a dental care product composed of a mixture of powdered and liquid substances containing ingredients such as cleansers, polishers, moisturizers, sweeteners/flavorings, detergents, preservatives, binders, and buffers. Toothpaste ingredients are divided into two categories: active ingredients with therapeutic effects and inactive ingredients without therapeutic effects, which affect consistency, taste, stability, abrasiveness, and appearance.

Typically, all toothpaste products contain the same basic ingredients such as binders, moisturizers, preservatives, and water. Toothpaste is composed of several key components, each serving a specific purpose to maintain oral health. Abrasive materials (30-40%) are the primary elements, including silica, calcium carbonate, dicalcium phosphate, sodium metaphosphate, hydrated alumina, zirconium silicate, or calcium pyrophosphate. These abrasives effectively remove biofilm and stains while polishing and cleaning the tooth surface without damaging the enamel. Binders (1-5%) are used to hold all ingredients together, providing the desired texture of the toothpaste. Examples of binders include alginate, xanthan gum, carbopol, or cellulose derivatives such as carboxymethyl cellulose (CMC) and hydroxyethyl cellulose, which prevent the separation of liquid and solid components. Moisturizers (10-30%) are added to retain moisture and prevent the toothpaste from hardening when exposed to air, with common examples being glycerol, sorbitol, and propylene glycol. To ensure the product's stability and safety, preservatives (0.05-0.5%) are included to inhibit bacterial growth within the organic and moisturizing components. Frequently used preservatives are alcohol, benzoate, formaldehyde, and dichlorinated phenols. Additionally, active therapeutic ingredients (0-2%) are incorporated to address specific oral health concerns, such as preventing cavities, reducing sensitivity, controlling tartar buildup, or combating harmful bacteria. These therapeutic agents, including fluoride, desensitizing agents, anti-tartar agents, and antimicrobial agents, are carefully formulated to avoid interactions with other components, ensuring the toothpaste's overall effectiveness.

Streptococcus mutans is a non-motile, facultative anaerobic Gram-positive bacterium with a characteristic cocci shape, appearing as spherical or oval cells arranged in chains. It is a significant contributor to the development of dental caries, which makes it a critical target for antimicrobial studies in the development of toothpaste formulations. S. mutans thrives in the oral cavity as a normal resident but can become pathogenic under conducive conditions. These conditions typically include an environment rich in fermentable carbohydrates, which lead to acid production and the establishment of a cariogenic biofilm. Once established, S. mutans colonies adhere to tooth surfaces and contribute to the formation of carious lesions. These colonies primarily form on non-shedding surfaces like dental plaque, making them difficult to remove and exacerbating the risk of dental caries (Chamlagain et al., 2024).

The pathogenic potential of S. mutans is further enhanced by its ability to survive in low pH environments, produce extracellular polysaccharides, and resist antimicrobial agents. This resilience makes it an ideal target for natural and effective antimicrobial agents, especially in toothpaste formulations designed to inhibit or reduce its growth. The development of antibacterial toothpaste using natural extracts, such as Ambon banana stem, is particularly promising as these products aim to counteract S. mutans biofilm formation and acid production, thus preventing caries development.

Additionally, S. mutans has been extensively studied due to its role in dental health, making it a reliable model organism for evaluating the antimicrobial efficacy of various natural and synthetic agents in dental care products. Its well-characterized pathogenic mechanisms provide a solid foundation for assessing the effectiveness of new formulations aimed at improving oral hygiene and preventing oral diseases. Antibacterial activity can be studied using several methods, such as dilution methods, agar diffusion methods, and diffusion-dilution methods. Diffusion methods are widely used to study antibacterial activity (Hossain et al., 2024). There are three types of diffusion methods: cylinder, well, and disc methods (Zaeneldin, 2024). The principle behind diffusion methods involves the diffusion of antibacterial compounds into solid media, in which the test microbes have been inoculated. Observations yield a clear area formed around the discs, indicating the inhibition zone of bacterial growth.

The disc diffusion method involves using a paper disc as a medium to absorb antimicrobial substances, which are then saturated in the test material. The paper discs are placed on the surface of agar media that have been inoculated with the test microbial culture. The plates are then incubated for 18-24 hours at 35°C. The clear area or inhibition zone around the disc is observed to indicate microbial growth inhibition. The diameter of this clear zone is proportional to the amount of the test microbial culture on the paper disc (Bubonja-Šonje et al., 2020). The disc method's advantage is that it allows for faster testing by preparing the discs.

In contrast, the well diffusion method involves creating wells in agar media that have been inoculated with bacteria, then adding the test solution into the wells. The media are incubated for 18-24 hours at 35°C. The inhibition of microbial growth is observed by noting the presence of clear zones around the wells (Retnaningsih et al., 2019). This method’s advantage lies in its ease of application to samples in paste or gel form. Flavonoids exert antibacterial activity by forming complexes with bacterial cell walls, thereby destabilizing bacterial membranes and inhibiting nucleic acid synthesis. Saponins exhibit antimicrobial properties by altering cell membrane permeability, leading to cell lysis. Meanwhile, tannins act by precipitating bacterial proteins and inhibiting vital enzymes required for bacterial survival. A study by Hafizha et al. (2019) demonstrated that Ambon banana stem extract at concentrations of 70% and 67.5% could inhibit the growth of Enterococcus faecalis by 7.44% and 14.53%, respectively. Furthermore, Rosmainar and Heriprayogi (2021) reported that sap extract from the Ambon banana bulb at 20% and 25% concentrations effectively inhibited the growth of Staphylococcus aureus, with inhibition zone diameters of 0.8 cm and 0.11 cm. These findings suggest the potential of Ambon banana stem sap as a natural antimicrobial agent, making it a promising ingredient for developing antibacterial toothpaste to enhance oral hygiene while reducing waste from banana stem residues.

The extraction of banana sap can be carried out using maceration, which is simple, easy, and relatively quick. The best sap is extracted from the bulb, which is cut diagonally, blended until smooth, soaked in ethanol, and filtered. The filtrate is then evaporated using a rotary evaporator (Hafizha et al., 2019; Budi, 2020). This method ensures efficient removal of moisture, producing a concentrated extract with a higher secondary metabolite content, suitable for various agricultural and industrial applications. Additionally, the rotary evaporation process helps maintain the quality of the extract by reducing thermal exposure, preserving its essential properties for further use.

Based on the above discussion, the author intends to research the effectiveness of herbal toothpaste made from the stem sap extract of the Ambon banana plant (*Musa paradisiaca var. sapientum*) in inhibiting the growth of *Streptococcus mutans* Serotype-d (OMZ-176). The research questions formulated in this study aim to address three primary concerns. First, it seeks to determine the optimal concentration of Ambon banana stem sap extract required to inhibit the growth of Streptococcus mutans serotype-d (OMZ-176). Second, it aims to explore the physicochemical characteristics of herbal toothpaste formulated with Ambon banana stem sap extract. Lastly, it evaluates the effectiveness of this herbal toothpaste in inhibiting the growth of S. mutans serotype-d (OMZ-176).

To achieve these objectives, the study focuses on determining the optimal concentration of Ambon banana stem sap extract for antibacterial efficacy, identifying the physicochemical characteristics of the formulated herbal toothpaste, and assessing its antibacterial effectiveness against S. mutans. The research offers both theoretical and practical benefits. Theoretically, it provides valuable insights into the antibacterial properties of herbal toothpaste containing Ambon banana stem sap extract, contributing to academic literature and serving as a reference for students and researchers. Practically, this study benefits the general public by increasing awareness of the health advantages of Ambon banana sap as a safe and effective active ingredient in herbal toothpaste. Additionally, it informs banana plantation owners about the potential economic value of utilizing Ambon banana stem sap as an alternative ingredient for high-value products like herbal toothpaste.

2. Methods

This research was conducted from June 2022 to September 2022 at various locations, including the Biology and Chemistry Laboratories of MAN Insan Cendekia Pekalongan, the Regional Technical Implementation Unit/*Unit Pelaksana Teknis Daerah* (UPTD) Herbal Medicine Service and Scientific Validation Center/*Balai Pelayanan dan Saintifikasi Jamu* (BPSJ) Pekalongan, the Phytochemistry Laboratory, and the Chemistry Laboratory of Universitas Muhammadiyah Pekajangan Pekalongan. Additionally, the study utilized the facilities at the Health Analyst Academy (AAK) Pekalongan and collected samples from banana plantations in Wonopringgo and South Pekalongan Districts, Pekalongan City. This study employed an exploratory-experimental research design, collecting primary data through experiments related to the extraction process of Ambon banana stem sap, toothpaste formulation, antibacterial testing, and herbal toothpaste quality testing. Secondary data were gathered from academic references, scientific journals, textbooks, theses, dissertations, and online articles, which provided foundational knowledge and context for the study.

The tools utilized in this research were essential for the various experimental stages and included saws, knives, basins, hybrid dryers, beakers, analytical balances, sieves, rotary evaporators, autoclaves, mortars and pestles, and measuring cylinders (100 mL). Additionally, test tubes, 250 mL beakers, watch glasses, spectrophotometers, Petri dishes, 250 mL Erlenmeyer flasks, volumetric flasks (25 mL, 10 mL, and 50 mL), and volumetric pipettes (10 mL) were employed for precise measurements and observations. Graduated pipettes (5 mL), thermometers, funnels, stirring rods, droppers, microscope slides, calipers, pH meters, Brookfield viscometers, strainers, and blenders were also used to support the experimental procedures and ensure accurate data collection.

The materials used in this study varied depending on the experimental phase. For the banana stem extraction, Ambon banana stems and 96% ethanol were utilized. In phytochemical testing, various reagents were employed, including DMSO, methanol, concentrated hydrochloric acid, $FeCl_3$, magnesium powder, 2N HCl, chloroform, acetic anhydride, and concentrated sulfuric acid. Antibacterial testing involved the use of *Streptococcus mutans* culture, MHA medium, physiological NaCl, Ambon banana stem sap extract, and DMSO as the solvent. For toothpaste formulation, the ingredients included Ambon banana stem sap extract, calcium carbonate ($CaCO_3$), xanthan gum, sorbitol, sodium lauryl sulfate (SLS), peppermint essence, and distilled water. The extraction process for Ambon banana stems was carried out using the maceration method. The stems were first powdered and then soaked in 96% ethanol within a sealed container for 4-5 days, during which the mixture was occasionally stirred to enhance extraction efficiency. After the soaking period, the mixture was filtered to separate the extract from the solid residue. The resulting filtrate was then concentrated using a rotary evaporator, effectively separating the extract from the ethanol solvent, as outlined by Ilmi (2017).

2.1 Phytochemical testing of ambon banana stem extract

Qualitative and quantitative testing of the Ambon banana stem extract were conducted to analyze its phytochemical components. In the qualitative tests, several methods were employed to identify the presence of specific compounds. For flavonoids, the extract was added to a test tube with 2 mg of magnesium powder and 3 drops of concentrated HCl, then shaken. The appearance of red, orange, or yellow color indicated the presence of flavonoids, as noted by Bhernama (2020). To test for saponins, 0.5 grams of the sample was mixed with 10 mL of hot water, allowed to cool, and then shaken vigorously for 10 seconds to produce foam. The addition of a drop of 2N HCl was used to assess foam stability, where persistent foam indicated the presence of saponins (Marjoni, 2016). For tannins, 1 gram of the extract was combined with 10 mL of hot water, cooled, and filtered. The filtrate was then mixed with a 1% FeCl3 solution in a 1:2 ratio. A color change to dark blue or greenish-black signaled the presence of tannins (Rahmawati et al., 2018). Terpenoids were identified by dissolving the extract in chloroform, followed by the addition of 0.5 mL of acetic anhydride and 2 mL of concentrated sulfuric acid along the test tube wall. The formation of a brown or violet ring indicated terpenoids, while a blue-green ring suggested steroids (Bhernama, 2020).

In the quantitative analysis, the total flavonoid content of the Ambon banana stem extract was determined using UV-Vis spectrophotometry. Standard quercetin was utilized as it contains keto groups on C-4 and hydroxyl groups on C-3 and C-5 in its flavonol structure, as described by Mutingatun et al. (2022). The wavelength was measured in the range of 400-800 nm to construct a standard calibration curve using concentrations of 5, 10, 20, 40, 60, and 80 μg/mL in 10 mL volumetric flasks (Sari et al., 2021). This approach allowed for precise quantification of the flavonoid content within the extract.

2.2 Antibacterial testing of ambon banana stem extract

The bacterial suspension was prepared by inoculating a loopful of Streptococcus mutans into physiological NaCl and matching it to McFarland standard 0.5. Antibacterial testing was carried out using the disc diffusion method with extract concentrations of 10%, 20%, 40%, 50%, 60%, and 70%. Positive controls used amoxicillin, and negative controls used DMSO.

The suspension was spread on MHA plates, and discs with extract, positive control, and negative control were placed on the plates (Azizah & Artanti, 2019). Measurements were repeated four times based on Frederer's formula (t-1)(r-1)≥15. Inhibition zones were measured with calipers, and data were analyzed using one-way ANOVA with a 95% confidence level.

2.3. Quality evaluation of herbal toothpaste with ambon banana stem

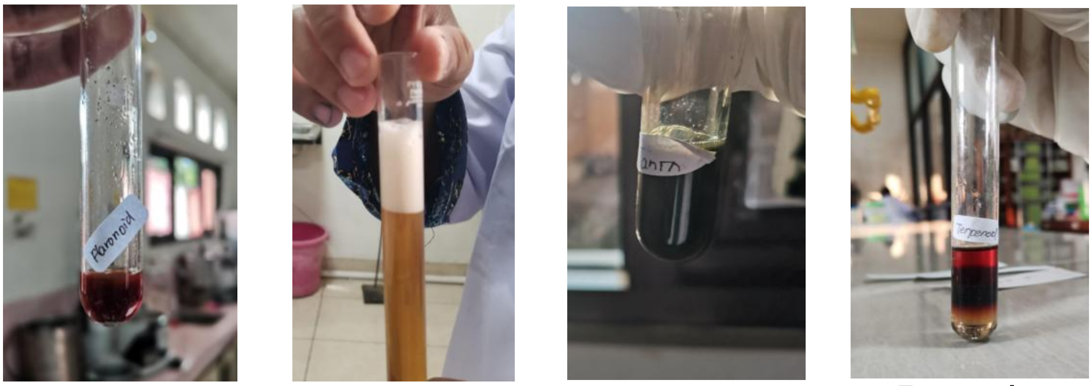
Quality Quality evaluation and antibacterial testing of herbal toothpaste containing Ambon banana stem extract were conducted to ensure compliance with Indonesian National Standard/*Standar Nasional Indonesia* (SNI) criteria and to determine its antibacterial effectiveness. The quality evaluation, modified from Ilmi (2017), included tests for homogenization, stability, viscosity, pH, foaming, and spreadability. The homogeneity test involved spreading 0.1 g of toothpaste on a glass slide; the absence of rough particles indicated homogeneity. pH testing was performed using a pH meter, with acceptable pH values ranging from 6 to 10, as stipulated by SNI 8861:2020. Viscosity was measured using a Brookfield viscometer with a spindle speed of 30 rpm. The foaming test assessed foam generation by diluting 0.025 g of toothpaste in 25 mL of distilled water, shaking the mixture 200 times in 100 seconds, and recording the foam scale. For the spreadability test, 1 g of toothpaste was placed between two transparent glass plates, with a 200 g weight applied for 1 minute, after which the spread diameter was measured.

The antibacterial testing of herbal toothpaste containing Ambon banana stem extract was conducted using the well diffusion method. This procedure was carried out under sterile conditions, with samples including toothpaste containing 60% and 70% extract concentrations, a commercial toothpaste as a positive control, and a plain toothpaste as a negative control. The bacterial suspension was prepared as previously described and spread on Mueller-Hinton Agar (MHA) plates. Wells were made in the agar, filled with the toothpaste samples, and the plates were incubated for 24 hours at 37°C. Inhibition zones were measured using calipers and analyzed using one-way ANOVA with a 95% confidence level, following the method by Widyastuti & Taslima (2019). In terms of data processing and analysis methods, data from the phytochemical and physicochemical tests were presented in graphs or tables and interpreted using descriptive and visual analysis. The antibacterial test results were analyzed using one-way ANOVA with a 95% confidence level, allowing for statistical comparison of the effectiveness of different toothpaste formulations.

3. Results and Discussion

3.1 Results of ambon banana stem extract testing on inhibition of streptococcus mutans Serotype-d (OMZ-176)

Samples of Ambon banana stems were collected from the Pekalongan area, amounting to 5 kg. The samples were washed, chopped, and dried in a hybrid dryer at 55°C for four days. The dried stems were ground to obtain 514 grams of dried simplisia with a moisture content of 7.10%. The extraction method used was maceration with 96% ethanol as a solvent. Ethanol was chosen for its universality, polarity, and ease of availability. 96% ethanol is preferred due to its selectivity, non-toxicity, good absorption, and high extraction capability, enabling it to extract non-polar, semi-polar, and polar compounds more effectively (Serang & Wijayani, 2022). The ratio of solvent to sample was 1:5, with 500 grams of simplisia soaked in 2.5 liters of 96% ethanol for 4x24 hours with occasional stirring. The maceration liquid was filtered and concentrated using a vacuum rotary evaporator at 60°C, yielding 7.2 grams of thick extract with a 1.44% yield. Secondary metabolite compounds were qualitatively identified to ascertain the presence of compounds with potential antibacterial properties (Azizah & Artanti, 2019).



Flavonoids Positive (+) | Flavonoid Positif (+) | Tannins Positive (+) | Terpenoids Positive (+)

Fig. 1. Secondary metabolite identification result of ambon banana stem extract

The Figure 1 indicates that the extract contains flavonoids, saponins, tannins, and terpenoids. The flavonoid content was further quantified using UV-Vis spectrophotometry. Quercetin was used as the standard, with an optimum wavelength of 441.5 nm. The calibration curve was established with concentrations of 5, 10, 20, 40, 60, and 80 μg/mL. A linear regression equation of y = 0.0091x + 0.0544 was obtained, with an R^2 value of 0.9993.

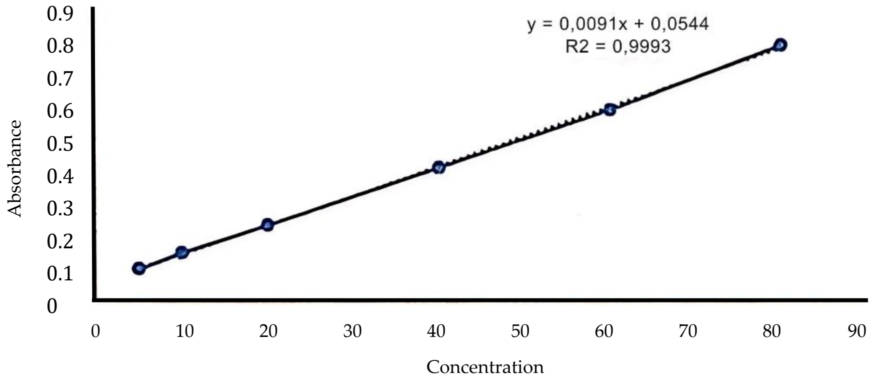


Fig. 2. Standard curve of quercetin at maximum wavelength of 441.5 nm

This Figure 2 shows the standard curve of Quercetin which illustrates the relationship between the concentration of Quercetin and the measured absorbance value. The graph shows a strong linear relationship between concentration and absorbance, as indicated by the regression line equation $y = 0.0091x + 0.0544$ and the value of the coefficient of determination $R^2 = 0.9993$. Value R^2 which is close to 1 indicates that this regression model is very accurate in explaining the variation of absorbance data based on Quercetin concentration. This linear relationship indicates that the method used is consistent and reliable for the quantitative analysis of Quercetin in the samples tested.

The determination of total flavonoid content was calculated by inserting the sample's absorbance value into the linear regression equation, derived from the quercetin calibration curve, to obtain its concentration (Sari et al., 2021). The total flavonoid content in the Ambon banana stem extract sample was found to be 14.794 μgQE/g extract. This result exceeds the findings of previous research by Riyani and Adawiah (2015), who measured the flavonoid content of Ambon banana stem extract using the Soxhlet extraction method with different solvents, reporting the highest flavonoid content at 8.4301 μgQE/g. In contrast, a study by Agusta et al. (2021) measured the flavonoid content of Ambon banana stem extract using 70%, 80%, and 90% solvents over three days, yielding flavonoid contents of 52.56, 27.39, and 18.96 μgQE/g, respectively. Differences in flavonoid content among studies are presumed to arise from variations in treatment methods and environmental factors at the sample collection site.

These findings demonstrate that Ambon banana stem sap extract is suitable for antibacterial testing against *Streptococcus mutans* in vitro. The antibacterial activity of Ambon banana stem extract against *S. mutans* serotype-d (OMZ-176) was evaluated using the disk diffusion method. This method was chosen due to its simplicity, practicality, and suitability for liquid samples, as the process of saturating the sample onto the disk allows for easier measurement of the inhibition zone (Azizah & Artanti, 2019). The results of the antibacterial testing are presented in Table 1.

Table 1. Antibacterial testing of ambon banana stem extract against Streptococcus mutans Serotype-d (OMZ-176)

Sample	Concentration Code	Concentration Code	p-value
10 A	100 mg/ml	0	0
20 A	200 mg/ml	0	
40 A	400 mg/ml	0	
50 A	500 mg/ml	0	
60 A	600 mg/ml	9.405±0.0613	
70 A	700 mg/ml	10.623±0.0044	
K+	Amoxicillin	40.39±0.1268	
K-	DMSO	0	

Based on the table above, it is evident that the concentration of Ambon banana stem sap extract has a significant effect on the inhibition zone diameter, as indicated by the p-value < 0.05. The extract was first detected to have antibacterial inhibition at concentrations of 600 mg/mL and 700 mg/mL. The inhibition zone diameters obtained were 9.405 ± 0.0613 mm at 600 mg/mL and 10.623 ± 0.0044 mm at 700 mg/mL. These results do not yet surpass the positive control, which is the antibiotic amoxicillin. A previous study by Noor and Apriasari (2014) also reported that Kalimantan Mauli banana stem sap extract at a concentration of 800 mg/mL exhibited an inhibition zone of 15 mm against S. mutans. The differences in concentration may be attributed to variations in secondary metabolite levels in different banana plant varieties. The concentration of secondary metabolites can be influenced by factors such as topography, climate, soil type, rainfall, fertilization, and the solvent used in the extraction process (Budi, 2020).

3.2 Physicochemical characteristics of herbal toothpaste with ambon banana stem extract

Following the antibacterial results, toothpaste was formulated with extract concentrations of 600 mg/mL and 700 mg/mL. Figure 3 shows toothpaste preparations with banana ambon stem extract at concentrations of 600 mg/mL (left), 700 mg/mL (center), and plain preparations (right) without extract. Visually, the preparation with the extract showed a more intense yellowish brown color compared to the plain preparation. All three preparations had a thick consistency and homogeneous texture. The difference in color and texture among the various extract concentrations indicates the effect of extract concentration on the physical characteristics of toothpaste preparations. Further physicochemical tests were conducted to measure the homogeneity, pH, viscosity, and foaming ability of each preparation.

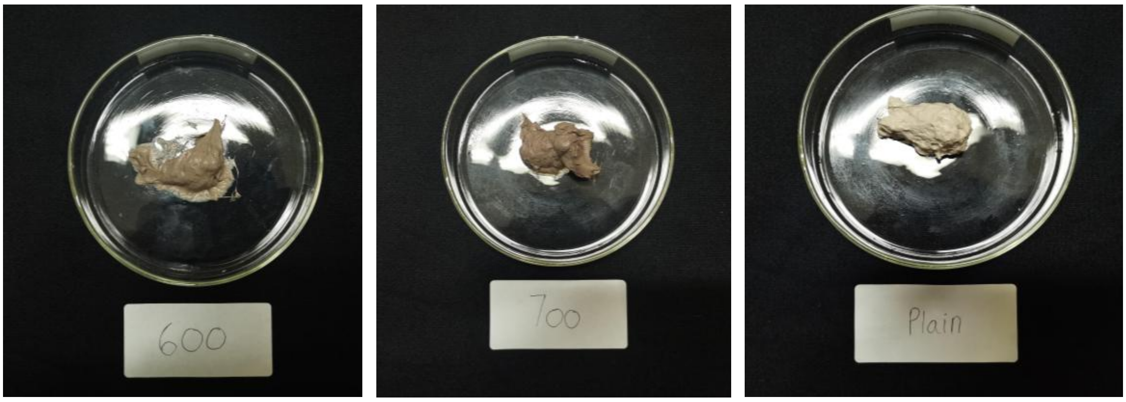


Fig. 3. 600 mg/ml (left), 700 mg/ml (center), and plain (right) banana stem extract toothpaste preparations

Homogeneity testing was conducted to determine whether the toothpaste was homogeneous or evenly mixed. The results of the homogeneity test showed that both formulations of Ambon banana stem extract toothpaste were homogeneous (Figure 4). This was evidenced by the absence of coarse particles when the preparation was placed on the microscope slide.

Fig. 4. Homogeneity test results of 600 mg/ml (left) and 700 mg/ml (right) banana stem extract toothpaste.

Next, pH testing was conducted to determine the pH of the toothpaste formulation and its compliance with the quality standards for toothpaste pH according to SNI 8861:2020, which is 6-10. Based on Table 2. it is evident that the Ambon banana sap toothpaste formulation has a basic pH in both formulations, yet it still meets the SNI standard. The slightly basic pH observed is likely due to the high flavonoid content in the extract. This is supported by a study by Devi et al. (2020), which stated that extracts containing a high level of flavonoids generally have a pH around 9. Toothpaste with a low pH (4.5-5.5) can cause irritation and promote the growth of acidogenic bacteria, such as *Streptococcus mutans*, which thrive in acidic environments. Additionally, a pH below 5.5 has the potential to cause tooth demineralization and enamel damage, leading to dental caries (Widodo et al., 2015). Toothpaste with a high pH can inhibit the growth of bacteria like *S. mutans*, promote remineralization of teeth, and strengthen the enamel layer, thus preventing dental caries (Khamisli et al., 2019).

Table 2. pH measurements of herbal toothpaste with ambon banana stem extract

Formulation extract concentration	pH testing			
	Repetition 1	Repetition 2	Repetition 3	Average
700 mg/ml	9.4	9.5	9.5	9.46
600 mg/ml	9.4	9.5	9.3	9.4

In addition, Table 2 shows the results of pH measurement of toothpaste preparations with banana ambon stem extract at concentrations of 600 mg/mL and 700 mg/mL. Based on three repetitions, the preparation with a concentration of 700 mg/mL has an average pH of 9.46, while the preparation with a concentration of 600 mg/mL has an average pH of 9.4. The relatively small difference in pH value indicates that the increase in extract concentration does not significantly affect the pH of the preparation. Both formulations are in the appropriate pH range for toothpaste products, which is in the mild alkaline range that can help maintain pH balance in the oral cavity.

Moving on to the next test, foam formation, this test was conducted to determine whether foam is produced from a solution containing toothpaste. The results of the test showed that the ethanol extract of Ambon banana stem sap toothpaste is capable of producing foam when used. The 600 mg/mL formulation was found to generate more foam. According to Vranić (2004), the denser the toothpaste formulation, the less foam it produces; conversely, if the formulation is more liquid, it generates more foam.

Table 3. Foaming test results of herbal toothpaste with ambon banana stem extract

Formulation extract concentration	Foam height (cm)			
	Repetition 1	Repetition 2	Repetition 3	Average
700 mg/ml	0.5	0.5	0.5	0.5
600 mg/ml	1	0.9	0.8	0.8

Overall, the table above shows the results of the foam formation test on toothpaste preparations with ambon banana stem extract at concentrations of 600 mg/mL and 700 mg/mL. Based on three repetitions, the preparation with a concentration of 700 mg/mL produced an average foam height of 0.5 cm, while the preparation with a concentration of 600 mg/mL produced an average foam height of 0.8 cm (Table 3). These results indicate that formulations with lower extract concentrations (600 mg/mL) tend to produce more foam than those with higher concentrations (700 mg/mL). This difference is likely due to differences in the composition of active compounds that affect the surfactant properties in the preparation.

Viscosity testing was conducted to determine the thickness of the toothpaste formulation. Viscosity serves as a parameter that describes the resistance of a liquid to flow. The higher the viscosity, the greater the resistance. According to the SNI standard, the viscosity should be between 200-500 dPa·s. The results of the study show that both formulations did not meet the standard, but they nearly reached the minimum threshold, with viscosity values ranging from 196.58 to 196.65 dPa·s. This viscosity value can be influenced by the type of thickening agent used, as well as the temperature during mixing and storage. According to Lacner (2001), the viscosity of a formulation is primarily influenced by temperature, pressure, and the mixing of ingredients. The increase in viscosity may be caused by improper temperature control during storage.

Table 4. Viscosity test results of ambon banana stem extract toothpaste formulations

Formulation	Viscosity			
	Repetition 1	Repetition 2	Repetition 3	Average
700 mg/ml	196.66	196.66	196.65	196.65
600 mg/ml	196.53	196.57	196.65	196.58

Overall, the table presents the results of the viscosity test on toothpaste formulations with ambon banana stem extract at concentrations of 600 mg/mL and 700 mg/mL. Based on the data obtained, the formulation with a concentration of 700 mg/mL showed an average viscosity value of 196.65 dPa.S which was consistent in all repetitions. Meanwhile, the formulation with a concentration of 600 mg/mL had a slightly lower average viscosity value of 196.58 dPa.S (Table 4). This small difference in viscosity may be related to variations in the content of active substances or thickening components in the extracts used.

The spreadability test of the toothpaste aims to determine how well the formulation spreads when applied by consumers, such as when used as a toothbrushing material. The larger the spreadability value, the greater the surface area of the teeth that the toothpaste will cover (Adeleye et al., 2021). A good toothpaste formulation has a spreadability range similar to that of commercial toothpaste, which is between 2.61 and 5.32 cm. However, excessively high spreadability indicates that the toothpaste has a very liquid consistency, is prone to breaking down or dissolving like lotion, and is difficult to apply during use. The spreadability of toothpaste can be influenced by the water content in the formulation; the more water it contains, the greater the spreadability. In this study, the Ambon banana sap toothpaste formulation met the spreadability range of commercial toothpaste.

Tabel 5. Scatter test results of banana ambon stem extract toothpaste formulations

Formulation	Spreadability (cm)
700 mg/ml	3.38
600 mg/ml	3.65

Overall, the table above tries to show the results of the spreadability test of toothpaste formulations using ambon banana stem extract at two different concentrations, namely 600 mg/mL and 700 mg/mL. Based on these data, the formulation with a concentration of 600 mg/mL has a higher spreadability of 3.65 cm compared to the 700 mg/mL formulation which has a spreadability of 3.38 cm (Table 5). This difference in spreadability is likely due to differences in viscosity, where higher extract concentrations tend to increase viscosity thereby reducing the spreadability of the toothpaste.

4. Conclusions

The Ambon banana stem extract demonstrated effective inhibition of *Streptococcus mutans* growth at concentrations of 600 mg/mL and 700 mg/mL, showing inhibition zones of 9.405±0.0613 mm and 10.623±0.0044 mm, respectively. Both concentrations exhibited significant antibacterial activity, with the 600 mg/mL formulation producing an inhibition zone of 13.6233±0.8519 mm, and the 700 mg/mL formulation yielding an inhibition zone of 11.565±0.8674 mm. These results indicate that Ambon banana stem extract possesses strong antibacterial properties against *S. mutans*, a key pathogen involved in dental caries.

The 600 mg/mL formulation displayed desirable physical properties, including a cream color, thick consistency, homogeneity, and a pH of 9.4. Additionally, it had a foam height of 0.8 cm, a viscosity of 196.58 dPa.S, and good spreadability (3.65 cm). Meanwhile, the 700 mg/mL formulation showed similar characteristics, with slight variations in foam height (0.5 cm) and spreadability (3.38 cm), along with a pH of 9.43 and viscosity of 196.65 dPa.S. These findings underscore the potential of Ambon banana stem extract in the formulation of natural antibacterial toothpaste, particularly for managing oral health and combating dental plaque. The antibacterial efficacy observed supports its use in oral care products aimed at reducing *S. mutans* colonization and associated oral health issues.

Acknowledgement

The authors would like to express sincere gratitude to the reviewers for valuable insights and constructive feedback, which have significantly contributed to the improvement of this manuscript.

Author Contribution

Conceptualization, Y. A., G. W., and F. R. N.; Methodology, Y. A. and G. W.; Data Collection, Y. A. and G. W.; Writing – Original Draft Preparation, Y. A. and G. W.; Data Analysis, Y. A., G. W., and F. R. N.; Interpretation of Results, Y. A., G. W., and F. R. N.; Writing – Review & Editing, Y. A., G. W., and F. R. N.

Funding

This research received no external funding.

Ethical Review Board Statement

No available.

Informed Consent Statement

No available.

Data Availability Statement

No available.

Conflicts of Interest

The authors declare no conflict of interest.

Open Access

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Biographies of Authors

Yochidamai Akhsanitaqwim, Applied Natural Medicine, Graduate School of Pharma-Medical Sciences, Toyama University, Toyama City, Toyama Prefecture 930-0151, Japan; Science Hunter Indonesia, PT Riset Prestasi Indonesia.

- Email: yochidamai31@gmail.com
- ORCID: N/A
- Web of Science ResearcherID: N/A
- Scopus Author ID: N/A
- Homepage: N/A

Galang Wirasat, Teknik Sipil, Fakultas Teknik, Universitas Sebelas Maret; Science Hunter Indonesia, PT Riset Prestasi Indonesia.

- Email: 06galang@icp.sch.id
- ORCID: N/A
- Web of Science ResearcherID: N/A
- Scopus Author ID: N/A
- Homepage: N/A

Fahim Rafa Nugraha, Science Hunter Indonesia, PT Riset Prestasi Indonesia.

- Email: ahimrafanugraha@gmail.com
- ORCID: N/A
- Web of Science ResearcherID: N/A
- Scopus Author ID: N/A
- Homepage: N/A