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EFFECTIVENESS OF BREADFRUIT LEAF EXTRACT IN MOUTHWASH FORMULA TO INHIBIT THE BACTERIA OF *Streptococcus mutans* THAT CAUSES CARIES

Efektivitas Ekstrak Daun Sukun Dalam Formula Mouthwash Untuk Menghambat Bakteri Streptococcus mutans Penyebab Karies Gigi

Nur Khairi[1*], Andi Nur Aisyah[1], Maulita Indrisari[2], Sukmawati Sukmawati[3], Michrun Nisa[1]

[1]Bagian Farmasetika dan Teknologi Farmasi, Fakultas Ilmu Kesehatan, Universitas Almarisah Madani, Makassar, Indonesia

[2]Bagian Farmakoterapi, Fakultas Kedokteran, Universitas Palangkaraya, Palangkaraya, Indonesia

[3]Program Studi Kebidanan, Fakultas Ilmu Kesehatan, Universitas Almarisah Madani, Makassar, Indonesia

*Email: nurkhairijalil@gmail.com

ABSTRAK

Karies gigi merupakan masalah kesehatan mulut yang disebabkan oleh bakteri Streptococcus mutans, bakteri ini membentuk biofilm dan menghasilkan asam perusak enamel gigi. Upaya pencegahan sering dilakukan dengan menggunakan sediaan mouthwash yang mengandung bahan antibakteri. Produk mouthwash komersial umumnya mengandung bahan kimia yang dapat menyebabkan efek samping. Daun sukun diketahui mengandung senyawa bioaktif flavonoid dan tannin yang berpotensi sebagai antibakteri alami alternatif bahan aktif pada sediaan mouthwash. Penelitian ini bertujuan mengevaluasi aktivitas antibakteri ekstrak daun sukun dan memperoleh formula sediaan mouthwash yang efektif terhadap Streptococcus mutans. Desain penelitian menggunakan true experimental dengan pretest-posttest control group desain. Metode penelitian dengan mengekstraksi daun sukun menggunakan metode maserasi dengan pelarut etanol 96%. Formulasi sediaan mouthwash dengan variasi konsentrasi gliserin (15%, 20% dan 25%). Aktivitas antibakteri ekstrak dan formula mouthwash dengan menggunakan metode paper disk. Analisis data efektivitas dilakukan menggunakan Kruskall walls dan Mann-Whitney test. Hasil penelitian menunjukkan ekstrak daun sukun konsentrasi 2-4% memiliki aktivitas antibakteri kategori sangat kuat dengan zona hambat terbesar 20,25-23,81 mm dan berbeda signifikan (p<0,05) dengan kontrol negatif. Formula mouthwash ekstrak daun sukun secara organoleptik berbentuk cair, berwarna hijau kekuningan, bau khas sukun dan mint, formula memiliki pH dengan rentang 5,5-7,9 sedangkan viskositas diperoleh mendekati 1cPs, pH dan viskositas ketiga formula tidak menunjukkan perbedaan antar kelompok. Efektivitas mouthwash menunjukkan kategori kuat dengan zona hambat antara 18,81±1,5 mm hingga 18,94±0,5 mm dan secara statistik tidak menunjukkan perbedaan antar ketiga formula (p>0,05). Ekstrak daun sukun 2% dan gliserin 15% merupakan formula mouthwash stabil dan mampu menghambat bakteri penyebab karies gigi.

Kata kunci: aktivitas antibakteri, *Artocarpus altilis,* karies gigi, obat kumur herbal, *Streptococcus mutans*

ABSTRACT

Dental caries is caused by *Streptococcus mutans*, which forms biofilms and produces acid that damages tooth enamel. Prevention is often achieved through mouthwashes containing antibacterial agents, but commercial products may have side effects due to chemical ingredients. *Artocarpus altilis* (breadfruit) leaves contain bioactive compounds like flavonoids and tannins, which have potential as natural antibacterial agents. This

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study aimed to evaluate the antibacterial activity of *Artocarpus altilis* leaf extract and formulate an effective mouthwash against *Streptococcus mutans*. The study used a true experimental design with a pretest-posttest control group. The extract was obtained by maceration with 96% ethanol. Three mouthwash formulations were prepared with varying glycerin concentrations (15%, 20%, and 25%). Antibacterial activity was assessed using the paper disk method, and data were analyzed using Kruskal-Wallis and Mann-Whitney tests. Results showed that the 2-4% extract had strong antibacterial activity, with inhibition zones ranging from 20.25–23.81 mm, significantly different from the negative control ($p<0.05$). The mouthwash formulations were liquid, yellow-green in color, with a characteristic breadfruit and mint scent, pH ranging from 5.5 to 7.9, and viscosity of 1 cPs. The mouthwash exhibited strong antibacterial activity, with inhibition zones between 18.81±1.5 mm and 18.94±0.5 mm, showing no significant differences across formulas ($p>0.05$). The 2% *Artocarpus altilis* extract and 15% glycerin formula was stable and effectively inhibited *Streptococcus mutans.*

Keywords: antibacterial activity, *Artocarpus altilis*, dental caries, herbal mouthwash, *Streptococcus mutans*

INTRODUCTION

Dental caries is one of the most common oral health problems, with a growing prevalence worldwide. According to the World Health Organization (WHO), nearly 90% of the human population experiences dental caries at some point in their lives. The primary cause of this disease is the bacterium Streptococcus mutans, which plays a key role in the formation of dental plaque and tooth decay. This bacterium has the ability to bind to the tooth surface and metabolize carbohydrates into acids that can damage tooth tissue[1], [2], [3]. The presence of high levels of *S. mutans* in saliva is closely related to an increased risk of caries[1]. Efforts to prevent and control the growth of these bacteria are very important to reduce the prevalence of dental disease.

Mouthwash is a product often used to maintain oral hygiene, reduce plaque, and kill bacteria that cause dental disease. Conventional mouthwashes generally contain chemicals like alcohol, chlorhexidine, and cetylpyridinium chloride (CPC), which can cause side effects on the oral mucosa, such as irritation, altered taste perception, and tooth staining.[4], [5]. One increasingly popular alternative is the use of alcohol-free herbal mouthwash. Research on the use of plant extracts, including guava (*Psidium guajava*), propolis, starfruit (*Averrhoa bilimbi*), and several other plants, has been shown to be effective in inhibiting the growth of S. mutans and reducing plaque formation.[6], [7], [8]. In addition, herbal plants such as Triphala, green tea leaves, and Andrographis paniculata also show antibacterial and anti-inflammatory potential [9]. One herbal plant that has not been widely studied for use as an active ingredient in mouthwash is breadfruit leaves (*Artocarpus altilis*). Breadfruit leaves are known to contain several potential bioactive compounds, such as flavonoids, tannins, saponins, and alkaloids, which are known to have antibacterial activity [10]. These compounds function to fight pathogenic bacteria, including S. mutans, which is the main cause of tooth decay. Previous research has shown that the flavonoid and tannin compounds in breadfruit leaves can inhibit bacterial growth by disrupting the metabolism of microorganisms and reducing biofilm formation. Breadfruit leaves also have anti-inflammatory effects that can help maintain gum health and reduce inflammation in the oral cavity, which often occurs due to bacterial infections [10]. These anti-inflammatory and antimicrobial effects are important reasons why breadfruit leaves are worthy of further exploration as an active ingredient in herbal mouthwash formulations. Several previous studies have also demonstrated the potential of breadfruit leaves in traditional medicine, such as their reported use in treating various skin conditions and respiratory infections [11], [12].

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The use of herbal mouthwash preparations promises safer benefits. The effectiveness of the preparation often depends on the type of active ingredient used and the correct formulation method. Further research is needed to better understand the chemical composition of effective plants and to explore the types of plant extracts that can be optimized in oral care products. The diversity of plants that can provide antibacterial effects indicates great potential for developing natural mouthwash products that are not only safe but also effective in reducing oral infections caused by pathogenic bacteria such as S. mutans. This study aimed to assess whether breadfruit leaf extract can be used as an active ingredient in an effective mouthwash product and compare its effectiveness with conventional mouthwash products containing chemicals. Through this research, it is hoped that new solutions will be found in the development of natural and safe oral care products for dental and oral health.

METHODS

This study used an experimental design with a laboratory experimental method, which applied a pretest-posttest control group design. This study was conducted at the Pharmaceutical Biology Laboratory and Microbiology Laboratory of the Makassar College of Pharmaceutical Sciences from February to August 2023. The samples used were breadfruit leaves of the *Artocarpus altilis* species, obtained from the area around Makassar. The sampling technique used was purposive sampling, by selecting breadfruit leaves that were still fresh and free from contamination. The variables observed in this study included the independent variable, namely the concentration of breadfruit leaf extract (2%, 4%, and 6%), and the dependent variable, which was antibacterial activity against *Streptococcus mutans*, which was measured based on the diameter of the inhibition zone formed. The instruments used for this study were paper disks for antibacterial testing, a pH meter for measuring the pH of the mouthwash preparation, and a viscometer for measuring the viscosity of the preparation. All instruments used have been standardized and have laboratory accuracy.

Sample Collection and Processing

Breadfruit leaf samples were obtained from Bone Regency, Makassar, South Sulawesi. A total of 500 g of green breadfruit leaves were wet sorted to separate them from the rest of the plant and cleaned with water to remove dirt. The leaves were then cut into pieces, dried, dry sorted, and powdered.

Making Breadfruit Leaf Extract

2 kg of breadfruit leaf powder was extracted with 96% ethanol solvent for 3 x 24 hours at room temperature (±24^{o}C) using the maceration method. The use of 96% ethanol solvent in the extraction refers to the effectiveness of extracting flavonoid and tannin compounds without damaging the chemical structure of the compound, and is relatively fast in the evaporation process, in addition to this solvent has a lower toxicity level and is more affordable. The macerate obtained was collected and then concentrated with a rotary evaporator at a temperature of 40 °C until a thick extract was obtained. The extract was then fresh-dried to produce a dry extract[10].

Antibacterial Activity Testing of Breadfruit Leaf Extract

The antibacterial activity of breadfruit leaf extract was tested using a 6 mm piper disk. Breadfruit leaf extract was tested at concentrations of 1%, 2%, and 4%, with tetracycline as a positive control and 10% DMSO as a negative control. The paper disk was placed in a petri dish containing NA medium and a suspension of *Streptococcus mutans* bacteria. The petri dish was incubated at 37 ˚C for 24 hours. The resulting inhibition zone was measured using a caliper and recorded. All tests were performed in triplicate[13].

Formula Design

The breadfruit leaf extract mouthwash formula design can be seen in table 1 below.

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Table 1. Breadfruit Leaf Extract Mouthwash Formula Design

Composition	F1 (%)	F2 (%)	F3 (%)	Function
Breadfruit leaf extract	2	2	2	Active ingredient
Glycerin	15	20	25	Humectant
Sorbitol	5	5	5	Sweetener
Menthol	0.10	0.10	0.10	Cooling Agents
Peppermint oil	0.5	0.5	0.5	Fragrance
Sodium benzoate	0.15	0.15	0.15	Preservative
Aquadest	Ad 100 ml	Ad 100 ml	Ad 100 ml	Solvent

Mouthwash Making Procedure

Breadfruit leaf extract was dissolved using distilled water. Glycerin was added to each formula concentration and then dissolved. Then, sorbitol was added and dissolved until homogeneous. Menthol was dissolved with a little alcohol while stirring, then added Na.benzoate dissolved in distilled water was added until homogeneous, then stirred until it can be poured. The solution was mixed, then filtered and put into a bottle. Aquadest was added up to 100 ml, then peppermint oil was dropped, homogenized with a magnetic stirrer at 100 rpm for 15 minutes. The prepared solution was put into a tightly closed container and stored in a cool place for evaluation [14].

Physical Stability Evaluation of Mouthwash

Organoleptic

Evaluation of the mouthwash preparation was conducted by observing its shape, color, taste, and aroma. The preparation was tested for accelerated stability in a climatic chamber at 40°C and 75%±2°C humidity for 4 weeks, with organoleptic observations.

Viscosity

The viscosity of a mouthwash formulation significantly affects its consistency when used as a mouthwash. The closer the viscosity of a mouthwash formulation is to that of water, the easier and more comfortable it is to use as a mouthwash. The standard viscosity for water viscosity calculations is approximately ±1 cP.[14]. The preparation was tested for accelerated stability with a climatic chamber at a temperature of 40 °C and humidity of 75%±2°C for 4 weeks with viscosity observations.

pH test

The pH of the test solution was measured using a pH meter that had been calibrated with a standard buffer solution. The resulting mouthwash pH should be within the oral pH range of 5.5–7.9, so that it does not irritate the oral mucosa when consumed.[14]. The preparation was tested for accelerated stability with a climatic chamber at a temperature of 40 °C and humidity of 75%± 2°C for 4 weeks with pH observations.

Antibacterial Effectiveness Test of Breadfruit Leaf Extract Mouthwash

NA medium was aseptically added into a sterile petri dish as much as 10 ml, allowed to solidify, then streaked with a suspension culture of *Streptococcus mutans* bacteria well so that the bacteria were evenly distributed. Then the paper disk was dipped into the mouthwash test sample solution formulas 1, 2, and 3, negative control (mouthwash base), and positive control (product "X"). The paper disk that had been dipped into the test sample was placed on the surface of the media that had solidified aseptically using sterile tweezers, with a distance of 2-3 cm from the edge of the petri dish, incubated at 37°C for 1 x 24 hours. The inhibition area formed was measured with a caliper[13].

RESULT

Breadfruit Leaf Extract Results

The results of breadfruit leaf extraction using the maceration method using 96% ethanol can be seen in table 2.

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Table 2. Breadfruit Leaf Extraction Results (*Artocarpus altilis*).

Sample	Simple Weight (g)	Solvent solution (L)	Extract Weight (g)	% Yield
Breadfruit leaves (*Artocarpus altilis*)	350	9	77.39	22.11

Breadfruit leaf samples were obtained from Bone Regency, Makassar, South Sulawesi. Extraction was carried out using the maceration method using 96% ethanol, resulting in an extract yield of 22.11%. The resulting extract was then tested for activity against *Streptococcus mutans* bacteria. Three concentrations of extracts were tested: 1%, 2%, and 4%. The test solution was prepared by weighing 0.05 g, 1.1 g, and 1.2 g of extract, respectively, dissolved in 5 mL of 10% DMSO. The positive control used tetracycline, and the negative control used 10% DMSO. The test results can be seen in Table 3.

Antibacterial Activity Test Results

Table 3. Results of the Inhibition Test of Breadfruit Leaf Extract on the Growth of *Streptococcus mutans* Bacteria

No.	Concentration	Average (mm)	Category
1.	Extract 1%	18.11±1.0a	Strong
2.	Extract 2%	20.25±0.8 b	Very strong
3.	Extract 4%	23.81±0.6 c	Very strong
4.	Control (+) Tetracycline	32.27±0.9 d	Very strong
5.	Control (-) DMSO 10%	0±0.0 e	Weak

Statistical testing using one-way ANOVA
[a, b, c, d, e]is a significant difference with LSD analysis ($p<0.05$, n=3)

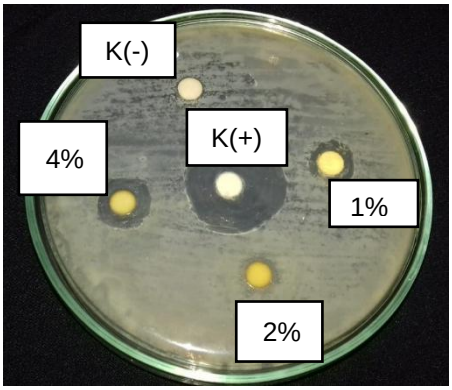

Figure 1. Antibacterial Activity Test of *Streptococcus mutans*

Antibacterial activity test data using the One-Way Anova method showed a significant difference between the concentrations of breadfruit leaf extract on the inhibition zone of Streptococcus mutans. Post-hoc analysis using the LSD test showed that each extract concentration group had a significant difference with the negative control ($p < 0.05$). The 4% concentration had a significant difference compared to the 1% and 2% concentrations ($p < 0.05$), indicating that antibacterial effectiveness increased with increasing extract concentration. Although the 4% extract concentration was significantly different from the 2% extract concentration, the inhibition zone category of the extract concentrations was the same, namely the very strong category. Therefore, the 2% concentration was chosen for use in the mouthwash formulation.

Breadfruit leaf extract was formulated into three mouthwash formulas with variations in glycerin as a humectant, the formula results are shown in Figure 2. The three formulas obtained were then further evaluated using a climatic chamber.

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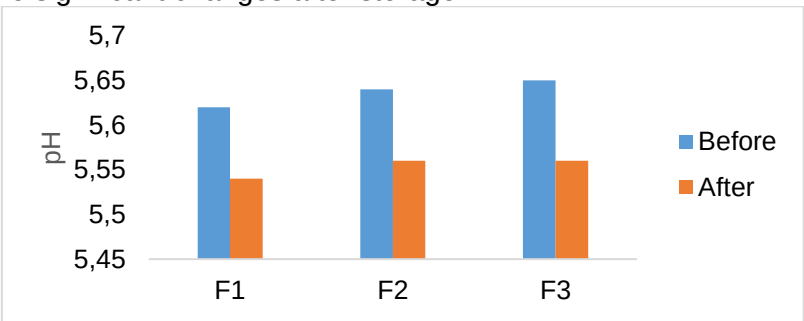
Figure 2. Breadfruit Leaf Extract Mouthwash

Table 4. Results of Organoleptic Observations of Breadfruit Leaf Extract (*Artocarpus altilis*) Before and After Storage

Formula	Characteristics	Before	After
F1	Form	Liquid	Liquid
	Color	Yellowish green	Yellowish green
	Smell	The distinctive aroma of breadfruit leaves, mint	The distinctive aroma of breadfruit leaves, mint
	Flavor	Mint taste and a refreshing sensation	Mint taste and a refreshing sensation
F2	Form	Liquid	Liquid
	Color	Yellowish green	Yellowish green
	Smell	The distinctive aroma of breadfruit leaves, mint	The distinctive aroma of breadfruit leaves, mint
	Flavor	Mint taste and a refreshing sensation	Mint taste and a refreshing sensation
F3	Form	Liquid	Liquid
	Color	Yellowish green	Yellowish green
	Smell	The distinctive aroma of breadfruit leaves, mint	The distinctive aroma of breadfruit leaves, mint
	Flavor	Mint taste and a refreshing sensation	Mint taste and a refreshing sensation

Note:
F1: Glycerin Concentration 15%
F2: Glycerin Concentration 20%
F3: Glycerin Concentration 25%

Organoleptic testing in Table 4 shows the preparation has a characteristic yellowish-green color, a distinctive aroma of breadfruit leaves with mint, and a refreshing taste. There were no significant changes after storage.



Note: F1: Glycerin Concentration 15%; F2: Glycerin Concentration 20%; F3: Glycerin Concentration 25%

Figure 3. Graph of pH test results for Breadfruit Leaf Extract Mouthwash preparation

The pH values of all preparations were within the safe range for the oral cavity (5.5–7.9), namely 5.62–5.65 before storage and 5.54–5.56 after storage. The results are shown in Figure 3.

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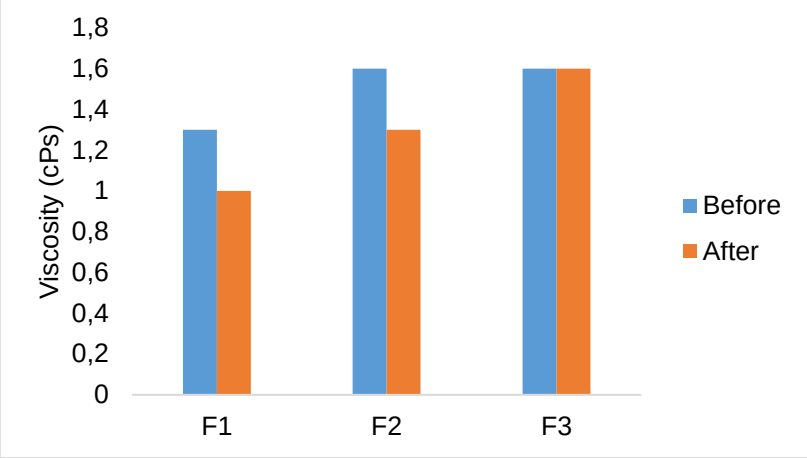

Note: F1: Glycerin Concentration 15%; F2: Glycerin Concentration 20%; F3: Glycerin Concentration 25%

Figure 4. Graph of Viscosity Test Results of Breadfruit Leaf Extract Mouthwash

The viscosity value of the preparation after testing is presented in Figure 4, showing that the mouthwash preparation is close to the viscosity value of water, namely 1 cPs. This indicates that the preparation meets viscosity requirements both before and after storage.

Table 5. Results of the Effectiveness Test of Breadfruit Leaf Extract Mouthwash against *Streptococcus mutans*

Preparation	Average (mm)	Category
F1	18.81±1.5[a]	Strong
F2	18.94±0.5[a]	Strong
F3	18.75±1.1[a]	Strong
Control (+) Product X	31.15±0.4[b]	Very strong
Control (-) Mouthwash base	0±0.0[c]	Weak

Statistical testing using Kruskal-Wallis,
[a, b, c, d, e] is a significant differenceMann-Whitney test($p<0.05$, n=3)
Note: F1: Formula 1 Glycerin concentration 15%
F2: Formula 2 Glycerin concentration 20%
F3: Formula 3 Glycerin concentration 25%

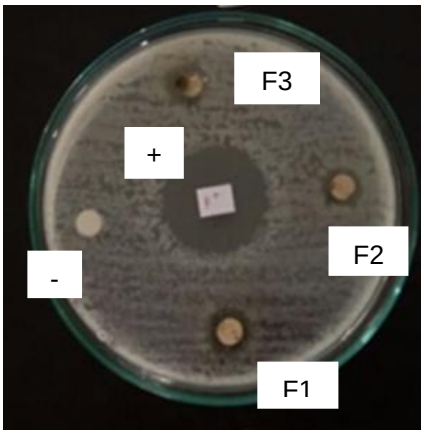

Figure 5. Testing the Effectiveness of Breadfruit Leaf Extract Mouthwash against *Streptococcus mutans* bacteria

The results of the effectiveness test of mouthwash preparations against *Streptococcus mutans* bacteria by measuring the diameter of the inhibition zone against the three breadfruit leaf extract formulas, control (+) and control (-) can be seen in Figure 5. The

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antibacterial activity test data using the One-Way ANOVA method, shown in Table 5, shows no difference between the three breadfruit leaf extract formulas against the inhibition zone of *Streptococcus mutans*. While the negative control and extract formula after post-hoc analysis using the LSD test showed that the extract formula group had a significant difference with the negative control ($p < 0.05$). And the mouthwash formula had a significant difference compared to the positive control ($p < 0.05$), however, the mouthwash formulation was still included in the strong category in inhibiting *Streptococcus mutans* bacteria.

DISCUSSION

The results of this study indicate that breadfruit leaf extract has significant antibacterial activity against *Streptococcus mutans* bacteria. A concentration of 1% obtained an inhibition zone of 18.11 ± 1.0 mm with a strong category, while at a concentration of 2% the inhibition zone was obtained 20.25 ± 0.8 mm, and at a concentration of 4% it was 23.81 ± 0.6 mm, with a very strong inhibition zone category. The results of this study are in line with the findings of Artaningsih et al. (2018)[15], which reported that the ethanol extract of gamal leaves has a similar ability to inhibit the growth of *Streptococcus mutans* in vitro. The positive control used to test the antibacterial activity in this study was tetracycline; the results of the study showed a significant difference between the extract and tetracycline. Tetracycline is a broad-spectrum antibiotic used in the treatment of infections by inhibiting protein synthesis, while breadfruit leaf extract still contains a mixture of bioactive compounds that have more varied mechanisms. Although breadfruit leaf extract is not as strong as tetracycline, it still has great potential as a safe natural active ingredient and an alternative to synthetic materials. The antibacterial effect in plants can be attributed to the bioactive content in breadfruit leaves, such as flavonoids, tannins, and saponins. These compounds are known to damage bacterial cell membranes, cause cytoplasmic leakage, and inhibit the activity of essential enzymes[16]. Other research has also shown that polyphenolic compounds in herbal plants can interfere with biofilm formation by *Streptococcus mutans*, which is the primary mechanism by which this bacterium causes caries. Flavonoids in breadfruit leaf extract also act as antimicrobial agents through several mechanisms. According to Bhagavathy et al. (2019),[17]Flavonoids can inhibit the enzyme glucosyltransferase (GTF), a key enzyme in biofilm formation by *Streptococcus mutans*. Without biofilm, bacteria cannot adhere to tooth surfaces, thus reducing the risk of caries formation. A study by Veloz et al. (2016)[7]. This is supported by showing that polyphenolic compounds can inhibit Gtf activity at subinhibitory concentrations, significantly reducing bacterial viability. The tannin compounds contained in breadfruit leaves also function as protein-precipitating agents that can damage bacterial cell membranes and inhibit their vital functions[18]. The combined effect of flavonoids and tannins may be the main reason for the strong antibacterial activity of breadfruit leaf extract, as evidenced by the larger inhibition zone at higher concentrations.

Several studies have investigated the antibacterial activity of *Streptococcus mutans* with other herbal plants, including those by Gabriella et al. (2022)[16], which showed that extracts of starfruit (*Averrhoa bilimbi*) and red betel (*Piper crocatum)* leaves produced an inhibition zone of 18 mm at certain concentrations. This activity was comparable to breadfruit leaf extract at a concentration of 1%, but lower than at concentrations of 2% and 4%. This indicates that breadfruit leaves have competitive antibacterial potential compared to other herbal plants. Another study by Ravi et al. (2017)[19]showed that herbal extracts such as Syzygium cumini can produce inhibition zones of up to 20 mm on *Streptococcus mutans*, supporting the finding that bioactive compounds from herbal plants have an effective mechanism of action against oral pathogens.

Breadfruit leaf extract-based mouthwash was formulated with varying concentrations of glycerin (15%, 20%, and 25%), which acts as a humectant to maintain physical stability

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and comfort. This study showed that the pH of the mouthwash was within a safe range for the oral cavity (5.5–7.9). This pH stability is important to prevent irritation of the oral mucosa and maintain antibacterial activity. Lindawati et al. (2020)[20]reported that changes in pH in the oral cavity can affect the bacterial ecosystem, so it is important for mouthwash to have a pH that supports microbial balance. Furthermore, the viscosity of mouthwash is close to that of water, which ensures user comfort when rinsing. According to Barata et al. (2023)[21], too high a viscosity can reduce the effectiveness of mouthwash because it makes it difficult to reach all areas of the oral cavity. With the appropriate viscosity, a mouthwash based on breadfruit leaf extract can provide maximum antibacterial benefits and is comfortable to use.

The effectiveness test results showed that the breadfruit leaf extract mouthwash formula had an inhibition zone between 18.81 ± 1.5 mm to 18.94 ± 0.5 mm, which was categorized as strong. However, compared to the positive control (commercial product X), which had an inhibition zone of 31.15 ± 0.4 mm, the effectiveness of the breadfruit leaf-based mouthwash was still lower. According to Kasraei et al. (2014)[22],this difference may be due to the silver or zinc oxide nanoparticles in commercial products, which have broad-spectrum antibacterial activity. However, breadfruit leaf extract-based mouthwash offers a safety advantage, as it does not contain chemicals that can irritate the oral mucosa, as reported by Satpathy et al. (2013)[4]. The results of this study provide evidence that breadfruit leaf extract can be used as an active ingredient in herbal mouthwash formulations. With the increasing demand for natural and environmentally friendly products, breadfruit leaf extract-based mouthwash has great potential to be developed as a natural alternative to oral care products. Although its effectiveness is lower than that of commercial chemical-based products, its advantages in terms of safety and natural characteristics make it a promising alternative. Other studies have shown that the combination of several plant extracts can increase antibacterial effectiveness through synergistic effects, so the development of new formulations by combining breadfruit leaf extract with other natural active ingredients could be a future research direction [23].

Several factors influence the antibacterial effectiveness of breadfruit leaf extract, which need to be considered in further product development. The first factor is extract concentration, where antibacterial effectiveness tends to increase with increasing concentration. Too high a concentration may not always be ideal, as it can cause irritation to the oral mucosa [24]. Further research is needed to determine the optimal concentration that provides a balance between effectiveness and ease of use. The second factor is the extraction method. In this study, maceration with 96% ethanol was used, but other extraction methods, such as ultrasonication or supercritical fluid extraction (SFE), can produce higher concentrations of active compounds and improve their bioavailability.[25]The use of nanoencapsulation technology can also be a way to increase the effectiveness of breadfruit leaf extract by facilitating the penetration of active compounds into bacterial cells. Another factor that can influence antibacterial effectiveness is the quality of the breadfruit leaves used. The content of bioactive compounds in breadfruit leaves can vary depending on the planting location, harvest time, and processing method. Leaves harvested at the right time and processed correctly are likely to contain higher concentrations of active compounds. Furthermore, the formulation of the mouthwash product also plays a crucial role. The blending of ingredients such as glycerin serves to increase product comfort and stability, but the concentration of these ingredients must also be optimized to avoid compromising the antibacterial effectiveness of the breadfruit leaf extract[25], [26], [27].

Overall, although the effectiveness of breadfruit leaf extract in inhibiting *S. mutans* is still lower than commercial chemical-based products, breadfruit leaf-based products still have enormous potential as a natural alternative for oral health care, particularly as a

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preventative against *Streptococcus mutans* bacteria, which cause dental caries. With further research and optimization, this product could become a primary choice for consumers who prioritize safety, sustainability, and effectiveness in maintaining oral health.

CONCLUSION

Breadfruit leaf extract concentration of 2-4% has a very strong antibacterial activity with the largest inhibition zone of 20.25-23.81 mm. Formula 1 of 2% breadfruit leaf extract and 15% glycerin is a stable mouthwash formula and can inhibit bacteria that cause dental caries with an inhibition zone between 18.81 ± 1.5 mm to 18.94 ± 0.5 mm in the strong category. This study shows the potential of breadfruit leaf extract mouthwash as a natural alternative against *Streptococcus mutans* bacteria that cause dental caries. This study has not explored the impact of long-term use of mouthwash preparations, so further research is needed to confirm its use.

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