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RESEARCH **Open Access**

The Development of *Spondias pinnata (L.f) Kurz*. Leaf Extract Toothpaste: Formulation, Physical Properties Evaluation and its Antibacterial Efficacy Against *Streptococcus mutans* Bacteria

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Abstract

Cemcem (*Spondias pinnata (L.f) Kurz*) is a native Indonesian plant that has long been used as a traditional medicine. The ethanol extract of *S. pinnata* contains alkaloids, carbohydrates, flavonoids, triterpenoids, steroids, tannins, resins, and saponins. It has proven antibacterial activity against gingivitis and periodontitis bacteria, such as Streptococcus mutans. This study aims to develop a toothpaste formula with the active ingredient of *S. pinnata* leaf extract with various concentrations of 5, 15, and 30%; evaluate its properties; and measure its antibacterial effectiveness against *Streptococcus mutans*. The research method used a quasi-experimental method with a post-test-only control group design. The S. pinnata leaves were extracted with 96% ethanol solvent using the maceration technique. The properties of the toothpaste were evaluated based on homogeneity, spreadability, foam height, viscosity, and pH. The liking test was conducted to assess color, taste, scent, and texture based on the perception of 30 panelists. Antibacterial activity was tested using the well diffusion method. The test results confirm that all preparation formulas are homogeneous, the foam height is in the range of 4.33 - 21.33 cm, the spreadability is in the range of 3.44 - 4.53 cm2, the viscosity of toothpaste is in the range of 20767-67600 cps, and the pH value is in the range of 4.82 - 7.59. The results showed that toothpaste containing 5% of *S. pinnata* leaf extract met the physical and pH requirements and was most favored by panelists. This formula also showed antibacterial activity against *S. mutans* with an average inhibition zone diameter of 7.72 mm ± 0.439. Based on the study's results, it can be concluded that toothpaste containing 5% *S. pinnata* leaf extract effectively inhibits the growth of *S. mutans* bacteria by 54.33% compared to the positive control. Further research is suggested to optimize the concentration of *S. pinnata* leaf extract to maximize its antibacterial activity, without affecting its physical quality and organoleptic acceptance.

Keywords: Antibacterial, Extract, *Spondias pinnata*, *Streptococcus mutans*, Toothpaste.

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1. INTRODUCTION

Cemcem (*Spondias pinnata* (L.f) Kurz.), a native Indonesian plant, is gaining attention for its unique properties that make it a potential solution in healthcare. Cemcem (*S. pinnata*) was found in the Kintamani area of Bangli and now spreading to Gianyar and Klungkung, Bali. Cemcem is a key ingredient in the traditional Balinese drink, known as loloh cemcem, made from its leaves (Laksemi, 2019). Local Balinese have long used the plant as an appetite enhancer and traditional medicine for various ailments, including toothache (Dharmawati et al., 2022; Hazra et al., 2008).

In terms of ethnomedicine, *S. pinnata* skin is used to treat dysentery, vomiting, and TB; roots are used to control menstruation; leaves are used as a medication for dysentery; unripe fruit is used as an aphrodisiac; ripe fruit is used to treat constipation and as an anti-scorbutic medication. The flowers are used to treat gonorrhea, dyspepsia, obesity, hemorrhagic illness, and vomiting (Jain et al., 2014; Poojary et al., 2018; Kishore et al., 2016; Laksemi, 2019; Panda et al., 2014; Sujarwo et al., 2015; Sujarwo et al., 2017).

The pharmacological activity of *S. pinnata* varies according to the phytoconstituents in these plants. The phytochemical screening revealed that *S. pinnata* ethanol extract contains alkaloids, carbohydrates, flavonoids, triterpenoids, steroids, tannins, resins, and saponins. Essential oils from pulp contain carboxyl acid, esters, alcohol, and scenttic hydrocarbons (Khatoon et al., 2015; Laksemi, 2019; Sujarwo et al., 2015). Other research mentioned triterpenoids, saponins, essential oils, amino acids, and polysaccharides (Poojary et al., 2018). Phytochemical analysis from different research found 24-methylene cyclopentanone, lignoceric acid, sitosterol, and D-glucoside has been isolated from *S. pinnata* (Attanayake et al., 2015; Khatoon et al., 2015).

S. pinnata is rich in phenol and flavonoid components. This component is essential in antioxidant activity that can fight oxidative stress related to cancer, atherosclerosis, inflammation, diabetes, hair loss, ischemic heart disease, and neurodegenerative disorders, including Alzheimer's and Parkinson's (Jain et al., 2014). Some previous reports revealed that 70% methanol extract of *S. pinnata* stem bark has cytotoxic activity against cancer cells of human lung adenocarcinoma with IC50 147.84 µg/ ml, whereas for human breast adenocarcinoma of 149.34 µg/ml and also has a hypoglycemic effect equivalent to glibenclamide. In contrast, ethanol extract has an analgesic effect equivalent to acetylsalicylic acid. Ethanol extract of *S. pinnata* fruit has antibacterial activity against *P.aeruginosa* and *S. epidermidis* at a dose of 500 µg / disc with a disc diffusion method, potent cytotoxicity at IC50 2.12 µg / ml. Leaf extract of *S.pinnata* has antibacterial activity against gram-positive bacteria, including *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Sarcina lutea*, and gram-negative *Salmonella paratyphi, Vibrio parahimolyticus, Escherichia coli, Pseudomonas aeruginosa*. Other studies also show that *S.pinnata* has anti-TB activity (Sameh et al., 2018; Laksemi, 2019).

Ethanol and acetone extracts of *S. pinnata* stem bark as a hepatoprotector in liver damage caused by alcohol in mice (Kishore et al., 2016). The combination of whey and *S. pinata* has a neuroprotective effect on mice receiving etoposide chemotherapy, which causes mucositis as a side effect (Rao et al., 2018). In addition, ethanolic extract of *S.pinnata* leaf also has antibacterial activity against *S. mutans* and *P. gingivalis* in the strong category (Bekti et al., 2022; Dharmawati et al., 2022). *Spondias pinnata* has shown notable antibacterial effects against oral pathogens, including *Streptococcus mutans*, which is a primary contributor to dental caries (Tzimas et al., 2024). In vitro studies indicate that extracts can rival the efficacy of antibacterial agents like chlorhexidine (Tzimas et al., 2024). Previous research demonstrates that *Spondias pinnata* bark extract significantly increases collagen density in gingival tissues post-curettage in Wistar rats, outperforming both negative and positive control groups and also

promotes angiogenesis, facilitating better blood vessel formation in gingival wounds, which is crucial for effective healing (Ramadhany, Adibah & Dewi, et al., 2023; Ramadhany, Adibah & Yanta, 2024).

S. mutans is the main microorganism that plays a role in the formation of dental plaque. Due to the ability to produce acid, *S. mutans* causes tissue demineralization and infection in the oral cavity, whereas *P. gingivalis* is the primary pathogenesis of periodontitis found in dental plaque. Periodontal disease is an inflammatory disease of the tissue around the teeth that begins with gingival inflammation and continues to damage the structure of other tooth-supporting tissue, such as cementum, periodontal tissue, and alveolar bone (Nugraha, et al., 2023; Nugraha, et al., 2023). According to the 2018 National Basic Health Research report in Indonesia, 73.1%–75% of the population have periodontal disease. The most common periodontal disease is gingival inflammation or gingivitis, with 13.7%–14.1% of patients experiencing bleeding gum (Kementerian Kesehatan Republik Indonesia, 2018).

Dental plaque is a biofilm containing many bacteria in both hard and soft tissues. Dental plaque is a common etiologic factor for gingivitis. Dental plaque accumulation is prevented by controlling plaque mechanically, namely by brushing teeth with toothpaste. Using toothpaste in the community has become a daily necessity because using toothpaste regularly can maintain dental and oral health. A new development in the prevention of gingivitis is using natural ingredients. One natural ingredient that holds great promise in reducing gingivitis and periodontitis is *S. pinnata*. Phytochemical compounds such as phenol and flavonoids have antibacterial activity against gingivitis and periodontitis bacteria. Previous studies have shown that phytochemical compounds such as phenols and flavonoids in *S. pinnata* plants have the potential to enhance healing processes and prevent dental caries. However, the development and evaluation of toothpaste with the active ingredient of *S. pinnata* leaf extract has not been widely carried out. Therefore, this research will develop *S. pinnata* leaf extract toothpaste, evaluate its physical properties and pH value, and study its antibacterial effectiveness against *S. mutans*. *Spondias pinnata* leaf extract toothpaste can be formulated using carbopol as the formula base. Carbopol has several advantages because it can form a paste that is stable in extended storage, non-toxic, and has the lowest irritating effect (Fujiastuti & Sugihartini, 2015; Rahayu et al., 2016; Tunjungsari, 2012). Other studies proved that using carpobol as the formula base on the toothpaste formulations can produce toothpaste that meets organoleptic requirements, spreadability, foam height, storage stability, pH, and viscosity (Hafizah, 2019). To prove the effectiveness of toothpaste containing cemcem leaf extract as an antibacterial toothpaste, we develop the formulation of *S. pinnata* leaf extract toothpaste, conducting the properties evaluation and liking test, and the efficacy of the toothpaste against *S. mutans* compared with the positive control. The results of this study are expected to produce natural antibacterial toothpaste so that it can be an alternative solution for handling oral health problems.

2. RESEARCH METHOD

The research used a quasi-experimental method with a post-test-only control group design. It was conducted from February to August 2024 at the Chemistry Laboratory of the Department of Medical Laboratory Technology, Health Polytechnic of Denpasar, Bali, and the Microbiology Laboratory of Warmadewa University. The research comprised four main stages: sample collection and preparation, toothpaste formulation, testing of the resulting formula, and an antibacterial efficacy test.

Spondias pinnata leaf extract was produced by immersing the simplicia powder into 96% ethanol. Subsequently, toothpaste with different concentrations of *S. pinnata* leaf extract was developed to produce a natural antibacterial toothpaste candidate. The properties of this formulation were evaluated using homogenity, spreadability, foam height, pH, and viscosity tests. The most favored formula, as determined by the panelists, was then evaluated using a hedonic test based on color, taste, scent, and texture. The selected formula was used as the test

formula, and further testing was conducted using the well diffusion method to study the antibacterial activity. Antibacterial activity is indicated by the diameter of the inhibition zone in the well diffusion method test.

The diameter of the inhibition zone was tested descriptively to determine the mean and standard deviation. Furthermore, the data normality is analyze using the Shapiro Wilk Test. The difference in inhibition zone diameter between the test formula group and the positive control group was analyzed by Independent T-Test if the data were normally distributed and Mann Whitney Test if the data were not normally distributed. The antibacterial efficacy was determined by comparing the inhibition zone diameter of the test formula with the positive control and expressed as a percentage (%). The data was recorded, processed, and presented as narratives and tables. Ethical approval was obtained from the Ethics Commission of Health Polytechnic of Denpasar, DP.04.02/F.XXXII.25/0385/2024.

Preparation of *S. pinnata* leaf extract. *S. pinnata* leaves were obtained from Kintamani, Bangli, Bali, Indonesia, with the authentication number TL.02.04/D.XI.5/16536.323/2023 from Testing Laboratory, Functional Implementation Unit for "Traditional Health Services" Tawangmangu, Indonesia. Each *S. pinnata* stems selected leaves that met the sample requirements, including light-dark green leaves with no holes, rot, or dryness. Next, the selected leaves were washed thoroughly with tap water; then, the leaves were air-dried to remove residual water from washing. Next, the leaves were dried using an oven at a temperature of 40 ± 1˚C for approximately 20-40 hours until completely dry. After drying, the leaves were sorted to separate them from the stems and other parts involved in drying. Leaves that had passed the dry sorting stage were then crushed using a blender and sieved to obtain simplicia powder of relatively the same size (Habibah et al., 2023).

The obtained *S. pinnata* leaves powder was then used in the extraction process. The extraction process was performed by weighing 400 g of dried leaves, which were then dissolved in 96% ethanol at a ratio of 1:5. The re-maceration process was carried out two times to increase the effectiveness of the extraction process. In addition, agitation was performed for 15 minutes each day so that the ethanol could reach all parts of the leaf powder. The obtained extract was filtered with filter paper. The obtained filtrate was concentrated in the rotary evaporator vacuum at 30˚C (Habibah et al., 2023).

Formulation of toothpaste containing cemcem leaf extract. The preparation of toothpaste containing cemcem leaf extract was conducted on (Ilmi, 2017) with some modifications. A 2 g of carbopol was added to the water and continuously stirred for about 30 minutes, then added 2 g of TEA to obtain the A gel. Next, a weight of cemcem leaf extract, 0.2 g sodium saccharin, 0.18 g methylparaben, 20 g glycerin, and 0.1 g oleum menthol was mixed and stirred continuously until homogenous. The 20 g of $CaCO_3$ was added and stirred for 10 minutes to obtain the B paste. The A gel and B paste were mixed well via stirring. Finally, 1 g of Na-lauryl sulfate was added to the mixture and stirred continuously for about 10 minutes.

Properties evaluation and liking test. The properties of a natural antibacterial toothpaste candidate were evaluated through various tests including homogeneity, spreadability, foam height, viscosity, and pH. The homogeneity test involved applying the toothpaste formula on a piece of transparent glass, which was observed visually in an upside-down position. The spreadability test was conducted by applying the toothpaste formula on a piece of transparent glass and measuring the spread diameter. Foam height was evaluated by dissolving 1g of toothpaste formula, shaking for a while, and then measuring the foam height after standing for about 5 minutes. The pH test was carried out using a pH meter by dissolving 1g of toothpaste in 100 ml of distilled water. The viscosity test was conducted using a Brookfield viscometer (Aris et al., 2022; Hafizah, 2019; Ilmi, 2017).

The liking test was conducted using the hedonic method with the organoleptic test. The most favored formula was evaluated by 30 panelists aged 18-30 years through their preference for the toothpaste formula on the parameters of color, taste, scent, and texture. Ratings of liking and disliking for organoleptic parameters were expressed on a 5 Likert scale: 1: very poor; 2: poor; 3: neutral; 4: like; and 5 excellent (Ratih & Habibah, 2022).

The antibacterial activity and efficacy against *S. mutans* bacteria. The bacterial strains used were *S. mutans* ATCC. *S. mutans* were cultured into BHI-A with vitamin K. The agar media was made by 10 µl vitamin K, 50 µl hemin solution, BHI-A 37 g in 100 ml sterile distilled water, and 500 µl yeast extract. The bacteria were inoculated from the ATCC bacterial stock and then incubated at 37˚C for 24h.

S. mutans suspension was made by incorporating one colony of *S. mutans* from BHI-A into liquid media with a total volume of 10 ml containing 0.37 g BHI-B, 5 µl hemin, 1 µl vitamin K, and 50 µl yeast extract. Then, the suspension was incubated for 24 hours, and the concentration was measured to obtain turbidity equivalent to 1.5 x 106 CFU/ml. For antibacterial activity, the disc diffusion method was used. The *S. mutans* suspension was swabbed on the entire MH agar surface. Wells were made using a 6 mm cork-borer. The samples of the test formula and positive control were inserted into the wells that had been made and then incubated for 24 hours at 37˚C. Next, the clear zone around the wells was observed and measured using a calliper (Dharmawati et al., 2022).

The diameter of the inhibition zone obtained was tested descriptively to determine the mean and standard deviation. Then, proceed with the data normality test using the Shapiro Wilk Test. The difference in inhibition zone diameter between the test formula group and the positive control group was analyzed by Independent T-Test if the data were normally distributed and Mann Whitney Test if the data were not normally distributed. The antibacterial efficacy was determined by comparing the inhibition zone diameter of the test formula with the positive control and expressed as a percentage (%). The antibacterial efficacy was determined with the following equation (Equation 1):

$$\text{Antibacterial efficacy (\%)} = \frac{\text{The average of the inhibition zone diameter of the test formula (FU)}}{\text{The average of the inhibition zone diameter of the positive control}} \times 100\%$$

3. RESULTS AND DISCUSSION

The study utilized the maceration method with 96% ethanol solvent to obtain *S. pinnata* leaf extract. The concentrated extract obtained in this study weighed 122.18g, resulting in an extract yield of 30.57% compared to the simplicia powder used. Calculating the extract yield is crucial for determining the ratio of the extract obtained to the initial weight of the simplicia. It also helps qualitatively assess the number of bioactive compounds in the extracted materials (Utami et al., 2020).

Samples were prepared in powder form to increase their surface area, thereby enhancing the efficiency of the extraction process and ultimately leading to a greater extract yield. The particle size of the material used plays a significant role in ensuring a smooth and efficient extraction process. This is because a larger particle size increases the contact area between the material and the solvent, leading to improved extraction efficiency (Habibah et al., 2023; Ningsih et al., 2019).

The maceration method utilized in this study offers numerous advantages for isolating compounds from natural materials. During the soaking process, the pressure difference inside and outside the cell causes the cell wall to break, allowing the compounds in the cytoplasm to dissolve into the solvent. The duration of soaking can be adjusted to optimize the extraction process. The maceration method also offers advantages such as not requiring the plant in the form of fine powder, not demanding special skills, and minimizing distillate loss (Atun, 2014).

The solvent used for maceration was 96% ethanol, chosen for its ability to extract polar compounds with bioactivity, such as phenolic compounds and flavonoids, from cemcem leaf samples (Fauziyah et al., 2022; Habibah, 2024; Riwanti et al., 2020). Ethanol, being a polar

solvent, is selective, neutral, less toxic, and capable of dissolving various secondary metabolites while evaporating quickly. After the maceration process, the filtrate is separated from the residue through filtration. Then, it undergoes an evaporation process, essential for subsequent analysis to minimize matrix intervention, especially from the solvent (Habibah et al., 2023).

Toothpaste containing *S. pinnata* leaf extract was formulated by dissolving and mixing gelling agents, developers, preservatives and *S. pinnata* leaf extract at various mass variations according to Table 1. The various ingredients in the toothpaste formulation have their respective functions. The active ingredient is *S. pinnata* leaf extract with 5, 15, and 30% b/w concentration. Carbopol acts as a gelling agent that makes the texture of toothpaste softer. Calcium carbonate ($CaCO_3$) acts as an abrasive that functions as a tooth surface cleaner and polisher and has the largest proportion in toothpaste formulations. Glycerin is a humectant that acts as a moisturizer and increases the softness of toothpaste. Sodium lauryl sulfate acts as a detergent that acts as a foam builder and helps the cleaning motion of the toothbrush. Peppermint oil is an scent giver, methylparaben is a preservative, and distilled water is a solvent (Hafizah, 2019; Widyastuti et al., 2019). The result shows that that all three toothpaste formulas (F1, F2, and F3) made from *S. pinnata* leaf extract have a semi-solid texture with no syneresis. The formula containing *S. pinnata* leaf extract is green in color with a deeper green color as the concentration of extract increases. The green color is influenced by the concentration of the added extract. Additionally, the mint scent comes from the added peppermint oil during the synthesis process.

Table 1. Toothpaste properties evaluation

Parameter	Requirements	Base Formula	F1 (5%)	F2 (15%)	F3 (30%)
Homogeneity	**Homogene**	**Homogene**	**Homogene**	**Homogene**	**Homogene**
Spreadability	5-7 cm^2	3.443±0.004	3.977±0.012	4.190±0.005	4.530±0.005
Foam height	-	21.33±1.53	15.67±0.58	6.33±0.58	4.33±0.58
Viscosity	20000-30000 cPs	67600±854	39433±1677	26500±1311	20767±702
pH	4.5-10.5	7.59±0.02	6.16±0.04	5.33±0.01	4.82±0.01

Table 1 confirms that all preparation formulas are homogeneous. It was proved by the absence of grains, lumps, or coarse particles during the testing and observation, which indicates the good physical stability of the extract toothpaste preparation. The main factor influencing homogeneity is the particle size distribution. A uniform particle size produces a homogeneous preparation (Marlina & Rosalini, 2017).

Based on the results presented in Table 1, it is known that the foam height of the toothpaste is in the range of 4.33 - 21.33 cm. This range indicates that the toothpaste is able to produce a satisfactory amount of foam during use. Based on the results, it is known that the greater the concentration of *S. pinnata* leaf extract added, the lower the foam height. At higher extract concentrations, the addition of Na-lauryl to the preparation is not enough to emulsify the entire extract (Afni et al., 2015).

Based on the results presented in Table 1, it is known that the spreadability of toothpaste is in the range of 3.44 - 4.53 cm2. The spreadability increased with the increase of concentration of *S. pinnata* leaf extract added. A wider spreadability will cause the toothpaste to be more easily distributed in the target area when used. Conversely, if the value of spreadability is smaller, the toothpaste will have more difficulty reaching the target area, thus affecting consumer comfort. The value of spreadability is inversely proportional to the viscosity. This can be seen in Table 2, which shows that the higher the viscosity of the preparation, the lower the spreadability value, and vice versa. The amount and type of gelling agent used during toothpaste formulation will also affect the consistency of the paste (Hafizah, 2019).

Based on the results presented in Table 1, it is known that the viscosity of toothpaste is in the range of 20767-67600 cps. The increasing concentration of *S. pinnata* leaf extract causes a decrease in toothpaste viscosity. This can be caused by decreased pH when adding *S. pinnata* leaf extract to the toothpaste formulation. The greater the concentration of *S. pinnata*, the lower the pH value. The toothpaste will become thinner at a more acidic pH, producing a toothpaste with a lower viscosity.

Based on the Indonesian National Standard (SNI), the pH quality requirements for toothpaste preparations range from 4.5 to 10.5 (Afni et al., 2015; Hafizah, 2019). Based on the results presented in Table 1, it is known that all toothpaste preparations in this study meet the pH value requirements with a value range of 4.82 - 7.59. The pH test in this study showed that the pH value of toothpaste preparations decreased with an increasing concentration of *S. pinnata* leaf extract. The bioactive compounds in *S. pinnata* leaf extracts, such as flavonoids, tannins, and polyphenols, have an acidic pH, causing *S. pinnata* leaf extract also to be acidic. So, the greater the concentration of *S. pinnata* leaf extract added to the toothpaste preparation causes, the lower the pH value of the toothpaste.

The level of liking test was conducted to determine the most acceptable toothpaste containing *S. pinnata* leaf extract. The most favored formula was evaluated by 30 panelists aged 18-30 years based on the parameters of color, taste, scent, and texture.

Table 2. The liking test result

Number of panelists	The average of organoleptic test *		
	F1 (5%)	F2 (15%)	F3 (30%)
30	3.52	2.98	2.88

Description:*1: very poor; 2: poor; 3: neutral; 4: like; and 5 excellent*

Table 2 shows that the liking test was conducted on 30 panelists aged 18-30 years, both male and female. The organoleptic parameters tested included color, taste, scent, and texture. Color is the first impression that appears and is assessed by panelists. Color is the first impression because it uses the sense of sight. An attractive color will invite panelists to try a product. Apart from color, taste is one of the factors that can determine whether a product is acceptable to panelists. Taste is something that is received by the tongue. In sensing human taste, there are four primary tastes, namely sweet, bitter, sour, and salty. Another parameter that determines the liking test is the scent. Scent is one of the parameters used to test sensory properties (organoleptic) using the sense of smell. The scent is acceptable if the material produced has a specific scent. Furthermore, scent is a subjective sensation produced by smell (odor). Constituents that can cause scent are volatile compounds contained in a product. Texture is a sensation associated with touch. Texture is a parameter of preference test that is as important as color, taste, and scent because it affects the image of a product (Fatima, 2020; Lamusu, 2018; Issusilaningtyas, Rochmah, & Farabi, 2024).

In this study, the panelists' preference for toothpaste preparations was expressed on a 5 Likert scale: 1: immensely dislike; 2: dislike; 3: neutral; 4: like; and 5 very like. Furthermore, the scores on the four parameters were averaged so that the organoleptic score of each formula was obtained. The liking test results presented in Table 3 show that F1 with a 5% concentration of cemcem leaf extract is the most acceptable toothpaste preparation formula for panelists. The highest mean organoleptic score, among other formulas, is indicated by 3.52. Based on the results of the liking test, F1 (5%) was chosen as the following test formula to determine its antibacterial efficacy against *S. mutans* bacteria.

The antibacterial activity and efficacy against *S. mutans* bacteria. The antibacterial activity of the test samples and positive control against *S. mutans* bacteria was carried out in-vitro by the good diffusion method. Antibacterial activity was measured based on the diameter of the inhibition zone indicated by the clear zone formed around the wells after treatment and

expressed in mm. The observation results of the inhibition zone diameter against *S. mutans* bacteria can be seen in Figure 1.

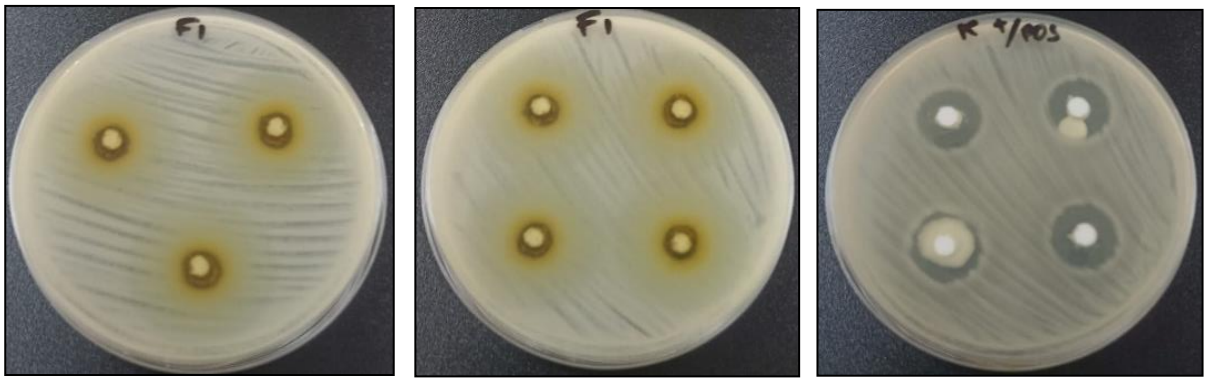

Figure 1. The inhibition zone of test formula (F1) and positive control

The results of the diameter inhibition zone of formula F1 and positive control are presented in Table 3. The diameter of the inhibition zone obtained was tested descriptively to determine the mean and standard deviation.

Table 3. The diameter inhibition zone of formula F1 and positive control

Group	Replication	The average of diameter inhibition zone	Dev. Std.
F1 (5%)	1	8.21	0.38
	2	7.31	0.32
	3	7.33	0.13
	4	7.95	0.63
	5	7.88	0.19
	6	8.07	0.27
	7	8.13	0.25
	8	7.33	0.13
	9	7.39	0.14
	10	7.61	0.18
Positive control	1	15.17	0.41
	2	13.93	0.29
	3	14.82	0.54
	4	14.06	0.15
	5	14.22	0.72
	6	15.44	0.43
	7	13.92	0.17
	8	14.34	0.30
	9	14.46	0.38
	10	13.89	0.21

Table 3 shows that the results of descriptive analysis of the diameter of the inhibition zone, it is known that formula F1 can inhibit the growth of *S. mutans* bacteria with an average inhibition zone diameter of 7.72mm ± 0.439, while the average inhibition zone diameter of positive control of 14.43mm ± 0.633. The average diameter of the inhibition zone of formula F1 and positive control is presented in Figure 4.

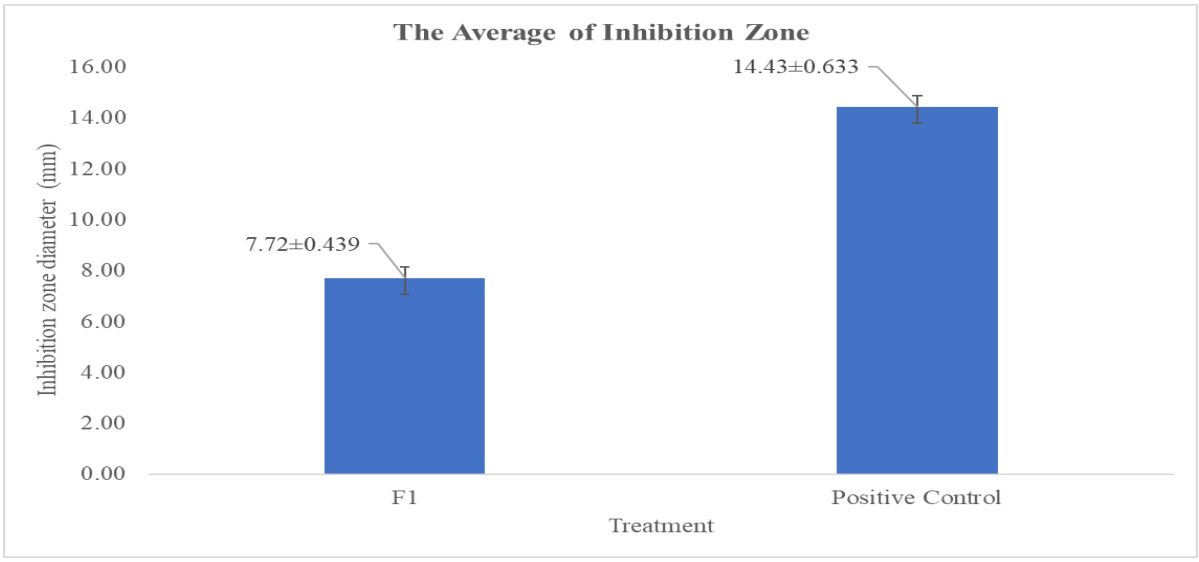

Figure 2. The average of the inhibition zone of the test formula (F1) and positive control

The data normality test using the Shapiro-Wilk Test showed that the data of formula F1 were normally distributed with a significance value of 0.138 and the positive control was not normally distributed with a significance value of 0.002 ($p < 0.05$). Hence, the statistical analysis was continued with a non-parametric test using the Mann-Whitney Test. The results of the difference test using Mann-Whitney non-parametric analysis prove that there is a difference in the diameter of the growth inhibition zone of *S. mutans* bacteria in formula F1 against the positive control used with a value of sig. 0.000 (<0.05).

Furthermore, the antibacterial effectiveness of the F1 test preparation against *S. mutans* bacteria was determined by comparing the diameter of the inhibition zone to the positive control by using Equation 1 and expressed in percent. The results showed that formula F1 in this study was effective in inhibiting the growth of *S. mutans* bacteria compared to the positive control with an effectiveness of 54.33%.

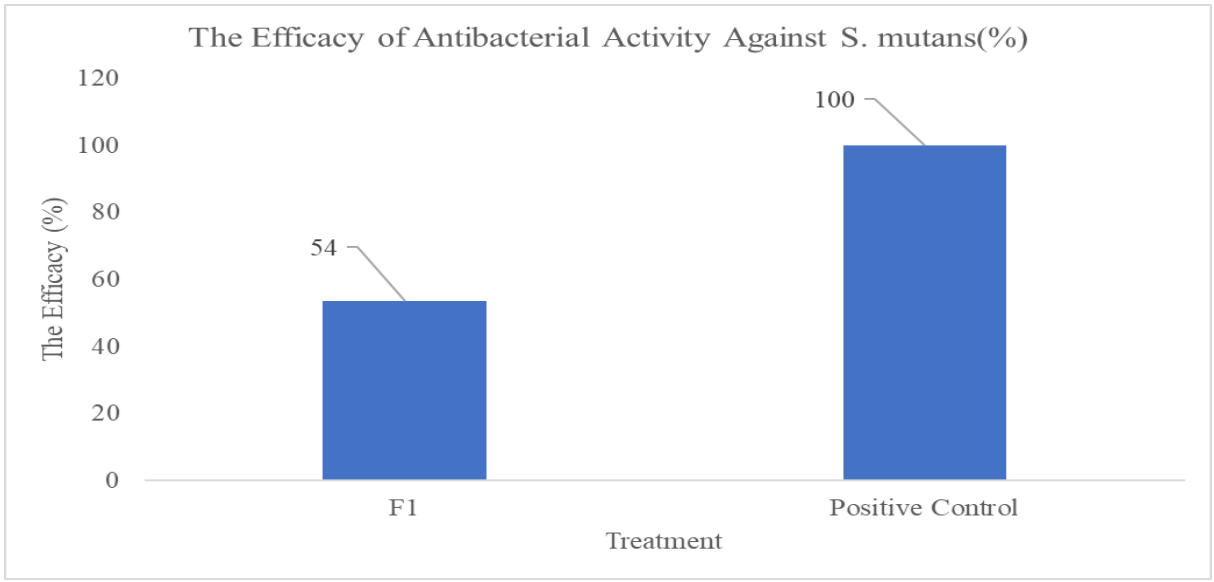

Figure 3. The efficacy of antibacterial activity against *S. mutans* of the test formula (F1) and positive control

The antibacterial test in this study used the good diffusion method. The good diffusion method was chosen because the samples tested in this study were semi-solid. The good diffusion method was chosen so that the active compounds in the toothpaste can diffuse more easily into the growth medium of *S. mutans* bacteria. The antibacterial activity of the toothpaste test preparation made from F1 cemcem leaf extract against *S. mutans* bacteria is expressed by

the diameter of the inhibition zone in mm. Based on the results, it is known that the F1 formula has antibacterial activity, as indicated by the formation of an inhibition zone diameter with a mean area of 7.72mm ± 0.439. The results of this study are in line with the research of Dharmawati et al., 2022, which states that *S.pinnata* leaf extract can inhibit the growth of *S. mutans* with a diameter of 12.95 mm at a concentration of 60% and increased to 15.77 mm at a concentration of 80%. However, in that study, antibacterial activity was carried out directly on the extract preparation, with a higher concentration so that the diameter of the inhibition zone was also wider.

In this study, the test was carried out on toothpaste preparations made from *S. pinnata* leaf extract so that the concentration of extracts used was smaller. In the development of natural antibacterial toothpaste, the extracts cannot be added in too large a concentration. The addition of extract concentrations to toothpaste preparations can cause changes in the physical properties and stability of toothpaste, such as increasing viscosity and changing pH values. The addition of the extract can affect the level of panellist preference, which is a crucial factor in consumer acceptance of the product. Therefore, finding the optimal concentration that maximizes antibacterial effects while maintaining desirable physical properties and consumer acceptance is very important in the development of natural antibacterial toothpaste. In general, the concentration of active ingredients used in the development of natural antibacterial toothpaste formulas generally ranges from 1-5% (Abidin et al., 2023; Afni et al., 2015; Nuraskin et al., 2021) .

The formation of the diameter of the inhibition zone by the F1 test preparation proves that the bioactive compounds contained in the test preparation can diffuse into the bacterial growth medium and act as antibacterial agents against *S. mutans*. Based on previous research, it is known that cemcem leaf extract contains bioactive compounds, including alkaloids, flavonoids, steroids, and tannins (Asnani et al., 2017; Bekti et al., 2022; Dharmawati et al., 2022). Each of these bioactive compounds has the ability as an antibacterial agent through its respective mechanism.

The bioactive compounds such as phenols, flavonoids, and tannins will form a complex on the bacterial cell wall that inhibits bacterial growth to cause bacterial cell death (Bekti et al., 2022; Dharmawati et al., 2022). Alkaloids have antibacterial abilities because they contain aromatic quaternary compounds and can form intercalates with DNA so that they can cause cells to undergo mutations or genetic damage (Nurhartanti & Masduqi, 2020). Flavonoid compounds act as antibacterial agents because they can damage bacterial cell walls by binding to proteins and lipids, resulting in cell lysis to death, disrupt cell metabolism by denaturing protease enzymes in cells, inhibit nucleic acid synthesis, inhibit cytoplasmic membrane function, inhibit attachment and biofilm formation, and inhibit the work of porins on cell membranes (Dharmawati et al., 2022; Widyastuti et al., 2019). Steroids play a role in antibacterial activity because they can interact with cell membrane phospholipids, causing membrane integrity to decrease, changing membrane morphology so that it can eventually cause cell membrane lysis. Tannins act as antibacterials through the mechanism of destroying bacterial permeability, inactivating enzymes and destroying the function of genetic material by forming protein complexes through hydrogen bonds and hydrophobic bonds, dissolving the lipid layer of the bacterial cell wall to cause cell fluid leakage, binding metal ions involved in the metabolic process of bacterial cells and affecting cell wall permeability to interfere with the absorption of essential elements needed for bacterial growth (Nurhartanti & Masduqi, 2020; Widyastuti et al., 2019).

S. mutans bacteria are gram-positive bacteria that are normal oral cavity flora. *S. mutans* bacteria can synthesize polysaccharides such as dextran from sucrose, a sticky polysaccharide that plays an important role in the formation of dental caries. Prevention and control of dental

caries can be done by brushing teeth with toothpaste that has antibacterial ability against *S. mutans*. The results of this study prove that the F1 test preparation, which is a toothpaste made from 5% cemcem leaf extract, has an antibacterial ability against *S. mutans* so that it can be developed as an alternative to the prevention, control, and treatment of dental caries caused by *S. mutans* bacteria.

While the development of toothpaste from *S. pinnata* leaf extract in this study has shown promising potential, it has several limitations. These include the need to optimize the concentration of *S. pinnata* leaf extract to maximize its antibacterial activity, without affecting its physical quality and organoleptic acceptance by consumers. Furthermore, it is crucial to test the physical stability, pH, and activity against product storage duration. The antibacterial activity is currently limited to *S. mutant* bacteria. Therefore, it is important to expand the testing to other strains of periodontopathogenic bacteria such as *Porphyromonas gingivalis*, *Treponema denyicola, Tannerella fosythia Aggregatibacter Actinomycetemcomitans.* This further testing is a critical step in the development of natural antibacterial toothpaste.

4. CONCLUSION

Based on the research results, it can be concluded that toothpaste with a concentration of *S. pinnata* leaf extract of 5% meets the physical and pH requirements and is most acceptable based on organoleptic parameters of color, odor, taste, and texture. This toothpaste formula demonstrates significant antibacterial activity against *S. mutan*s, with an average inhibition zone diameter of 7.72 mm ± 0.439. It effectively inhibits the growth of *S. mutans* bacteria by 54.33% compared to the positive control. This proves that the toothpaste containing *S. pinnata* leaf extract of 5% can be developed as a natural antibacterial toothpaste. Further research is suggested to optimize the concentration of *S. pinnata* leaf extract to maximize its antibacterial activity, without affecting its physical quality and organoleptic acceptance, to test its physical stability, pH and activity against storage duration and to assess its effectiveness against other gingivitis and periodontitis bacteria.

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