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Formulation And Antibacterial Effectiveness Test Leaf Magrove Ethanol Extract Toothpaste (*Rhizophora Mucronata)* Against The Bacteria *Streptococcus Mutans*

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Abstrak

Mangrove plants that grow abundantly along the coast are widely known for blocking waves and protecting coastal ecosystems. However, the use of mangrove plants is still limited and tends to focus on the function of physical protection against coastal abrasion so that parts of the mangrove plant such as masi leaves are rarely known about their benefits among the public. This research aims to formulate ethanol extract of mangrove leaves (*Rhizophora mucronata*) into a toothpaste preparation and test its antibacterial activity against the growth of *Streptococcus mutans bacteria* using the paper disc method. In this research, 4 toothpaste formulations were made with different concentrations, namely F0 (without extract), FI (10%), FII (15%) and FIII (20%). Then a physical stability test was carried out on the toothpaste preparation for 4 weeks including organoleptic tests, homogeneity, pH, spreadability, foam height, viscosity of each of the 4 formulas, showing that the results met the stability requirements of the toothpaste preparation. Furthermore, testing the antibacterial activity of toothpaste preparations with concentrations of 10%, 15%, 20% and K-, K+ showed that the average diameter of the inhibition zone formed was 3mm, 3.38 mm, 5.75 mm and 1.66 mm. , 5.16mm. It can be concluded that the ethanol extract of mangrove leaves (*Rhizophora mucronata*) can be formulated into a toothpaste preparation and can provide maximum antibacterial effect, namely at a concentration of 20% at 5.75 mm.

Key words : Mangrove leaf ethanol extract, toothpaste, *Streptococcus mutans*

1. Introduction

Dental caries is caused by a buildup of dental plaque which contains a lot of bacteria. Dental plaque is a soft layer consisting of a collection of microorganisms or bacteria, saliva components and food residue on the surface of the teeth. This disturbance in the teeth will facilitate the process of breaking down the layers of the teeth caused by acid and excreted by oral bacteria. Caries or plaque on teeth is the result of the enzymatic activity of bacteria that multiply on the surface and defects of teeth due to the presence of nutritional substrates (which are not clean when cleaned), especially from food waste. Caries causes the surface of the tooth to which it is attached to thicken. This thickening of the tooth surface is followed by brittle tooth enamel. Continuous brittleness results in tooth loss (Pratama, 2014). *Streptococcus mutans* bacteria are the main causative bacteria in the oral cavity which cause the formation of dental plaque (Klai *et al.*, 2014) .

Streptococcus bacteria *mutans* is a microorganism that causes dental caries which plays a major role in the onset of dental caries. *Streptococcus mutans* is able to synthesize the extracellularpolysaccharide glucan, can produce lactic acid through a homofermentation process, forms colonies that adhere closely to the tooth surface, and is more acidogenic than

other *Streptococcus species* . Therefore, *Streptococcus mutans* has become the main target in efforts to prevent dental caries (Kuddus, 2019) .

According to (MOH RI, 1979) paste is a semisolid preparation containing one or more medicinal ingredients intended for topical use. Toothpaste is a semisolid product consisting of a mixture of abrasive agents, cleaning agents, and additives that is used with a toothbrush to clean inaccessible places. Pasta Teeth are a semi-solid material which is used together with a toothbrush to clean all teeth. Toothpaste used at the time Brushing teeth functions to reduce plaque formation, strengthen teeth against caries, clean and polish tooth surfaces, eliminate or reduce bad breath as well as maintain dental health and maintain dental aesthetics (Khairi *et al.* , 2019) .

The mangrove plant (*Rhizophora mucronata*) is a species of mangrove that has antibacterial, antiviral and antifungal properties. Antibacterials are substances that can inhibit or kill bacteria that cause infections. Infection is caused by pathogenic bacteria or microorganisms, where the microbes enter the body's tissues and multiply in the tissues (Ernawati & Hasmila, 2015) .

Based on research (Devi *et al* , 2022) reporting that mangrove plants have antibacterial potential, the results of the research show that mangrove leaf extract mouthwash preparations have *Streptococcus mutans bacterial activity* in F1 producing an inhibition zone of 3.83 mm, F2 of 4.48 mm. , and F3 of 4.71 mm. Controlling pathogenic bacteria uses mangrove plants as an antibacterial agent. Mangrove leaves (*Rhizophora mucronata*) contain secondary metabolite compounds in the form of alkaloids, tannins and flavonoids. According to (Egra *at al.*, 2019) antibacterial activity was found in the *Rhizophora mucronata plant* containing the active compounds triterpenoids, flavonoids, alkaloids and tannins.

Mangrove trees that grow abundantly along the coast of the city of Palopo are widely known for blocking waves and protecting coastal ecosystems. However, the use of mangrove trees is still limited and tends to focus on the function of physical protection against coastal erosion. Based on the above background, researchers are interested in making toothpaste formulations from mangrove leaf (*Rhizophora mucronata*) ethanol extract with concentrations of 10%, 15% and 20% with evaluation of the stability of the preparation including organoleptic tests, homogeneity tests, pH tests, spreadability tests, height tests. foam, viscosity test and antibacterial effectiveness test.

2. Methodology

1. Sampling
   Samples were taken directly from mangrove trees (*Rhizophora mucronata*) in the coastal area of Palopo City, South Sulawesi Province.
2. Making simplicity
   a. The mangrove leaves (*Rhizophora mucronata*) that have been taken are washed using running water until clean
   b. Mangrove leaves that have been washed are then drained

c. Then the mangrove leaves (*Rhizophora mucronata*) are cut into thin pieces or chopped
d. Then dry it in the sun covered with a black cloth until it becomes dry simplicia
e. The dried black mangrove leaves are ground using a blender
f. Then sifted using mesh sieve no. 40 (Maulidah *et al.* , 2022) .

3. Sample Extraction
   a. The extraction process is carried out by maceration
   b. Using 96% ethanol solvent, 500 grams of simplicia powder
   c. Soaked using 5000 ml of ethanol solvent. Soaking is carried out for 3x24 hours, in a maceration container with occasional stirring
   d. Filter the soaking results for the first 3 days using a funnel lined with filter paper (filtrate 1)
   e. Simplicia is again soaked for 2 days in 500 ml of 96% ethanol and then filtered (filtrate 2)
   f. The results of filtrate 1 and filtrate 2 are mixed
   g. The filtrate obtained was then evaporated using an evaporator at a temperature of 70 ^{o} C until a liquid extract was obtained
   h. The liquid extract is put into a porcelain cup for
   i. Next, it was evaporated using *a water bath* at a temperature of 60 $^{\circ}$C
   j. Until a thick extract is obtained (Paputungan & Yamlean, 2014) .
4. Making Eggshell Powder (Calcium Carbonate)
   a. Egg shells are cleaned and soaked in hot water
   b. Then dried in the oven at 105 ^{o}C for 30 minutes
   c. After that, the egg shells are crushed using a mortar and pestle until they become a fine powder.
5. Making Toothpaste
   a. Prepare tools and materials
   b. Put Na-CMC in hot (boiling) water and leave for 30 minutes, then grind evenly until it forms a gel base I
   c. Grind the egg shells then add sorbitol to form a gel mass (mass II)
   d. Mass II is mixed with mangrove leaf extract (*Rhizophora mucronata*), crushed until slightly moistened. Then add mass I and grind until homogeneous (mass III)
   e. Dissolve sodium saccharin in distilled water, add to mass III and grind until homogeneous (mass IV)
   f. Dissolve *methylparaben* and *propylparaben* in the remaining hot water and stir until evenly distributed, then add to (mass IV) then grind until evenly distributed
   g. Add sodium lauryl sulfate and stir gently until a uniform mass is obtained until the paste expands, put it in a toothpaste container (Marlina & Rosalini, 2017) .
6. Toothpaste Evaluation
   a. Organoleptic Testing

      Organoleptic testing is testing that is based on sensing. Organoleptic testing can be done by observing shape, color, smell and taste (Marlina & Rosalini, 2017) .

b. Homogeneity Test

The paste to be tested apply up to 100 mg of the paste to be tested on a glass object and observe its homogeneity. If there are no grains on the glass object. So toothpaste meets the requirements of the homogeneity test (Marlina, & Rosalini, 2017) .

c. Test pH

Testing is carried out using universal pH. The pH test of the preparation is measured using a universal pH stick by dipping it into the preparation. The standard pH of toothpaste is 4.5-10.5 (Marlina & Rosalini, 2017) .

d. Spreadability test

A total of 1 gram of the preparation is placed in the center of a round scale glass, then covered with another round glass. The diameter of the distribution of the preparation is measured longitudinally and transversely, and is carried out for each additional load of 50 grams up to a total weight of 150 grams. The spreading power that meets the requirements is 5-7 cm (Yusuf *et al.* , 2017)

e. Test foam height

A total of 1 gram of toothpaste preparation is added with distilled water and then put into a 100 ml measuring cup. Shake for 20 seconds by turning the measuring cup evenly and leave for 5 minutes. Then measure the height of the foam using a ruler (Marlina & Rosalini, 2017) .

f. Viscous test

The viscosity test is carried out by dipping the viscometer spindle in the preparation that has been placed in a glass beaker. The preparation viscometer is seen on the scale in the display tool. The number that shows the viscosity of the tool is high viscosity and then look at the *Stomer viscosity table* (Lachman, 1994).

7. Antibacterial Effectiveness Testing

a. Tool Sterilization

All tools and materials that will be used are washed until clean and continued with drying. The next step was sterilization using an autoclave for 20 minutes at a temperature of 121°C with a pressure of 2 atm, as well as the media used in the form of *Nutrient Agar* (NA) media (Mulyadi *et al* ., 2017) .

b. Creating Positive Control and Negative Control

The positive control will be made by making a positive control solution using herbal toothpaste on the market, while the negative control will use a distilled water solution.

c. Rejuvenation Bacteria

Each *Streptococcus mutans bacteria* was taken using a sterile tube, 1 sterile tube from the pure culture. Then inoculated by streaking on slanted *nutrient agar* (NA) medium, incubated in an incubator at 37 ^{o} C for 1x24 hours.

d. Preparation of Test Bacterial Suspensions

Put 10 ml of 0.9% NaCl solution into a test tube. Bacteria are collected with a sterile tube needle. Suspended in 10ml solution NaCl 0.9% sterile, after that homogenized (Afriani, 2017).

8. Making Test Media
   a. Prepare 20 gr of NA and 150 ml of distilled water.
   b. Then put it in an Erlernmeyer flask.
   c. NA (*Nutrient agar*) is heated on *a hot plate* , and inserted into a magnetic stirrer in an Erlenmeyer flask until it boils.
   d. Then sterilized using hot steam sterilization in an autoclave at 121°C for 20 minutes.
   e. Then pour it into a sterilized petri dish
   f. Wait until the media solidifies (Khoir unnisak *et al* ., 2020) **.**
9. Antibacterial Effectiveness Test
   a. A total of 0.1 ml of the test bacterial suspension was put into a petri dish containing sterile NA media.
   b. The disc paper used is 0.5 cm in diameter.
   c. Place a paper disc that has been smeared with toothpaste with a concentration of 10%, 15% and 20% on the surface of the NA media that has been planted with bacteria.
   d. Place a paper disc that has been smeared with herbal toothpaste on the market as a positive control and sterile distilled water as a negative control.
   e. Incubated for 24 hours at 37 ˚C.
   f. The diameter of antibacterial effectiveness was observed based on the inhibitory diameter which was indicated by the clear blood that formed around the paper disc and was measured using a ruler.
   g. Measuring results are recorded.

3. Results and discussion

3.1 Simplicia Standardization Results

1. Determination of Water Content

The results of determining the water content of mangrove leaf simplicia (*Rhizophora mucronata*) can be seen in table 4.1.

Table 4.1 Results of Water Content Determination

Parameter	Amount of Loss	Standard
Water content	9.1%	≤ 10%

Determination of water content aims to determine the percentage of water content that remains in the simplicia. It is important to know the maximum limit of water content in simplicia because if the amount of water contained is too high it will become a medium for the growth of bacteria and fungi which can damage the quality of simplicia (Ministry of Health of the Republic of Indonesia, 2000).

Based on the table above, the test results for determining the water content of mangrove leaf simplicia powder (*Rhizophora mucronata*) are 9.1%. In line with research (Desiani *et al.* , 2022), the water content of simplicia powder obtained was 5.79%. Based on the amount of water content obtained, it meets the good water content, namely <10%. If the water content is more than 10%, it becomes a good

medium for microbes, fungi and insects to grow and develop, which can damage the quality of simplicia (BPOM RI, 2019) .

2. Determination of Drying Losses

The results of determining the drying loss of mangrove leaf simplicia (*Rhizophora mucronata*) can be seen in table 4.2.

Table 4.2 Results of Determination of Drying Losses

Parameter	Amount of Loss	Standard
Drying Shrinkage Test	10%	≤ 10%

Drying loss is a non-specific parameter which aims to provide a maximum limit (range) of the amount of compounds lost in the drying process. Basically, drying loss is a substance after drying at a temperature of 105 ^{o} C so that the weight is constant then expressed in percent.

Based on the table above, the drying shrinkage obtained from the powder is 10%. These results show that it has met the drying shrinkage requirements in general, namely no more than 10% (Ministry of Health of the Republic of Indonesia, 2000).

3. Determination of Rendament

The results of determining the soaking of mangrove leaf ethanol extract (*Rhizophora mucronata*) can be seen in table 4.3.

Table 4.3 Results of Determination of Soakment

Plant	Powder (gr)	Condensed Extract (gr)	Soaking (%)
Mangrove Leaves	500	92.1	18.42

Yield is a comparison of the w eight of the extract obtained with the initial weight of the sample used. Yield expresses the effectiveness of certain solvents against the materials of an extraction system, but does not indicate the activity level of the extract (Alhaddad *et al* ., 2019) . Based on the table above, the results of determining the marinade obtained from the maceration results are 18.42%. Meanwhile, in the results of research (Kurnianingsih *et al* , 2021) the results of soaking the ethanol extract of mangrove leaves (*Rhizophora mucronata*) with a yield value of 7.3%. The factors that can influence low or high soaking values are temperature and length of maceration time. Because the higher the temperature can cause the movement of particles to the solvent to be faster, thus affecting the value of the mass transfer coefficient of a component and making it easier for the solvent to extract the active substance, and the longer the maceration, the longer the heating effect and the longer the contact between the solid and the solvent, which will increase the number of cells. which is broken and the active ingredients are dissolved (Chairunnisa *et al* , 2019).

3.2 Antibacterial test

1. Organoleptic Test

The results of organoleptic testing carried out on preparations by observing changes

in shape, color, odor and taste can be seen in table 4.4.

Table 4.4 Organoleptic Test Results

Parameter	Formulation	Observations (Week to)			
		I	II	III	IV
Form	F0	Half solid	Half solid	Half solid	Half solid
	F1	Half solid	Half solid	Half solid	Half solid
	F2	Half solid	Half solid	Half solid	Half solid
	F3	Half solid	Half solid	Half solid	Half solid
Color	F0	White bone	White bone	White bone	White bone
	F1	Chocolate	Chocolate	Chocolate	Chocolate
	F2	Light brown	Light brown	Light brown	Light brown
	F3	Dark brown	Dark brown	Click here	Click here
Bau	F0	This mint	This mint	This mint	This mint
	F1	This mint	This mint	This mint	This mint
	F2	This mint	This mint	This mint	This mint
	F3	This mint	This mint	This mint	This mint
Rasa	F0	Manis	Manis	Manis	Manis
	F1	Sweet	Sweet	Sweet	Sweet
	F2	Sweet	Sweet	Sweet	Sweet
	F3	Sweet	Sweet	Sweet	Sweet

Information :
F0 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*)
F1 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*) 10%
F2 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*) 15%
F3 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*) 20%

Physical testing of toothpaste containing ethanol extract of mangrove leaves (*Rizosphora mucronata*) was carried out to determine the stability and suitability of the toothpaste. Organoleptic testing is testing that is based on sensing. Organoleptic testing can be done by observing shape, color, smell and taste (Marlina & Rosalini, 2017) .

The results of organoleptic testing of mangrove leaf ethanol extract toothpaste were carried out on four formulas by observing the shape, color, smell and taste of each formulation. Organoleptic testing of all formulas was carried out based on shape, color, smell and taste, all formulas retained their shape and taste. In the organoleptic test based on the color of each preparation, it was stable from the first week to the fourth week of testing. Meanwhile, research *(* Afni *et al.*, 2015) showed that the higher the concentration of areca nut extract used, the color of the toothpaste produced was more

brown (dark) . The reason why each formula has a different color is influenced by the amount of mangrove leaf (*Rhizospora mucronata*) ethanol extract added and the addition of food coloring to each formula. In terms of organoleptics based on smell, each formula has a distinctive mint smell because each formula has *peppermint aroma added* . Based on research [18] Regarding toothpaste preparations that use miswak powder, it has a distinctive mint smell, because peppermint oil is added to this toothpaste . In terms of organoleptic taste, each formula has a sweet taste due to the addition of sorbitol to the toothpaste preparation. Based on organoleptic testing, each formula provides good stability because it maintains shape, color, smell and taste during 4 weeks of storage.

2. Homogeneity Test

The results of testing the homogeneity of toothpaste preparations with ethanol extract of mangrove leaves (*Rhizophora mucronata*) can be seen in table 4.5.

Table 4.5 Homogeneity Test Results

Formulas	Observations (Week to)				Standard
	I	II	III	IV	
F0	Homogeneous	Homogeneous	Homogeneous	Homogeneous	SNI No.12-3524-1995: homogeneous, no air bubbles, lumps and separate particles (Gratia *et al.*, 2021)
F1	Homogeneous	Homogeneous	Homogeneous	Homogeneous	
F2	Homogeneous	Homogeneous	Homogeneous	Homogeneous	
F3	Homogeneous	Homogeneous	Homogeneous	Homogeneous	

Information :
F0 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*)
F1 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*) 10%
F2 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*) 15%
F3 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*) 20%

Homogeneity testing is carried out to determine whether during the process of making toothpaste the active drug ingredients and other additional ingredients are mixed homogeneously. The results of the homogeneity test can be seen in the table above, homogeneous results were obtained for each formula. In line with research (Afni *et al.* , 2015), testing the homogeneity of each areca seed extract (*Areca catechu* L.) toothpaste, no coarse particles were seen, resulting in a homogeneous toothpaste preparation. Based on the homogeneity test results obtained, it can be concluded that it has fulfilled the requirements that each toothpaste must be homogeneous so that the resulting toothpaste is easy to use and evenly distributed on the surface of the teeth

(Masduqi & Anggoro, 2016) . The toothpaste that is made does not experience separation and is stable as indicated by the absence of coarse grains on the slide and does not experience separation between mangrove leaf extract (*Rizosphora mucronata*) and propolis with the paste or between the additional ingredients of the paste itself and does not experience separation between the solids. water for 4 weeks of storage at room temperature (Masduqi & Anggoro, 2016) .

3. **Test pH**

Testing of the acidity level of the ethanol extract toothpaste preparation of mangrove leaves (*Rhizophora mucronata*) was carried out using universal pH. The pH test results can be seen in table 4.6.

Table 4.6 Ph Test Results

Replication	Observations (Week to)				Standard
	I	II	III	IV	
F0	7	7	7	7	Ph 4.5- 10.5 (Marlina & Rosalini, 2017).
F1	7	6	7	7	
F2	7	7	7	6	
F3	7	7	7	6	

Information :
F0 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*)
F1 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*) 10%
F2 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*) 15%
F3 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*) 20%

Based on the table of pH measurement results above, the pH value obtained from toothpaste preparations with a pH of 6-7. The results of pH measurements showed that toothpaste preparations F1 in the second week and F2, F3 experienced changes in pH values in the fourth week, but the concentration of each toothpaste was in accordance with the pH of the oral mucosa so it was safe for use. In line with research (Aris *et al.* , 2022) mulberry leaf extract toothpaste preparation (*Morus alba L*) Based on the results of pH testing during 4 weeks of storage, the pH value obtained was pH 6.26-8.56. The pH value obtained from each formula showed that from the first week to the fourth week, the pH obtained was relatively stable. Based on the pH test results obtained, it can be concluded that it has met the requirements by meeting the pH standard for toothpaste, which is 4.5-10.5 (Marlina & Rosalini, 2017) . Meanwhile, the quality requirements for toothpaste preparations must be in accordance with the pH of the oral mucosa between 4.5–11.0 (Indonesian National Standards, 2016) . Changes in the pH value of each formula are caused by environmental factors such as changes in temperature because storage is carried out at room temperature and the storage container is less tight, allowing air to enter (Afni, N., & Said, N., 2015) .

4. **Spreadability Test**

The results of observations of the spreadability test of toothpaste preparations with ethanol extract of mangrove leaves (*Rhizophora mucronata*) can be seen in table 4.7.

Table 4.7 Spread Power Results

Replication	Observations (Week to)				Standard
	I	II	III	IV	
F0	5.4 cm	5.2 cm	6.4 cm	5 cm	
F1	5 cm	5.3 cm	5.7 cm	5.3 cm	5-7 cm
F2	5.4 cm	5.3 cm	5.5 cm	5.4 cm	(Yusuf *et al.* , 2017)
F3	5.7 cm	5.4 cm	5.4 cm	5.3 cm	.

Information :
F0 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*)
F1 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*) 10%
F2 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*) 15%
F3 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*) 20%

The purpose of the spreadability test is to find out how much the paste spreads when used. Spreadability is an important characteristic in a formulation because it influences the transfer of the active ingredient to the target area in the right dose, ease of use, the pressure required to get it out of the package and acceptance by consumers (Yusuf *et al.* , 2017) .

Based on the table of dispersion power measurement results above, the dispersion power has a value of 5 cm - 6.4 cm. In line with research (Mahdalin *et al.*, 2017) regarding gambier toothpaste, the spreadability was obtained with the smallest value of 7.8. In the measurement the spread power varies at the four formulas but the spreadability still meets the requirements, namely 5-7 cm (Yusuf *et al.* , 2017) . The difference in spreadability produced by a toothpaste can be influenced by the addition of water in the preparation, the more water, the wider the spreadability (Mahdalin *et al.* , 2017) .

5. Foam Height Test

The results of observations of the foam height test for toothpaste preparations with ethanol extract of mangrove leaves (*Rhizophora mucronata*) can be seen in table 4.8.

Table Foam Height Results

Replication	Observations (Week to)				Standard
	I	II	III	IV	
F0	4.5cm	4.5 cm	4 cm	4.5 cm	The foam height test
F1	5 cm	5.5 cm	5.5 cm	5.5 cm	results depend on
F2	3.2 cm	3.1 cm	3 cm	3 cm	the level of
F3	3.5 cm	3.7 cm	3.5 cm	3.5 cm	consumer preference
					(Daud *et al.* , 2016) .

Information :
F0 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*)
F1 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*) 10%
F2 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*) 15%
F3 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*) 20%

A toothpaste preparation is said to be good if foam forms. The foam formation test aims to see the amount of foam produced by toothpaste to remove dirt and clean the mouth when brushing your teeth .

Based on the foam height test table, the foam height above ranges from 3-5.5 cm. In line with research (Nadza *et al* ., 2016) the high foam test results for toothpaste preparations from Arabic bidara leaf extract (*Ziziphus spina-christi* L.) made has a spreadability value of 5-5.5 cm. The foam height test results for each formula vary every week This is due to manual shaking carried out by the researcher so that the height of the foam produced is unstable. There are no specified requirements for the foam height in toothpaste preparations. Based on the test results, the foam height depends on the level of consumer preference (Daud *et al.* , 2016) . There is a decrease in the foam height parameter because the foam height parameter is very dependent on the surfactant used, water hardness, room temperature at the time of measurement and standing time (Ministry of Health of the Republic of Indonesia, 1995) .

6. Viscosity Test

The results of observations of the viscosity test of toothpaste preparations with ethanol extract of mangrove leaves (*Rhizophora mucronata*) can be seen in table 4.9.

Table 4.9 Viscosity Test Results

Formulation	Observations (Week To)				Standard
	I	II	III	IV	
F0	1200	2268	3144	3732	
F1	2232	2340	2208	3240	2000-50000 cP
F2	3456	3552	3516	3720	(SNI 1995).
F3	4392	4320	4452	4608	

Information :

F0 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*)
F1 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*) 10%
F2 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*) 15%
F3 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*) 20%

The purpose of viscosity testing is to determine the viscosity of the formulation. The higher the viscosity, the thicker the formulation and vice versa. The thick formulation makes it difficult to brush your teeth. When making toothpaste, viscosity needs to be considered because toothpaste is a semi-solid preparation which has a high concentration of solid substances. If the toothpaste has a low viscosity, the toothpaste will be very soft, causing the toothpaste to sink into the bristles of the toothbrush and drip from the toothbrush. However, if the toothpaste has a viscosity that is too high, it will be difficult for the toothpaste to come out of the tube and will not be able to disperse well in the mouth (Rowe *et al.*, 2012). Based on the viscosity test table above, the test for each formula has met the viscosity standard, for the F0 preparation in the first week of testing it did not meet the standard because the preparation was a little runny, this was caused by the absence of added extract. The resulting viscosity values in the formula provide different viscosities from 1200- 4608 cP. In line with research

(Aris *et al.* , 2022) regarding the results obtained from the viscosity test of mulberry leaf extract toothpaste (*Morus alba* L) has a value between 10745-28875 cP. These results are in accordance with (Indonesian National Standards, 1995) The viscosity value of toothpaste ranges from 2000-50000 cP.

7. Antibacterial test

Results of antibacterial testing of toothpaste preparations from ethanol extract of mangrove leaves (*Rhizophora mucronata*) against *Streptooccus bacteria mutans* can be seen in table 4.10.

Table 4.10 Antibacterial Test Results

Bacteria	Replication	Resistance Diameter (mm)			Average (mm)	Standard
		I	II	III		
S. mutans	F0	1,2	1.7	2	1.66	Inhibitory zone diameter 5 mm: categorized as weak, 5-10 mm: moderate, 10-20 mm: strong, 20 mm very strong. According to Davis and Stout (1971) *in* [31]
	F1 10%	2	3.75	3.25	3	
	F2 15%	2.5	4	5	3.83	
	F3 20%	5.25	5.75	6.25	5.75	
	KP	4	5.25	6.25	5.16	

Information :
F0 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*)
F1 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*) 10%
F2 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*) 15%
F3 = Formulation without ethanol extract of mangrove leaves (*Rhizophora mucronata*) 20%
KP = Positive Control

The antibacterial effectiveness test was carried out to determine the antibacterial effectiveness of the ethanol extract toothpaste preparation of mangrove leaves (*Rizosphora mucronata*) against the growth of *Streptococcus mutans bacteria* . Based on the table of antibacterial effectiveness test results for toothpaste preparations with ethanol extract of mangrove leaves (*Rhizophora mucronata*) above, it can be seen that each concentration can form an inhibitory zone for the growth of *mutant Streptococcus bacteria* . The 10% concentration extract with an average diameter of 3 mm had a weak inhibitory response, the 15% concentration extract with an average of 3.83 mm diameter has a weak inhibitory response, and a concentration of 20% with an average of 5.75 mm has a strong inhibitory response. From the three concentrations above, it can be seen that the average inhibition zone formed is categorized according to Davis and Stout (1971) . (Harita, 2019) The criteria for antibacterial strength are as follows: the diameter of the inhibition zone is 5 mm or less, then the inhibitory activity is categorized as weak, the diameter of the inhibition zone is 5-10 mm, then it is categorized as moderate. The positive control used in this study was toothpaste (brand x) on the market with antibacterial claims containing the active substance fluoride. Fluoride is a substance commonly used in toothpaste products. Fluoride works by converting hydroxyapatite in enamel into fluorapatite. Fluoride makes enamel resistant to acid dissolution thereby

inhibiting the demineralization process and promoting remineralization which improves and stops carious lesions (Sabrina & Hartomo, 2020) . Meanwhile, the negative control used, namely F0 (without extract), can inhibit the growth of bacteria in the weak category. The inhibition zone formed in F0 (without extract) can be caused by the presence of ingredients in F0 which have antibacterial activity. This ingredient, namely a combination of methyl paraben and propyl paraben, can increase the preservative effect, methyl paraben is effective for fungi and propyl paraben is effective for bacteria. Propyl paraben (0.02% w/v) along with methyl paraben (0.18% w/v) has been used to preservation of various parenteral pharmaceutical formulations (Rowe *et al* ., 2009) . The Food and Drug Monitoring Agency (BPOM) issued BPOM Regulation Number 23 of 2019 concerning Technical Requirements for Cosmetic Ingredients, where it is explained that the use of methylparaben and propylparaben as preservatives in cosmetic preparations is limited to 0.4% w/v for single use and 0.8% b/v for mixed/combination use (BPOM 2019). The phytochemical compounds contained in the *Rhizophora mucronata plant* can be used as antibacterial agents . Mangrove plants (*Rhizophora mucronata*) have secondary metabolite compounds in the form of alkaloids, tannins and flavonoids (Kasitowati *et al.* , 2017) . According to (Egra *et al.* , 2019) antibacterial activity was found in the *Rhizophora mucronata plant* containing the active compounds triterpenoids, flavonoids, alkaloids and tannins .The mechanism of action of terpenoids is by disrupting the process of membrane or cell wall formation, the membrane or cell wall is not formed or is formed imperfectly (Ristiansyah, 2018) . Flavonoids work as antibacterials with several mechanisms of action, including inhibiting nucleic acid synthesis, inhibiting cytoplasmic membrane function and inhibiting energy metabolism in bacteria (Delyana *et al.* , 2014) . Compound alkaloids own mechanism inhibition with method destroy component peptidoglycan on cell bacteria, so that wall cell No formed intact And cause bacteria become damaged (Endarini, 2016) . Tannins are polymers of phenolic compounds which have the ability to activate attachment to bacterial cells, deactivate enzymes and prevent the transport of cell covering proteins. Apart from that, tannins also attack cell wall polypeptides, which means that cell wall formation is imperfect. This causes bacterial cells to rupture due to osmotic pressure and physical stress, resulting in bacterial cell death . Data from research on the antibacterial activity of several extract concentrations were continued with analysis of the one-way test data for ethanol extract of mangrove leaves (*Rhizophora* mucronata) *One Way Anova* . The one-way test (*One Way Anova*) is a type of parametric statistical test which aims to find out whether there is a difference in averages between samples.

4. Conclusion

a. Mangrove leaves (*Rhizophora mucronata*) can be formulated into toothpaste preparations that meet physical stability test standards including organoleptic tests that do not change shape, color, smell and taste. The homogeneity test of each toothpaste formula obtained homogeneous results. The pH test of each formula meets the pH standards of toothpaste and oral mucosa. Testing the spreadability of each formula obtained results that were in accordance with the standards for the spreadability of

toothpaste. Foam height test for each formula meets the criteria. The viscosity test for each formula obtained results that were in accordance with the toothpaste viscosity standards.

b. The ethanol extract of mangrove leaves (*Rhizophora mucronata*) which is formulated into toothpaste preparations with different concentrations can inhibit the growth of *Streptococcus mutans bacteria* with antibacterial effectiveness test results resulting from concentrations of 10%, 15% and 20% with weak to moderate inhibition zone categories.
c. Test the antibacterial effectiveness of toothpaste preparations from ethanol extract of mangrove leaves (*Rhizophora mucronata*) using three different concentrations, 10% concentration with an average value of 3 mm, 15% concentration with an average value of 3.83 mm, and 20% concentration with an average value of 5.75 mm, so It can be concluded that the concentration with maximum antibacterial effectiveness of ethanol extract of mangrove leaves (*Rhizophora mucronata*) is at a concentration of 20%.

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