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FORMULATION AND TEST OF ANTIBACTERIAL ACTIVITY PREPARATION PAPER SOAP STARFRUIT LEAVES EXTRACT (*Averrhoa bilimbi* L.) AGAINST BACTERIA *Staphylococcus epidermidis*

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Abstract: Along with the progress of the times, innovations in the field of soap making continue to develop. One form of innovation that is now popular in the community is paper soap. On the other hand, starfruit plants (*Averrhoa bilimbi* L.) is known to have potential as an antibacterial agent. This study aims to evaluate the concentration of paper soap preparations made from starfruit leaves extract (*Averrhoa bilimbi* L.) which are most effective in inhibiting the growth of bacteria. This study uses an experimental method. The leaves are then extracted by the maceration method using 96% ethanol. The extract is further defractionated into n-hexane, ethyl acetate, and water fractions. Each extract and fraction is formulated into a paper soap preparation with concentrations of 7.5%, 15%, and 22.5%. For comparison, positive control in the form of *Travelling Paper Soap* and negative control in the form of soap-based. The antibacterial activity test used the disc diffusion method against *Staphylococcus epidermidis* bacteria. The results showed that paper soap preparations with a concentration of 22.5% ethyl acetate fraction produced the largest inhibition zone, which was 22.80 mm and the result is close to the positive control inhibition zone of 23.45 mm.

1. INTRODUCTION

Based on the Regulation of the Minister of Health of the Republic of Indonesia Number 17 of 2024, the term pharmaceutical preparations covers various products, ranging from drugs, drug ingredients, natural medicines, cosmetics, health supplements, to quasi-drugs. According to the definition from the Food and Drug Monitoring Agency (BPOM) RI, soap is included in the cosmetic category with the main function of cleaning. Antiseptic soap, this function is extended to not only remove dirt, but also eliminates bacteria. Antiseptic soap products are available in various forms, including solid, liquid, and transparent. The latest innovation is paper soap, a thin sheet of soap that is lightweight, practical, easy to carry, and designed for single-use, ensuring cleanliness and quality (Ayu Adri et al., 2023).

Washing hands with soap is one of the simplest but most effective preventive measures in preventing disease transmission. The combination of soap and water is able to remove dirt and dust from the surface

of the skin, while reducing the number of pathogenic microorganisms such as bacteria, viruses, and parasites that stick to it (Ayu Adri et al., 2023).

With increasing public awareness of the importance of hand hygiene, there is a need for hand soap products that are compact, efficient, and easy to carry. This development encourages the presence of various innovations in the form of antiseptic soap preparations. Today, common forms on the market include solid, liquid, and transparent soaps (Awaluddin, 2022).

The domestic soap industry showed significant growth. As technology advances and consumer demands, innovations in soap manufacturing are becoming more diverse. One popular innovation is paper soap, which is trending thanks to its advantages—practical, lightweight, hygienic in storage, easy to carry, and more environmentally friendly than conventional forms (Awaluddin, 2022).

One of the natural ingredients that has the potential to be used in making antibacterial soap is starfruit (*Averrhoa bilimbi* L.). This plant has long been known to inhibit the growth of bacteria and has traditionally been used by Indonesian people to relieve pain, lower blood sugar levels, and as a natural source of vitamin C through the juice of the fruit (Chaniago et al., 2024).

Flavonoids, polyphenol compounds found in many plants, have been shown to have antibacterial activity through mechanisms such as inhibition of nucleic acid synthesis, disruption of cytoplasmic membranes, and suppression of bacterial cell energy metabolism. This compound also reduces the ability of bacteria to adhere (adhesion) and form biofilms, as well as affect porine structure, membrane permeability, and pathogenicity. In starfruit leaves, the types of flavonoids identified include luteolin and apigenin (Rante et al., 2024).

According to the Indonesian Herbal Pharmacopoeia, the total flavonoid content in starfruit leaves must be at least 2.12%, calculated as equivalent to routine. Flavonoids work through three main pathways: inhibit the formation of Reactive Oxygen Species (ROS), neutralize already formed ROS, and provide cell protection through their antioxidant properties (Ayu et al., 2024).

Staphylococcus epidermidis is a Gram-positive bacterium that can trigger various infections, including abscesses, acne, skin infections, urinary tract, and kidney disorders. These bacteria are generally sensitive to antibiotics such as erythromycin, clindamycin, and tetracycline. In topical therapy, the use of soap is one of the practical and effective forms, in addition to other forms such as ointments and masks (Rahasasti et al., 2024).

2. RESEARCH METHODS

2.1 Tools and Ingredients

2.1.1 Tools

The tools used in this study include a measuring flask, erlenmeyer 100 ml, erlenmeyer 250 ml, beaker cup 250 ml, measuring cup 500 ml, measuring cup 250 ml, analytical scale, funnel, split funnel, rotary evaporator, dropper pipette, petri dish, aluminum foil, filter paper, stirring rod, horn spoon, parchment paper, ruler, spoon, pH meter, napkin, test tube and its shelves, water baths, porcelain cups, glass bottles, jars, wooden tongs, gloves, bunsen, calipers, chandeliers, blenders, hotplates, incubators, round

ose needles, watch glass, and ovens.

2.1.2 Ingredients

The ingredients used in this study include star fruit leaves (*Averrhoa bilimbi* L.), aquadest, n-hexane, ethyl acetate, chloroform, aluminum foil, ethanol 96%, jasmine essential oil, phenolphthalein indicator, HPMC, cotton, paper disk, water-soluble paper, filter paper, KOH 30%, VCO oil, NA (Nutrient Agar) media, NaCl 9%, glycerin, magnesium (Mg), sodium lauryl sulfate, and glycerin.

3. RESEARCH PROCEDURE

3.1 Sample Collection

The sample in this study is in the form of starfruit leaves (*Averrhoa bilimbi* L.) obtained from Jalan Manaf Lubis, Marbau District, North Labuhanbatu Regency. Sampling was carried out purposively, without comparing with similar samples from other locations.

3.2 Making of Simplicia

The leaves of starfruit (*Averrhoa bilimbi* L.) are cleaned of dirt, washed with running water, then drained and weighed by wet weight. The sample is then dried using an incandescent lamp until it is completely dry. Once dry, the leaves are mashed using a blender until a suitable simplicia powder is obtained, then stored in a tightly sealed container in a room protected from sun exposure.

3.3 Simplicia Characterization Checks

3.3.1 Determination of The Moisture Content

Moisture content is a parameter that indicates the amount of water contained in a material and can be determined by the right methods, such as titration, distillation, or gravimetry. This measurement aims to establish the minimum limit or range of permissible moisture content. The maximum limit set is closely related to the purity level of the material as well as the potential contamination that can occur due to excess moisture (Depkes RI, 2000).

3.3.2 Determination of water-soluble pollen levels

A total of 5 grams of samples were extracted using the maceration method for 24 hours in 100 ml of water-chloroform solvent (at a ratio of 2.5 ml of chloroform per 1 liter of distilled water) in a closed flask. In the first 6 hours, the mixture is periodically shaken, then left to rest for 18 hours without stirring. After that, the mixture is filtered, then the first 20 ml of filtrate is evaporated to dry in a flat-bottomed evaporator cup that has been marked. The resulting residue is heated at 105°C until it reaches a constant weight. The percentage of the extract that is soluble in water is calculated based on the dry weight of the starting ingredient (Depkes RI, 1995).

3.3.3 Detemination of Soluble Essence Levels in Ethanol

A total of 5 grams of dry samples were macerated in 100 ml of 96% ethanol for 24 hours using a stuffed flask, with occasional shaking for the first 6 hours, then left for 18 hours. The filtrate is immediately filtered to prevent ethanol evaporation, then as much as 20 ml of filtrate is evaporated to dry in a flat-bottomed evaporative cup that has been heated and marked. The resulting residue is heated at 105°C until it reaches a constant weight. The percentage of soluble ethanol juice 96% is calculated based on the weight of the initial dry sample (Depkes RI, 1995).

3.3.4 Determination of Total Ash Content

A total of 2 grams of carefully rubbed and weighed samples are placed in a porcelain cross that has been pointed and marked with its syllable, then flattened. The swelling is carried out gradually until all the charcoal is gone, then continued at 600 °C for 3 hours. After that, the cross is cooled and weighed until it reaches a fixed weight. The determination of ash content is carried out based on the initial weight of the dry material (Depkes RI, 1995).

3.3.5 Determination of Acid Insoluble Ash Levels

The ash resulting from the determination of ash content is boiled in 25 ml of diluted HCl for 5 minutes. The acid-insoluble part is then filtered using ash-free filter paper, washed with hot water, rinsed, and then cooled and weighed until it reaches a constant weight. The acid insoluble ash content is calculated based on the weight of the initial dry material (Depkes RI, 1995).

3.4 Making of Starfruit Leaves Extract (*Averrhoa bilimbi* L.)

Starfruit Leaves Extract (*Averrhoa bilimbi* L.) is carried out by the maceration method. A total of 1 kg of starfruit leaves simplicia powder (*Averrhoa bilimbi* L.) was extracted using 96% ethanol solvent in a ratio of 1:10. The simplicia powder is put in a dark glass container, then macerated with 75 parts of solvent (7.5 liters) of 96% ethanol and left for 5 days protected from light while frequent stirring. Then filtration is carried out so that filtrate and residue are obtained. Then the residue obtained earlier was soaked again using 25 parts of solvent (2.5 liters) of 96% ethanol. The maserat is housed in a dark bottle, left in a cool place and protected from sunlight for 2 days then filtered. Then the extract is concentrated using a rotary evaporator until a thick extract is obtained.

3.5 Preparation of Fractionation of N Hexane (Non-Polar), Ethyl Acetate (Semi-Polar) and Water (Polar) Extract of Starfruit Leaves (*Averrhoa bilimbi* L.)

3.5.1 N-Hexane Fractionation (Non Polar)

A total of 10 g of thick extract of starfruit leaves (*Averrhoa bilimbi* L.) is dissolved in 10 ml of 70% ethanol, then put in a separate funnel. Next, add 100 ml of n-hexane, shake, and let stand until the two solvents separate. This process is repeated three times. The n-hexane fraction obtained is then vaporized using a rotary evaporator until it is concentrated (Sholichatun et al., 2023).

3.5.2 Ethyl Acetate Fractionation (Semi Polar)

A total of 10 g of samples are weighed and dissolved in 10 ml of 70% ethanol, then put into a separation funnel. Next, 100 ml of ethyl acetate is added, shaken, and then let stand until the two solvents separate. This process is repeated three times. The ethyl acetate fraction obtained is then evaporated until it is concentrated using a rotary evaporator (Sholichatun et al., 2023).

3.5.3 Water Fractionation (Polar)

A total of 10 g of samples are weighed and dissolved in 10 ml of 70% ethanol, then put into a separation funnel. After that, add 100 ml of water solvent, shake, and let sit until the two solvents separate. This process is repeated three times. The water fraction obtained is then evaporated until it is concentrated using a rotary evaporator (Sholichatun et al., 2023).

3.6 Making Reagents

3.6.1 Mayer Reagents

A total of 1.36 g of HCl was dissolved in 60 ml of aquadest. Paxda other parts of KI are dissolved in 10 ml of aquadest. Then mix the two lalxu solution dilute with aquadest until 100 ml (Dwi Aristyawan et al., 2024).

3.6.2 Dragendorff Reagents

A total of 8 g of KI is dissolved in 20 ml of aquadest. In the other part 0.85 g of bismuth sub nitrate is dissolved in 10 ml of glacial acetic acid and 40 ml of aquadest. Then the two solutions are mixed. In its use, this solution is diluted with 2/3 part of a solution of 20 ml of glacial acetic acid in 100 ml of aquadest (Dwi Aristyawan et al., 2024).

3.6.3 Lieberman-Burchard Reagents

A total of 2 ml of concentrated H₂SO₄ and 2 ml of anhydrous acetic acid. The two are mixed then the solution is stored in a dark bottle (Dwi Aristyawan et al., 2024).

Iron (III) Chlorixda (FeCl₃) Reagent Solution Reagent 1%.

A total of 1 g of iron(III) chlorixda (FeCl₃) is dissolved in distilled water up to 100 mL and then filtered (Dwi Aristyawan et al., 2024).

3.6.4 HCl 2N Reagent Solution

A total of 17 mL of concentrated HCl diluted with aquadest up to 100 ml (Dwi Aristyawan et al., 2024).

3.7 Phytochemical Screening

3.7.1 Alkaloid

A total of 0.5 grams of sample was weighed, then 1 mL of HCl 2N x and 9 mL of distilled water were added, heated over a water bath for 2 minutes, cooled and then filtered, the filtrate was used for the following experiments: 1. Take 3 drops of filtrate, then add 2 drops of Mayer reagent to produce

white/yellow deposits. 2. Take 3 drops of filtrate, and add 2 drops to produce a brown-black deposit. 3. Take 3 drops of filtrate, then add 2 drops of dragendroff reagent to produce a brick-red precipitate (Lailatul Qodri, 2023).

3.7.2 Flavonoid

A total of 0.3 g of extract is weighed, then 3 mL of n-hexane is added until the solution becomes colorless. The mixture is filtered, and the residue is dissolved with 3 mL of 96% ethanol, then divided into three test tubes. The first tube is used as a blank. In the second tube, 0.5 mL of concentrated and heated HCl is added; A positive result is indicated by a change in color to bright red, which indicates the presence of leucoanthocyanin compounds. In the third tube, 0.5 mL of concentrated HCl and 0.1 g of magnesium are added; The change in color to orange-red indicates the presence of flavone compounds, pale red indicates flavonols, while dark red indicates flavanones (Lailatul Qodri, 2023).

3.7.3 Saponin

The extract was weighed as much as 0.3 grams and then added 10 mL of hot aquadest, the sample was shaken for 2-3 minutes the foam formed approximately 1-10 cm high and stable in 10 minutes showed the presence of saponin compounds (Lailatul Qodri, 2023).

3.7.4 Tannin

The extract was weighed as much as 0.5 grams then added 2 mL of 70% ethanol, then added 3 drops of FeCl₃. Positive results are shown by the presence of a change in blue-green color and the presence of deposits (Lailatul Qodri, 2023).

3.7.5 Terpenoid

The terpenoid test can be done by taking an extract of 0.5 grams added 1 ml of glacial acetic acid then adding 3 drops of concentrated H₂SO₄. Positive results are characterized by a change in color to red or brown (Lailatul Qodri, 2023).

3.8 Basic Formulation of Paper Soap Preparation

Virgin Coconut Oil	10 %
KOH 30%	2,57 %
HPMC	1,5 %
Gliserin	2,4 %
SLS	1 %
Essential Oil Jasmine	q.s
Water Souble Paper	1
Aquadest	ad 100

	Ethyl Acetate Extract and Water Extract Concentration 22.5%
K +	: Positive Control (<i>Travelling Paper Soap</i>)
K -	: Negative Control (Soap Base).

3.9 Procedure for Making Paper Soap Preparation

Paper soap is made by developing HPMC first using cold water. Heat VCO oil, dissolve SLS with aquades to taste, and dissolve KOH with 6 mL aquades. Put the KOH solution in the VCO oil that is being heated, then stir until a soap paste forms. After that, add SLS and glycerin, then put it in the HPMC while stirring until homogeneous. Finally, add jasmine essential oil to taste and aquades (Cahyaningrum et al., 2024).

Liquid bath soap extracted from starfruit leaves extract (*Averrhoa bilimbi* L.) is put into a prepared container, then applied using a brush evenly on water-soluble paper. Next, the paper is dried in the oven and cut to the desired size (Lailiyah & Rahayu, 2022).

3.10 Antibacterial Activity

3.10.1 Sterilization of Tools and Ingredients

The equipment to be used is first washed, dried, and then sterilized. Glassware such as measuring cups, beakers, measuring gourds, test tubes, and erlenmeyer were closed at the mouth using cotton plugs wrapped in sterile gauze, then wrapped in parchment paper. All of the equipment was sterilized using an autoclave at 121 °C at a pressure of 15 lbs for 15–20 minutes. The laminar air flow is cleaned of dust, then sprayed with 70% ethanol for sterilization. The ose needles are sterilized by the flambing method by passing them on the flame

3.10.2 Making of Nutrient Agar

NA media is made by weighing 2.8 g of the material, then dissolved in 100 mL of aquadest. The mixture is heated on a hot plate while stirring until homogeneous, then sterilized using an autoclave at 121°C for 15 minutes. Next, the media is aseptically poured into a petri dish and left at room temperature until it hardens (Ayu Adri et al., 2023).

Table 1: Formulation of Paper Soap Preparation

No .	Ingred- ients	Functions	Formulations (%)				
			K-	F1	F2	F3	K +
1	Travelling Paper Soap	antibacterial active substances	-	-	-	-	1
2	EESFL	antibacterial active substances	-	7,5	15	22,5	-
3	N-Hexane Extract of SFL	antibacterial active substances	-	7,5	15	22,5	-
4	Ethyl Acetate Extract of SFL	antibacterial active substances	-	7,5	15	22,5	-
5	Water Extract of SFL	antibacterial active substances	-	7,5	15	22,5	-
6	Virgin Coconut Oil	Oil phase on soap	10	10	10	10	-
7	KOH 30 %	Alkali	2,57	2,57	2,57	2,57	-
8	HPMC	Gelling Agent	1,5	1,5	1,5	1,5	-
9	Glyserin	Humectant	2,4	2,4	2,4	2,4	-
10	Sodium Lauryl Sulfate	Surfactant	1	1	1	1	-
11	Essential Oil Jasmine	Fragrance	q.s	q.s	q.s	q.s	-
12	Water Souble Paper	Carrier Media	1 pcs	1 pcs	1 pcs	1 pcs	-
13	Aqua dest	Solvent	ad 100	ad 100	ad 100	ad 100	-

(Cahyaningrum et al., 2024)

Remaks :

EESFL : Ethanol Extract of Starfruit Leaves (*Averrhoa bilimbi* L.)

SFL : Starfruit Leaves (*Averrhoa bilimbi* L.)

F1 : Ethanol Extract of Starfruit Leaves (*Averrhoa bilimbi* L.) , N-Hexane Extract, Ethyl Acetate Extract and Water Extract Concentration 7.5%

F2 : Ethanol Extract of Starfruit Leaves (*Averrhoa bilimbi* L.) , N-Hexane Extract, Ethyl Acetate Extract and Water Extract Concentration 15%

F3 : Ethanol Extract of Starfruit Leaves (*Averrhoa bilimbi* L.) , N-Hexane Extract,

3.10.3 Bacterial Rejuvenation

The test bacteria were grown on the Nutrient Agar (NA) medium by scraping the bacteria from pure cultures using an ose needle on an inclined agar surface. The bacteria that have been scratched on the media are then incubated at a temperature of 37°C for 24 hours.

3.10.4 Making of Bacterial Suspension

The test bacteria on the oblique media were taken with sterile oce wire and then suspended into a tube containing 2 ml of 0.9 % NaCl solution until the same turbidity standard as the Mc. Farland solution turbidity standard was obtained.

3.11 Evaluation Test of Paper Soap Preparations

3.11.1 Organoleptic Test

Evaluation of paper soap preparations is carried out by observing the shape, color, and aroma of the preparation (Wahid & Wahid Sulaiman, 2024).

3.11.2 pH Test

A sheet of paper soap is put into 9 mL of water, then a pH meter that has been rinsed with distilled water is dipped in the sample solution. After that, the pH is measured using a pH meter. The ideal pH range for paper soap is between 8 to 11 (Wahid & Wahid Sulaiman, 2024).

3.11.3 Foam Height Test

Weigh 1 sheet of soap sample then dissolve 10 mL of aquades. Whipped by flipping the test tube, the test tube is shaken and measured the height of the foam formed (Wahid & Wahid Sulaiman, 2024).

3.11.4 Weight Uniformity Test

Uniformity of weight was carried out by weighing 20 pieces of paper soap one by one, then calculating the weight one by one, and calculating the average weight and comparing each average (Wahid & Wahid Sulaiman, 2024).

3.11.5 Wash Time Test

The wash time test is carried out by taking a sheet

of paper soap, adding a little water, rubbing both palms until the foam comes out. While calculating how long it takes for hand soap to be able to wash hands (Wahid & Wahid Sulaiman, 2024).

3.12 Antibacterial Activity Test

The method used in this study is the disc diffusion method, which is the most commonly used method. The working principle is antibacterial from ethanol extract and the tested fraction is absorbed on disc paper, then attached to an agar medium that has been homogeneously mixed with bacteria. Next, the media is incubated until an inhibition zone is formed around the disc. This method has advantages, including being more practical, requiring no special equipment, and relatively low cost (Retnaningsih et al., 2023).

The research procedure was carried out using starfruit leaves extract (*Averrhoa bilimbi* L.) and fractions at concentrations of 7.5%, 15%, and 22.5%. A total of 0.1 ml of inoculum is placed in a sterile petri dish, then 20 ml of liquid NA media at 40–50 °C is added. The media is homogenized and allowed to solidify. Disc paper is dipped in each sample, then attached to the compacted NA media. The petri dish is then incubated for 24 hours. The inhibition zone formed around the disc paper was observed and measured using calipers (Wahid & Wahid Sulaiman, 2024).

4. RESULTS AND DISCUSSION

4.1 Sample Identification Results

The results of sample identification were carried out at the Medanese Herbarium (MEDA) of the University of North Sumatra, Medan, Department of Biology, Faculty of Mathematics and Natural Sciences, the sample obtained was starfruit leaves (*Averrhoa bilimbi* L.) with the family *Oxalidaceae*.

4.2 Results of Taking and Making Simplicia

Sampling of starfruit leaves (*Averrhoa bilimbi* L.) was obtained in the Marbau area, North Labuhanbatu Regency, with a sample weight of 5.4 kg which was taken directly from the tree, fresh starfruit leaves (*Averrhoa bilimbi* L.) were washed clean, then dried and obtained a dry

weight of 1.6 kg. The leaves of starfruit (*Averrhoa bilimbi* L.) are declared dry when they appear brownish-green. Then it is made into powder by blending it until the powder weight is 1 kg.

4.3 Results of Simplicia Characterization Checks

Table 2: Results of Simplicia Characterization Examination

No.	Characterization checks	Results	Requirements (FHI, 2022)	Conclusions
1	Determination of the moisture content	9,38%	<10%	Qualify
2	Determination of water-soluble pollen levels	8,89%	<10,5%	Qualify
3	Determination of Soluble Essence Levels in Ethanol	10,54%	>4,0%	Qualify
4	Determination of total ash content	0,49%	<6,0%	Qualify
5	Determination of acid insoluble ash levels	0,36%	< 0,7%	Qualify

The determination of the moisture content in the simplicia of starfruit leaves (*Averrhoa bilimbi* L.) was carried out to determine the amount of water contained in the simplicia. Results of determining the moisture content of starfruit leaves (*Averrhoa bilimbi* L.) shows a value of less than 10%, which is 9.38%. The yield of water-soluble juice obtained was 8.89%. The determination of the content of water-soluble juice aims to determine the amount of polar compounds that can be extracted in water.

The result of ethanol soluble juice obtained was 10.54%. The determination of ethanol soluble juice levels aims to determine the levels of compound juice in simplicia that can be soluble in ethanol. The total ash content obtained was 0.49%. The determination of the total ash content was carried out to provide an overview of the internal and external mineral content contained in the simplicia of starfruit leaves (*Averrhoa bilimbi* L.).

The result of acid insoluble ash was 0.37%. The determination of acid insoluble ash levels is carried out to determine the amount of minerals that are insoluble in acid. This value is no more than 0.7%, so it meets the requirements of Herbal Pharmacopoeia Edition II (Nur et al., 2025).

4.4 Simplicia Extraction Results

Table 3: Simplicia Extraction Results

No.	Powder	extraction	Yield	Requirement (FHI, 2022)	Conclusion
1	1000 gr	300 gr	30%	>13,1%	Qualify

In this study, starfruit leaves (*Averrhoa bilimbi* L.) were used extracted using a 96% ethanol refiner. The extraction process is carried out using the maceration method. The simplicia of starfruit leaves (*Averrhoa bilimbi* L.) used is 1000 gr. A total of 7.5 liters of 96% ethanol solvent were put into a glass vessel then macerated for 5 days and stirred occasionally. Then filtering is carried out and the residue obtained is remacerated for 2 days using 2.5 liters of 96% ethanol solvent. Furthermore, the filtrate obtained is evaporated with a rotary evaporator and then thickened so that a thick extract of 300 grams is obtained.

4.5 Phytochemical Screening Results

Table 4: Phytochemical Screening Results

No.	Secondary Metabolites	Reactants	Results	Conclusion
1	Flavonoid	0.5 mL concentrated HCL and 0.1 gram Magnesium	Dark Red	(+) Flavonoid
2	Alkaloid	Mayer reagent (Yellow/White)	Yellow	(+) Alkaloid
3		Bouchart reagent	Blackish Brown	
4		Dragendorff reagent	Brick Red Precipitate	
5	Saponin	Aquades and shake vigorously	Foam	(+) Saponin
6	Tanin	2 mL of 70% ethanol, then 3 drops of FeCl ₃ are added.	Blackish Green	(+) Tanin
7	Terpenoid	1 ml of glacial acetic acid then add 3 drops of concentrated H ₂ SO ₄	Brown	(+) Terpenoid

In the phytochemical screening test of starfruit leaves (*Averrhoa bilimbi* L.) was carried out to determine the content of secondary metabolites found in starfruit leaves extract (*Averrhoa bilimbi* L.). Examination has been carried out on starfruit leaves extract (*Averrhoa bilimbi* L.) containing secondary metabolite compounds of alkaloids, flavonoids, saponins, tannins and terpenoids that have been attached.

4.6 N-Hexane Fraction Yield Results

Table 5: N-Hexane Fraction Yield Results

No.	Repetitions	Extract (gr)	Fractions Results (gr)	Yield Results	Requirement	Conclusion
1	1	10,023	2,25	22,4%	>10%	Qualify
2	2	10,016	2,28	22,7%		
3	3	10,031	2,31	23,0%		

The N-Hexane fraction is repeated 3 times so that the results are marked with blackish-green color. This color indicates the presence of tannins, and terpenoid compounds.

4.7 Ethyl Acetate Fraction Yield Results

Table 6: Ethyl Acetate Fraction Yield Results

No.	Repetitions	Extract (gr)	Fractions Results (gr)	Yield Results	Requirement	Conclusion
1	1	10,021	2,18	21,7%	>10%	Qualify
2	2	10,036	2,29	22,8%		
3	3	10,071	2,35	23,3%		

Ethyl acetate fractionation is repeated 3 times so that results are marked by brownish-yellow color. This color indicates the presence of flavonoid compounds.

4.8 Water Fraction Yield Results

Table 7: Water Fraction Yield Results

No.	Repetitions	Extract (gr)	Fractions Results (gr)	Yield Results	Requirement	Conclusion
1	1	10,025	2,24	22,3%	>10%	Qualify
2	2	10,041	2,18	21,3%		
3	3	10,021	2,28	22,7%		

Water fractionation is repeated 3 times so that results are marked by greenish and pale yellow colors. This color indicates the presence of flavonoid and carotene compounds (plant color pigments). In the results of the fractionation, the yield obtained meets the requirements, which is >10%.

4.9 Evaluation Test Results of Paper Soap Preparations

4.9.1 Organoleptic Tests

Organoleptic tests are carried out by observing the smell, color and shape of the preparation. The results can be seen in the following table.

Table 8: Organoleptic Test Results

No.	Formulations	Concentration (Shape, Smell and Color)			
		K+ and K-	F1	F2	F3
1	K-	Thin as paper, Jasmine Essential Oil Aroma	-	-	-
2	EESFL	-	Thin as paper, Extract Aroma, Light Brown	Thin as paper, Aroma Extract, Brown	Thin as paper, Aroma Extract, Dark Brown
3	N-Hexane	-	Thin as paper, Extract Aroma, Greenish White	Thin as paper, Extract Aroma, Greenish White	Thin as paper, Aroma Extract, Light Green
4	Ethyl Acetate	-	Thin as paper, Extract Aroma, Light Brown	Thin as paper, Aroma Extract, Brown	Thin as paper, Extract Aroma, Dark Brown
5	Water	-	Thin as paper, Extract Aroma, Brownish White	Thin as paper, Extract Aroma, Slightly darker brown	Thin as paper, Aroma Extract, Brown
6	K+	Thin as paper, Unscented, White	-	-	-

Examination of organoleptic evaluation test on K- thin like paper, aroma essential oil jasmine, 7.5% thin extract like paper, aroma extract, light brown, 15% thin extract like paper, aroma extract, brown, and 22.5% thin extract like paper, aroma extract, dark brown. N-Hexane 7.5% paper-thin, aroma extract, light brown, 15% paper-thin n-hexane, aroma extract, greenish-white, and n-hexane 22.5% paper-scented, aroma extract, light green. In 7.5% ethyl acetate thin like paper, aroma extract, light brown, ethyl acetate 15% thin like paper, aroma extract, brown, and ethyl acetate 22.5% thin like paper, aroma extract, dark brown.

In the water fraction 7.5% thin like paper, aroma extract, brownish white, 15% thin water like paper, aroma extract, chocolate slightly darker and water 22.5% thin like paper, aroma extract, chocolate, as well as the last K+ thin like paper, unflavored, white.

4.9.2 pH Test Results

Table 9: pH Test Results

No.	Formulations	Concentration and pH				Requirements (4085:1996) 8-11
		K+ and	F1	F2	F3	

		K-				
1	K-	8,42	-	-	-	Qualify
2	EEDBW	-	8,04	9,75	9,05	
3	N-Heksan	-	8,79	8,93	8,92	
4	Etil Asetat	-	8,45	9,44	8,66	
5	Air	-	9,49	9,34	8,43	
6	K+	8,10	-	-	-	

The purpose of pH testing is to find out if the pH of the soap preparation is suitable for the pH of the skin ranging from 8-11 and to see the pH changes during 2 weeks of storage. The pH results of each formula still meet the pH requirements of soap which ranges from 8-11. However, there is a decrease in pH, this is due to the storage temperature that is not fixed or changes every day. A drop in pH can occur due to environmental conditions during poor storage (Wahid & Wahid Sulaiman, 2024).

4.9.3 Foam Height Test Results

The foam height test is performed by measuring the height of the foam in liquid soap resulting from shaking for 5 minutes. The foam height requirement based on SNI 1996 is the foam height requirement of 1.3-22 cm.

Table 10: Foam Height Test Results

No.	Formulations	Foam Concentration and Height Results				Requirements (SNI 4085:1996) 1,3 cm – 22 cm
		K- and K+	F1	F2	F3	
1	K-	1,6 cm	-	-	-	Qualify
2	EESFL	-	1,5 cm	1,5 cm	1,7 cm	
3	N-Hexane	-	1,5 cm	1,6 cm	1,6 cm	
4	Ethyl Acetate	-	1,8 cm	1,7 cm	1,7 cm	
5	Water	-	1,5 cm	1,7 cm	1,5 cm	
6	K+	2,0 cm	-	-	-	

The results of the foam height test meet the specified requirements. This stabilizer is affected by the surfactants, foams, and oils used. The resulting foam should not be too high because it will strongly cleanse the fat on the skin so that it will cause the skin to become dry (Cahyaningrum et al., 2024).

4.9.4 Weight Uniformity Test Results

Table 11: Weight Uniformity Test Results

No.	Formulations	Concentration and Weight Uniformity Results				Maximum Deviation Limit
		K+ and K-	F1	F2	F3	
						≤ 80 mg (0,080 g) =

1	K-	0,554	-	-	-	±10% 80 mg – 250 mg (0,080–0,250 g) = ±7,5% > 250 mg (> 0,250 g) = ±5%
2	EESFL	-	0,364	0,306	0,306	
3	N-Hexane	-	0,546	0,294	0,386	
4	Ethyl Acetate	-	0,426	0,428	0,374	
5	Water	-	0,418	0,436	0,444	
6	K+	0,106	-	-	-	

At the time of weighing there are several paper soaps that have different weights but with a small number spacing this can be caused by the uneven process of applying liquid soap and the process of cutting paper soap which is done manually and produces preparations that have different sizes, so that paper soap preparations have different or non-uniform weights (Awaluddin, 2022).

4.9.5 Washing Time Test Results

Table 12: Washing Time Test Results

No.	Formulations	Concentration and Wash Time Test Results (Seconds)			
		K+ and K-	F1	F2	F3
1	K-	10,17	-	-	-
2	EESFL	-	10,37	10,69	10,92
3	N-Hexane	-	11,09	11,27	11,52
4	Ethyl Acetate	-	10,49	10,75	11,40
5	Water	-	10,41	10,91	10,96
6	K+	10,19	-	-	-

In the test of the washing time of paper soap preparations of starfruit leaves (*Averrhoa bilimbi* L.) obtained results as in table 4.10 showing that the washing time in this test is longer compared to other soap preparations, this is because the paper soap preparation must be dissolved first when the paper preparation is washed using running water (Wahid & Wahid Sulaiman, 2024).

4.10 Antibacterial Activity Test Results of *Staphylococcus epidermidis* on Paper Soap Preparation

Based on the results obtained in Table 13, it is known that 22.5% ethyl acetate extract of starfruit leaves (*Averrhoa bilimbi* L.) has the largest inhibitory diameter between the concentration of extracts and fractions in *Staphylococcus epidermidis* bacteria which is 22.80 mm and close to the positive control of 23.45 mm.

It can be seen in this table of contents, with the following categories of bacterial inhibition zones:

Weak (<5 mm)
Medium (6-10 mm)
Strong (11-20 mm)
Very Strong (>21 mm).

The following are the results of the antibacterial activity test against *staphylococcus epidermidis* bacteria on paper soap extract preparations and leaves fractions of starfruit (*Averrhoa bilimbi* L.).

Table 13: Antibacterial Activity Test Results of *Staphylococcus epidermidis* on Paper Soap Preparations

No.	Formulations		Repetitions			Mean
			1	2	3	
1	K-	K-	0	0	0	0
2	F1	N-Hexane 7,5%	13,45	13,85	14,15	13,82 ± 0,35
3		Water 7,5%	15,00	15,20	15,75	15,32 ± 0,38
4		EESFL 7,5%	16,05	16,35	16,55	16,32 ± 0,25
5		Ethyl Acetate 7,5%	16,70	16,72	16,90	16,77 ± 0,11
6	F2	N-Hexane 15%	17,25	17,50	17,70	17,48 ± 0,22
7		Water 15%	18,05	18,40	18,75	18,40 ± 0,35
8		EESFL 15%	19,00	19,10	19,35	19,15 ± 0,18
9		Ethyl Acetate 15%	19,45	19,60	19,75	19,60 ± 0,15
10	F3	N-Hexane 22,5%	19,85	19,95	20,05	19,95 ± 0,1
11		Water 22,5%	20,15	20,30	20,60	20,35 ± 0,22
12		EESFL 22,5%	20,90	21,20	21,60	21,23 ± 0,35
13		Ethyl Acetate 22,5%	22,60	22,80	23,00	22,80 ± 0,2
14	K+	K+	23,10	23,30	23,95	23,45 ± 0,44

These results show that the secondary metabolite content in the ethyl acetate extract of starfruit leaves (*Averrhoa bilimbi* L.) can be extracted optimally due to the semi-polar solvent properties of ethyl acetate. This property allows it to dissolve polar and non-polar compounds, making it more effective at attracting flavonoids than fully polar water. Flavonoids themselves are polar but have non-polar groups, so they are compatible with ethyl acetate.

Based on the results of the inhibition zone measurement, the largest to smallest order was K+, ethyl acetate fraction 22.5%, ethanol extract 22.5%, water fraction 22.5%, n-hexane fraction 22.5%, ethyl acetate fraction 15%, ethanol extract 15%, water fraction 15%, n-hexane fraction 15%, ethyl acetate fraction 7.5%, ethanol extract 7.5%, water fraction

7.5%, n-hexane fraction 7.5%, and K-, with an average of 13.82–23.45 mm (Table 13).

Analysis using SPSS 22 showed that all data met the assumptions of normality (Shapiro-Wilk, Sig. > 0.05) and homogeneity (Levene, Sig. 0.177 > 0.05), so that ANOVA tests could be performed. The ANOVA results gave a Sig. < value of 0.05, indicating a significant difference between groups. A subset homogeneous test showed the ethyl acetate fraction of 22.5% had the highest antibacterial activity and did not differ significantly from the positive control.

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5. CONCLUSIONS AND SUGGESTIONS

5.1 Conclusions

1. Paper soap preparation of starfruit leaves extract (*Averrhoa bilimbi* L.) has activity as an antibacterial against *Staphylococcus epidermidis* bacteria.
2. Preparation of paper soap extract of starfruit leaves (*Averrhoa bilimbi* L.) meet the requirements for preparation evaluation test.
3. At a concentration of 22.5%, paper soap preparation, ethyl acetate, leaves extract, starfruit (*Averrhoa bilimbi* L.) was more effective than the extract, and other fractions were also close to positive control and had an inhibition zone of 22.80 mm.

5.2 Suggestions

1. It is recommended to the next researcher to investigate more deeply related to the antibacterial activity of other herbaceous parts of starfruit (*Averrhoa bilimbi* L.).
2. It is recommended for the researcher to then conduct a physical stability test of paper soap

preparation of starfruit leaves extract (*Averrhoa bilimbi* L.).

3. It is recommended for the next researcher to test the preparation of paper soap extract of starfruit leaves extract (*Averrhoa bilimbi* L.) using volunteers or hedonic tests.

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