Cleanse and Protect: Harnessing the Antibacterial Power of Guava Leaves in Liquid Soap Antiseptic Formulation

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Abstract. Patients with diabetes mellitus are at a higher risk of developing complications in the form of open wounds on the skin's surface. Guava leaves contain secondary metabolite substances that have been used in the treatment of wounds. This study aimed to test the antibacterial activity of guava leaves extract-based antiseptic liquid soap for the treatment of diabetic wounds. The study involved creating preparations for antiseptic liquid soap using different concentrations (0.5%, 3%, and 5%) of guava leaves extract, and conducting organoleptic, pH, and antibacterial activity tests on Escherichia coli and Staphylococcus aureus bacteria. Results showed that the guava leaves extract antiseptic liquid soap was physically stable and had antibacterial activity. The best antibacterial effectiveness was observed at a concentration of 0.5%. This study suggests that guava leaves extract-based antiseptic liquid soap could be a potential treatment option for diabetic wounds.

Keywords: wounds, diabetes, guava leaves, liquid soap, antibacterial.

1 Introduction

Infectious diseases, particularly in tropical areas like Indonesia, have a high mortality rate worldwide [1]. One issue that has long plagued the health sector in Indonesia is infectious diseases, which can occasionally worsen over time. These diseases are caused by numerous types of bacteria, such as Escherichia coli, Staphylococcus aureus, Salmonella thyphi, Pseudomonas aeruginosa, and Streptococcus mutans, and affect a large number of people [2].

Open wounds on the skin's surface that are associated with local tissue death are known as diabetic wounds, which are a type of chronic complication of diabetes mellitus [3]. Patients with diabetes mellitus have a 29-fold higher risk of developing diabetic wound complications. Diabetic wounds are chronic complications of diabetes mellitus characterized by open wounds on the skin's surface that result from macroangiopathy, neuropathy, and vascular insufficiency. Diabetes mellitus facilitates the entry of germs or bacteria into wounds and leads to infections, while high blood sugar levels provide an ideal environment for germ growth. [4].

Natural ingredients are being promoted as a potential solution for diabetic wound cleansing treatments due to their low side effect profile and effective and affordable

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outcomes. Natural materials have many benefits, including their easy accessibility, affordability, and ability to be self-grown. Consequently, natural ingredients offer a distinct approach to disease prevention and treatment. One such example is guava leaves, which are a type of plant used in wound care due to their antibacterial properties.

The Indonesian people have long been aware of the benefits of guava leaves (Psidium guajava L). These leaves have been traditionally used as a mouthwash, astringent for toothaches, anti-spasmodic, treatment for cholera-related diarrhea and vomiting, and a local remedy for rheumatism. Guava leaves are rich in various phytochemicals such as tannins, essential oils, flavonoids, carotenes, vitamins B1, B2, B3, B6, and C, as well as resin. These phytochemicals provide guava leaves with their therapeutic properties, including antimicrobial, anti-inflammatory, antioxidant, and wound healing effects. Therefore, guava leaves are a promising natural ingredient for diabetic wound cleansing treatments [5]. The tannin content in the ethanol extract of guava leaves has been found to have antifungal and antibacterial effects against A. niger and Candida albicans, both of which are examples of microorganisms that can contaminate food and are susceptible to the effects of tannins. In addition, guava leaves were screened for their antibacterial activity against gram-positive S. aureus and gram-negative E. coli and P. aeruginosa. Quercetin and its glycosides were found to have antibacterial activity against these microorganisms as well as antifungal activity against C. albicans [6]. Liquid soap is currently being produced in large quantities as it serves a more practical purpose than other types of soap. It can be used to treat diseases caused by bacteria and fungi on the skin by cleaning the body and the environment, and can be used as a form of medicine to reduce the likelihood of disease [7].

Antiseptics are chemicals that are applied to living tissues to prevent or control the growth of microorganisms. They are commonly used to clean and disinfect wounds, cuts, and other injuries, as well as to sterilize medical instruments and surfaces. Antiseptics work by disrupting the cell membranes and other structures of microorganisms, making them unable to survive or reproduce. By using antiseptics, healthcare providers can limit the spread of infections and promote healing in patients [8]. Liquid soap with added antiseptic ingredients, such as triclosan, chlorhexidine, or benzalkonium chloride, can effectively reduce the number of bacteria on the skin and prevent the spread of infections. However, it is important to use these products correctly and not overuse them, as overuse can lead to the development of antibiotic-resistant bacteria [9]. Based on the background, the purpose of this study was to determine the best formulation for producing guava leaves antiseptic liquid soap and to evaluate the antibacterial activity of the resulting product. The study aimed to utilize the antibacterial properties of guava leaves, particularly their tannin and quercetin contents, to produce a natural and effective antiseptic soap. By doing so, the study sought to contribute to the development of affordable and accessible antiseptic products that can help prevent the spread of infectious diseases, particularly in resource-limited settings like Indonesia.

2 Method

2.1 Extracting guava leaves

To prepare the guava leaves extract, 250 grams of powdered guava leaves simplicia were macerated using 96% ethanol solvent for 5 days with occasional stirring. The resulting extract was then evaporated to obtain a thick extract.

2.2 Phytochemical screening

Saponin. To conduct a saponin test, 1 g of guava leaves extract was added to a test tube containing 10 ml of hot water. The mixture was vigorously shaken for 10 seconds. A positive saponin result was indicated by the formation of a significant amount of foam that remained for at least 10 minutes and reached heights of 1 to 10 cm. The foam did not disappear after the addition of one drop of 2N HCl [10].

Tanin. To test for the presence of tannins, 1 g of extract was boiled in 10 ml of distilled water for 3 minutes, then cooled and filtered. The resulting filtrate was diluted until it was almost colorless, and then 1-2 drops of FeCl3 reagent were added. The occurrence of a blue-black or black-green color indicates a positive tannin result [10].

Flavonoid. 1 g of extract was added to 10 ml of ethanol and heated over a water bath, 0.1 mg of Mg powder was added, and 5 drops of concentrated HCl were added. A positive reaction of flavonoids will be indicated by the formation of a yellow color [10].

2.3 Preparation of Antiseptic Liquid Soap Formulation of Guava Leaves Extract

Table 1 presents the formulation design for the Antiseptic Liquid Soap Formulation of Guava Leaves Extract.

Table 1. Formulation Design of Antiseptic Liquid Soap Formulation of Guava Leaves Extract

Name	Negative control (%)	F1 (%)	F2 (%)	F3 (%)	Information
Guava leaves Extract	-	0.5	3	5	Active substance
Potassium hydroxide	6	6	6	6	Emulsifier
Sodium lauryl sulfate	17	17	17	17	Foam Forming
Glycerin	3	3	3	3	Emollient
Stearic Acid	1	1	1	1	Emulsifier
Citric Acid	0.3	0.3	0.3	0.3	pH regulator
Castor Oil	10	10	10	10	Emollient
Distilled water	Ad 100	Ad 100	Ad 100	Ad 100	Solvent

2.4 Evaluation of the Quality of Antiseptic Liquid Soap Preparations of Guava Leaves Extract

Organoleptic Test. The organoleptic test is a sensory evaluation that assesses the physical characteristics of a product such as appearance, color, odor, texture, and taste. In the case of antiseptic liquid soap, the organoleptic test would evaluate the color, odor, and texture of the soap [11].

pH test. The pH of all antiseptic liquid soap formulations of guava leaves extract was measured using a pH meter [8].

Homogeneity Test. Each formulation of the antiseptic liquid soap preparation of guava leaves extract is weighed at 0.5 g and then applied to a glass object or other suitable transparent material. The preparation must have a homogeneous composition and not contain any coarse grains. This is a test for the physical appearance of the soap, ensuring that it has a smooth and even texture. [12].

Foam Height Test. The sample, weighing 1 g, was placed into a test tube and 10 ml of distilled water was added. The test tube was vigorously shaken for 10 seconds, and the height of the foam formed was immediately measured. The tube was then allowed to stand for 10 minutes, and the height of the foam was measured again after 10 minutes [11].

Spreadability Test. The spreadability test was carried out by placing 0.5 g of the preparation on a glass plate, then placing another glass plate on top of the preparation. A load of 1 kg was then placed on the upper plate for a period of 1 minute. The distance that the preparation had spread between the two plates was then measured. The process was repeated with increasing loads until the preparation stopped spreading within a certain time period. The spreadability was determined based on the maximum load that allowed the preparation to spread without breaking or tearing[12].

Viscosity Test. The viscosity test was carried out by measuring the viscosity with a Brookfield viscometer. Antiseptic liquid soap of guava leaves extract was poured into the container, and a size four spindle was attached to the viscometer. The rotor was then run at a speed of 30 rpm. After the speed showed a stable number, the results were recorded and multiplied by a factor of 200 to obtain the final viscosity measurement in centipoise (cP) [12].

2.5 Antibacterial Activity Test of Staphylococcus aureus and Escherichia Coli Bacterial Antiseptic Soap Preparation of Guava Leaves Extract

Sterilization of Tools and Materials. Good hygiene practices such as tool sterilization are essential in laboratory settings to prevent contamination and ensure accurate results.

Autoclaving at 121°C for 15 minutes is a common method of sterilization that effectively kills microorganisms and spores on laboratory equipment. Wrapping the equipment in paper before autoclaving helps to maintain sterility and prevent contamination during storage and transport [8].

Preparation of Nutrient Agar (NA) Media. A total of 10.5 grams of Nutrien Agar was dissolved in 375 ml Aquadest while heated on a hot plate. The solution was sterilized in an autoclave for 15 minutes at 121°C; this preparation is ready to be used in Petri dishes [8].

Preparation of Bacterial Suspensions. Test bacteria that have been inoculated are taken with a sterile wire loop and then suspended into a tube containing 2 ml of 0.9% NaCl solution. The suspension is adjusted to a turbidity that matches the standard McFarland solution. This turbidity is used as a reference for the bacterial concentration in the solution [13].

Antibacterial Activity Testing. Testing the antibacterial activity of the antiseptic liquid soap preparation of guava leaves extract involved several steps, including preparing the bacterial suspension, inoculating the bacteria onto the NA media, adding the preparation onto sterile disc paper, and incubating the media for 24 hours. The diameter of the inhibition zone was measured to determine the effectiveness of the preparation against the bacteria. The results were compared to a positive control for chloramphenicol. This agar diffusion method is a commonly used technique for testing the antimicrobial activity of various substances [14].

3 Result and Discussion

In this study, the maceration method was chosen for the extraction process due to its simplicity and ability to preserve heat-sensitive compounds. The choice of solvent plays a critical role in the success of the extraction process, as it needs to have similar polarity properties to the compounds being extracted. In this study, 96% ethanol was used as it is a selective, non-toxic, and efficient solvent for extracting non-polar, semi-polar, and polar compounds. The extract was then evaporated using a rotary evaporator to remove any residual solvent and obtain a concentrated extract. The resulting extract was subjected to phytochemical screening to identify the presence of various bioactive compounds. Overall, this approach enables the extraction of potentially beneficial compounds from guava leaves using a simple and effective method.

3.1 Phytochemical Screening

To evaluate the presence of bioactive compounds in guava leaves extract that act as antibacterial, a phytochemical screening was conducted by testing for three key com-

pounds: saponins, tannins, and flavonoids. The presence of these compounds was determined by performing the Phytochemical Test of Guava Leaves Extract, the results of which are presented in Table 2.

Table 2. Phytochemical Test Results of Guava Leaves Extract

Extract	Secondary Metabolites	Method	Results
Guava Leaves	Saponins	HCl	+++
	Tannins	FeCl3	++
	Flavonoids	Concentrated HCl and Mg powder	+++

Information: (+) : Weak positive test; (++) : Strong positive test; (+++) : The positive test is very strong

Table 2 provides evidence that guava leaves extract contains multiple secondary metabolite compounds. Natural extracts comprise a range of secondary metabolites that can be identified using reagents that reveal the unique characteristics of each group of these metabolites [15]. Based on the results of the phytochemical screening, it showed that guava leaves extract contained saponins, tannins and flavonoids [5].

3.2 Preparation of Antiseptic Liquid Soap Formulation of Guava Leaves Extract

Making antiseptic liquid soap from guava leaves extract (Psidium guajava L.) involves the use of various ingredients such as stearic acid as an emulsifier, castor oil as an emollient, potassium hydroxide as an emulsifier, sodium lauryl sulfate as a foam former, citric acid as a pH regulator, and glycerin as an emollient. In order to ensure the quality of the preparation, several tests were carried out, including organoleptic, pH, homogeneity, foam height, spreadability, and viscosity tests. These tests are essential in determining the quality of the antiseptic liquid soap preparation and whether it meets the liquid soap standard set by SNI.

3.3 Physical Quality Test of Antiseptic Liquid Soap Preparations on Guava Leaves Extract

Table 3 presents the results of the physical quality test conducted on the antiseptic liquid soap preparation, which included the organoleptic test, pH test, homogeneity test, foam height test, spreadability test, and viscosity test. The antiseptic liquid soap preparation underwent various physical quality tests, including organoleptic, pH, homogeneity, foam height, spreadability, and viscosity tests. The organoleptic test results indicated that all three formulations (F1, F2, and F3) had a characteristic extract odor and a thick texture, with F1 being light greenish-brown, F2 being brown, and F3 being dark brown. The pH test results showed that all formulations fell within the range specified by SNI (8-11). The homogeneity test revealed that all formulations were homogeneous. The foam height test results were within the range of 25-60 mm, while the spreadability test results met the requirements of 3-5 cm. Additionally, the viscosity test results met the SNI requirement of 500-20000 mPas.

Table 3. The physical quality of the antiseptic liquid soap preparation of guava leaves extract

Formula	Organoleptic	pH	Homogeneity	Foam Height (cm)	Spreading Power (cm)	Viscosity (mPas)
F1	Greenish brown, typical extract, thick	9	Homogeny	4	4.5	7363
F2	Brown, typical extract, thick	8	Homogeny	3.5	3.4	7827
F3	Dark brown, typical extract, thick	8	Homogeny	2.5	3.3	13823

3.4 Evaluation of Physical Quality of Antiseptic Liquid Soap Preparations for 6 Cycles

Organoleptic test. The purpose of evaluating antiseptic liquid soap preparations is to determine their physical quality stability, ensuring that they meet the requirements of liquid soap preparations. After conducting the evaluation of antiseptic liquid soap preparations in the 1st cycle, it was observed that F1, F2, and F3 had stable organoleptic preparations in terms of color. F1 appeared as light greenish-brown, F2 was brown, and F3 was dark brown. The color became darker with an increase in the concentration of guava leaves extract. All three formulas had a distinct extract odor and thick texture. In the 6th cycle, the evaluation of the storage of antiseptic liquid soap preparations yielded consistent results. F1 appeared as greenish-light brown, F2 was brown, and F3 was dark brown. The concentration of guava leaves extract affected the color, with higher concentrations resulting in darker colors. All three formulas had a distinct extract odor and thick texture. Table 4 shows the evaluation results of the organoleptic test for antiseptic liquid soap preparations of guava leaves extract.

Table 4. Evaluation of the organoleptic test

Observation	Formula	Storage Time 6 Cycles					
		1	2	3	4	5	6
Color	F1	Greenish Brown	Greenish Brown	Greenish Brown	Greenish Brown	Greenish Brown	Greenish Brown
	F2	Brown	Brown	Brown	Brown	Brown	Brown
	F3	Dark Brown	Dark Brown	Dark Brown	Dark Brown	Dark Brown	Dark Brown
Smell	F1	Typical extract	Typical extract	Typical extract	Typical extract	Typical extract	Typical extract
	F2	Typical extract	Typical extract	Typical extract	Typical extract	Typical extract	Typical extract
	F3	Typical extract	Typical extract	Typical extract	Typical extract	Typical extract	Typical extract
Texture	F1	Thick	Thick	Thick	Thick	Thick	Thick
	F2	Thick	Thick	Thick	Thick	Thick	Thick
	F3	Thick	Thick	Thick	Thick	Thick	Thick

Homogeneity Test. To ensure the stability of physical quality that meets the requirements of liquid soap preparations, an evaluation of antiseptic liquid soap preparations is carried out. Based on the evaluation of the storage of antiseptic liquid soap preparations in three formulas from cycle 1 to cycle 6, the homogeneity of the preparation remained stable, and there were no coarse particles. The results of the Homogeneity Test for antiseptic liquid soap preparations of guava leaves extract can be seen in Table 5. The results of the evaluation of the storage of antiseptic liquid soap preparations in the three formulas from Cycle 1 to Cycle 6 were stable, homogeneity of the preparations with the union of the soap preparations, and the absence of small particles in the preparations. That means the soap has undergone a saponification process.

Table 5. Evaluation of homogeneity test

Formula	Homogeneity					
	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
F1	homogeneous	homogeneous	homogeneous	homogeneous	homogeneous	homogeneous
F2	homogeneous	homogeneous	homogeneous	homogeneous	homogeneous	homogeneous
F3	homogeneous	homogeneous	homogeneous	homogeneous	homogeneous	homogeneous

pH Test. The pH (acidity) test is one of the quality requirements for liquid soap. This is because the antiseptic liquid soap comes in direct contact with the skin and can cause problems if the pH does not match the skin's pH. In general, liquid soap products have a pH that tends to be alkaline. This is caused by the basic ingredients of the liquid soap, KOH, which is used to produce a saponification reaction with fat or oil, or synthetic detergents with a pH value above a neutral pH. According to SNI, liquid soap pH is between 8 and 11. Evaluation of the pH test for antiseptic liquid soap preparations of guava leaves extract can be seen in Table 6.

Table 6. Evaluation of the pH test

Formula	pH					
	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
F1	9	9	9	9	9	9
F2	8	8	8	8	8	8
F3	8	8	8	8	8	8

Evaluation of antiseptic liquid soap preparations is carried out to determine the stability of the physical quality that meets the requirements of liquid soap preparations. Based on the evaluation of the storage of antiseptic liquid soap preparations in the three formulas from cycle 1 to cycle 6, the pH of the preparation was stable because it did not experience a change in pH. F1 has a pH of 9, F2 has a pH of 8, and F3 has a pH of 8. The three formulas meet SNI requirements, namely having a pH range of 8-11. The results show that all the antiseptic liquid soap formulas produced meet the criteria for

good liquid soap. Cosmetic products with a very high pH can increase the absorption capacity of the skin, causing skin irritation.

High foam test. One of the attractions of soap is its foam content. Evaluation of antiseptic liquid soap preparations is carried out to determine the stability of the physical quality that meets the requirements of liquid soap preparations. Based on Table 7, the results of the evaluation of liquid soap storage in the three formulas, cycle 1 to cycle 6, the height of the foam preparation was stable because it did not change. Namely, F1 has a foam height of 4.5 cm or the equivalent of 45 mm, F2 has a foam height of 3.5 cm or 35 mm, and F3 has a foam height of 2.5 cm or 25 mm. This proves that the higher the concentration, the higher the foam will be because the amount of SLS used in each formula is the same, and SLS cannot emulsify guava leaves extract. The three formulas meet SNI requirements, namely having a foam height range of 25-60 mm.

Table 7. Evaluation of the foam height test

Formula	High foam (mm)					
	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
F1	45	45	45	45	45	45
F2	35	35	35	35	35	35
F3	25	25	25	25	25	25

Spreadability Test. The spreadability test was carried out by placing 0.5 g of an antiseptic liquid soap sample on a round glass with a diameter of 15 cm, then placing another glass on top and letting it stand for 1 minute, then recording the diameter of each additional load until it was constant. Based on the test results for six cycles, the results of the spreading power meet the range. The test for good spreadability is according to the requirements, namely 3-5 cm [10]. Evaluation of the spreadability test for antiseptic liquid soap preparations of guava leaves extract can be seen in Table 8.

Table 8. Evaluation of the spreadability test

Formula	Spreadability (cm)					
	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
F1	4.5	4.5	4.5	4.5	4.5	4.5
F2	3.4	3.4	3.4	3.4	3.4	3.4
F3	3.3	3.3	3.3	3.3	3.3	3.3

Viscosity Test. Data on the viscosity measurements on all antiseptic liquid soap formulations of guava leaves extract can be seen in Table 9. Viscosity is a parameter of concern in liquid soap preparations. Viscosity testing aims to determine the consistency of the preparation, which will later affect the application of the preparation, such as being easily poured from the container, but not easily spilled flowing from the hand [16]. In table 9, it can be seen that the three preparations have different viscosities even though the thickener concentration is the same. The three preparation formulas show results that meet the range. According to SNI 06-4085-1996, the viscosity requirements

for liquid soap are in the range of 500-20000 mPas. So it can be concluded that the viscosity of liquid soap meets the SNI quality requirements.

Table 9. Evaluation of the viscosity test

Formula	Viscosity (mPas)					
	Cycle1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
F1	7363	7363	7363	7363	7363	7363
F2	7827	7827	7827	7827	7827	7827
F3	13823	13823	13823	13823	13823	13823

3.5 Antibacterial activity test of guava leaves extract on liquid soap preparation

The antibacterial activity test of guava leaves extract showed good results on both test bacteria, Escherichia coli and Staphylococcus aureus, marked by the clear zone formed on the agar media after incubation. The clear zone formed can be seen in Fig. 1 and Fig. 2.

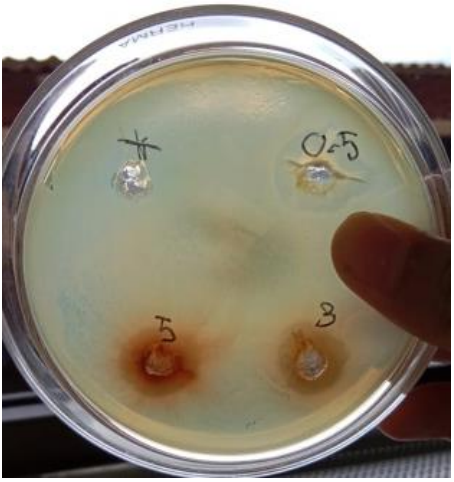

Fig. 1. A Clear zone of Escherichia coli isolate test

The results of the antibacterial activity test against Escherichia coli are presented in Table 10.

Table 10. Results of the antibacterial activity test against Escherichia coli

Bacteria	Formulation	Inhibition Zone Diameter (mm)
Escherichia coli	F1	11.02
	F2	12.10
	F3	15.02

Fig. 2. A clear zone of Staphylococcus aureus isolate test

The results of the antibacterial activity test against Staphylococcus aureus are presented in Table 11.

Table 11. Results of the antibacterial activity test against Staphylococcus aureus

Bacteria	Formulation	Inhibition Zone Diameter (mm)
Staphylococcus aureus	F1	13.06
	F2	15.02
	F3	21.12

Antiseptic liquid soap of guava leaves extract can inhibit the growth of E. coli bacteria and the growth of S. aureus bacteria. Furthermore, antiseptic liquid soap preparations of guava leaves extract on F1, F2, and F3 could inhibit the growth of E. coli bacteria, namely 11.2, 12.1, and 15.02 mm. The preparations were also tested against S. aureus bacteria, with inhibition values of 13.06, 15.02, and 21.12 mm. The greater the concentration of guava leaves extract in antiseptic liquid soap, the greater the inhibition of bacterial growth. The inhibition of guava leaves extracts liquid soap had a greater inhibition than the positive control, namely chloramphenicol, which contained an active antibacterial compound that had an inhibition of 11.02 mm against E. coli and 12.18 mm against S. aureus.

The criteria for antibacterial power were categorized based on the diameter of the inhibition zone formed, namely the diameter of the inhibition zone of 5 mm or less was categorized as weak, the inhibition zone of 5-10 mm was categorized as moderate, the inhibition zone of 10-20 mm was categorized as strong, and the inhibition zone of 20 mm or more was categorized as very strong [10]. Based on these categories, the antibacterial power of antiseptic liquid soap guava leaves extract on Staphylococcus aureus

F1 (13.06 mm) was categorized as strong, F2 (15.02 mm) was categorized as strong, F3 (21.12 mm) was categorized as very strong. on Escherichia coli bacteria, with F1(11.02 mm) categorized as strong, F2 (12.1 mm) categorized as strong, F3 (15.02 mm) categorized as strong.

4 Conclusion

This study shows that guava leaves extract can be utilized to produce a liquid soap preparation that meets the appropriate preparation evaluation requirements. Additionally, the antiseptic liquid soap preparation of guava leaves extract on F1, F2, and F3 showed inhibition zones against Escherichia coli ranging from 11.02 to 15.02mm and inhibition zones against Staphylococcus aureus ranging from 13.06 to 21.12mm. These results suggest that the antiseptic liquid soap preparation of guava leaves extract has potential as an antibacterial agent.

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