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Antibacterial Activity of Formulated Guava Leaf Extract (Psidium Guajava L.) Hand Wash Soap on Staphylococcus Aureus

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Abstract−During the Covid-19 pandemic, the use of hand-washing soap increased. Hand-washing soap using natural ingredients as active ingredients is still not widely developed. One plant that has been proven to inhibit the growth of bacteria is guava leaves. This research aimed to formulate and test the antiseptic activity of liquid hand-washing soap from guava leaf extract. The research method used was laboratory experimental, with stages including; preparation of guava leaf extract, liquid soap formulation with a preparation concentration of FI 5%, FII 6%, FIII 7%, FIV 8%, then the physical properties of the soap will be tested to determine its quality and quality. Apart from the physical test, an antibacterial activity test was also carried out to determine the antibacterial effectiveness of the liquid hand wash soap formulation using the disc diffusion method to determine the diameter of inhibition or area of the inhibition zone. The preparation formula of 7% to 8% concentration of the preparation can inhibit growthStaphylococcus aureus. The diameter of the inhibition zone formed is included in the strong category. Based on research, it can be concluded that guava leaves have the potential to inhibit bacteria S. aureus, and evaluation of the preparations of all formulas shows stability and good characteristics. Each concentration has a good antibacterial effect, where the higher the extract concentration, the greater the diameter of the bacterial growth inhibition zones. aureusresulting from.

Keywords: Antioxidants; Handwash; Psidium Guajava L; Staphylococcus Aureus

1. INTRODUCTION

During the Covid-19 pandemic, the use of hand-washing soap increased. This increase occurred due to adapting to new habits of always washing hands before and after activities. Assistance and counseling regarding adapting the new habit of washing hands during the Covid-19 pandemic aims to make people understand that washing hands is very important to prevent the entry of germs, viruses, and bacteria through the mouth, nose, and eyes Soap is a material derived from natural oils or fats reacting with caustic soda in a process known as the saponification reaction (Mahdi et al., 2022). The need for soap used every day costs money . The method of making soap is not as difficult as you imagine. The benefits of soap as a cleaning agent are related to the surfactant properties it contains (Dalimunthe et al., 2024). Surfactants are molecules that have a polar group that likes water (hydrophilic) as well as a non-polar group that likes fat/oil (lipophilic), so these two groups can unite a mixture containing oil and water so that it can be removed with water (Kusumayanti et al., 2018). Hand-cleaning liquid soap is soap for cleaning made using a saponification process using the addition of other substances or without the addition of different substances which does not irritate the skin of the hands (Puteri, Ningtias, et al., 2024). Today's modern society usually finds it more practical to use liquid hand-washing soap in small packages that are easy to carry everywhere. In contrast to people who want to use it practically or without running water, quite a few choose antiseptics in the form of Hand Sanitizer (Abdilah et al., 2022).

However, other studies report that the use of alcohol-based hand sanitizers has bad effects if used continuously, besides that alcohol is not recommended for use on irritated skin and not even in cosmetics, because it can cause toxicity to the skin (Mahdi et al., 2022). Using liquid soap is better than gel hand sanitizer because hand sanitizer does not remove dirt or organic substances, so if your hands are very dirty or contaminated with blood or body fluids, you must first wash your hands with water using soap (Sianipar et al., 2021). As time goes by, innovation regarding herbal plants continues to develop and many antibacterial studies have been carried out, but few people use them and process them into pharmaceutical products, especially liquid hand-washing soap (Robiatun et al., 2022).

Research conducted by Wahid, et al., (2024), shows that guava leaf extract (*Psidium guajava* L.) can be developed into a physically stable hand soap. And the preparation has antibacterial activity against Staphylococcus aureus with the most effective concentration at a concentration of 25% of 20.5 mm ± 0.63 which is included in the very strong category (Wahid et al., 2024). Similar research also shows that guava leaves (*Psidium guajava* L.) have the potential to inhibit E. coli bacteria. The preparation formula of 6% to 10% concentration of the preparation can inhibit the growth of E. coli. The diameter of the inhibition zone formed is included in the strong category (Puteri, Ginting, et al., 2024). In addition, Handarni, et al., (2020) have also conducted a qualitative screening study of phytochemicals of antibacterial compounds in guava leaf extract (*Psidium guajava* L.). The results showed that the extract contains antibacterial compounds, namely saponins, tannins, and flavonoids (Handarni et al., 2020). As a study of the antibacterial activity of guava leaf extract at concentrations of 5%, 10%, and 15% with 70% ethanol solvent and chloroform extract against Staphylococcus aureus based on the diameter of the inhibition zone. The results obtained showed antibacterial activity in each solvent where the concentration of 15% in both extracts had the highest antibacterial activity in inhibiting the growth of Staphylococcus aureus (Fijriati et al., 2022).

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The provision of hand wash soap using natural ingredients as active ingredients which have activity both bacteriostatic (inhibits bacterial growth) and bactericidal (kills bacteria) has not yet been widely developed (VH et al., 2021). Therefore, it is necessary to develop alternative antiseptics derived from plants. One plant that has been proven to inhibit bacterial growth is guava leaves. The author's aim in conducting this study was to determine whether the ethanol extract of guava leaves (*Psidium guajava* L.) can be made into a hand wash soap preparation that meets the requirements for physical stability and to determine at what concentration the ethanol extract of guava leaves (*Psidium guajava* L.) hand wash soap is effective in inhibiting *Staphylococcus aureus.* Research on hand wash soap from guava leaves has several important contributions to the development of science, both in terms of health, biotechnology, and science in general.

2. RESEARCH METHODS

2.1 Research Parameters

The research method used was laboratory experimental. The research was carried out by making liquid hand-washing soap from guava leaf extract with varying concentrations of FI 5%, FII 6%, FIII 7%, and FIV 8%. Next, the liquid hand wash soap is tested for its physical properties to determine its quality and quality, and an antibacterial activity test is also carried out to determine the antibacterial effectiveness of the liquid hand wash soap formulation using the disc diffusion method to determine the diameter of the inhibition or area of the inhibition zone. The places used in this study include the Cosmetics Laboratory, the Pharmaceutical Preparation Formulation and Technology Laboratory for the manufacture and evaluation of liquid hand soap preparations and the Pharmaceutical Microbiology Laboratory of Al Washliyah Muslim Nusantara University to test the activity of antibacterial compounds from guava leaf extract.

2.2 Tools

The equipment used in this research is analytical scales, filter paper, Erlenmayer, rotary evaporator, measuring cups, chemical glasses, test tubes, tube racks, aluminum foil, maceration vessels, tube needles, tweezers, Eppendorf pipettes, calipers, spirit lamps, object glass, cover glass, autoclave, incubator, refrigerator, and light microscope.

2.3 Materials

The materials used in this research were fresh guava leaves, Staphylococcus aureus isolates obtained from the Faculty of Medicine, University of North Sumatra, Nutrient agar (NA) media with 0.9% NaCl ethanol solvent, sterile distilled water, empty paper discs, cotton wool, cotton buds. , alcohol 96%, Sodium Lauryl Sulfate, glycerin, cocomide dea, As. Citrate, As. Stearate, carbopol, KOH 40%, TEA, Perfume.

2.4 Sample collection

Guava leaves were taken from stems using a controlled random sampling technique (random purposive sampling) taken from Purwodadi Village, Sunggal District, Deli Serdang Regency. The leaves taken are leaves that are light green or dark green.

2.5 Sample processing

A 2 kg sample of guava leaves was cleaned with clean running water to remove impurities attached to the guava leaves. Then it is air-dried for 1 month at room temperature so that it is not exposed to direct sunlight because plant parts containing flavonoids, quinones, curcuminoids, carotenoids and several alkaloids can change when exposed to direct sunlight (Syahputra et al., 2021). Next, the simplicia is ground using a blender and sieved using a number 40 sieve to obtain a coarse powder and then weighed (Pulungan et al., 2022).

2.6 Characterization of Simplicia

Simplicia characterization examination includes: determination of water content, determination of water soluble essence content, determination of ethanol soluble essence content, determination of total ash content, determination of acid insoluble ash content.

2.6.1 Determination of Water Content

Put 200 ml of toluene and 2 ml of distilled water into a round bottom flask, then distill for 2 hours, let it cool for 30 minutes and then read the volume of water in the collection tube. Next, put 5 g of simplicia powder which has been carefully weighed into the flask, then heat it carefully for 15 minutes. After the toluene starts to boil, the drip speed is adjusted to 2 drops per second until some of the water is distilled, then the distillation speed is increased to 4 drops per second. Once all the water is distilled, the inside of the cooler is rinsed with toluene. Distillation was continued for 5 minutes, then the receiving tube was allowed to cool to room temperature, after the water and toluene had completely separated the volume was read to an accuracy of 0.05 ml. The difference between the two volumes of water read corresponds to the water content contained in the material being examined. Water content is calculated in percent (Nurmazela et al., 2022).

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2.6.2 Determination of water soluble essence content

Weighed 5 g of simplicia powder, macerated for 24 hours in 100 ml of water chloroform (2.5 chloroform in distilled water to 1 liter) in a stoppered flask, shaking occasionally for the first 6 hours, then left for 18 hours then filtered, the first 20 ml of filtrate was evaporated until dry in a flat-bottomed evaporator dish that has been heated at 105°C until the weight remains constant. The percentage of water-soluble essence is calculated for the material that has been dried in air(Rani et al., 2023).

2.6.3 Determination of Essence Content Soluble in Ethanol

Weighed 5 g of simplicia powder which had been dried in air, macerated for 24 hours in 100 ml of 96% ethanol in a stoppered flask while shaking repeatedly for the first 6 hours and then left for 18 hours. Then filtered, 20 ml of the filtrate was evaporated until dry in a shallow cup based on the level that had been tared and the remainder was heated at a temperature of 105°C until the weight remains constant. The concentration of soluble essence in ethanol was calculated for the material that had been dried in air(F. A.-U. Nasution et al., 2023).

2.6.4 Determination of Total Ash Content

A total of 2 g of simplicia powder, which is carefully weighed, is put into a porcelain dish that has been fired and tarized. The exchange rate is heated at a temperature of 600°C until the charcoal runs out, cool and weigh until a constant weight is obtained, the ash content is calculated from the material that has been dried in air(Septiana et al., 2024).

2.6.5 Determination of Acid Insoluble Ash Content

The ash obtained from determining the total ash content was added with 25 ml of dilute hydrochloric acid and boiled for 5 minutes, the part that did not dissolve in the acid was collected, filtered through ash-free filter paper, then washed with hot water. The residue and filter paper were heated at a temperature of 600°C until constant weight, then cooled and weighed. The acid insoluble ash content is calculated for the material that has been dried in air(Ridwanto et al., 2023).

2.7 Making Extracts

The dry coarse powder of guava leaves obtained was then macerated using 2 L of ethanol solvent, covered and left for 5 days, stirring repeatedly. Next filtered. The filtered dregs are macerated again and left for 2 days, stirring repeatedly. Next filtered. The filtrate obtained is then concentrated using a rotary evaporator at a temperature of 40ºC, so that a concentrated extract is obtained and separated from the solvent [12]. The concentrated extract of guava leaves is then diluted with ethanol solvent to obtain a concentration of 5%; 6%; 7%; and 8%. Dilution is carried out using the formula V1 x N1 = V2 x N2. Where V1 = initial volume, V2 = desired volume, N1 = initial concentration, N2 = desired concentration [13].

2.8 Phytochemical Screening

Phytochemical screening carried out in this study included examination of alkaloids, flavonoids, saponins, tannins and steroids/triterpenoids.

2.8.1 Alkaloid Examination

Simplicia powder and extract were weighed at 0.5 g, added with 1 ml of 2 N HCl, added with 9 ml of distilled water, then heated over a water bath for 2 minutes, cooled and then filtered, the filtrate was used to examine alkaloids. 3 drops of filtrate are added with 2 drops of Mayer's reagent, a white or yellow lumpy precipitate will form. 3 drops of filtrate are added with 2 drops of Bouchardat's reagent, a brown to black precipitate will form. 3 drops of filtrate added with 2 drops of Dragendroff reagent will form brown or orange. If sediment or turbidity occurs in at least 2 test tubes in the experiment above, then the alkaloid is positive (Suryani et al., 2024).

2.8.2 Flavonoid Examination

A total of 10 g of simplicia and guava leaf extract was weighed, then 100 ml of hot water was added, boiled for 5 minutes and filtered while hot. Then 5 ml of the filtrate obtained was taken, then 0.1 g of Mg powder was added, 1 ml of concentrated hydrochloric acid and 2 ml of amyl alcohol were then shaken and then allowed to separate. Flavonoids are positive if a red, yellow or orange color forms in the alcohol layer (Ridwanto et al., 2023).

2.8.3 Tannin Examination

A total of 0.5 g of simplicia and guava leaf extract was put into a test tube, 10 ml of distilled water was added, shaken and filtered. The filtrate was diluted with distilled water until it was colorless. Take 2 ml of the solution and add 1 to 2 drops of iron (III) chloride reagent. If a blackish blue or blackish green color occurs, it indicates the presence of tannins (Ningtias & Rani, 2023).

2.8.4 Saponin Examination

A total of 0.5 g of simplicia and guava leaf extract was put into a test tube, 10 ml of hot water was added, cooled and shaken for 10 seconds. If foam is formed as high as 1-10 cm which is stable and does not decrease after 10 minutes and

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does not disappear with the addition of 1 drop of 2 N hydrochloric acid, this indicates the presence of saponin (Rambe et al., 2021).

2.8.5 Examination of Steroids/Triterpenoids

A total of 1 g of simplicia and guava leaf extract was macerated with 20 ml of ether for 2 hours, then filtered. The filtrate was evaporated in an evaporating cup and then 5 drops of anhydrous acetic acid and 5 drops of concentrated sulfuric acid (Liberman-Burchardad reagent) were added. The formation of a purple to purple-red color indicates the presence of triterpenoids and the formation of a blue-green color indicates the presence of steroids (Kaban et al., 2022).

2.8.6 Glycoside Examination

Samples of simplicia and guava leaf extract were weighed at 3 g then added with 10 ml of a mixture of 95% ethanol and distilled water (7:3) and 10 ml of 2 N hydrochloric acid, refluxed for 10 minutes, cooled and filtered. Take 20 ml of filtrate, add 25 ml of distilled water and 25 ml of 0.4 M lead (II) acetate, shake, let stand for 5 minutes then filter. The filtrate was put into a separating funnel and filtered with 20 ml of a mixture of isopropanol and chloroform (2:3) 3 times. The water layer and the isopropanol-chloroform mixture layer are separated and anhydrous sodium sulfate is added to each, filtered and evaporated at a temperature of not more than 50ºC. The residue was dissolved in 2 ml of methanol and used for the following experiments: 0.1 ml of the filtrate from the water layer (sugar component) in a test tube was evaporated over a water bath, 2 ml of water and 5 drops of molish reagent were added. Concentrated sulfuric acid was added slowly through the tube wall, a purple ring formed at the boundary of the two liquids indicating the presence of a sugar component (glycone). The filtrate from the chloroform-isopropanol mixture layer (non-sugar component) of 0.1 ml plus 5 ml of anhydrous acetic acid and 10 drops of concentrated sulfuric acid produces a blue or green color (Liberman-Bourchard reaction) indicating the presence of a non-sugar component (aglycone) (Yuza et al., 2023).

2.9 Liquid Soap Formulation

The formulation of the ingredients used in liquid soap has been modified from Mahdi's research, 2021. The formula can be seen in Table 1 below.

Table 1. Liquid Hand wash Soap Formula

Material	FI	FII	FIII	FIV
Guava leaf extract	5%	6%	7%	8%
TEA	4%	85%	85%	85%
SLS	85%	85%	85%	85%
Glycerin	15%	15%	15%	15%
Stearic acid	5%	5%	5%	5%
Citric acid	1.5%	1.5%	1.5%	1.5%
Cocamide DEA	5%	5%	5%	5%
Castor oil	50%	50%	50%	50%
Perfume	qs	qs	qs	qs
Aquadest	qs	qs	qs	qs

2.10 Evaluation of the Quality of Liquid Soap Preparations

Evaluation of the quality of liquid hand wash liquid preparations is carried out using several examinations, including organoleptic examination, homogeneity, pH and spreadability tests.

2.10.1 Organoleptic observations

Organoleptic observations of hand wash soap preparations include changes in color, odor and shape during storage (Fitri et al., 2022).

2.10.2 Homogeneity Testing

A certain amount of the preparation is applied to two pieces of glass or other suitable transparent material, the preparation must show a homogeneous composition with no visible coarse grains. Tests are carried out during storage (Ridwanto et al., 2024).

2.10.3 pH measurement

The sample is put into a vial then dipped in pH litmus paper, then the color change of the litmus paper is seen. The number shown by universal pH is the price of the pH of the preparation (Kaban et al., 2022).

2.10.4 Spreadability

The spreading power was measured with two glass plates, one glass plate was covered with a millimeter block base to make it easier to observe and measure and the other plate was used as a cover. Spreadability was measured by placing 1 g of hydrogel in the middle of the glass. Cover the preparation with a cover glass and a weight with a total weight of 125

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g for 1 minute, calculated by the diameter of the distribution area. Measurements were carried out in 3 replications (Septiana et al., 2024)

2.11 Antibacterial Test

This test looked at the inhibitory power of liquid hand wash soap which was formulated with guava leaf extract as the main ingredient using the pour plate technique, namely the bacterial suspension was taken using a micro pipette and mixed into the still liquid MHA medium. The petri dish containing MHA and bacteria was divided into 5 parts, each of which was placed with a paper disc containing liquid hand wash soap with a concentration of 5; 6; 7; 8 %. Then, on the surface of the other MHA media, divided into two parts, a paper disc containing brand X liquid soap was placed as a positive control and a paper disc containing ethanol solvent as a negative control. This test was carried out with 3 repetitions. Then the MHA medium was incubated at 37ºC for 24 hours in an inverted cup position and the bacterial growth inhibition zone in each group was observed by measuring the diameter of the clear zone formed using a caliper. Discs containing liquid hand wash soap preparations of various concentrations were made by taking 0.1 mL of preparation at each concentration using a micro pipette and dropping it onto a sterile blank paper disc (Nasri et al., 2023).

2.12 Data analysis

Data from research results were analyzed descriptively. The research data is the average clear zone formed around the paper disc.

3. RESULTS AND DISCUSSION

3.1 Simplisia and Ethanol Extract of Guava Leaves

Fresh guava leaves weighing 2 kg are cleaned with running water and then air-dried at room temperature for 1 month. The guava leaf simplicia obtained weighed 500 g, then ground it using a blender and sieved to obtain coarse simplicia powder weighing 290 g (Figure 1).

(1) (2) (3)

Figure 1. Simplicia and Ethanol Extract of Guava Leaves (1), Fresh Guava Leaves (2), Coarse Powder of Simplicia and (3) Concentrated Extract

3.2 Characterization of Simplicia

The results of simplicia characterization are that in macroscopic testing, the physical form of guava leaf simplicia is green in color, odorless and has a slightly sour taste. The results of microscopic tests on guava leaf simplicia powder showed that there were thickened vascular bundles and covering hairs. The results of the characterization of guava leaf simplicia can be seen in Table 2.

Table 2. Characterization of Guava Leaf Simplicia

No	Parameter	Check up result (%)
1	Water content	9.1
2	Water Soluble Essence Content	5, 9
3	Ethanol Soluble Essence Content	1.5
4	Ash Content	20.1
5	Acid Soluble Ash Content	22.1

Determination of water content is carried out to provide a minimum limit or range for the amount of water content in the material. The results of determining the water content of guava leaf simplicia obtained a percentage level of 9.1%. The water content in the sample (simplisia) should not be more than 10% because excess water in the simplicia will encourage the growth of microorganisms and molds (fungi), putrefaction reactions, enzymatic reactions, which are ultimately followed by hydrolysis reactions of chemical compounds in the simplicia. The method used to determine water content is the azeotroph distillation method. The principle of this distillation method is to combine two solvents that have

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different boiling points and different polarities. Distillation is used to separate mixtures consisting of two or more components that are difficult to separate. The solvent used in determining water content is toluene. Toluene has a lower specific gravity than water, the specific gravity of toluene is 0.866 g/ml. Using a solvent that has a lighter specific gravity than water aims to keep the water at the bottom of the container glass so that volume measurements are easier. Water and solvent (toluene), condense so that condensation occurs and falls on the scale tube, marked by the formation of a layer of two phases, namely water and toluene. The water phase is at the bottom while the toluene is at the top. Water is at the bottom because the specific gravity of water is greater than toluene (H. M. Nasution et al., 2024).

In testing the water soluble essence content of guava leaf simplicia powder, the concentration percentage was 5.9%, while for testing the water soluble essence content of guava leaf simplicia powder, the concentration percentage was 1.5%. The concentration results obtained meet the requirements where the content of essence that is soluble in water is not less than 7% and the concentration of essence that is soluble in ethanol is not less than 2.5% (Depkes, 1989). Water and ethanol soluble essence content is a test to determine the amount of compounds that can be dissolved in water (water soluble essence content) and the content of compounds that can be dissolved in ethanol (ethanol soluble essence content) (Fauzi et al., 2024).

To determine the water and ethanol soluble essence content, the simplicia is first macerated for ± 24 hours with water and ethanol (96%). When determining the level of water soluble essence, chloroform is added to simplicia first, the addition of chloroform is intended as an antimicrobial agent. Because during maceration only water is used, perhaps the extract will be damaged because water is a good medium for microbial growth and it is feared that a hydrolysis process will occur which will damage the extract, thereby reducing the quality and quality of the extract. Meanwhile, when determining the soluble essence content of ethanol, chloroform is not added, because ethanol already has antibacterial properties so there is no need to add chloroform. Data on the essence content in a particular solvent is usually needed to determine the solvent that will be used to extract a particular compound so that more substances are extracted from the simplicia that will be extracted (Alviana et al., 2024).

In testing the total ash content of guava leaf simplicia powder, the percentage content was 20.1% and for testing the acid insoluble ash content, the percentage content was 22.1%. The results of determining the ash content still meet the requirements where the total ash content is not more than 10% and the ash content that is not dissolved in acid is not more than 1% (Depkes, 1989). Determination of ash content aims to provide an overview of the internal and external mineral content originating from the initial process until the formation of the extract. In principle, the extract is heated until the organic compounds and their derivatives are destroyed and evaporate until only mineral and inorganic elements remain. The large total ash content in each extract indicates that the extract obtained from the maceration process contains a lot of minerals. Meanwhile, the presence of ash levels that are insoluble in acid indicates the presence of sand or other impurities that are still present (Indriyanti et al., 2018).

3.2 Phytochemical Screening of Simplicia and Guava Leaf Extract

Phytochemical screening is carried out to test the presence or absence of secondary metabolite compounds such as alkaloids, flavonoids, saponins, steroids/triterpenoids, tannins and glycosides. The results of phytochemical screening of simplicia and ethanol extract of guava leaves can be seen in Table 3.

Table 3. Phytochemical Screening

No.	Inspection	Powder Yield	Extract Results
1	Alkaloids	+	+
2	Flavonoids	+	+
3	Saponin	+	+
4	Tannin	+	+
5	Steroids	-	-
6	Triterpenoids	+	+
7	Glycosides	+	+

Description: (+): contains secondary metabolite compounds
(-): does not contain secondary metabolite compounds

Based on the table above, it shows that in examining alkaloids there are 3 tests, namely testing with Mayer, Bouchardat and Dragendorf reagents. The first test with Mayer's reagent showed positive results because a white or yellow lumpy precipitate was formed. Then the second test with the Dragendorf reaction showed positive results, marked by the formation of an orange color. The third test with Bouchardat's reagent also showed positive results because it formed a brown precipitate. From the results obtained above, it can be concluded that simplicia and guava leaf extract positively contain alkaloids. This is in accordance with the literature that alkaloids are considered positive if sediment or turbidity occurs in at least 2 reactions from 3 experiments. Basically, the principle of testing alkaloid compounds in precipitation reactions with heavy metal ions. The nitrogen atom (basic) in the alkaloid structure has the ability to interact with metal ions. The addition of 2 N HCl is intended to attract alkaloid compounds in the extract because alkaloids are basic, so by adding acids such as HCl salts will be formed, so that the alkaloids will separate from other components of the plant cells that are also extracted by distributing them into the acid phase (Rachmawati & Suriawati, 2019).

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Flavonoid testing showed positive results for simplicia and guava leaf extract due to the formation of an orange color in the amyl alcohol layer. Flavonoid compounds containing hydroxyl groups will interact with Mg2+ ions to form colored complex compounds. The reaction of flavonoid compounds with Mg and HCl metals will form flavilium salts which are red or orange in color (Yanti & Vera, 2019).

The tannin examination showed positive results for simplicia and guava leaf extract. This is due to the formation of complex compounds between phenolic compounds containing hydroxyl (OH) groups and iron (III) ions. The oxygen atom has a lone pair of electrons so it is reactive towards positive ions to form complex compounds(Djindadi et al., 2020).

Saponin examination showed positive results for simplicia and guava leaf extract because the foam formed after shaking lasted a long time and did not disappear with the addition of 1 drop of 2N HCl. Foam is formed because saponin has properties that can reduce the surface tension of water. Like soap, saponins have large molecules containing hydrophilic and lipophilic groups. In water, saponin molecules align themselves vertically on the surface, with the lipophilic groups facing away from the water (Sahputra et al., n.d.).

The triterpenoid test is based on the ability of triterpenoid and steroid compounds to form color by concentrated H2SO4 in glacial acetate solvent which forms a purple to purple-red color indicating the presence of triterpenoids and the formation of a blue-green color indicating the presence of steroids. Based on the results of the phytochemical screening that has been carried out, it is known that simplicia and guava leaf extract are positive for containing triterpenoid compounds and negative for containing steroids (Djindadi et al., 2020).

The glycoside examination showed positive results for simplicia and guava leaf extract because no purple ring was formed at the liquid boundary of the remaining solution after adding Molisch reagent and concentrated sulfuric acid. The mechanism for the formation of purple rings comes from carbohydrates being hydrolyzed by sulfuric acid into monosaccharides then both are condensed to form furtural which reacts to form purple rings (Nurbaity, 2020).

3.3 Making Guava Leaf Extract Liquid Hand Soap

Making liquid soap. Making antiseptic liquid soap from guava leaf extract is made in 2 ways. First, the oil phase is made, namely dissolving stearic acid, castor oil and cocamid DEA by heating it to a temperature of 70°C. Then the second water phase is made, namely sodium lauryl sulfate, and citric acid by heating at the same temperature. Next, mix the oil phase, guava leaf extract and the water phase. After the two phases are homogeneous, add glycerin until an emulsion is formed, then add TEA until it is homogeneous and a liquid soap mass is formed (Figure 2.)

Figure 2. Preparation of Liquid Hand wash Soap in Each Formula

3.4 Evaluation of Physical Quality Test of Liquid Hand Soap Preparations

3.4.1 Organoleptics and Homogeneity

Organoleptic testing on liquid hand wash soap preparations can be carried out by observing color, shape and odor which are organoleptic test parameters. Based on SNI provisions, the results of organoleptic testing on liquid hand wash soap with ethanol extract of guava leaves have a soap texture in liquid form, an attractive color and a distinctive soapy smell with a perfume aroma. The organoleptic results in this study are shown in Table 4.

Table 4. Organoleptic Results and Homogeneity

Organoleptic	FI	FII	FIII	FIV
Form	Liquid	Liquid	Liquid	Liquid
Color	Brownish yellow	Yellowish-brown	Light brown	Dark brown
Aroma	Not scented	Typical extract	Typical extract	Typical extract
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous

According to SNI, which is a reference in soap making, it explains that the organoleptic test for liquid soap is based on the form, namely liquid, smell and color, namely having a smell and color that is typical of the extract. The results of this study are in accordance with established standards, while the homogeneity test aims to determine how

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evenly distributed the active substances contained in the extract and additional ingredients used to support the preparation of soap formulations are. The result of homogeneity or even distribution in a composition will affect the effectiveness of a product. The homogeneity test was carried out by placing the liquid soap preparation on a glass slide and then observing it using a microscope. The homogeneity test of the liquid hand wash soap formulation with ethanol extract of guava leaves was homogeneous in each formula. According to the literature, it is stated that a preparation can be said to be homogeneous, if all the compounds and ingredients added show no lumps on microscope observation.

3.4.2 Check pH and Spreadability

pH testing of guava leaf extract liquid hand wash soap preparations aims to obtain an optimal pH that is appropriate to the condition of the skin and palms so that it does not cause irritation and makes the palms dry. Determining the pH value in liquid soap preparations can be done using a pH meter that has been calibrated with a buffer which aims to produce a maximum constant value (Figure 3). Basically, pH is very important in testing the standards and quality of a soap preparation, because a pH value that is suitable for the skin will produce good effectiveness without causing irritation problems on the skin. The pH value specified for liquid hand wash soap preparations based on SNI 16-4399-1996 specifications ranges from 6-8

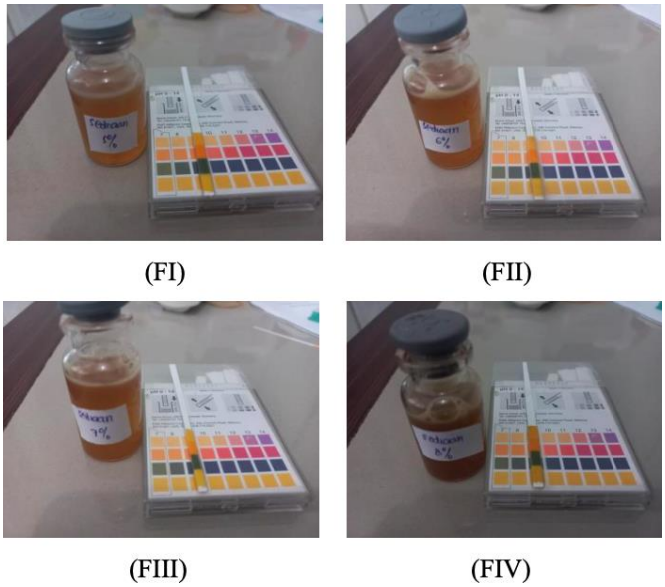

Figure 3. pH Test Results for Each Formula

Spreadability testing was carried out by pressing 0.5 g of the preparation with a load of up to 50 g for 1 minute, then recording the diameter for each additional load until it was constant. Test good spreading power according to the requirements, namely 3 – 5 cm. The results of the pH and spreadability tests for liquid hand wash soap preparations can be seen in Table 4 as follows.

Table 5. pH and Spreadability Test Results

Formulas	pH	Spreading Power (mm)
FI	8	49.9
FII	8	49.1
F III	8	48.9
F IV	8	42.1

3.5 Antibacterial Activity Test Results

The antibacterial activity test was carried out to determine the activity of the guava leaf extract liquid hand wash soap as an antibacterial in inhibiting the growth of S. Aureus. The presence of this resistance can be determined by the formation of an inhibition zone around the paper disc which is calculated using a caliper in mm. The inhibition zone is a zone formed due to the antibacterial power of the extract used (Nasri et al., 2024). The diameter of the inhibition zone formed in each treatment can be seen in Table 6.

Table 6. Mean Inhibition Zone Diameter (mm)

Formula (%)	Barrier Diameter (mm)				Inhibitory Response
	1	2	3	Average	
FI	8.5	7.6	8.4	8.1	Currently
FII	9.6	9.5	8.2	9	Currently
F III	12.6	12.4	11.8	12	Strong

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Formula (%)	Barrier Diameter (mm)				Inhibitory Response
	1	2	3	Average	
F IV	12.3	12.7	12.2	12.4	Strong

Based on Table 5, it shows that the preparation formula of 5% to 8% concentration of the preparation can inhibit the growth of Staphylococcus aureus. The diameter of the inhibition zone formed in the 5% and 6% formulas is classified as medium (6-10 mm). However, the formula is 7% and the average diameter of the inhibition zone formed is included in the strong category (11-20 mm). If we look at the size of the inhibition zone formed in each liquid hand wash soap preparation formula, the higher the concentration of guava leaf extract given, the greater the diameter of the inhibition zone formed. The effectiveness of an antimicrobial substance is influenced by the concentration of the substance given, where increasing the concentration of the extract causes a greater number of antimicrobial compounds to diffuse into the agar medium so that the inhibition zone formed will increase. Apart from concentration factors, the type of plant used as an antibacterial and differences in the cell wall structure of a bacteria can also determine the ability to inhibit bacterial growth . Based on Table 5, it shows that increasing the extract concentration results in a higher content of active ingredients which function as antibacterials so that their ability to inhibit bacterial growth is also greater. The ability of antibacterial compounds such as flavonoids is greatly influenced by the biological activity of flavonoid compounds to damage bacterial cell walls. The constituents of bacterial cell walls consist of peptidoglycan, lipids and amino acids which will react with the alcohol groups in flavonoid compounds so that the cell walls are damaged due to damage to the bacterial DNA structure which ultimately results in bacterial cells lysis and the bacteria will die (Nasri et al., 2024). Increasing the extract concentration results in a higher content of active ingredients which function as antibacterials so that their ability to inhibit bacterial growth is also greater. The ability of antibacterial compounds such as flavonoids is greatly influenced by the biological activity of flavonoid compounds to damage bacterial cell walls. The constituents of bacterial cell walls consist of peptidoglycan, lipids and amino acids which will react with the alcohol groups in flavonoid compounds so that the cell walls are damaged due to damage to the structure of the bacterial DNA, which ultimately results in lysis of the bacterial cells and the bacteria will die. Alkaloid compounds as antibacterials work by utilizing the reactive properties of the base groups in alkaloid compounds that contain nitrogen. When this base group comes into contact with the constituents of the bacterial cell wall, it causes a change in the balance of the constituents of the bacterial cell wall, especially DNA, which is the main constituent of the cell nucleus. As a result, the bacterial cell nucleus experiences damage and lysis so that the bacteria will die. Apart from flavonoids and alkaloids, saponins also have a role in inhibiting the growth of S. aureus through permeability of bacterial cell membranes.Saponins and steroids work by binding to proteins and lipids found in cell membranes and causing cell lysis. Damage to the cell membrane causes disruption of the transport of nutrients (compounds and ions) through the cell membrane so that bacterial cells experience a lack of nutrients necessary for their growth (Nasri et al., 2023).

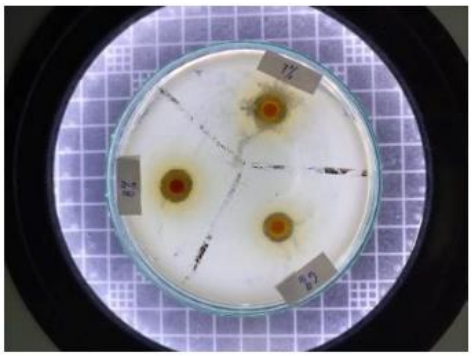

Figure 4. Diameter of Staphylococcus aureus Inhibition Zone after administering liquid hand wash soap with guava leaf extract (Psidium guajava L.)

Figure 4 shows the average diameter of the inhibition zone of the treatment group where the results obtained have the inhibitory power of each formula tested. The antibacterial activity of the guava leaf extract liquid hand wash soap preparation in the study can be seen by the formation of an inhibition zone in each treatment. Even though there are formulas that are categorized as medium, liquid hand wash soap and guava extract have activity in inhibiting the growth of S. aureus in formulas with concentrations: 5; 6; 7 and 8%.

4. CONCLUSION

Based on the results of the study, it can be concluded that guava leaves (*Psidium guajava* L.) have the potential to inhibit *Staphylococcus aureus* bacteria and the evaluation of all formulations showed good stability and characteristics. Formulas III and IV of the preparation with a concentration of 7% to 8% were able to inhibit the growth of *Staphylococcus aureus*, where the higher the concentration of guava leaf extract, the greater the diameter of the inhibition zone against the growth of *Staphylococcus aureus* bacteria produced. The diameter of the inhibition zone formed is included in the strong category. Research on guava leaf-based hand wash soap brings great benefits to consumers, producers, and the environment. By

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https://ejurnal.seminar-id.com/index.php/jharma

offering a more natural, effective, and environmentally friendly product, this research can open up new market opportunities, support sustainability, and provide a healthier alternative in maintaining cleanliness and health.

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