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Original Research Article

Physicochemical and antibacterial pathogenic characterization of papaya leaf extract liquid soap (*Carica papaya* L.)

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ABSTRACT

This research aims to identify the physicochemical characteristics and pathogenic antibacterial activity of papaya leaf extract liquid soap (*Carica Papaya* L.). The sample used was papaya leaf extract whose mass was varied. The data acquisition process was obtained from the results of physicochemical testing of liquid soap formulations and from the results of antibacterial activity tests. This research began by extracting the sample using maceration technique. The extract was then formulated to be the liquid soap formulation 1, 2 and 3 with various masses i.e. three, four and five grams of extract, respectively. The physicochemical test showed that the organoleptic test, pH test, foam height test, specific gravity test, water content test and free alkali test met the SNI physical quality standards for liquid soap and the antibacterial activity test carried out on *S. aureus* and *E. coli* bacteria was obtained for *S. aureus* bacteria. formulations 1, 2 and 3 produce resistance of 2.06 mm, 2.21 mm and 2.46 mm respectively. Meanwhile, for *E. coli* bacteria, the inhibitory power produced by formulations 1, 2 and 3 was 2.84 mm, 3.41 mm and 3.71 mm, respectively. Based on the results of this research, it can be concluded that the liquid soap formulation from papaya leaf extract meets the physical standards for liquid soap quality according to SNI and has antibacterial activity.

Introduction

Soap is a mixture of surfactants that can be used with water to clean dirt and wash, where soap has a long chemical structure of C¬12 to C16 carbon chains and has amphiphilic properties where in the head there is a polar hydrophilic group and in the tail there is a non-polar hydrophobic group (Sukeksi et al., 2017). The function of soap is to help remove germs and dirt from the surface of the skin pores (Faikoh, 2017). Soap consists of two types, namely solid soap and liquid soap, each of which has physicochemical characteristics, where physicochemical characteristics are properties that lead to the characteristic physical properties of a chemical compound (Kurniawati, 2021).

One of the characteristics of using soap is that it kills bacteria which is obtained by adding active substances such as triclosan. However, the use of triclosan is thought to have many negative impacts, such as interfering with brain growth and reproductive hormones, causing antibiotic resistance which will later hinder the working of drugs, being able to result in the creation of superbugs or bacteria that have undergone many cell changes or mutations which can later be making bacteria no longer able to be killed. It can be seen from the alleged negative impact caused by the substance triclosan that it is necessary to think about other substitute materials, such as using natural ingredients or plants that can be antibacterial (Gusviputri et al., 2013).

Papaya (*Carica papaya* L.) is a very profitable horticultural plant. A plant that originates from Central America and the West Indies and comes from the Caricaeae family. The parts of the papaya plant that have medicinal properties are the stem, fruit, sap and leaves (Oktafani & Suwandi, 2019). Papaya leaves contain various secondary metabolites such as flavonoids, tannins, saponins and alkaloids, all of which have antibacterial properties such as flavonoids which can cause interference in the formation of bacterial cells so that they experience damage that causes bacterial cell death (Rahayu, 2013). Tannins can activate adhesion enzymes which can inhibit the process of bacterial attachment to their host (Juliastuti et al., 2014). Saponin compounds are able to damage the permeability properties of bacterial cell walls by forming complex compounds and cell membranes through hydrogen bonds which can ultimately cause bacterial cell death (Nor et al., 2018). As well as alkaloids which can cause bacterial cell death by damaging the peptidoglycan components of bacterial cells, in which case the cell wall will not form completely (Nor et al., 2018). Antibacterials are compounds that can be used to treat infections caused by bacteria. Including pathogenic bacteria which have the ability to cause infection or disease in their hosts (Pulungan & Brata, 2017). In humans, bacteria that are commonly found are *S. aureus* and *E. coli*.

S. aureus is a gram-positive bacteria found in around 20-75% of the hands, respiratory tract, vagina, hair and face (Razak et al., 2013). Meanwhile, *E. coli* bacteria are gram-negative bacteria. This bacteria is very easy to spread by polluting water and contaminating materials that are touched by it, which can later cause digestive disorders and disruption of the working system of the stomach (Hamidah et al., 2019). *E. coli* is a normal flora found in the digestive organs of animals and humans which has lipopolysaccharide in its cell walls. The presence of *E. coli* in water or food can also be considered to have a high association with the discovery of disease germs in food (Kurniadi et al., 2013). This bacteria is very easy to spread by polluting water and contaminating materials that are touched by it, which can later cause digestive disorders and disruption of the working system of the stomach (Hamidah et al., 2019). Infections caused by Escherichia coli bacteria include urinary tract infections, diarrhea, urinary tract infections and bile duct infections (Elliott et al., 2002).

Based on research conducted by (Zega et al., 2021; Nasri et al., 2022) with the title "antibacterial activity of ethanol extract of papaya leaves (*Carica papaya* L.) against Pseudomonas aeruginosa bacteria", (Nor et al., 2018) entitled "antibacterial activity test of ethanol extract of papaya Leaves (*Carica papaya* L.) against the growth of *Escherichia coli* bacteria in vitro" and (Sudarwati & Fernanda., 2018) with the title "antibacterial activity of papaya leaves (*Carica papaya* L.) using ethanol solvent against bacillus subtilis bacteria" that papaya leaf extract has antibacterial activity or can inhibit bacterial growth. Based on the research background that the author has stated above, the author is interested in conducting research on the physicochemical characteristics and antibacterial activity of soap with the addition of papaya leaf extract.

Methods

This research was conducted from March 2023 to June 2023, for physicochemical tests carried out at the Chemistry Laboratory, Faculty of Teacher Training and Education, Bengkulu University, while for antibacterial tests carried out at the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Bengkulu University, Bengkulu.

Materials and Chemicals

The tools used in this research were a set of glassware available in the laboratory, blender, spatula, filter paper, Whatman No.1, hot plate, rotary vacuum evaporator (RE-52A), petri dish, wire mesh, spirit glass, stirring rod, aluminum foil, laminary air flow, spray bottle, Memmet oven, Vortex, analytical balance (Pioneer), autoclave (HVA-85), incubator. The ingredients used are papaya leaf extract, papaya leaf extract soap formulation, PP indicator, KOH 40%, 0.1 N HCl, *Staphylococcus aureus*, *Escherichia coli*, ethanol 96% solvent, distilled water, Nutrient agar media, Nutrient Broth.

Making Papaya Leaf Simplicia

Take 2 kg of old papaya leaves, clean and slice them, then dry them without direct sunlight until dry, then blend the dried papaya leaf slices.

Phytochemical Test

Phytochemical tests were carried out to determine the compounds contained in papaya leaves. Four phytochemical tests were carried out on the samples, namely alkaloid, flavonoid, saponin and tannin tests (Handayani et al., 2023). Weighed 0.5 grams of papaya leaf simplicia then added 10 ml of ethanol 96% then heated for 2 minutes in a water bath then filtered.

Alkaloid Identification

A total of 0.5 ml of papaya leaf extract was added with 1 ml of ammonia, 1 ml of chloroform and 0.5 ml of 2 N sulfuric acid. Shake until two layers were formed. The top layer was analyzed with 3-5 drops of Mayers' reagent. The formation of a white precipitate indicates that the extract contains alkaloids (Sangi et al., 2019).

Flavonoid Identification

A total of 2 ml of extract was added with 1 ml of concentrated HCl and 0.5 mg of Mg ribbon. A positive result is indicated by a dark red (magenta) to orange color within 3 minutes.

Saponin Identification

Add 1 ml of hot distilled water to 1 ml of papaya leaves, then shake and let sit for 2 minutes. Positive results are indicated by the presence of stable foam.

Tanin Identification

A total of 1 ml of papaya leaf extract was then put into a test tube and then 3 drops of 10% $FeCl_3$ solution were added. Positive results are indicated by a color change to dark blue black.

Extraction

Extracted 500 grams of papaya leaf powder with ethanol 96% in a jar covered with aluminum foil, soaked for 3 days while stirring occasionally, filtered the sample using filter paper, then evaporated the solvent using a rotary vacuum evaporator.

Papaya Leaf Extract Liquid Soap Formulation

The liquid soap formulations that will be used are made in different masses, namely concentrations of 3 grams, 4 grams and 5 grams, can be seen in Table 1.

Table 1. Papaya leaf extract liquid soap formulation (Patmawati et al., 2021)

Ingredients	Liquid Soap Base	Formula 1	Formula 2	Formula 3
Papaya Leaf Extract	0 g	3 g	4 g	5 g
Olive oil	15 ml	15 ml	15 ml	15 ml
KOH	8 ml	8 ml	8 ml	8 ml
Stearic Acid	0.25 ml	0.25 ml	0.25 ml	0.25 ml
Citric Acid	1 g	1 g	1 g	1 g
CMC	0.5 g	0.5 g	0.5 g	0.5 g
Aquadest	50 ml	50 ml	50 ml	50 ml
Fragrance	1 ml	1 ml	1 ml	1 ml

Organoleptic Test

Organoleptic Tests are observed using the five senses, such as color, texture and smell.

pH Test

pH measurements were carried out using a pH meter on all liquid soap preparations (Mawarni, 2021).

Foam Height Test

Put 1 ml into the measuring cup, add 10 ml of distilled water, shake the measuring cup, leave the measuring cup for 5 minutes then measure the height of the foam.

Specific Gravity Test

The pycnometer was dried and weighed. Add distilled water and leave at 25°C. The pycnometer is removed and weighed. Repeated using a liquid soap formulation (Muthmainnah, 2020):

$$\text{Specific Gravity} = \frac{W2 - W0}{W1 - W0}$$

Information:
W0 : empty pycnometer weight
W1 : distilled water pycnometer weight
W2 : sample pycnometer weight

Water Content Test

Weighed 1 gram of liquid soap preparation in an evaporation cup of known weight, heated in an oven at 105°C for 2 hours until the weight remained constant.

$$\text{Water content} = \frac{W1 - W2}{W} \times 100\%$$

Information:
W : weight of soap
W1 : weight of container + soap
W2 : container weight + heated soap

Free Alkali Test

Put 5 grams of liquid soap into the Erlenmeyer flask then add 100 ml of distilled water and phenolphthalein indicator until it turns red or purple. Next, titrate using 0.1 N HCl until the color disappears (Rinaldi et al., 2021).

$$\text{Alkali content} = \frac{v \times N \times 0{,}056}{gr} \times 100\%$$

Information:
V : titration volume HCl (mL)
N : normality of HCl (N)

Antibacterial Test

The antibacterial test that will be carried out is an inhibition test. The bacteria used are *S. aureus* and *E. coli*. The test was carried out using the disc diffusion method, namely using disc paper as a medium for antibacterial substances and using Nutrient Agar (NA) media. Paper discs in petri dishes were dripped with all the preparations and then incubated in the best conditions at a temperature of 37°C for 48 hours under anaerobic conditions.

Data Analysis

For the physicochemical test, descriptive data analysis techniques were used, while for the antibacterial activity test, the Analysis of Variance (ANOVA) test was used with the Static Package for Social Science (SPSS) software.

Results and Discussion

Papaya leaves made from simplicia have a mass loss of 72%. Simplicia is then macerated using ethanol 96%. The advantages of the maceration method are that it is relatively cheap, does not use complicated equipment and is able to avoid evaporation of compound components because there is no heating, while the disadvantages are that it takes quite a long time and uses a lot of solvent so it is not efficient (Kiswandono, 2017). The extract obtained was sticky and thick, blackish green in color, amounting to 35.5787 grams with an extract yield presentation of 7.11574%. For phytochemicals, papaya leaf extract contains secondary metabolite compounds such as alkaloids, flavonoids, tannins and saponins.

Organoleptic Test

Organoleptic tests are carried out to see the physical properties of liquid soap in the form of color, smell and texture. The standards set by SNI 06-4085-1996, liquid soap must have a distinctive color, texture and smell. Organoleptic Test Results can be seen in Table 2.

Table 2. Organoleptic test results

Preparation	Collors	Texture	Smell
Liquid Soap Base	White	Liquid and Thick	Lily
Formula 1	Green	Liquid and Thick	Lily
Formula 2	Green	Liquid and Thick	Lily
Formula 3	Deep Green	Liquid and Thick	Lily

The color of the liquid soap produced in this study was a white soap base, in formulas 1 and 2 it was green, while formula 3 changed color to dark green due to the addition of papaya leaf extract. The texture of the liquid soap produced in this research was liquid and thick. The smell of liquid soap produced in this research is the smell of lilies which comes from perfume or essential oil.

pH Test

The pH test is carried out to determine the pH of liquid soap preparations that meet the quality requirements for liquid soap. According to SNI 06-4085-1996 the permitted pH of liquid soap is in the range 8-11. pH test results can be seen in Table 3.

Table 3. pH test results

Preparation	pH
Liquid soap base	9.34
Formula 1	8.76
Formula 2	9.90
Formula 3	10.91

From the research, data was obtained for liquid soap base having a pH of 9.34, formulation 1 having a pH of 8.76, formulation 2 having a pH of 9.90 and formulation 3 having a pH of 10.91. From the data it can be seen that the higher the concentration of the extract added, the higher the pH produced, this is due to the addition of papaya leaf extract which is alkaline (Sahambangung et al., 2019).

Foam Height Test

The foam height test is one of the quality requirements for liquid soap. This is because the foam functions to prevent redeposition, which means that dirt particles that have been dissolved in the water by the soap do not settle again so that the dirt can be thrown away with the previous water. According to SNI 06-4085-1996, the foam height requirement for liquid soap is 13-220 mm. Foam height test results can be seen in Table 4.

Table 4. Foam height test results

Preparation	Foam Height
Liquid soap base	16 mm
Formula 1	25 mm
Formula 2	22 mm
Formula 3	15 mm

From the research, data on foam height was obtained, namely for soap base 16 mm, formulation 1 obtained a foam height of 25 mm, formulation 2 obtained a foam height of 22 mm and formulation 3 obtained a foam height of 15 mm. This is thought to be due to the influence of the viscosity of the papaya leaf extract.

Specific Gravity Test

The specific gravity test was carried out to determine the effect of the ingredients used in liquid soap formulation on the specific gravity produced (Dimpudus et al., 2017; Tutik et al., 2022). According to SNI 06-4085-1996, the quality requirement for liquid soap is to have a specific gravity of 1.01 – 1.1 g/ml. Specific gravity test results can be seen in Table 5.

From Table 5, the specific gravity of the soap base is 1.050 g/ml, formulation 1 is 1.029 g/ml, formulation 2 is 1.036 g/ml and formulation 3 is 1.023 g/ml. The specific gravity value is influenced by the constituent materials, such as the presence of fat in the environment (Gaman & Sherrington, 1992).

Table 5. Specific gravity test results

Preparation	Specific Gravity
Liquid soap base	1.050 g/ml
Formula 1	1.029 g/ml
Formula 2	1.036 g/ml
Formula 3	1.023 g/ml

Water Content Test

The water content test is used to determine the percentage of water content contained in liquid soap preparations. According to SNI the maximum water content for liquid soap preparations is <60%. Water content test results can be seen in Table 6.

Table 6. Water content test results

Preparation	Water Content
Liquid soap base	60%
Formula 1	57%
Formula 2	52%
Formula 3	48%

From the water content data for liquid soap base it is 60%, for formula 1 it is 57%, for formula 2 it is 52% and for formula 3 it is 48%. Based on research data, it can be seen that the greater the extract concentration, the smaller the water content obtained. This is due to the addition of extracts to the liquid soap formulation which results in a reduction in the amount of distilled water.

Free Alkali Test

The free alkali test is carried out to measure the amount of alkali that is not bound to fatty acids or oils (Legi et al., 2021). According to SNI 06-3532-1994, the free alkali content in liquid bath soap is 0.14%. Free alkali test results can be seen in Table 7.

Table 7. Free alkali test results

Preparation	Free Alkali Content
Liquid soap base	0.14%
Formula 1	0.11%
Formula 2	0.11%
Formula 3	0.13%

From the research data, it was found that free alkali for liquid soap base was 0.14%; formulation 1 of 0.11%; formulation 2 is 0.11% and formulation 3 is 0.13%. Based on research data, it appears that the higher the concentration of papaya leaf extract, the higher the alkali content. This is due to the presence of secondary metabolite compounds, namely alkaloids, which have special alkaline properties, so the soap will become alkaline (Rahayu, 2022).

Antibacterial Test

The antibacterial activity test of the papaya leaf extract liquid soap preparation against Staphylococcus aureus and Escherichia coli bacteria was carried out to determine its antibacterial activity. Inhibition zone diameter categories are ≤ 5 mm including weak, 6-10 mm including moderate, 11-20 mm including strong and ≥ 21 mm including very strong (Surjowardojo et al., 2015).

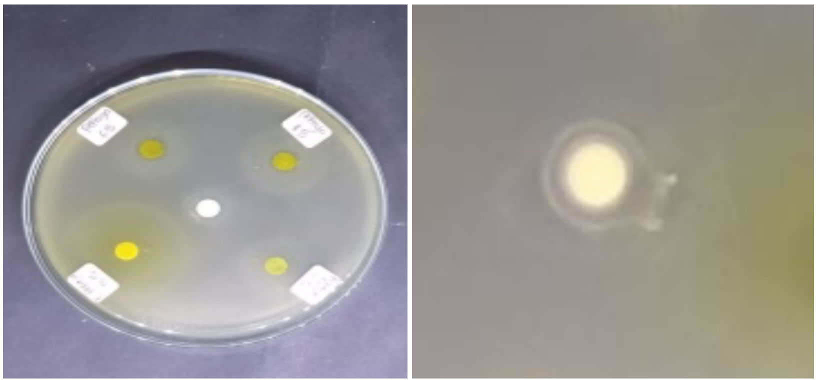
Antibacterial Activity Test on S. aureus Bacteria

Antibacterial activity test results on *S. aureus* can be seen in Table 8. Based on research data, it can be seen that the soap base has an inhibition zone of 3.31 mm in the weak category, formula 1 (3 gram extract) has an inhibition zone of 2.06 mm in the weak category, formula 2 (4 gram extract) has an inhibition zone of 2.21 mm is in the weak category, formula 3 (5 gram extract) has an inhibition zone of 2.46 mm in the weak category, 50% papaya leaf extract has an inhibition zone of 28.20 mm in the very strong category and the positive control has an inhibition zone of 6.01 mm is in the medium category.

Table 8. Antibacterial activity test results on *S. aureus* bacteria

Repetition	Liquid soap base	Formula 1	Formula 2	Formula 3	Extract 50%	Positive Control
1	2.8	2.85	2.45	2.95	29	6.25
2	3	1.45	2.25	2.35	29.45	6
3	3.7	2.7	2.25	2.05	26.25	7.3
4	3.75	1.25	1.9	2.5	28.1	4.5
Average Resistance Power (mm)	3.31	2.06	2.21	2.46	28.20	6.01

From the data above, it can be seen that the inhibitory power produced by the liquid soap base is greater than the inhibitory power produced by formulas 1, 2 and 3 which added papaya leaf extract. This is thought to be due to the weak antibacterial ability of secondary metabolite compounds in papaya leaf extract. From the data, it can also be seen that the difference in the inhibitory power of the liquid soap base and the positive control is very different, which is caused by the composition added to each preparation. The positive control has antibacterial compositions such as myristic acid (Idrus et al., 2014), lauric acid (Sulastri et al., 2016), Cocamidopropyl betaine (Afifah, 2015) and thymol (Warongan et al., 2015). Meanwhile, the liquid soap base composition which has antibacterial properties is olive oil (Tumbel et al., 2017) which proves that the inhibitory power produced by the positive control (Lifebouy liquid soap) is greater than the liquid soap base. Antibacterial Test on *S. aureus* can be seen in Fig-1.

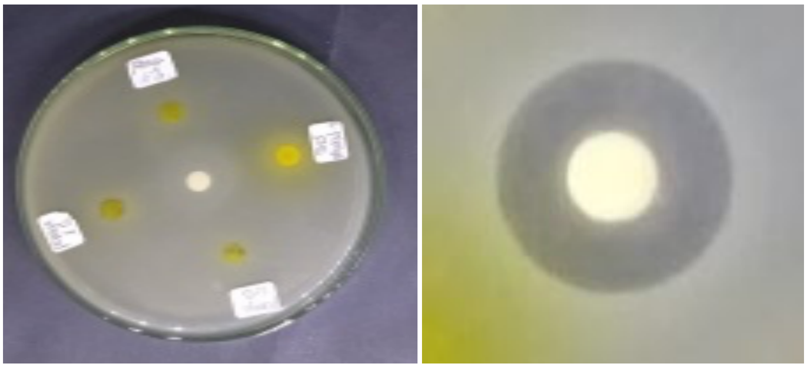


(a) (b)

Fig-1. Antibacterial Test on S. aureus Bacteria. (a) base, Formulation 1, Formulation 2 and Formulation 3; (b) positive control

Antibacterial Activity Test Results on E. coli Bacteria

Antibacterial activity test results on *E. coli* can be seen in Table 9. Based on the research data above, it can be seen that the soap base has an inhibition zone of 4.30 mm which is in the weak category, formulation 1 (3 gram extract) has an inhibition zone of 2.84 mm which is in the weak category, formulation 2 (4 gram extract) has a The inhibitory zone of 3.41 mm is in the weak category, formulation 3 has an inhibitory zone of 3.71 mm which is in the weak category, 50% papaya leaf extract has an inhibitory zone of 9.59 mm which is in the medium category and the positive control has an inhibitory zone of 8.06 mm is in the medium category. The data on the antibacterial activity test for *E. coli* produced was not much different from the data on the antibacterial activity test for *S. aureus*, in which the inhibitory power value of the liquid soap base produced was greater than the inhibitory power value of the soap formulation which added papaya leaf extract. Antibacterial test on *E. coli* can be seen in Fig-2.



(a) (b)

Fig-2. Antibacterial test on *E. coli* bacteria. (a) base, Formulation 1, Formulation 2 and Formulation 3; (b) positive control

From the antibacterial research that has been carried out, it turns out that papaya leaf extract added to the liquid soap formulation only has a slight effect on the antibacterial activity, which is due to variations in the concentration of papaya leaf extract added to the liquid soap formulation, namely less than 20%, which is explained in the journal. Sahambangung et al. (2019), that the concentration of papaya leaf extract added to liquid soap formulations with varying concentrations of 20%, 30% to 40% can have a very strong inhibitory effect on antibacterial activity. However, if the variation in concentration of the extract used is greater, it will affect the organoleptic properties produced. From the results obtained, papaya leaf extract cannot be used as the sole basic ingredient in making liquid soap, therefore it is necessary to formulate additional natural ingredients in the composition of liquid soap in the form of adding a mixture of papaya leaf extract and other plant extracts which have antibacterial activity high levels while improving its organoleptic physical properties.

Table 9. Antibacterial activity test results on *E. coli* bacteria

Repetition	Liquid soap base	Formula 1	Formula 2	Formula 3	Extract 50%	Positive Control
1	5.7	2.85	4	4.05	10.3	7.95
2	4.55	2.55	2.85	3.1	9.25	8.75
3	4.05	3.05	3.5	3.15	9.75	7.8
4	2.9	2.9	3.3	4.55	9.05	7.75
Average Resistance Power (mm)	4.30	2.84	3.41	3.71	9.59	8.06

Normality Test

The results of the normality test on the antibacterial activity of *S. aureus* bacteria obtained a normality test with a significance of 0.205 (base); 0.166 (formulation 1); 0.487 (formulation 2); 0.911 (formulation 3); 0.522 (50% papaya leaf extract) and 0.779 (positive control) which means that all preparations have a significant value > 0.05 or are normally distributed. Normality test on the antibacterial activity of *E. coli* bacteria obtained 0.987 (base); 0.305 (formula 1); 0.650 (formula 2); 0.981 (formula 3); 0.701 (50% papaya leaf extract) and 0.068 (positive control) which means that all preparations have a significant value > 0.05 or are normally distributed.

Homogeneity Test

The homogeneity test on *S. aureus* bacteria was found to be significant at 0.110, which means the data obtained was also homogeneous. Meanwhile, the homogeneity test on *E. coli* bacteria was found to be significant at 0.140, which means the data obtained was also homogeneous.

Anova Test

The anova test for *S. aureus* bacteria obtained a significant value of 0.000 < 0.05. Meanwhile, the ANOVA test on *E. coli* bacteria obtained a significant value of 0.000 < 0.05, so it can be concluded that variations in papaya leaf extract have significant differences in each liquid soap preparation in terms of the antibacterial activity of *S. aureus* and *E. coli*.

Conclusion

Based on the research that has been carried out, it can be concluded that papaya leaf extract (*Carica papaya* L.) which is added to the liquid soap formulation has organoleptic physicochemical characteristics, pH, foam height, specific gravity, water content, and free alkali which meets SNI quality and has antibacterial properties with bacterial inhibitory power in the weak category.

Conflict of Interests

The author(s) declares that there is no conflict of interest in this research and manuscript.

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