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MES SURFACTANT-BASED LIQUID SOAPS ADDED WITH ECO-ENZYME AND PANDAN WANGI LEAF EXTRACT (Pandanus amaryllifolius Roxb) ON PHYSICO-CHEMISTRY PROPERTIES, AND ANTIBACTERIAL ACTIVITY

Riawati, Diah Mardiana, Arie Srihardyastutie*

Department of Chemistry, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang, East Java, Indonesia

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ABSTRACT

The growing demand for liquid soap has spurred innovations in soap formulations, particularly using methyl ester sulfonate (MES) as a surfactant base combined with natural ingredients like eco-enzyme and fragrant pandan leaf extract. This study aimed to determine the optimal liquid soap formulation by evaluating physicochemical properties and antibacterial activity against *Staphylococcus aureus*. The research was conducted in two stages. First, liquid soap was produced at different temperatures (20°C, 50°C, and 100°C) to identify the optimal temperature based on maximum lipase activity. In the second stage, various formulations were prepared, incorporating eco-enzyme and fragrant pandan leaf extract at the identified optimal temperature. The six formulations tested were: F1 (MES-based soap), F2 (20% eco-enzyme), F3 (15% eco-enzyme and 5% fragrant pandan leaf extract), F4 (10% eco-enzyme and 10% fragrant pandan leaf extract), F5 (5% eco-enzyme and 15% fragrant pandan leaf extract), and F6 (20% fragrant pandan leaf extract). The formulations were assessed for lipase activity, pH, density, and viscosity. The most effective formulation was further tested for antibacterial activity using the disc diffusion method with six treatments, including MES-based soap and controls. Statistical analysis using One-Way ANOVA revealed that adding eco-enzyme and fragrant pandan leaf extract significantly affected the soap's properties. The optimal formulation, containing 5% eco-enzyme and 15% fragrant pandan leaf extract, exhibited a lipase activity of 15,778 U/mL, a pH of 5.02, a density of 1.06 g/mL, a viscosity of 3.59 cP, and an antibacterial zone of 37.22 mm, making it the best candidate for further development.

**Corresponding Author Email:* arie_s@ub.ac.id
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INTRODUCTION

Soap is an essential cleanser in daily life, used to remove impurities such as dust and grease that accumulate on the skin through direct interaction with the environment [1]. The demand for soap made from natural and organic ingredients is on the rise, driven by consumers' growing awareness of the environmental impact of their choices. Today's consumers increasingly prefer soap with eco-friendly packaging and products produced through sustainable practices. Factors such as heightened hygiene awareness, demand for eco-friendly products, technological innovations, and lifestyle changes have all contributed to the widespread use of soap [2].

While dust is easily removed with water, grease, a non-polar compound, is insoluble in water and requires surfactants to be effectively cleaned. Surfactants are active compounds that can dissolve grease in water due to their dual nature, with polar (hydrophilic) and non-polar (lipophilic) groups, which allows them to reduce surface tension [4]. Anionic surfactants, the primary components in detergents and other cosmetic cleaning products, are widely used as cleansers [5][6].

However, the widespread use of synthetic surfactants in households and industry raises environmental concerns. These surfactants, such as alkyl benzene sulfonate (LAS), Sodium Lauryl Sulfate (SLS), and triclosan, often end up in wastewater treatment plants or are directly discharged into the environment, causing ecological damage [7]. Therefore, there is a need for more environmentally friendly surfactants. Methyl ester sulfonate (MES) is a renewable surfactant that has gained attention for its environmental benefits, tolerance to water hardness, and excellent detergency and foaming properties, making it valuable in the soap and detergent industry [8][9]. MES is particularly favoured for its effectiveness as a cleaner and its biodegradability. Despite its stability in water and low foaming tendency, MES's straight carbon chain is biodegradable, making it an ideal component in eco-friendly soap and detergent formulations [4].

Various soap forms are available today, including cream, solid bars, powder, and liquid [10][11]. Among these, liquid soap is particularly favoured, whether natural or synthetic, due to its perceived hygienic and practical benefits [12]. Liquid soap dissolves more readily in water than solid or bar soap, making it more convenient [13]. As a result, natural liquid soap has gained popularity because it is considered more environmentally friendly and is generally free from harmful side effects [14]. The growing demand for natural and organic ingredients in soap reflects consumers' increasing awareness of the environmental impact of their choices, leading them to prefer products with eco-friendly packaging and sustainable production practices. Factors such as heightened hygiene awareness, the demand for eco-friendly products, technological advancements, and lifestyle changes have all contributed to the widespread use of soap in daily life [15]. This shift has intensified industrial competition as consumers increasingly seek environmentally friendly natural liquid soap [16].

Soap is typically produced through cold or hot processes, with temperature being a key differentiating factor. Temperature significantly influences the activity of the lipase enzyme, which is crucial for the soap's ability to clean. At higher temperatures, enzymes may undergo conformational changes that hinder substrate access to the enzyme's active site, thereby reducing effectiveness [17]. Maintaining an optimal temperature is essential to ensure the maximum performance of the lipase enzyme in cleaning products, thereby preserving the product's stability and effectiveness over time. In addition to its cleansing function, soap serves various purposes, including as an antibacterial agent [18], medication [19], beauty soap [20], antioxidant [21], and skin moisturiser [22].

This research focuses on producing an antibacterial soap using eco-enzymes combined with fragrant pandan leaf extract [23]. The fermentation process of eco-enzymes produces ethanoic acid and alcohol, which have germicidal properties, making them effective against viruses and bacteria [24]. Additionally, eco-enzymes contain lipase, protease, and amylase enzymes, which act as biocatalysts to reduce pollutants in wastewater and inhibit the growth of pathogenic bacteria [25]. Studies have shown that eco-enzyme production offers multiple benefits: it reduces organic waste, acts as an antibacterial agent, and can inhibit bacterial growth by approximately 99% at a 20% concentration. Eco-enzymes are environmentally friendly, easy to produce, and user-friendly [26][27].

Previous studies have demonstrated that the lipase enzyme produced from eco-enzyme fermentation can catalyse lipid hydrolysis, enhancing soap's effectiveness as both a detergent and antibacterial agent [28]. The antibacterial properties of soap can be further improved by incorporating additional components such as fragrant pandan leaves (*Pandanus amaryllifolius* Roxb), which have similar antibacterial roles. Pandan leaf extract is widely used for its distinctive aroma and as a natural dye. The leaves are readily accessible, often found in household gardens, and are rich in bioactive compounds such as saponins, alkaloids, flavonoids, and polyphenols, which have notable antibacterial potential. Therefore, combining these two natural ingredients—eco-enzyme and pandan leaf extract—is expected to enhance the efficacy of liquid soap production [29].

Fragrant pandan leaves contain secondary metabolites, including saponins, flavonoids, polyphenols, and alkaloids, contributing to their antibacterial properties [30][31]. Saponins function by altering cell permeability and disrupting bacterial cell membranes. Alkaloids interfere with the peptidoglycan components of bacterial cell walls, leading to imperfect cell wall formation and cell death. Phenolic compounds damage cell membranes and walls, disrupting the active transport system and affecting cell metabolism. Flavonoids inhibit nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism in bacteria.

Research has shown that pandan leaf extract exhibits significant inhibitory effects against various bacterial strains, including *Staphylococcus aureus* [32][33], *Staphylococcus epidermidis* [23], *Bacillus cereus* ATCC 11778, *Enterococcus faecalis*, *Shigella dysenteriae* ATCC 11835, *Vibrio cholerae* ATCC 14033, and *Escherichia coli* [29].

METHODS

1. Materials and Equipment

This research utilised a rotary evaporator (IKA® HB 10 Digytal) to concentrate the filtrate from the maceration of fragrant pandan leaf powder. The equipment used for soap production included analytical scales (Mettler Toledo), a hotplate stirrer (PMC), a beaker (Pyrex), a measuring flask (Pyrex), and a thermometer (Precision). An oven (Memmert), stands, clamps, and a burette (Duran) were employed to test the lipase activity of the liquid soap using the titrimetric method. A pH meter (Laqua) was used to measure the soap's acidity, a 10 mL pycnometer (Pyrex) for density testing, and a Cannon-Fenske viscometer for viscosity testing. Additional tools such as a vortex mixer (Heidolph), autoclave, UV-vis spectrophotometer (Shimadzu), and incubator (Nüve) were used in the antibacterial activity tests.

The materials used in the research included eco-enzymes made from pineapple peel, brown sugar, and water, fermented in the Biochemistry Laboratory at Brawijaya University for three months. Fragrant pandan leaves were obtained from PT. Materia Medica Batu in Malang, East Java, Indonesia. Other materials included 96% ethanol, MES, distilled water, 0.05 N NaOH, Nutrient Agar (Oxoid), and *Staphylococcus aureus* bacteria obtained from the Faculty of Medicine, Brawijaya University.

2. Eco-Enzyme Fermentation

The eco-enzyme was produced by mixing 360g of pineapple peel, 120g of brown sugar, and 1200 mL of water (in a 3:1:10 ratio) and fermenting the mixture for three months under anaerobic conditions. Pineapple peel was chosen due to its richness in bromelain and phenol enzymes, which have antibacterial properties. Brown sugar was used as a substrate because the sugar content influences the amount of alcohol produced during fermentation, and alcohol significantly inhibits bacterial growth. Thus, the combination of pineapple peel and brown sugar in the eco-enzyme is expected to enhance the antibacterial activity of the liquid soap.

3. Pandan Wangi Leaf Extraction

The extraction process was conducted using the maceration method. One kilogram of fragrant pandan leaf powder was soaked in 2 litres of distilled water in a covered container for 24 hours, with occasional stirring. After 24 hours, the mixture was filtered using a funnel lined with filter paper to obtain the filtrate, which was then placed in a brown bottle. The filtrate was concentrated using a rotary evaporator, and the final extract was stored in a refrigerator until use in liquid soap production. The yield of the extract was calculated using the following formula:

$$\%\ \text{yield} = \frac{extract\ weight}{sample\ weight}\ x\ 100\% \qquad (1)$$

Distilled water was chosen as the solvent for pandan leaf extraction due to its high polarity, which makes it effective at dissolving a wide range of polar compounds from plants. Additionally, distilled water is non-toxic, non-flammable, and cost-effective. The extraction process involved careful control of time and temperature, as these parameters are crucial in optimising the dissolution of active ingredients while minimising oxidation and degradation. Stirring during maceration helped accelerate the equilibrium concentration of the extracted material in the solvent, and covering the extraction container with aluminium foil maintained the stability of the solvent concentration, preventing evaporation.

4. The Making of Liquid Soap and the Effect of Adding Eco-Enzyme and Fragrant Pandan Leaf Extract at Various Temperatures

7.5 g of MES was dissolved in 70 mL of distilled water to prepare the liquid soap and heated until fully dissolved. Following this, 5 mL of eco-enzyme and 5 mL of fragrant pandan leaf extract were added at temperatures of 100°C, 50°C, and 25°C, along with a sufficient amount of NaCl, and the mixture was stirred until homogeneous. The solution was then transferred to a 100 mL measuring flask, and distilled water was added to reach the final volume. The optimal temperature for the soap formulation was determined based on the highest lipase activity observed.

5. The Making of Liquid Soap by Varying the Composition of Eco-Enzyme and Fragrant Pandan Leaf Extract

In this step, 7.5 g of MES was again dissolved in 70 mL of distilled water and heated until completely dissolved. Eco-enzyme and fragrant pandan leaf extract were added in various compositions at the previously determined optimal temperature, along with NaCl. After thoroughly mixing all the ingredients, the mixture was transferred to a 100 mL measuring flask, and distilled water was added to reach the final volume. The resulting liquid soap formulations were analysed for lipase activity, and the data was recorded. The specific formulations based on the variations in composition ratios are detailed in Table 1.

Table 1. Soap Making Formulation Based on Composition Comparisons

The making of liquid soap using MES-based surfactants with a composition ratio of *eco-enzyme* and Pandan Wangi leaf extract at optimum temperature					
Formulation	MES (g)	*Eco-enzyme* (mL)	Pandan Wangi Leaf Extract (g)	Aquadest (mL)	Nacl (g)
Control	7.5	-	-	70	1.0
1	7.5	20	-	70	1.0
2	7.5	15	5	70	1.0
3	7.5	10	10	70	1.0
4	7.5	5	15	70	1.0
5	7.5	-	20	70	1.0

6. Lipase Activity Test

Lipase activity was tested using the titration method. First, the soap solution was incubated and then titrated with 0.05 N NaOH. The lipase activity was calculated using the following equation:

$$\text{Lipase Activity} = \frac{(A-B)x\ 100\ xN(NaOH)}{V\ x\ t} \quad (2)$$

Description:
A= mL NaOH for titration sample (mL)
B= mL NaOH for blanko titration (mL)
W= Sample volume (mL)
t = Incubation duration (minute)

7. pH Test

The pH test involved mixing 1.5 mL of the soap solution with 15 mL of distilled water and leaving it for 24 hours to ensure complete dissolution. The pH of each sample was then measured, and the pH value displayed on the pH meter was recorded.

8. Density Test

For the density test, a clean and dry pycnometer was first weighed, with the empty pycnometer's weight recorded as weight (a). Next, 10 mL of the sample was added to the pycnometer, which was then weighed again, recording this as weight (b). The density (ρ\rhoρ) was calculated using the formula:

$$\rho = \frac{b-a\ (gr)}{pycnometer\ volume\ (mL)} \qquad (3)$$

9. Viscosity Test

Viscosity testing was conducted using a Cannon-Fenske viscometer. The viscometer was cleaned with a solvent before adding 10 mL of soap solution using a measuring pipette. The viscometer was placed in a buffer and left for 10 minutes to reach equilibrium temperature. The time required for the soap solution to pass between the two marks on the viscometer was measured by drawing the solution through the capillary using a suction ball. The viscosity (η\etaη) was calculated using the formula:

$$\frac{\eta}{\eta_0} = \frac{t\rho}{t_0\rho_0} \qquad (4)$$

Description:
η and η_0 = solution and solvent viscosity
t and t_0 = flow time of solution and solvent
ρ and ρ_0 = density of solution and solvent

10. Antibacterial Test

The antibacterial activity test began with the preparation of the medium. First, 7 grams of Nutrient Agar (NA) were dissolved in 250 mL of distilled water and then heated while stirring until homogeneous and boiling. The mixture was sterilised in an autoclave at 121°C under 15 PSI pressure for 15 minutes. After sterilisation, 7 mL of NA media was poured into test tubes, sealed with sterile cotton and aluminium foil, and allowed to solidify at a slight angle.

Next, *Staphylococcus aureus* bacteria were rejuvenated by streaking them onto NA media using an inoculation loop and incubating them at 37°C for 24 hours. To prepare the bacterial suspension, 9 mL of 0.9% NaCl was added to a test tube, into which a loopful of the *Staphylococcus aureus* culture was added and vortexed until homogeneous. The optical density (OD) was measured using a UV-vis spectrophotometer at a wavelength of 600 nm, with a target cell density of 0.6, equivalent to 10610^6106 CFU/mL.

For the antibacterial activity test, sterile paper discs made from Whatman filter paper (approximately 6 mm in diameter) were immersed in 30 µL of the test solution for 10 minutes. The discs were then placed on an NA medium inoculated with *Staphylococcus aureus* and allowed to solidify. The plates were incubated at 37°C for 24 hours. The presence of a clear zone around the paper disc indicated antibacterial activity. The diameter of the clear zone was measured three times at different positions and averaged.

11. Data Analysis

` The liquid soap formulations were analysed using the One-Way ANOVA test, conducted with IBM SPSS statistical software at a 95% confidence level (α=0.05). A Tukey HSD (Honestly Significant Difference) post-hoc test was performed to determine differences between treatment groups. Experimental data are presented as mean ± standard deviation (SD). A p-value < 0.05 was considered statistically significant, indicating a significant difference between the soap formulations and the control after adding eco-enzyme and fragrant pandan leaf extract.

RESULTS AND DISCUSSION

1. Lipase Activity

Lipase is a water-soluble enzyme catalyses the hydrolysis of ester bonds in water-insoluble lipid substrates. When added to soap or other cleaning agents, lipase enhances the breakdown of ester bonds into simpler molecules, making them more soluble in water. This process improves the soap's effectiveness in removing fat and dirt from surfaces, thereby helping to prevent the growth of pathogenic bacteria on the skin [16].

a. The Effect of Temperature Variations, Addition of Eco-Enzyme, and Fragrant Pandan Leaf Extract in Liquid Soap

This study examined the effect of temperature variations on adding eco-enzyme and fragrant pandan leaf extract to liquid soap formulations using different surfactants. The soap was prepared at three different temperatures: 25°C, 50°C, and 100°C, with the addition of 5 mL of eco-enzyme and 5 mL of fragrant pandan leaf extract to each formulation. After thoroughly mixing all the ingredients, the solutions were homogenised in a 100 mL measuring flask. The soap rested for one day to enhance its stability before testing.

The optimal temperature for soap formulation was determined by measuring the highest lipase activity and assessed using titration (titrimetry). Acid-base titration was employed, with NaOH as the titrant. The endpoint of the titration was identified by a stable and consistent colour change of the indicator, signalling the completion of the reaction.

Table 2. Lipase Activity at Various Temperatures

No.	Temperature Variations	Lipase Activity (U/mL) ±SD	
		MES	MES + *Eco-enzyme* + Pandan Wangi Leaf Extract
1	25 ºC	0,33 ± 0,011^{b}	9,33 ± 0,222^{b}
2	50 ºC	0,43 ± 0,011^{c}	11,48 ± 0,339^{c}
3	100 ºC	0,27 ± 0,023^{a}	6,66 ± 0,222^{a}

Temperature is one of the key factors influencing lipase performance, with the enzyme exhibiting varying activity levels across different temperature ranges [34]. At 25°C and 100°C, lipase activity was less pronounced than at 50°C. At 25°C, the lipase may still be localised within the microorganism cells that produce it in the eco-enzyme [35].

At 50°C, lipase activity increased, likely due to hydrolysis in the lipase cell wall, which allowed the enzyme to mix more effectively with the soap. However, at 100°C, there was a significant decrease in lipase activity compared to the activity observed at 25°C and 50°C. This decline at higher temperatures can be attributed to the increased molecular movement of the lipase enzyme, destabilising hydrogen bonds and non-covalent interactions. These changes can cause the enzyme to denature, altering its shape and reducing its ability to interact with the substrate, thereby decreasing its overall activity [36].

b. Effect of Composition Variations, Addition of Eco-Enzyme, and Fragrant Pandan Leaf Extract in Liquid Soap

Liquid soap was also prepared with variations in the composition of eco-enzyme and fragrant pandan leaf extract under the optimal temperature conditions identified earlier to determine the composition ratio that yielded the highest lipase activity. The results of these experiments are presented in Table 3.

Table 3. Lipase Activity of Liquid Soap with variations in composition, addition of *Eco-Enzyme*, and Pandan Wangi Leaf Extract

No.	Variations in the additional compositions of *Eco-enzyme*: Pandan Wangi leaf extract	Lipase Activity (U/mL) ± SD
1	MES	0,085 ± 0,006[a]
2	20% *Eco-enzyme*	12,000 ± 0,222[c]
3	15% *Eco-enzyme*: 5% Pandan Wangi leaf extract	13,778 ± 0,222[d]
4	10% *Eco-enzyme*: 10% Pandan Wangi leaf extract	14,889 ± 0,222[e]
5	5% *Eco-enzyme*: 15% Pandan Wangi leaf extract	15,778 ± 0,222[f]
6	20% Pandan Wangi leaf extract	6,666 ± 0,222[b]

Based on Table 3, the results of the lipase activity test in liquid soap with varying compositions and the addition of eco-enzyme and fragrant pandan leaf extract show significant differences, as indicated by distinct notations for each variation. The data demonstrate that higher concentrations of eco-enzyme lead to increased lipase activity. Specifically, the liquid soap with a composition of 5% eco-enzyme and 15% pandan leaf extract exhibited the highest lipase activity.

This heightened activity may be attributed to the solution's thickness during the lipase activity test, where the higher percentage of pandan leaf extract contributed to a noticeable colour change to pink. Additionally, during the titration process, the increased volume of NaOH required was challenging to observe, which may have impacted the accuracy of the calculation results.

These findings are consistent with the theory that increasing enzyme concentration enhances the rate of enzymatic reactions. Increasing the substrate concentration can boost enzyme activity up to a certain maximum limit when enzyme concentration remains constant. However, once all enzymes become saturated with substrate, further increases in substrate concentration will no longer result in higher lipase activity [37].

Based on Figure 1, it is evident that liquid soap with MES as the primary ingredient produces a transparent white colour. In contrast, liquid soap containing eco-enzyme and fragrant pandan leaf extract exhibits a brown colour, with the shade deepening as the concentration of pandan leaf extract increases. The colour change in liquid soap formulated with eco-enzymes and fragrant pandan leaf extract is due to chemical interactions between the enzymes, active compounds from the pandan leaves, and the soap components. The pandan leaf extract also contains chlorophyll, which naturally imparts a green colour. However, changes in pH or other chemical interactions can alter chlorophyll's colour from green to brown or yellow [23].

(A) (B)

Figure 1. Liquid soap visualisation. (A) Basic and (B) soap with various compositions are added with *eco-enzyme* and pandan wangi leaf extract. Orderly from left to right: 20% of eco-enzyme, 15% of *eco-enzyme*: 5% of pandan wangi leaf extract, 10% of *eco-enzyme*: 10% of pandan wangi leaf extract, 5% of *eco-enzyme*: 15% of pandan wangi leaf extract, and 20% of pandan wangi leaf extract.

2. pH Test

Liquid soap made from MES-based surfactants, with the addition of eco-enzyme and fragrant pandan leaf extract, was subjected to a pH test to determine whether the soap was acidic, basic, or neutral. Proper pH testing is crucial, as the pH level can significantly impact the soap's cleaning performance and consumer comfort, ensuring that the soap does not cause irritation or other harmful side effects to the skin. The ideal pH range for liquid soap stability and skin compatibility is typically between 4.5 and 7.5, which is considered optimal for maintaining the stability of liquid soap formulations [10].

Table 4. Liquid Soap pH Test Results

No.	Variations in the additional compositions of *Eco-enzyme*: Pandan Wangi leaf extract	pH ± SD
1	MES	4,19 ± 0,0115^{b}
2	20% *Eco-enzyme*	3,28 ± 0,0100^{a}
3	15% *Eco-enzyme*: 5% of Pandan Wangi leaf extract	4,71 ± 0,0100^{c}
4	10% Eco-enzyme: 10% of Pandan Wangi leaf extract	4,91 ± 0,0100^{e}
5	5% Eco-enzyme: 15% of Pandan Wangi leaf extract	5,02 ± 0,0100^{f}
6	20% of Pandan Wangi leaf extract	4,87 ± 0,0152^{d}

Based on Table 4, significant differences in the pH test results are observed for each variation of liquid soap, as indicated by distinct notations for each formulation. The soap with the highest pH, at 5.02, was found in the formulation with a ratio of 5% eco-enzyme to 15% pandan wangi leaf extract. This suggests that the addition of pandan leaf extract can influence the pH of the soap, likely due to the alkaline properties of pandan leaves, which contain compounds such as alkaloids and saponins.

Consequently, as the concentration of pandan leaf extract increases, so does the pH of the soap.

The surfactant MES also affects the pH, typically lower (around pH 4) due to the sulfonic group releasing H+ ions more readily, resulting in a more acidic solution. The data in Table 4 show that the pH of liquid soap increases with the addition of fragrant pandan leaf extract, reflecting the alkaline nature of its components. However, the addition of eco-enzymes also influences the pH. Formulations with only pandan leaf extract exhibit lower pH levels, even when the concentration of pandan extract is higher, compared to the formulation with 5% eco-enzyme and 15% pandan leaf extract. It is important to note that if the soap's pH is too high (pH > 11), it may cause skin irritation, such as dryness and irritation. Conversely, a low or acidic pH (pH < 8) can also lead to skin discomfort, including itching and sores. The liquid soap produced in this study had a slightly acidic pH because it did not use alkali as a primary raw material. This formulation is categorised as mild soap in the cosmetics industry, which is particularly suitable for babies, children, and adults with sensitive skin. Previous research indicates that cleaning products safe for babies should have a pH close to 5.5. Furthermore, soap with this pH is recommended for individuals with skin barrier conditions, such as atopic dermatitis and ichthyosis. High-quality soap can exacerbate xerosis and compromise skin barrier function, making the skin more susceptible to pathogenic bacteria [39].

3. Density Test

The density test determines liquid soap's consistency and viscosity or density. Density also affects how the soap is distributed to clean the dirt and bacteria on the skin more effectively.

Table 5. Liquid Soap Density Test Results

No.	Variations in the additional compositions of *Eco-enzyme*: Pandan Wangi leaf extract	Density Value (gr/mL) ± SD
1	MES	0,98 ± 0,01528[a]
2	20% *Eco-enzyme*	0,99 ± 0,01528[b]
3	15% *Eco-enzyme*: 5% Pandan Wangi leaf extract	1,01 ± 0,01000[c]
4	10% *Eco-enzyme*: 10% Pandan Wangi leaf extract	1,02 ± 0,01000[d]
5	5% *Eco-enzyme*: 15% Pandan Wangi leaf extract	1,06 ± 0,01000[e]
6	20% Pandan Wangi leaf extract	1,08 ± 0,01000[f]

Based on Table 5, the density of the liquid soap increases as the amount of pandan leaf extract used in the formulation increases. This is likely because a higher extract concentration contributes to the overall weight of the soap, leading to an increase in density. The increased density suggests that the water content in the soap is decreasing, resulting in more closely packed particles [38]. The type of surfactant used also plays a role; for instance, MES has a longer carbon chain (C16) and a high boiling point, making it more challenging to dissolve [40]. Additionally, the inclusion of salt in the formulation affects the viscosity of the soap.

The liquid soaps produced with varying compositions exhibit significantly different densities, ranging from 0.99 to 1.08 g/mL, which aligns with findings from previous research [41]. The composition of the formula directly influences the specific gravity of the soap. Ideally, the specific gravity of liquid soap should be close to that of water, around 1.01 g/mL. A significant difference between the specific gravity of the oil and water phases can lead to emulsion instability. Viscosity is another factor that impacts the specific gravity of liquid soap. The viscosity also affects consumer acceptance, as consumers prefer thicker liquid soap [41].

4. Viscosity Test

The viscosity test is one part of the soap parameter test that determines the quality of the soap supply that is suitable for use. The test results can be seen in Table 6.

Table 6. Viscosity Test Results of Liquid Soap

No	Liquid soap with the addition of *Eco-enzyme*: Pandan Wangi leaf extract	Viscosity cP ± SD
1	20% *Eco-enzyme*	1,07 ± 0,010^{a}
2	15% *Eco-enzyme*: 5% Pandan Wangi leaf extract	1,22 ± 0,015^{b}
3	10% *Eco-enzyme*: 10% Pandan Wangi leaf extract	2,28 ± 0,402^{c}
4	5% *Eco-enzyme*: 15% Pandan Wangi leaf extract	3,59 ± 0,025^{d}
5	20% Pandan Wangi leaf extract	5,61 ± 0,010^{e}

According to the data in Table 6, the viscosity test results show an increase in thickness as the concentration of pandan wangi leaf extract increases. This is because viscosity and density are closely related; as the density of the soap increases, the viscosity also increases proportionally [42]. This finding aligns with previous research, where the viscosity of liquid soap, without the addition of thickening agents, ranged from 1.07 to 5.61 cP [39]. The increase in viscosity with higher concentrations of fragrant pandan leaf extract is attributed to the corresponding increase in specific gravity.

Viscosity plays a crucial role in the pourability of liquid soap. If the viscosity is too high, the soap may be difficult to dispense from its container. Conversely, if the viscosity is too low, the soap will flow too easily, leading to faster depletion [39].

5. Antibacterial Activity Test

Liquid soap is a crucial component of daily hygiene, effectively removing dirt, dust, and oil accumulated from environmental exposure [27]. To ensure its effectiveness and safety, an antibacterial test is necessary. The antibacterial activity of the soap was evaluated using the disc diffusion method against *Staphylococcus aureus* bacteria. The agar disc diffusion method is widely used in microbiology laboratories to assess antibacterial activity. The zone of inhibition observed in this test is critical, as it indicates the effectiveness of the antibacterial agent in inhibiting bacterial growth. A larger zone of inhibition suggests a more potent antibacterial effect, highlighting the agent's potential as an antimicrobial [43].

The area of the clear zone formed around the disc represents the inhibitory power of the antimicrobial compounds against bacterial growth [44]. The principle of this method is that antibacterial compounds diffuse into the agar and inhibit the growth of the test microorganism, resulting in a clear zone where bacterial growth is suppressed [45]. *Staphylococcus aureus* was selected for this test because it is commonly found on the skin and is a relevant target for antibacterial soap [46].

Based on the lipase activity test results and physicochemical properties, the liquid soap formulation containing 5% eco-enzyme and 15% pandan wangi extract was identified as the optimal preparation and was subsequently tested for antibacterial activity. The zone of inhibition in the antibacterial activity test is categorised into several criteria: an inhibition zone with a diameter of <5 mm is considered weak, 5-9 mm is medium, 10-19 mm is strong, and >20 mm is very strong [12]. Based on Table 7 and Figure 2, the antibacterial activity of liquid soap containing 5% eco-enzyme and 15% pandan wangi leaf extract demonstrated a very strong inhibition zone of 37.22 mm against *Staphylococcus aureus*. Liquid soap with the addition of 5% eco-enzyme produced an inhibition zone of 15.23 mm, and liquid soap with 15% pandan wangi leaf extract resulted in an inhibition zone of 17.13 mm. Both formulations provided strong bacterial inhibition.

Liquid soap containing only MES surfactant as the basic ingredient exhibited a medium-level antibacterial effect, with an inhibition zone of 6.3 mm. The positive control (Control +) had an inhibition zone of 25.86 mm, also categorised as very strong, while the negative control (Control -) showed no inhibition.

The antibacterial efficacy of the liquid soap can be attributed to the presence of flavonoids, which disrupt bacterial cell walls, and alkaloids, which interfere with the peptidoglycan component of bacterial cells, inhibiting protein synthesis and leading to bacterial death [46]. Saponins also contribute to antibacterial activity by interacting with membrane sterols, forming foam that can disrupt and kill bacteria by releasing proteins and enzymes from the cells [24]. Additionally, the eco-enzyme content, including acetic acid and enzymes like lipase, amylase, and protease, further enhances the antibacterial properties of the soap. These findings align with previous research on the antibacterial effects of eco-enzymes produced from fruit peel waste [47, 45, 46].

Table 7. Antibacterial Activity Test Results of Liquid Soap

No.	Treatments	Inhibitory power ± SD	Category
1	MES	6,33 ± 6,28	Medium
2	5% *Eco-enzyme*	15,23 ± 1.12	Strong
3	15% Pandan Wangi leaf extract	17, 13 ± 8,14	Strong
4	5% *Eco-enzyme*: 15% Pandan Wangi leaf extract	37,22 ± 1,56	Very Strong
5	control +	25,86 ± 7,61	Very Strong
6	control -	-	-

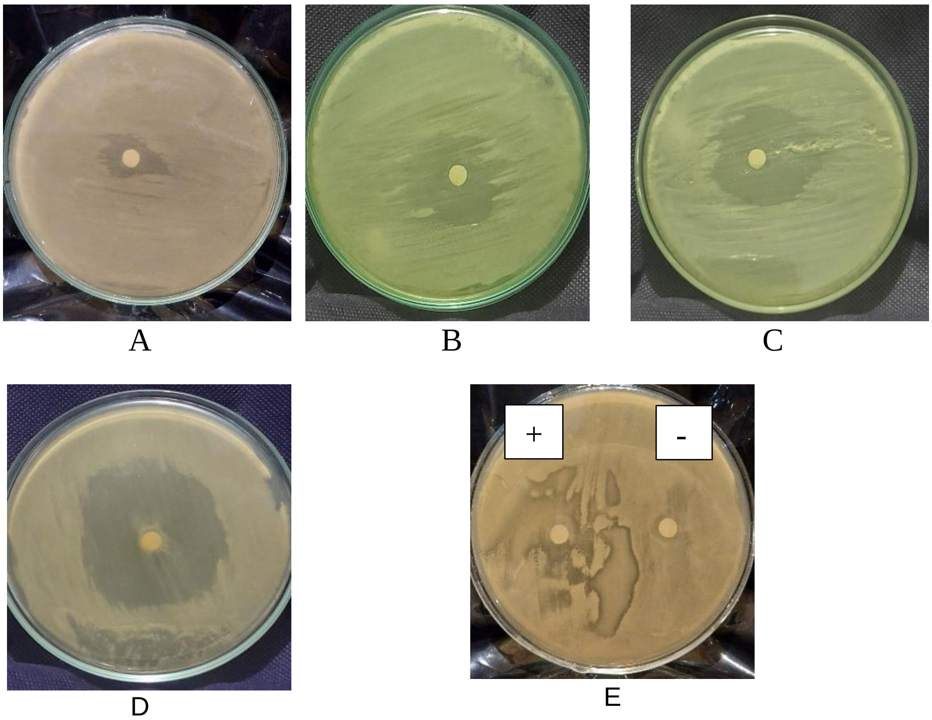

Figure 2. The samples of the antibacterial activity test of liquid soap against *Staphylococcus aureus* bacteria. (A) liquid soap made from MES, (B) liquid soap with the addition of 5% *eco-enzyme*, (C) liquid soap with the addition of 15% pandan wangi leaf extract, (D) liquid soap with the addition of 5% *eco-enzyme*: 15 % pandan wangi leaf extract, and (E) control (+ and -)

CONCLUSION

Based on the research results and discussion, it was concluded that MES surfactant-based liquid soap with the addition of eco-enzyme and pandan wangi leaf extract significantly influenced the properties of the liquid soap. The lipase activity test results indicated increased lipase activity with higher concentrations of eco-enzyme. The liquid soap exhibited an acidic pH in the pH test, as it did not use alkali as a raw material. The pH values for the eco-enzyme, MES, and pandan wangi leaf extract were approximately 3.4, 4.19, and 5.0, respectively. The addition of fragrant pandan leaf extract notably influenced the pH of the soap, making it more acidic with higher concentrations of the extract. The density and viscosity tests revealed that these properties were also affected by adding pandan wangi leaf extract, with higher concentrations leading to increased density and viscosity, demonstrating the direct proportionality between density and viscosity. The antibacterial activity test, conducted on the most optimal liquid soap formulation based on lipase activity and physicochemical tests, showed a strong antibacterial effect against *Staphylococcus aureus*, with an inhibition zone exceeding 20 mm. Consequently, the liquid soap formulation chosen for this study contained 5% eco-enzyme and 15% pandan wangi leaf extract, which most closely resembled typical liquid soap preparations. The final product demonstrated a lipase activity of approximately 15.778 U/mL, a pH of 5.02, a specific gravity of 1.06 g/mL, a viscosity of 3.59 cP, and an antibacterial activity with an inhibition zone of 37.22 mm against *Staphylococcus aureus*.

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REFERENCES

[1] C. Nurhayati, N. Susilawati, T. Susanto, W. Marthalia, and A. K. Nugroho, "Gambier leaf extract (Uncaria gambier rob) is used as an antibacterial and natural dye in making liquid bath soap with atrium lauryl ether sulfate," *IOP Conference Series: Earth and Environmental Science*, vol. 963, no. 1, 2022. doi:0.1088/1755-1315/963/1/012059.

[2] A. S. Yusuff, A. O. Gbadamosi, F. B. Bode-Olajide, and A. I. Igbafe, "Adsorption of methyl ester sulfonate surfactant on Berea sandstone: Parametric optimisation, kinetics, isotherm, and thermodynamics studies," *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, vol. 686, p. 133363, 2024. doi:10.1016/j.colsurfa.2024.133363.

[3] D. N. Afifah, R. F. Maulidina, N. Astuti, and R. A. Wahyadi, "Application of Saponins from Ambon Banana Petiole (Musa paradisiaca var. sapientum L.) as Natural Surfactants in Bio-Hand Soap," *Research in Chemical Engineering*, vol. 2, no. 1, pp. 08–13, 2023. doi:10.30595/rice.v2i1.80.

[4] I. F. Wulandari, F. Darusman, and M. L. Dewi, "Kajian Pustaka Surfaktan dalam Sediaan Pembersih," *Bandung Conference Series: Pharmacy*, vol. 2, no. 2, pp. 374–378, 2022. doi:10.29313/bcsp.v2i2.4203.

[5] K. Pawlak and K. Wojciechowski, "Precursor ion approach for simultaneous determination of nonethoxylated and ethoxylated alkyl sulfate surfactants," *Journal of Chromatography A*, vol. 1653, p. 462421, 2021. doi:10.1016/j.chroma.2021.462421.

[6] G. M. El Zokm, G. F. El-Said, and M. A. Okbah, "Impact of land development along the western Mediterranean coast of Egypt regarding surfactant sources, interfering elements and ecotoxicity," *Marine Pollution Bulletin*, vol. 203, p. 116372, 2024. doi:10.1016/j.marpolbul.2024.116372.

[7] G. Jena, K. Dutta, and A. Daverey, "Surfactants in water and wastewater (greywater): Environmental toxicity and treatment options," *Chemosphere*, vol. 341, p. 140082, 2023. doi:10.1016/j.chemosphere.2023.140082

[8] N. L. P. K. V. Nirmalayanti, "Skrining Berbagai Jenis Surfaktan Dan Kosurfaktan," *Ilmu Multidisiplin*, vol. 1, no. 3, pp. 158–166, 2021.

[9] A. F. Haque, E. Mulyani, and J. Hendick, "Formulasi Sabun Cair Cuci Tangan Antibakteri Ekstrak Etanol Daun Cabe Rawit (Solanum frutescens.L)," *Indonesian Journal of Pharmacy Education*, vol. 2, no. 2, pp. 152–160, 2022. doi: 10.37311/ijpe.v2i2.15510.

[10] P. Satrimafitrah, M. Afdal, Jusman, A. R. Razak, A. Ridhay, and N. I. Inda, "Viskositas dan Aktivitas Antibakteri Sabun Cair Berbasis VCO dengan Penambahan Ekstrak Etanol Daun Kelor (Moringa oleifera) Terhadap Bakteri Patogen," *KOVALEN: Jurnal Riset Kimia*, vol. 8, no. 1, pp. 74–82, 2022. doi:10.22487/kovalen.2022.v8.i1.15846.

[11] S. Widyaningsih, M. Chasani, H. Diastuti, and Novayanti, "Formulation of Antibacterial Liquid Soap from Nyamplung Seed Oil (Calophyllum inophyllum L) with Addition of Curcuma heyneana and its Activity Test on Staphylococcus aureus," *IOP Conference Series: Materials Science*

and Engineering, vol. 349, no. 1, 2018. doi:10.1088/1757-899X/349/1/012062.

[12] A. Rahman and V. Paramita, "Temperature Optimization and Addition of Aloe Vera Gel as Antibacteria In Coconut Oil-Based Liquid Soap," *Journal of Vocational Studies on Applied Research*, vol. 3, no. 2, pp. 52–55, 2021. doi:10.14710/jvsar.v3i2.12655.

[13] A. D. P. Zari, L. E. Wahyuningtyas, V. Nurhadianty, and S. Hidayat, "Fortifikasi Sabun Cair oleh Ekstrak Daun Salam," *Jurnal Rekayasa Bahan Alam dan Energi Berkelanjutan*, vol. 6, no. 1, pp. 34–41, 2022.

[14] T. Mahendra and N. Aryani, "Optimization of nanoparticle-based liquid soap," *Journal of Nanotechnology*, vol. 31, no. 10, pp. 2394–2396, 2019.

[15] A. Widyasanti, C. L. Farddani, and D. Rohdiana, "Pembuatan Sabun Padat Transparan Menggunakan Minyak Kelapa Sawit (Palm Oil) dengan Penambahan Bahan Aktif Ekstrak Teh Putih (Camellia sinensis)," *Jurnal Teknologi Agro-Industri*, vol. 5, no. 3, pp. 125–136, 2016.

[16] A. Asnani, E. V. Y. Delsy, and H. Diastuti, "Transfer Teknologi Produksi Natural Soap-Base untuk Kreasi Sabun Suvenir," *Jurnal Pengabdian kepada Masyarakat (Indonesian Journal of Community Engagement)*, vol. 4, no. 2, p. 129, 2019. doi:10.22146/jpkm.33581.

[17] M. Wathoni, A. Susanto, A. Kirana, and D. Putri, "Pemanfaatan Bahan Rumah Tangga dalam Pembuatan Sabun Cair dari Sabun Batang di Masa Pandemi," *Seminar Nasional Pengabdian Masyarakat LPPM UMJ*, pp. 1–6, 2019.

[18] C. Ndiaye, F. Maiga, D. Gueye, A. D. Gueye, and N. Ndiaye, "The impact of daily soap use in rural areas of Senegal on respiratory infectious diseases, fevers and skin microbiota," *International Journal of Infectious Diseases*, vol. 96, pp. 408–415, 2020. doi:10.1016/j.ijid.2020.04.076.

[19] H. Widiastuti and S. Maryam, "Sabun Organik: Pengenalan, Manfaat dan Pembuatan Produk," *Jurnal Pengabdian pada Masyarakat*, vol. 7, no. 1, pp. 46–55, 2022.

[20] W. Pangestika, S. Abrian, and R. Adauwiyah, "Pembuatan Sabun Mandi Padat dengan Penambahan Ekstrak Daun Avicennia Marina," *Jurnal Teknologi Agro-Industri*, vol. 8, no. 2, pp. 135–153, 2021. doi:10.34128/jtai.v8i2.146.

[21] D. Ramadian, H. Dewi, Zulhamidi, and R. A. Amris, "Pelatihan Pembuatan Sabun Cair Dan Sabun Transparan Di Kenagarian Pasie Laweh," *Journal of Science and Society Development*, vol. 2, no. 2, pp. 106–109, 2019.

[22] C. E. Hidayani, C. N. Ginting, and L. Chiuman, "Analysis of anti-bacterial activity of ethanol extract fragrant Pandan leaves (Pandanus amaryllifolius Roxb) against the growth of disease cause pathogen bacteria using the agar diffusion method," *Budapest International Research and Critics in Exact Sciences Journal (BirEx-J)*, vol. 3, no. 3, pp. 213–228, 2021. doi:10.33258/birex.v3i3.1177.

[23] S. I. Ratiani, R. Amir, S. B. Shapira, and N. S. Wahyuni, "Utilization of Eco-Enzyme Technology from Tomato Waste as Raw Material for Making Hand Sanitizer and Testing Its Inhibitory Power Against Staphylococcus Aureus Bacteria," *Proceedings of the International Conference on Religious and Science Education*, vol. 3, pp. 905–912, 2024.

[24] M. R. Rahayu, I. N. Muliarta, and Y. P. Situmeang, "SEAS (Sustainable Environment Agricultural Science) Acceleration of Production Natural Disinfectants from the Combination of Eco-Enzyme Domestic Organic Waste and Frangipani Flowers (Plumeria alba)," *Jurnal Rekayasa Bahan Alam dan Energi Berkelanjutan*, vol. 5, no. 1, pp. 15–21, 2021.

[25] D. A. Sipayung, N. M. Titiaryanti, Y. Theresia, and M. Astuti, "Pengaruh Konsentrasi dan Cara Aplikasi Eco Enzyme terhadap Pertumbuhan Bibit Kelapa Sawit di Pre Nursery," *Jurnal Rekayasa Bahan Alam dan Energi Berkelanjutan*, vol. 1, pp. 90–94, 2023.

[26] P. A. Handayani, D. R. Aditiya, and J. O. Almaidah, "Production of Liquid Soap from Virgin Coconut Oil with the Active Ingredient Citronella Oil," *Sainteknol: Jurnal Sains dan Teknologi*, vol. 21, no. 1, pp. 36–42, 2023. doi:10.15294/sainteknol.v21i1.44060.

[27] T. Fadlilla, M. S. Budiastuti, and M. M. A. R. Rosariastuti, "Potential of Fruit and Vegetable Waste as Eco-enzyme Fertilizer for Plants," *Jurnal Penelitian Pertanian Indonesia (JPPIPA)*, vol. 9, no. 4, pp. 2191–2200, 2023. doi:10.29303/jppipa.v9i4.3010.

[28] S. A. K. Natsir, Lahming, and R. F. Rauf, "Utilization of Pandan Leaf Extract (Pandanus Amaryllifolius) in Edible Film Substitute for Ready-to-Brew Coffee Packaging and Gives the Taste of Pandan Coffee," *Formosa Journal of Applied Sciences*, vol. 2, no. 10, pp. 2701–2710, 2023. doi:10.55927/fjas.v2i10.6378.

[29] Jacky, D. A. Putri, and M. Azizah, "Uji Aktivitas Anti Bakteri Ekstrak Etanol Daun Pandan Wangi (Pandanus Amaryllifolius Roxb) Terhadap Bakteri Penyebab Diare," *Jurnal Kesehatan Saelmakers Perdana*, vol. 2, pp. 91–98, 2019.

[30] P. Nancy, C. Bali, A. Raif, and S. B. Tarigan, "Uji Efektivitas Daun Pandan Wangi (Pandanus Amaryllifolius Roxb.) sebagai Antibakteri terhadap Salmonella Typhi," *BioLink Journal*, vol. 6, no. 1, 2019. doi:10.31289/biolink.v6i1.2218.

[31] A. Diningsih, "The Aktivitas Antibakteri Handsanitizer Ekstrak Daun Pandan Wangi (Pandanus amaryllifolius Roxb)," *Jurnal Kesehatan Ilmiah Indonesia (Indonesian Health Sciences Journal)*, vol. 7, no. 1, p. 244, 2022. doi: 10.51933/health.v7i1.801.

[32] F. Mursyida, H. Febriani, and R. Rasyidah, "Uji Efektivitas Antibakteri Ekstrak Daun Pandan Wangi (Pandanus amaryllifolius Roxb.) terhadap Pertumbuhan Bakteri Staphylococcus epidermidis," *KLOROFIL: Jurnal Ilmu Biologi dan Terapannya*, vol. 5, no. 2, p. 102, 2021. doi:10.30821/kfl.v5i2.10271.

[33] P. Chandra, Enespa, R. Singh, and P. K. Arora, "Microbial lipases and their industrial applications: A comprehensive review," *Microbial Cell Factories*, vol. 19, no. 1, 2020. doi:10.1186/s12934-020-01428-8.

[34] E. Demirkan, A. Aybey Çetinkaya, and M. Abdou, "Lipase from new isolate Bacillus cereus ata179: Optimization of production conditions, partial purification, characterization and its potential in the detergent industry," *Turkish Journal of Biology*, vol. 45, no. 3, pp. 287–300, 2021. doi:10.3906/biy-2101-22.

[35] C. O. Ezenwelu, O. A. Afeez, O. U. Anthony, O. A. Promise, C. U.-E. Mmesoma, and O. E. Henry, "Studies on Properties of Lipase Produced from Aspergillus sp. Isolated from Compost Soil," *Advances in Enzyme Research*, vol. 10, no. 2, pp. 49–60, 2022. doi:10.4236/aer.2022.102003.

[36] E. A. Saputra, et al., "Peran Enzim dalam Metabolisme Berdasarkan Al-Qur'an dan Hadist," *Peran Enzim dalam Metabolisme Berdasarkan Al-Qur'an dan Hadist*, vol. 1, pp. 27–35, 2022.

[37] B. Iskandar, R. S. Putri, G. Novita, M. D. C. Surboyo, Leny, and C. K. Lee, "Formulation and Activity Test of Sunflower Oil (Helianthus Annuus L.) Liquid Soap as Anti Acne," *International Journal of Applied Pharmaceutics*, vol. 14, Special Issue 3, pp. 55–59, 2022. doi: 10.22159/ijap.2022.v14s3.11.

[38] R. Hayati, A. Sari, F. Hanum, N. Nabilah, N. Earlia, and E.

Lukitaningsih, "Formulation and Antibacterial Activity of Averrhoa bilimbi L. Fruits Extract in Vegetable Oil-Based Liquid Hand Soap," *Malacca Pharmaceutica*, vol. 1, no. 1, pp. 30–36, 2023. doi:10.60084/mp.v1i1.35.

[39] K. D. Danov, et al., "Solubility of ionic surfactants below their Krafft point in mixed micellar solutions: Phase diagrams for methyl ester sulfonates and nonionic cosurfactants," *Journal of Colloid and Interface Science*, vol. 601, pp. 474–485, 2021. doi:10.1016/j.jcis.2021.05.147.

[40] R. Juninho, et al., "Papaya-Turmeric and Aloe Vera-Piper Betel-based liquid soaps: Formulation and antimicrobial properties," *Journal of Applied Sciences*, vol. 9, no. 1, pp. 26–37, 2024.

[41] S. Fatimah, F. Arifan, A. A. Herdiyanto, M. Rezky, and C. Ramadhani, "Research on CV. Rena Soap Nut Detergent as Future Organic Detergent in Indonesia," *IOP Conference Series: Earth and Environmental Science*, vol. 448, no. 1, 2020. doi:10.1088/1755-1315/448/1/012111.

[42] M. Balouiri, M. Sadiki, and S. K. Ibnsouda, "Methods for in vitro evaluating antimicrobial activity: A review," *Journal of Pharmaceutical Analysis*, vol. 6, no. 2, pp. 71–79, 2016. doi:10.1016/j.jpha.2015.11.005.

[43] A. Klančnik, S. Piskernik, B. Jeršek, and S. S. Možina, "Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts," *Journal of Microbiological Methods*, vol. 81, no. 2, pp. 121–126, 2010. doi:10.1016/j.mimet.2010.02.004.

[44] S. Hadi, Samsuar, H. I. Qudus, and W. Simanjuntak, "In vitro antibacterial activity of some dibutyltin (IV) chlorobenzoate derivatives against Staphylococcus aureus," *ARPN Journal of Engineering and Applied Sciences*, vol. 16, no. 15, pp. 1623–1629, 2021.

[45] L. Handrayani, R. Aryani, and Indra, "Liquid Bath Soap Formulation and Antibacterial Activity Test Against Staphylococcus aureus of Kecombrang (Etlingera elatior (Jack) R.M. Sm.) Flos Extracts," *Current Breakthrough in Pharmaceutical and Material Analysis*, pp. 17–22, 2014.

[46] S. Suliestyah, R. Aryanto, C. Palit, R. Yulianti, B. C. Suudi, and A. Meitdwitri, "Eco enzyme production from fruit peel waste and its application as an anti-bacterial and TSS reducing agent," *International Research Journal of Engineering, IT, and Scientific Research*, vol. 8, no. 6, pp. 270–275, 2022. doi:10.21744/irjeis.v8n6.2199.

[47] C. Vidalia, E. Angelina, J. Hans, L. H. Field, N. C. Santo, and E. Rukmini, "Eco-enzyme as disinfectant: A systematic literature review," *International Journal of Public Health Science (IJPHS)*, vol. 12, no. 3, pp. 1171–1180, 2023. doi:10.11591/ijphs.v12i3.22131.

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