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FORMULATION AND PHYSICAL QUALITY TESTING OF SOLID SOAP SANDALWOOD OIL (*Santalum album* L.) COMBINATION OF RAMBUSA LEAF EXTRACT (*Passiflora foetida* L.) AS AN ANTIBACTERIAL AGAINST *Staphylococcus aureus*

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Abstract

The skin is highly susceptible to infections and other skin diseases, one of which is caused by *Staphylococcus aureus* bacteria. *Staphylococcus aureus* is a Gram-positive bacterium, with the skin surface as its natural habitat. Sandalwood and rambusa oils are plants known for their antibacterial activity. The objective of this study was to determine the characteristics and physical quality of solid soap formulations made from sandalwood oil combined with rambusa leaf extract to inhibit *Staphylococcus aureus* bacteria. The method used in this research is experimental. Antibacterial testing was conducted using the disc diffusion method. The results indicated that the addition of sandalwood oil and rambusa extract in solid soap formulations significantly enhanced antibacterial activity against *Staphylococcus aureus*, with very strong inhibition. Specifically, the diameter of the inhibition zone in formulation 2 with a 50% concentration averaged 23 mm, while formulation 3 at 50% concentration showed an average of 19.8 mm. At a 100% concentration, formulation 3 showed an average inhibition zone of 26.5 mm. Furthermore, physical quality tests of the solid soap preparations confirmed that the results met the quality standards for solid soap as regulated by the Indonesian National Standard (SNI).

Keywords: Antibacterial; Solid Soap; Sandalwood Oil; Rambusa; Staphylococcus aureus

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INTRODUCTION

The skin is highly susceptible to infections and various skin diseases, particularly those caused by *Staphylococcus aureus*. *S. aureus* is a Gram-positive, oval-shaped bacterium with a diameter ranging from 0.7 to 1.2 µm. It typically forms irregular clusters resembling grapes and is non-motile. This bacterium grows optimally at 37°C and exhibits the most effective pigment formation at room temperature (20-25°C).[1,3] Transmission of *Staphylococcus aureus* frequently occurs through hand-to-hand contact.[4,5] To prevent infections caused by this bacterium, the use of antiseptics, such as soap preparations, is essential.

According to the Indonesian National Standard (SNI) 3532:2016, soap is defined as a solid cleansing preparation made through the saponification or neutralization of fats, oils, or fatty acids with alkali, intended for use with water. Soap also functions as a health aid by cleansing the body and reducing the risk of disease. Antibacterial soap is considered effective in cleaning the skin and has the potential to prevent bacterial infections. However, while triclocarban is commonly used as an antibacterial agent in solid body washes, the FDA has highlighted its potential to contribute to bacterial resistance due to its structural similarity to certain antibiotics.[6] Therefore, using natural ingredients as an alternative antibacterial agent can help avoid the negative impacts associated with triclocarban.

One natural ingredient that can be used as a substitute for triclocarban is the sandalwood plant (*Santalum album*) and the rambusa plant (*Passiflora foetida* L). Studies by Rahayu (2002) indicate that sandalwood oil can be used to treat various conditions, including stomach aches, asthma, skin problems, and inflammation, as a sedative, pain reliever, anti-cancer agent, antibacterial agent, and as an ingredient for aromatherapy.[7] Meanwhile, rambusa is known to exhibit antibacterial activity against *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. It also demonstrates antimicrobial properties.[8,9] According to Noviyanti's research (2014), rambusa leaves contain secondary metabolites such as alkaloids, steroids, and triterpenoids, which have significant potential as antibacterial compounds.[10] Based on the above description, researchers are interested in formulating and testing the physical quality of solid soap made from a combination of sandalwood oil (*Santalum album*) and rambusa leaf extract (*Passiflora foetida* L.). This study aims to highlight the antibacterial properties of these plant extracts, particularly against *S.aureus.*

METHODS

The tools used in this research included filter paper, beaker glass, analytical balance, dropper pipette, stir bar, test tubes, measuring cup, hand blender, test tube rack, cotton swabs, Petri dishes, autoclave, pH meter, hot plate, and silicone soap molds. The materials used in this study were sandalwood oil, rambusa leaf extract, coconut oil, olive oil, sodium hydroxide (NaOH), N-brand soap, nutrient agar, distilled water, and *Staphylococcus aureus* bacteria.

Collection and Preparation of Test Materials

The test equipment was prepared at the Microbiology Laboratory and the Natural Materials Chemistry Laboratory of Muhammadiyah University of East Kalimantan. Sandalwood oil was procured from e-commerce platforms, while rambusa leaves were collected from Tanjung Harapan Village, Sebulu District.

Extraction of Rambusa Leaf

The extraction process was conducted using the maceration method. Initially, 250 grams of rambusa leaf powder were placed in a macerator, followed by the addition of 2.5 liters of 96% ethanol solvent. The mixture was left for 3 days, with occasional stirring. The macerate was then separated using the filtration method. All the collected macerate was evaporated using a rotary evaporator to obtain a liquid extract. The evaporation process continued over a water bath to obtain a thick extract.[11,13]

Formulation and Manufacturing Method of Solid Soap

The formulation of the soap was adapted from previous studies, with modifications in the choice of active ingredients. In this study, the basic ingredients used were coconut oil, NaOH, and aquadest, similar to the original formulation.[14,15] However, the original extract was replaced with sandalwood oil and rambusa leaf extract, which were chosen for their potential antibacterial properties.

Prepare the necessary tools and materials, and weigh the ingredients according to the specified formulation. Add 22.08 grams of NaOH to distilled water and stir until fully dissolved. Next, add 120 grams of coconut oil to the NaOH solution and stir until the mixture is homogeneous. Then, add sandalwood oil and rambusa leaf extract, and blend the mixture using a hand blender until it thickens. Pour the soap mixture into a silicone mold and leave it at room temperature for 1-3 days to allow the soap to reach optimal hardness. Afterward, remove the soap from the mold, and it is ready for testing.

Table 1. Formulation of Sandalwood Oil Solid Soap with Rambusa Extract Combination

Composition	Formula 1	Formula 2	Formula 3	Function
Coconut oil	120g	120g	120g	Fatty acids
NaOH	22.08 g	22.08 g	22.08 g	Alkali
Aquadest	51.52 ml	51.52 ml	51.52 ml	Solvent
Sandalwood Oil	0 %	2 %	1%	Active ingredients
Rambusa Leaf Extract	0 %	1 %	2 %	Active ingredients

Physical Quality Testing of Solid Soap

According to research, physical quality tests conducted on solid soap include several parameters, such as;

a. Organoleptic Test
   Organoleptic testing involves a comprehensive physical assessment of the soap, focusing on its aroma, color, shape, and texture.
b. pH test
   For pH testing, 0.10 grams of solid soap is carefully weighed and soaked in 10 mL of distilled water. The pH is then measured using a pH meter. If the soap's pH falls within the range of 9-11, it meets the standard pH for bath soap, making it suitable for the skin.
c. Homogeneity Test
   Apply a small quantity of soap to a transparent glass surface and observe for the presence of any particles within the preparation. The standard for assessing the homogeneity of the soap is the complete absence of granules or particulates.
d. Foam Stability Test
   Weigh 1 gram of solid soap and place it in a measuring cup. Add 10 mL of distilled water, then shake for 30 seconds. Measure the resulting foam

using a ruler to determine the initial foam height. After 5 minutes, measure the foam height again to obtain the final foam height. To calculate the foam height, use the following formula:

$$\%\ Foam\ lost = \frac{initial\ foam\ height}{final\ foam\ height}\ X\ 100\%$$

Foam stability = 100% - % foam loss

The standard criterion for optimal foam stability is that the foam volume should remain between 60-70% within 5 minutes.

Bacterial Subculture Production

Measure 5 g of Nutrient Agar (NA) and add it to 250 mL of distilled water in an Erlenmeyer flask. Stir the mixture until homogeneous. Next, heat and sterilize the solution in an autoclave at 121°C for 15 minutes. After sterilization, allow the solution to cool. Pour the medium into petri dishes and leave it to solidify at room temperature.[16,17]

Staphylococcus aureus Bacterial Suspension

Staphylococcus aureus was collected using a sterile loop that had been heated with a Bunsen burner and then transferred into a test tube containing 9 mL of 0.9% NaCl solution. The mixture was shaken until homogeneous.[16]

Antibacterial Activity Test

The antibacterial activity test was conducted using the disc diffusion method. A cotton swab was used to collect the bacterial suspension, which was then evenly spread onto a plate containing Nutrient Agar (NA) media. Paper discs containing the test preparation, positive control, and negative control were placed onto the surface of the media, which had been inoculated with the bacterial suspension. The plate was incubated for 24 hours at 37°C. After incubation, the growth of the test bacteria was observed, and the inhibitory zone was measured by assessing the clear area around the paper disc using a ruler.[18,21]

RESULTS AND DISCUSSION

In this research, a solid soap preparation was made using sandalwood oil and rambusa leaf extract as active ingredients. Three different formulations of solid soap were prepared with varying concentrations. F1 was made from a soap base consisting of NaOH and coconut oil. F2 was made from the soap base with the addition of 2% sandalwood oil and 1% rambusa leaf extract, while F3 was made from the soap base with 1% sandalwood oil and 2% rambusa leaf extract. The results of the solid soap are presented in Figure 1.

A B C

Figure 1. Results of Solid Soap, (A) F1, (B) F2, (C) F3.

Rambusa Leaf Extraction Results

Table 2. Extraction Results

Plant Name	Simplicity Weight	Extract Weight	Rendeman
Rambusa	250 grams	30.11 grams	8.3%

In this study, the extraction method used for the Rambusa plant was the maceration technique. A total of 250 grams of simplicia was immersed in 2.5 liters of 96% ethanol. The maceration process was carried out for 3 days, with occasional stirring. Afterward, the macerate was filtered and then evaporated using a rotary evaporator and a water bath until it thickened. This process resulted in a thick extract weighing 30.11 grams, with an extract yield of 8.3%.

Organoleptic Test

Table 3. Organoleptic Test Resu

Formulas	Parameter	Observation time			
		Week 1	Week 2	Week 3	Week 4
F1	Appearance	Solid and smooth	Solid and smooth	Solid and smooth	Solid and smooth
	Color	Milk white	Milk white	Milk white	Milk white
	Odor	Typical soap scent	Typical soap scent	Typical soap scent	Typical soap scent
F2	Appearance	Solid and smooth	Solid and smooth	Solid and smooth	Solid and smooth
	Color	Light green	Light green	Light green	Light green
	Odor	The distinctive scent of sandalwood oil	The distinctive scent of sandalwood oil	The distinctive scent of sandalwood oil	The distinctive scent of sandalwood oil
F3	Appearance	Solid and smooth	Solid and smooth	Solid and smooth	Solid and smooth
	Color	Green	Green	Green	Green
	Odor	Scented with sandalwood oil and rambusa extract	Scented with sandalwood oil and rambusa extract	Scented with sandalwood oil and rambusa extract	Scented with sandalwood oil and rambusa extract

Based on the organoleptic test results of the three formulations of solid sandalwood oil soap combined with rambusa leaf extract over 4 weeks, it was found that there were no changes in texture, odor, or color from the first to the fourth week. This finding is consistent with research conducted by Pradana (2024), which also demonstrated no differences in organoleptic properties from the first to the fourth week (14).

pH test

Table 4. pH test results

Formulas	pH Result				Average
	Week 1	Week 2	Week 3	Week 4	
F1	9.0	9.0	9.2	9.5	9.1
F2	8.7	9.1	9.2	9.3	9.0
F3	8.8	9.2	9.2	9.3	9.1

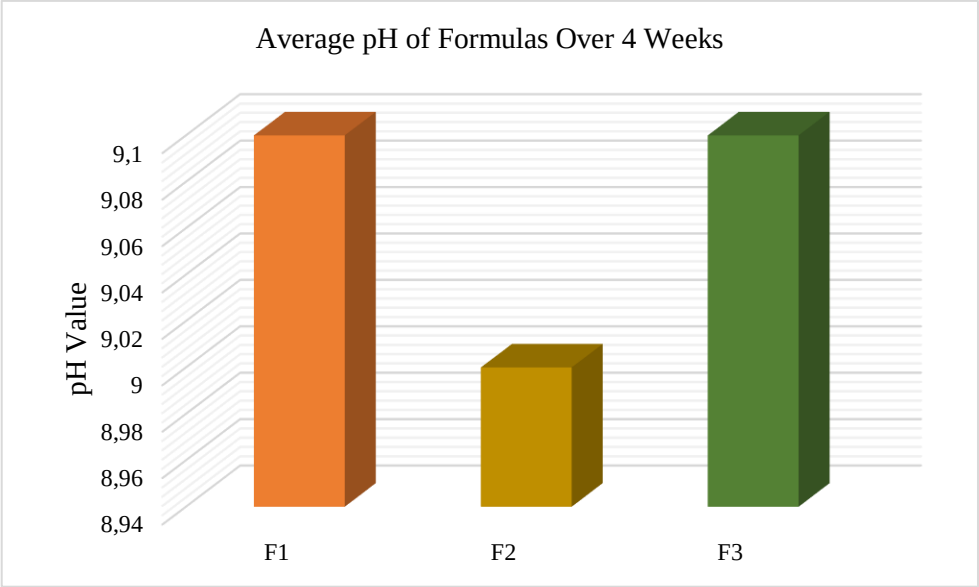


Figure 2. pH test graph

Based on Table 4, the results of the pH test evaluation for the solid soap show an average pH value ranging from 9.0 to 9.1, indicating that the soap remains within a pH range that is safe for the skin. The obtained results align with the standard pH requirements, which fall between 9 and 11, as outlined in previous research.[14]

Homogeneity Test

Table 5. Homogeneity Test Results

Formulation	Preparation Homogeneity			
	Week 1	Week 2	Week 3	Week 4
Formulation 1	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Formulation 2	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Formulation 3	Homogeneous	Homogeneous	Homogeneous	Homogeneous

In this test, the results demonstrated that the solid soap produced remained homogeneous and met the quality standards for solid soap preparations as specified by the Indonesian National Standard (SNI). This can be observed from the uniformity of color in the solid soap, indicating that each formula is evenly dispersed. According to research, the results still showed homogeneity, as there were no visible particles on the surface of the solid soap. This observation was clearly supported by examinations using a glass slide from the first week to the fourth week.

Foam Stability Test

Table 6. Foam Stability Test

Preparation Foam Height					
Formulation	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Average**
F1	71.42%	76.92%	62.3%	58.63%	67.32%
F2	64.72%	74%	73.08%	72.42%	71.05 %
F3	68.09%	77.78%	74.14%	71.19%	72.8%

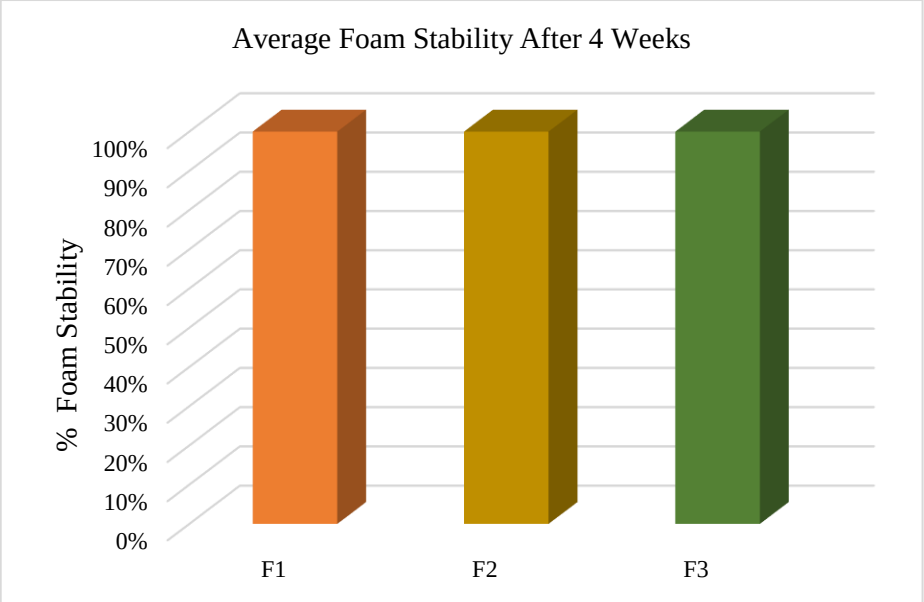


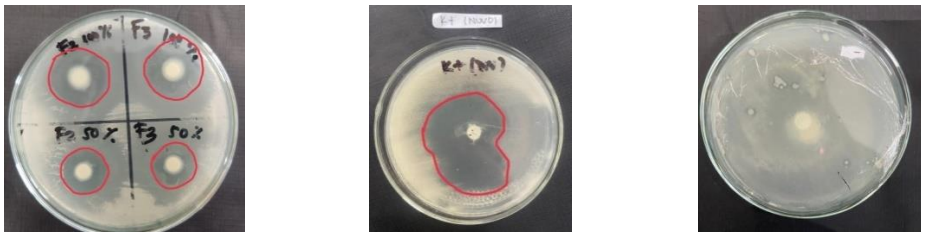
Figure 3. Foam Stability Test Graph

Based on the results presented in Table 6, foam stability testing of solid soap formulations with varying concentrations of sandalwood oil and rambusa leaf extract showed that the foam stability value for F1 was 66.39%, for F2 was 70.24%, and for F3 was 72.9%. These results indicate that the solid soap preparations meet the criteria for good foam stability. This is consistent with the foam stability criteria of 60-70% for acceptable foam stability, as reported by previous research, which found a foam stability of 68%.

Antibacterial Inhibition Test

Table 7. Antibacterial Inhibition Test

Formulas	Inhibition Zone Diameter			Average	Information
	R1	R2	R3		
F2 50%	12mm	27.5mm	29.5mm	23mm	Very strong
F2 100%	18.5mm	28mm	32mm	26.1mm	Very strong
F3 50%	12mm	24.5mm	29mm	19.8mm	Strong
F3 100%	19mm	27mm	33.5mm	26.5mm	Very strong
K+	48mm	48mm	48mm	48mm	Very strong
K-	0mm	0mm	0mm	0mm	-



A B C

Figure 4. Results of Observation of Inhibitory Power

A: Formula 2 (100% and 50% concentration), Formula 3 (100% and 50% concentration)
B : Positive control (Mek N soap) (100% concentration)
C : Negative control (Aquadest)

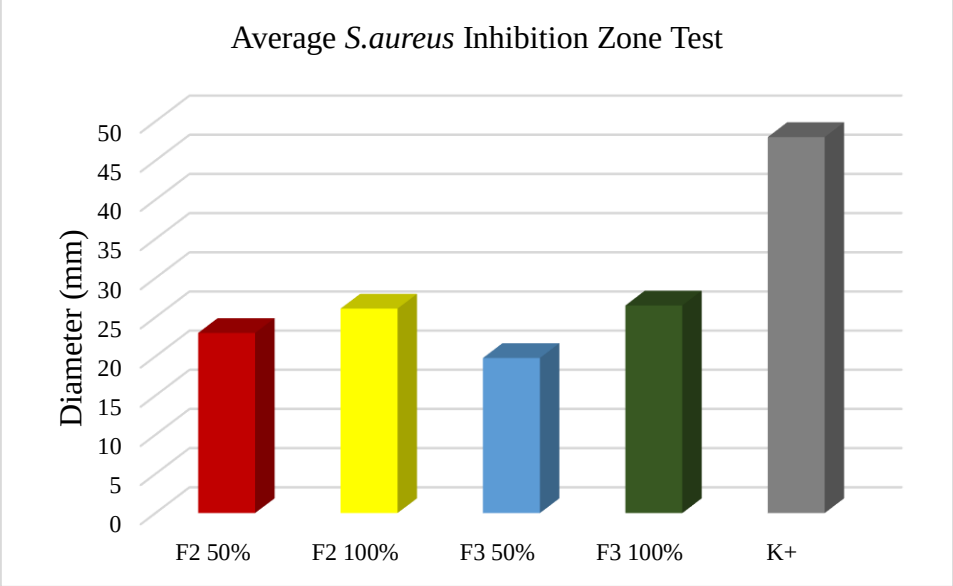


Figure 5. Inhibition Zone Test Graph for Staphylococcus aureus Bacteria

The strength of antibacterial activity is classified into four categories: weak, moderate, strong, and very strong resistance. Antibacterial activity is categorized as weak if the inhibition zone diameter is <5 mm, moderate if it falls within the range of 5-10 mm, strong if it is between 10-20 mm, and very strong if it is >20 mm. Based on the results of the inhibition zone test, which was measured by the clear zone around the disc, formulation 2 at a 50% concentration exhibited an average inhibition zone of 23 mm, falling into the very strong category. Formulation 3 at a 50% concentration showed an average inhibition zone of 19.8 mm, placing it in the strong category, while at a 100% concentration, the inhibition zone increased to an average of 26.5 mm, categorizing it as very strong.

In comparison to the positive control (brand N soap) at a concentration of 100%, the average inhibition zone was found to be 48 mm, which is classified in the very strong category. The negative control (aquadest) did not exhibit any antibacterial activity against Staphylococcus aureus. Research has shown that Rambusa leaf ethanol extract

exhibited antibacterial activity against Staphylococcus aureus with an inhibition zone of 12.7 mm at a 20% concentration. Additionally, studies have indicated that sandalwood extract can inhibit Staphylococcus aureus with an inhibition zone of 6.25 mm at a 12.5% concentration and 8.50 mm at a 100% concentration.

CONCLUSION

Based on the research findings, it can be concluded that both sandalwood and rambusa plant extracts can be used in solid soap formulations. The physical quality characterization of the preparations was conducted in accordance with established standards, and the solid soap formulations have been shown to inhibit the growth of Staphylococcus aureus bacteria.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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