

Formulation of Antibacterial Liquid Soap.....

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Formulation of Antibacterial Liquid Soap from Nyamplung Seed Oil (*Calophyllum inophyllum* L) with Addition of *Curcuma heyneana* and its Activity Test on *Staphylococcus aureus*

S Widyaningsih¹, M Chasani¹, H Diastuti¹, Novayanti¹

¹Chemistry Department, University of Jenderal Soedirman, Purwokerto, Indonesia

Email : sennysetiadi@yahoo.com

Abstract. The increasing demand of soap encourages new innovations of its quantity, quality, benefits and raw materials. One of the innovations that has been developed is the use of nyamplung (*C. inophyllum* L) seed oil as basic ingredients of soap, with addition of active substances such as antibacterial and fragrance. This research aims to determine best formulation of liquid soap according to SNI 06-4085-1996 and its antibacterial activity after addition of n-hexane fraction of *C. heyneana* rhizome and fragrance from lavender flower oil at various concentrations, then a hedonic test was conducted to evaluate the panelist's preference towards the soap with the best formulation. This research has been done through several stages of soap making with saponification method, soap characterization according to SNI 06-4085-1996, determination of best formulation of liquid soap based on the effectiveness index method and antibacterial activity test of liquid soap to *S.aureus* with disc diffusion method and hedonic test using some parameters such as colour, odor, foam, clean power and effects after being used. The results showed that the best characteristic soap according to SNI 06-4085-1996 was soap with 0.2% antibacterial content and 2.5% perfume. The best soap characteristic showed total fatty acid 67.49%, free fatty acid 1.01%, neutral fat 7.24%, weight of type 1.05 g/mL, pH of 9.85 and foam stability 76.69%. The results of the antibacterial activity test of the best formula soap showed the inhibitory zone of 14.92 mm.

1. Introduction

Consumer demand for body cleanser that is increase, promotes new innovations on soap products, in terms of quantity, quality and function. Liquid soap is a preferred product than solid soap, because it is more hygienic in storage and more practical in use.

Researchers tried to look for alternative raw material for soap production from non-edible oils with abundant availability. One of them that have the potential to produce soap is oil derived from nyamplung seeds (*Calophyllum inophyllum* L.).

The research on produce sodium soap based on nyamplung seed oil (*C. inophyllum* L.) was reported that it have antibacterial activity [1]. In order to improve the quality and gain the public interest, we need to add antibacterial and fragrances. This research was done to develop the form of soap that was made from nyamplung seed oil by adding antibacterial additives and perfumes.

The antibacterial additive that was used for soap formulation is the n-hexane fraction of the *Curcuma heyneana*. It was known that *Curcuma heyneana* containing sesquiterpen, a terpenoid compounds with germakran framework that are potentially used as an antibacterial agent [2]. The



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addition of perfumes to antibacterial soap formulations is intended to attract public interest. People love the fragrance of the flowers. The essential oil of the flowers gives a certain flavor. One of them is lavender oil (*Lavandula agustifolia*) [3].

The research started with saponification of nyamplung oil using KOH base, then added antibacterial substance from n-hexane fraction of *Curcuma heyneana* and fragrance from lavender flower oil. The composition of the antibacterial was varied at 0.2, 0.4, 0.6, 0.8 and 1% while the perfume was varied at 0.5, 1, 1.5, 2 and 2.5%. The results were characterized and compared with SNI 06-4085-1996. In order to test the antibacterial properties, the best characterized soap was tested with disc diffusion method against *Staphylococcus aureus* bacteria.

2. Experimental

2.1 Materials

The research used nyamplung seeds obtained from Gunung Selok Kroya, temu giring (*C. heyneana*), lavender flower oil, *S. aureus* bacteria, NA media (Nutrient Agar), NB media (Nutrient Broth) and tetracycline. Organic solvents n-hexane, acetone, methanol, sodium sulphate anhydrous, potassium hydroxide, carboxyl methyl cellulose (CMC), sodium lauryl sulfate (SLS), aquades, ethanol absolute, 70% alcohol, methyl red indicator, phenolphthalein (pp), hydrochloric acid and sulfuric acid.

2.2 Preparation of the n-hexane fraction of temu giring rhizomes [2]

Temu giring is cut into small pieces, dried, and smoothed with blender. The temu giring rhizome powder is macerated with acetone for 3x24 hours. The maceration results are filtered using a buchner funnel. The resulting filtrate is concentrated under low pressure using a rotary evaporator. The concentrated acetone extract was extracted by partitioning with n-hexane: methanol (1: 1). Partition extract result obtained two fractions of n-hexane fraction and methanol fraction. The obtained n-hexane fraction is concentrated at low pressure using a rotary evaporator. The n-hexane fraction of concentrated acetone extract was added anhydrous Na₂SO₄ to bind water and continued by filtration.

2.3 Preparation of soap

The nyamplung seed oil was stirred and heated until 70-80°C then was added KOH 30% (w/v). The reaction mixture is continuously heated while stirring at 500 rpm for 1 hour. The temperature is lowered to 60 and added aquadest (1:1 ratio of the reaction product) while stirring until homogeneous soap is formed. Furthermore carboxyl methyl cellulose (CMC) and sodium lauryl sulfate (SLS) were added to the product soap with various concentrations. Variations of n-hexane fraction of tilapia rhizome and natural perfume were added and remained distilled at 500 rpm for 10-15 min. Stirring is stopped and the magnetic stirrer is immediately taken from the soap solution. Soap products poured into containers and closed tightly. Variations of antibacterial and perfume concentrations can be seen in Table 1.

Table 1. Variations of antibacterial and perfume concentrations

n-hexane fraction of temu giring rhizome (w/w)	Lavender perfume (w/w)				
	P1 (0.5%)	P2 (1.0%)	P3 (1.5%)	P4 (2.0%)	P5 (2.5%)
A1 (0.2 %)	A1P1	A1P2	A1P3	A1P4	A1P5
A2 (0.4 %)	A2P1	A2P2	A2P3	A2P4	A2P5
A3 (0.6 %)	A3P1	A3P2	A3P3	A3P4	A3P5
A4 (0.8 %)	A4P1	A4P2	A4P3	A4P4	A4P5
A5 (1.0 %)	A5P1	A5P2	A5P3	A5P4	A5P5

2.4 Characterization of soap [4]

Characterization of soap includes parameters: total fatty acid, free fatty acids, unsaturated fats or neutral fats, pH test, weight type, foam stability

2.5 Data analysis result of soap characterization

Data analysis used was factorial completely randomized design. Factors tested were n-hexane fraction content of acetone extract of temu giring (*C. heyneana*) and perfume oil of lavender. The data obtained were analyzed using Statistical Product Services Solution (SPSS) 16.0 for windows. The results that showed a real difference will be analyzed by DMRT test (Duncan's Multiple Range Test) with 95% confidence level ($\alpha = 0,05$).

2.6 Determination of the best soap formulation

The best antibacterial soap formulations were determined by the effectiveness index method [6]. This method is done by sorting the variables by priority and contribution to the results. Each variable is a weight according to its contribution with relative numbers 0-1. The value weights are obtained from each variable by dividing the weight of the variable by the sum of all the variable weights. The best formulated soap is a soap that has the highest product value. The value of the product is calculated by multiplying the value of effectiveness (EV) by the weight of the value (WV).

2.7 Antibacterial activity test

The antibacterial activity test was performed by pouring 15 mL of Nutrient Agar (NA) medium at $\pm 40^\circ\text{C}$ into sterile petri dish then left at room temperature until the medium to solidify. A number of bacterial cultures in Nutrient Broth (NB) liquid medium are taken and dispersed in Nutrient Agar (NA) medium. The volume of bacteria taken based on the absorbance results at 600 nm. If the absorbance value is less or equal to 0.5 then is taken 100 μL bacterial culture, whereas if 0,6-1,0 is taken 50 μL . The suspension of the test bacteria on Nutrient Agar (NA) medium is streaked on the spread plate by using drugalsky, then allowed to dry for 15 minutes at room temperature. After drying, a paper disc with a diameter of ± 6 mm is placed over the NA medium. Samples and controls were taken as much as 10 μL and dripped onto paper disc then incubated for 24 hours at 37°C . Then it was measured the inhibitory diameter formed around the disc paper using calipers.

3. Results and Discussion

3.1 Antibacterial liquid soap

The process of producing antibacterial liquid soap is done using semi-hot method (semi-boiled). The soap produced from the saponification process that is diluted and added with the additive. The additives are carboxyl methyl cellulose (CMC), sodium lauryl sulfate (SLS), antibacterial substances from the n-hexane fraction of the temu giring rhizome (*C. heyneana*) and fragrance from lavender flower essential oil. The purpose of addition of temu giring rhizome (*C. heyneana*) and the perfume of the lavender flower oil is to give antibacterial and aromatic effects to the soap.

The characterization of liquid soaps are total fatty acids, free fatty acids, neutral fat, pH, type weight and foam stability. Then the result were compared with SNI 06-4085-1996 [4].

The highest of total fatty acid was shown by control soap without antibacterial and perfume addition of 70.52%. While total amount of fatty acid in formulation soap showed the range 66,30% - 69,70% (Fig.1). The lower amount of total fatty acids in the formulation soap is due to the higher n-hexane fractional content of temu giring rhizome (*C. heyneana*) and the fragrance of the lavender flower essential oil. The characterization results show that the total fatty acid amount in antibacterial liquid soap in accordance with SNI 06-4085-1996 [4].

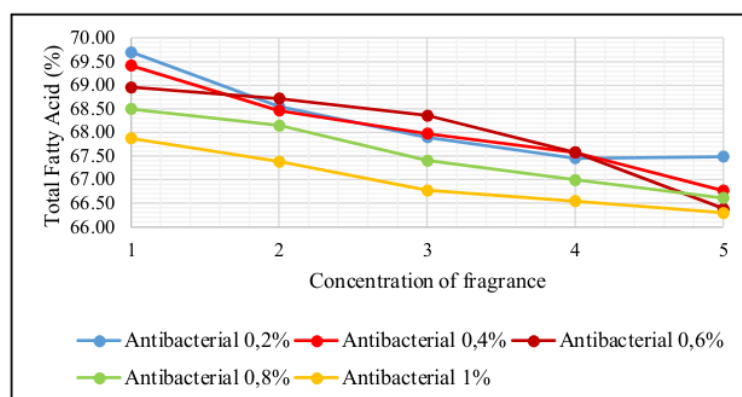


Figure 1. Total fatty acid at variation of fragrance concentration

The result of data analysis showed that there were significant differences on soap with 1% antibacterial addition and 2.5% perfume which showed total fatty acid amounting to 66.30%.

Formulated soaps have a range of free fatty acids 0.40% - 1.20% (Fig.2). The high amount of free fatty acids are caused by acidic terpenoid compounds [6,7] that were contained in additives. The terpenoids will react with the KOH solution, resulting a decrease of the alkaline content which will soap the free fatty acids. Result of free fatty acid amount on antibacterial liquid soap in accordance with SNI 06-4085-1996 [4].

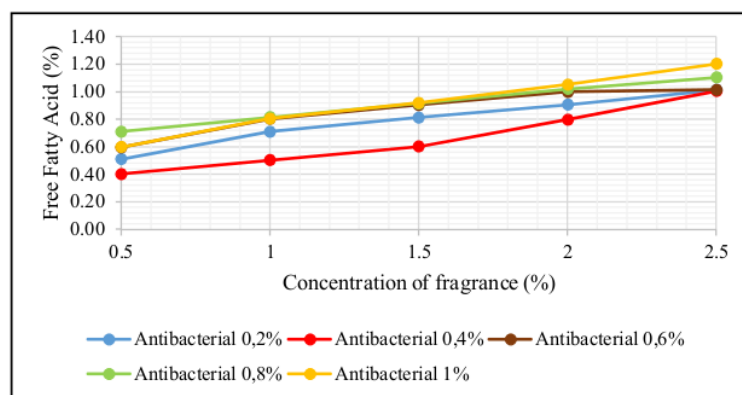


Figure 2. Free fatty acid at variation of fragrance concentration

The result of data analysis of free fatty acid showed a significant effect on the amount of free fatty acid in the formulation soap. The significant differences occurred in soap with 1% antibacterial addition and 2.5% perfume.

Soap formulations show the neutral fat in a range of 5.36% - 7.25% (Fig.3). The high amount of neutral fat in soap formulation is caused by the content of neutral fat components in the soap. The neutral fat components are generally sterols, dyestuffs, hydrocarbons and complex lipids [8]. The additives that is used contain terpenoid compounds. Terpenoid compounds are generally regarded as lipids and include

either neutral fat [6]. In addition, the n-hexane fraction of the temu giring rhizome (*C. heyneana*) contains a dye of curcumin which can affect the amount of neutral fat in the formulation soap. The result of the amount of neutral fat in antibacterial liquid soap is not in accordance with SNI 06-4085-1996 [4].

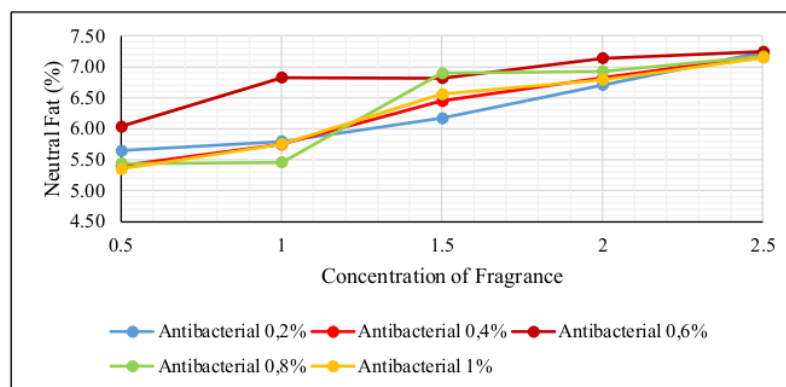


Figure 3. Natural fat at variation of fragrance concentration

Result of data analysis of neutral fat quantity showed a significant effect to neutral fat amount on soap formulation. The significant differences occurred in soap with 0.2% antibacterial addition and 2.5% perfume.

The pH measurement results show that the control soap has a pH range of 9.65 - 9.85 (Fig.4). While the formulation soap showed a pH range of 9.55 - 9.85. The higher pH value of the control soap is caused by the soap that is formed from the saponification process of triglycerides with alkaline base (KOH), where KOH is one of the strongest bases [9]. The lower pH value of the formulation soap are caused by the presence of terpenoid compounds in additive which can react with KOH. Terpenoid compounds are one of the acidic compounds because they contain hydroxyl and carbonyl groups [7,8]. The result of measurement of pH value in soap in accordance with SNI 06-4085-1996. pH value of soap that is safe for the skin is 8-11 [10].

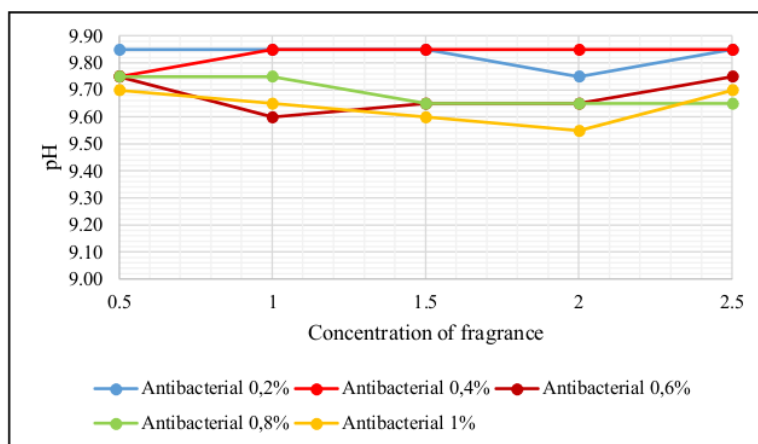


Figure 4. pH at variation of fragrance concentration

Result of data analysis showed a significant effect to pH on soap formulation. The significant differences occurred in soap with 0.8% antibacterial addition and 2.5% perfume.

The density of antibacterial liquid soap is influenced by the type and concentration of raw materials that was added. The higher the value of molecular weight of the raw material added, the higher the density [11]. The result shows that the density of soap in accordance with SNI 06-4085-1996. Result of data analysis showed a significant effect to density of soap formulation (Fig 5). The significant differences occurred in soap with 1% antibacterial addition and 2.5% perfume.

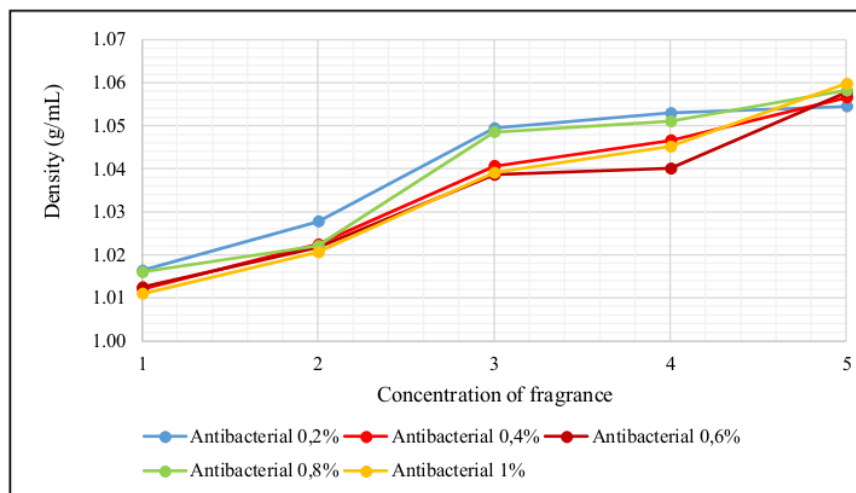


Figure 5. Density at variation of fragrance concentration

Soap formulation results show the value of foam stability with a range of 72.92% - 80.52% (Fig 6.). Addition of additive causes the foam stability value relatively higher. This is caused by the presence of saponin content in the additive that can produce foam [12]. One factor that affecting foam stability is the type of fatty acid in soap formulations. The nyamplung seed oil contains oleic acid which can produce a stable and soft foam, palmitic acid and stearic acid which has the stabilizing properties of the foam. Result of data analysis showed a non effect to foam stability.

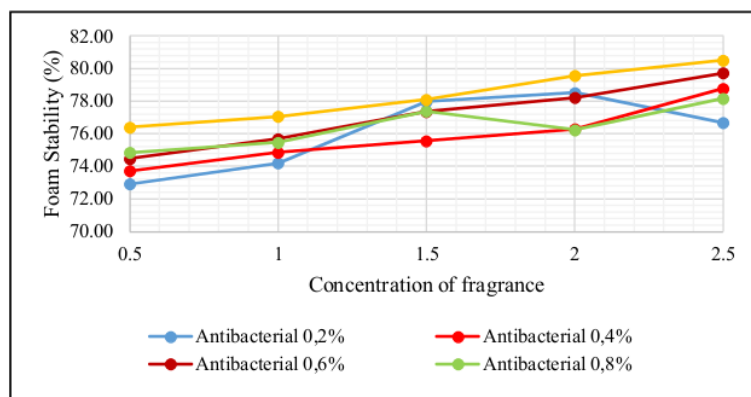


Figure 6. Foam stability at variation of fragrance concentration

3.2 The Best Soap Formulation

The best antibacterial liquid soap formulations were determined by the effectiveness index method [6]. This effectiveness index method can be done by sorting the variables based on the priority and contribution to the results, whereby the best antibacterial liquid soap formula has the highest product value. The order of priority and contribution to the results in this study were total fatty acids (%), free fatty acids (%), neutral fats (%), pH, type-weights and foam stability (%). Calculation result of soap product value of formula can be seen in Fig 7.

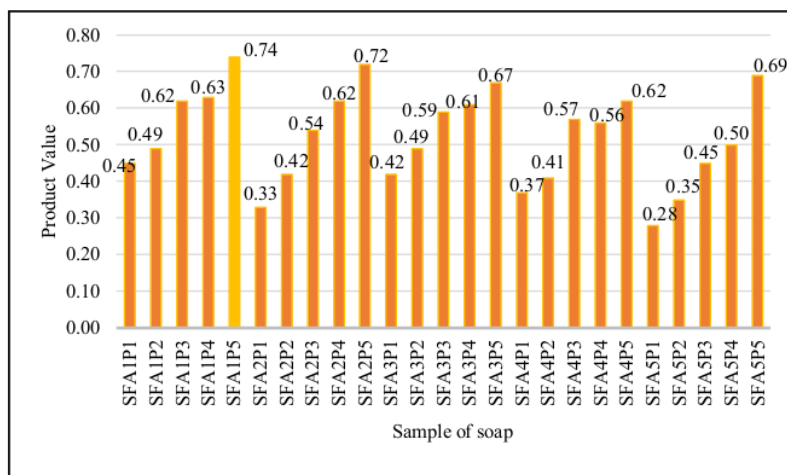


Figure 7. Product value of soap

Based on the Fig.7 shows that the best characterized soap formulation according to SNI 06-4085-1996 is soap with 0.2% antibacterial addition and 2.5% deodorizer with product value of 0.74.

The antibacterial activity test

The antibacterial activity test on the best soap formula according to SNI 06-4085-1996 was done on gram-positive bacteria i.e *S. aureus* bacteria. The n-hexane fraction of the temu giring rhizome (*C. heyneana*) showed an antibacterial activity with a inhibitory diameter of 5.34 - 15.23 mm. The highest antibacterial activity was shown by the n-hexane fraction of the temu giring rhizome (*C. heyneana*) at a concentration of 1% with a resistor diameter of 15.23 mm. Antibacterial activity on essential oil fragrance of lavender flower showed 13.19 mm inhibition diameter. This suggests that the higher concentrations of the added substances can increase antibacterial activity [13]. Some things that can affect antibacterial work include the concentration or intensity of antibacterial substances, the number of microorganisms, temperature, species of microorganisms and the presence of organic materials [14]. The terpenoids will accumulate in the lipid of bacterial cell membrane and cause disruption of the structure and function of the cell membrane that was caused by swelling and changes in the permeability of bacterial cell membranes [15,16].

The results of the antibacterial activity test on soap formulation showed that the addition of n-hexane fraction of temu giring rhizome (*C. heyneana*) and fragrance of lavender flower oil decreased antibacterial activity when compared in its single state. The effect of component interactions is due to the n-hexane fraction of the temu giring rhizoma (*C. heyneana*) and the fragrance of lavender flower oil that they become antagonistic because the combination of compounds has a smaller therapeutic effect or excludes the activity of other compounds [17,18].

Table 2. Result of antibacterial activity test on soap formulation

Sample	Inhibitory diameter (mm)
Aquades (-)	-
Tetracycline (+)	27.69
The fraction of n-hexane 0.2%	5.34
The fraction of n-hexane 0.4%	7.07
The fraction of n-hexane 0.6%	10.77
The fraction of n-hexane 0.8%	14.42
The fraction of n-hexane 1%	15.23
Essential oil of lavender 2.5%	13.19
SK	14.89
SKA1	14.43
SKA2	14.51
SKA3	14.80
SKA4	15.09
SKA5	15.54
SKP5	15.70
SFA1P5	14.92
SFA2P5	14.58
SFA3P5	14.25
SFA4P5	13.43
SFA5P5	13.04

4. Conclusion

Antibacterial liquid soap with the best characterization according to SNI 06-4085-1996 is antibacterial liquid soap with n-hexane fraction of temu giring rhizoma content of 0.2% and fragrant content of 2.5% lavender flower essential oil and has antibacterial activity against *S. aureus* of 14.92 mm.

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