Conference Paper

Formulation and Antibacterial Activity of Transparent Solid Soap Combination of Secang Ethanolic Extract and Clove Bud Oil Against Skin Disease Bacteria

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Abstract.

Secang wood ethanolic extract and clove bud oil have antibacterial properties against *Staphylococcus aureus, Propionibacterium acnes, and Pseudomonas aeruginosa*. The formulation of both in transparent bath soap is additional protection for the body. This study formulated soaps and determined their antibacterial activity against *Staphylococcus aureus, Propionibacterium acnes,* and *Pseudomonas aeruginosa*. Soap was made using the hot process and the antibacterial activity was tested using the agar diffusion. The soap formulas F0 (as the base), F1 (3% clove bud oil), and F2 (a combination of 3% clove bud oil and 0.5% sappan wood ethanolic extract) have good appearance and quality including pH, foam stability, free alkali, unsaponifiable fat, and mineral oil content. The water content in the soap is >15%. The inhibitory diameter values of the soap (F0, F1, and F2) against S. aureus, P. acnes, and P. aeruginosa were in the moderate to strong category. Based on the contact time test of 90 seconds, F0 cannot kill bacteria; F1 can kill *Staphylococcus aureus Propionibacterium acnes, and Pseudomonas aeruginosa*; F2 killed *Staphylococcus aureus* in 90 seconds and *Propionibacterium acnes, and Pseudomonas aeruginosa* in 60 seconds.

Keywords: soap, clove bud oil, secang, antibacterial

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1. INTRODUCTION

Socio-economic conditions, poor hygiene, an unclean environment, and behavior that does not support health can be a factor in the occurrence of skin diseases and their transmission [1]. Infectious skin diseases which often occur in the community are acne, pustulosis folliculitis, macular erythema, vesicopustular lesions, abscesses, and soft tissue infections which are usually caused by bacteria such as *Pseudomonas aeruginosa*, *Propionibacterium acnes,* and *Staphylococcus aureus* [2-4]. The skin has the first line of defense against microorganisms, such as the presence of an acid mantle layer and lipid barrier in the epidermis layer, above the stratum corneum. However, if there is a change in the pH of the mantle, it can cause an increase in the entry of bacteria and

various skin diseases [5]. Therefore, additional protection is needed to prevent bacterial infections in the body with natural ingredients that have antibacterial properties. Natural materials are believed to have better safety than synthetic materials.

Several studies have shown that Sappan wood extract (*Caesalpinia sappan*) and clove bud oil (*Syzygium aromaticum*) have been shown to have antimicrobial activity. Sappan wood ethanolic extract can inhibit bacterial growth with a percent decrease population of *P. aeruginosa* and *S. aureus*, respectively 99,88% and 96,28% [6]. Meanwhile, 15 mcg/disk clove bud oil could inhibit the growth of *P. acnes* by 25,3 mm [7], and 1,25% clove bud oil could inhibit the growth of *P. aeruginosa* by 20 mm and *S. Aureus* by 12 mm [8]. Furthermore, the combination (11

v/v) of Sappan wood methanolic extract (8 mg/mL) and clove bud oil (20 mcg/mL) was more effective than a single sample [9].

In this study, a solid bath soap formulation containing Sappan wood ethanolic extract and clove bud oil was used as an antibacterial. The transparent bath soap is chosen due to the product can be used every day by all circles of society as the first series of personal hygiene. This soap containing a combination of Sappan wood ethanolic extract and clove bud oil needs to be evaluated for its quality and also antibacterial activity against *Staphylococcus aureus*, *Propionibacterium acnes*, and *Pseudomonas aeruginosa*.

2. MATERIALS AND METHODS

2.1. Material

Simplicia of Sappan wood (Toko Herbal Bandung), clove bud oil, virgin coconut oil and olive oil (PT. Darjeeling Sembrani Aroma), stearic acid, NaOH, sucrose, coco DEA, and NaCl (Kimia Mart), glycerine, propylene glycol, and aquadest (Brataco); 70% ethanol and 95% ethanol (Onemed).

2.2. Methods

2.2.1. Preparation of Test Materials

Sappan wood simplicia was standardized, including organoleptic examination, determination of drying loss, moisture content, total ash content, acid insoluble ash content, water-soluble extract content, and ethanol-soluble extract content. Standardization of

simplicia needs to be carried out at stages in the research and development of nutritious ingredients from nature. This standardization is intended to ensure the quality and safety of the preparations produced. Furthermore, the simplicia of sappan wood was extracted by maceration method using 70% ethanol as a menstruum, it is hoped that the phenolic compounds in sappan wood can be extracted optimally. The resulting yield is 11,16% w/w. Phytochemical screening was carried out to detect the secondary metabolite compounds contained in simplicia and extracts. It was known that simplicia and extracts contained polyphenolics, flavonoids, anthraquinones, tannins, monoterpenes, sesquiterpenes, and triterpenoids [10,11]. While clove bud oil contains 80.32% eugenol.

2.3. The procedure of Making Transparent Solid Bath Soap

The method of making soap by heat will produce a soap with a smooth texture. The initial process is done by heating the fat fraction of VCO (virgin coconut oil), olive oil, and stearic acid until it melts in a water bath. The melted fat fraction reacted with an alkaline solution (NaOH) for the saponification reaction process. The mixture is heated over a water bath and stirred until it is completely mixed (trace) and thickens. Then glycerine, sugar solution, NaCl, coco DEA propylene glycol were added, then stirred until homogeneous. After that, propylene glycol and some 95% ethanol were added slowly to the mixture and stirred using a hand mixer for 10 minutes. Then cover the mixture container and leave it on the water bath for 20 minutes, stirring occasionally until the mixture melts again and forms a transparent liquid mass. Some ethanolic extracts of Sapan wood which had been dissolved with some 95% ethanol and clove bud oil were added to the mixture at 40^{o}C. The mixture was stirred again until homogeneous and poured into the mold. The soap will set in about 24 hours. After the soap is formed, the soap is stored for up to 2-4 weeks (curing phase) so that the saponification process is complete [12].

2.4. Quality Test (Soap Evaluation)

1. Acidity (pH). A total of 1 gram of soap was dissolved in 10 mL of distilled water and the pH was measured using a pH meter [12].

2. Foam Stability Test. A total of 1 gram of soap was put into a test tube containing 10 ml of aquadest, then homogenized with a vortex for 1 minute, and the height of the foam formed was measured using a ruler (initial height of the foam). The

foam height was measured again after 5 and 10 minutes. Foam stability can be calculated by the equation below [13].

$$Initialheightof foam = \frac{\%Foamstability}{Finalheightof foam} \times 100\% \quad (1)$$

3. Water content. The water content examination of soap was carried out by azeotropic distillation [14].

4. Free Alkali. Neutral alcohol was prepared by boiling 100 mL of alcohol and adding 0.5 mL of phenolphthalein indicator, then cooled to 70^{o}C and neutralized with 0.1N KOH in alcohol. As much as 5 grams of soap is put into neutral alcohol, boiled for 30 minutes using reflux over a water bath, then cooled and titrated with 0.1N HCl until the red color just disappears. Then it is calculated by the equation below [15].

$$Initialheightof foam = \frac{VxNx0,0561}{sampleweight(g)} \times 100\% \quad (2)$$

Where:

V = volume (mL) HCl used N = Normality (N) HCl used

5. Unsaponifiable Fat. The solution used for the free alkali examination was added with 5 ml of 0.5 N excess alcoholic KOH, boiled over a water bath for one hour, and cooled to a temperature of 70°C. Then 5 ml of the solution was pipetted and titrated with 0.5 N alcoholic HCl until the red color of the phenolphthalein indicator just disappeared (V1 ml). Next, a blank titration was carried out using 0.5 N alcoholic KOH pipetted as much as 5 mL and titrated with 0.5 N alcoholic HCl until the red color of the phenolphthalein indicator disappeared (V2 ml) [15].

$$Unsaponifiablefat = \frac{(V2-V1)xNx0,0561}{0,258W} \times 100\% \quad (3)$$

6. Mineral Oil Content. 5 grams of soap is put into a beaker glass, then added with water and heated until slightly dissolved. Then 10% HCl was added to it until the methyl orange indicator turned red, and all the fatty acids, neutral fats, and parts that could not be saponified would separate in the top layer. The mixture is put into a separatory funnel and the aqueous layer is removed. A total of 0,3 mL of a fat layer was pipette and added 5 mL of 0,5N KOH in alcohol, then heated until the saponification reaction was complete using reflux and water bath for 2 minutes. Then titrated with water. If there is turbidity, it indicates positive mineral oil and if the solution remains clear, it indicates negative mineral oil [15].

2.4.1. Antibacterial Test

A total of 30 mL of sterile agar medium and 200 µL of bacterial suspension equivalent to 0.5 McFarland were homogenized using a vortex, then poured into sterile petri dishes. After the agar solidifies, a well is made with a perforator with a diameter of 0.8 cm. The sample of bath soap that has been dissolved using aquadest (1:1) and sterilized using UV for 60 minutes, is poured into the well as much as 40 µL. Pre-incubated for 30 minutes, then incubated at 37° for 18-24 hours. The media used were Mueller Hinton Agar (for *P. aeruginosa*), Triple Sugar Iron Agar (for *P. acnes*), and Nutrient Agar (for *S. aureus*) [16].

2.4.2. Contact Time Test

A sterile petri dish was prepared and then 30 mL bacterial medium was poured into a petri dish. The media used were Mueller Hinton Agar (for *P. aeruginosa*), Triple Sugar Iron Agar (for *P. acnes*), and Nutrient Agar (for *S. Aureus*). The sample (bath soap) was dissolved using aquadest (1:1). As much as 5 mL of soap solution was put into a sterile test tube and sterilized using a UV lamp for 60 minutes, then 0.5 µL of bacterial suspension was added which was equivalent to 0.5 McFarland and homogenized using a vortex (contact time was calculated after the preparation was added with bacteria). Planting of the test sample was carried out by scratching the preparation using a sterile round loop, when the contact time was 15, 30, 45, 60, 75, and 90 seconds. Then pre-incubated for 30 minutes and incubated at 37° for 18-24 hours [17].

3. RESULTS AND DISCUSSION

3.1. Transparent Solid Bath Soap Formula

Solid soap can be made by cold process and hot process. The cold process requires a long saponification process and usually lasts up to 4 weeks, so that the alkali can be neutralized optimally. Meanwhile, soap making can take a short time by the hot process, so it is very suitable if applied to the solid soap industry. This study made transparent solid bath soap using the hot process.

Qualified soap is influenced by the material that used for making the soap. In this study, the main raw material used to make soap is virgin coconut oil. The most dominant fatty acid in coconut oil is lauric acid. The saturated fatty acid content contained in lauric

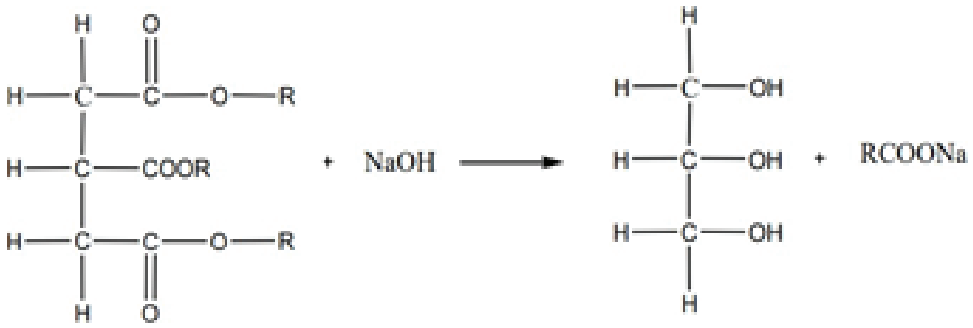

Figure 1: Saponification reaction [18].

acid can provide high solubility and good foaming properties in soap products. Soap is the result of saponification by reacting fats or glycerides with bases. Soap has a non-polar (-R) group and a polar (- COONa) group. Dirt that sticks to the skin, in general, is non-polar oil, fat, and sweat which cannot be washed off using water alone, therefore soap is needed to clean it [18,19]. The saponification process can be seen in Fig 1.

In base optimization, the selection of the base is based on the level of hardness and transparency/clarity of the resulting soap. The composition of the fat fraction, the amount of NaOH and water used to dissolve the NaOH in part A, was obtained from calculations using the soap calc application. The formula for transparent bath soap showed Table 1.

TABLE 1: The transparent solid bath soap formula.

Composition	**Concentration (%)**		
	F0	**F1**	**F2**
Part A			
VCO (Virgin coconut oil)	20.0	20.0	20.0
Olive oil	1.2	1.2	1.2
Stearic acid	6.8	6.8	6.8
NaOH	4.8	4.8	4.8
Water	10.8	10.8	10.8
Part B			
Glycerin	10.4	10.4	10.4
Sucrose	8.8	8.8	8.8
Water	7.2	7.2	7.2
NaCl	0.2	0.2	0.2
Cocamide diethanolamine	1.0	1.0	1.0
95% ethanol	12.8	12.8	12.8
Clove bud oil	0	3.0	3.0
Sappan Wood Ethanolic Extract	0	0	0.5
Propylene glycol	Ad 100		

3.2. Transparent Solid Bath Soap Evaluation

3.2.1. Physical Test

Physical tests of soap were carried out on the three formulas, including organoleptic tests, hardness, transparency, and pH tests. The results of the physical test of soap showed in Table 2.

TABLE 2: The result of physical test of bath soap formulas.

Test	Formula		
	F0	F1	F2
Organoleptic	Solid, slightly white, odorless	Solid, slightly yellowish, with a characteristic odor of clove bud oil	Solid, red in color, has a characteristic odor of clove bud oil
Hardness	+++	+++	+++
Transparency	Transparent	Transparent	Slightly transparent
pH	10,02 ± 0,03	10,02 ± 0,08	9,99 ± 0,06

Note: +++ : hard

3.2.2. Foam Stability Test

A foam stability test is carried out to find the durability of soap foam. The results showed in Table 3.

TABLE 3: The result of foam test dan foam retention time.

Formula	Foam Retention Time (cm)			Foam Stability (%)
	Initial	After 5 minutes	After 10 minutes	
F0	6.0	5.5	5.5	91.7
F1	5.8	4.9	4.9	84.5
F2	4.5	3.8	3.8	84.4

Foam is formed when the surface tension of water is reduced and air mixed, causing bubbles. Foam is gas trapped by a thin layer of liquid containing many soap molecules adsorbed on the thin layer. There is no absolute requirement in regulating the quality of foam in soap products because the height of the foam is not related to the ability of the cleaning process of a soap product but is related to consumer perception and aesthetics. The height of the foam in the soap can be influenced by the ingredients contained in the soap formula. The saturated fatty acids contained in coconut oil's lauric acid can provide good foam properties in soap products [19]. The results of testing

the foam stability of soap products show good criteria because good foam stability is achieved when within 5 minutes the foam stability value is not less than 70%. The use of cocamide DEA in the soap formula also affects the stability of the foam produced [13].

3.2.3. Quality Test (Soap Evaluation)

Soap quality test includes measurement of water content, free alkali, unsaponifiable fat, and mineral oil. The results of the soap quality test can be seen in Table 4.

Table 4: The result of soap quality test.

Test	Formula		
	F0	F1	F2
Water content	34.15%	33.43%	32.87%
Free alkali	0.007%	0.01%	-
Unsaponifiable fat	3.43%	3.98%	-
Mineral oil	Negative	Negative	Negative

Note (-): cannot be determined because the endpoint of the titration is not visible because the solution is red

The water content in soap will affect how the soap is stored. The value of the water content of all formulas exceeds 15%. So, if the water content in the soap is greater, the soap will shrink in shape faster than soap with a smaller water content value. The water content of the transparent soap that has been made is influenced by the ingredients of the soap which are hygroscopic and tend to absorb water, including glycerin, sugar, NaOH, cocamide DEA, and propylene glycol. The high humectant content in transparent soap has better moisturizing power than ordinary solid soap [13].

The Free alkali test showed the amount of NaOH that did not react with free fatty acids in the saponification reaction. If the free alkali analysis shows a 0% result, the resulting soap has good quality because all NaOH can react with free fatty acids to produce soap. Unsaponifiable fats are neutral fats or triglycerides that react during the saponification process. The limit of unsaponifiable fat is 2.5 – 7.5. The test resulted in F0 (3.43%), F1 (3.98%), while the amount of unsaponifiable fat in F2 could not be determined because the soap solution was red so that the endpoint of the titration was not visible [15].

Mineral oil cannot be saponified even though it has been saponified with a strong base (NaOH/KOH) in excess it will remain as oil and on the addition of water, there will be an emulsion between water and oil which is marked by turbidity. In the test, there was no mineral oil content in solid bath soap [15].

3.3. Antibacterial Activity of Transparent Solid Bath Soap

The antibacterial activity test on solid bath soap was carried out on bacteria that cause skin infections. There are *Staphylococcus aureus*, *Propionibacterium acnes*, and *Pseudomonas aeruginosa*. Antibacterial activity test using the agar diffusion method. The results of the antibacterial activity of solid bath soap can be seen in Table 5.

Table 5: The result of antibacterial activity of transparent solid bath soap.

Bacteria	Diameter of inhibition zone (mm) ± SD		
	F0	F1	F2
S. aureus	9.63 ± 0.21	11.80 ± 0.92	11.83 ± 0.92
P. acnes	10.63 ± 0.51	13.83 ± 1.15	13.67 ± 0.57
P. aeruginosa	9.73 ± 0.12	10.60 ± 0.35	11.03 ± 0.47

Based on Table 5, solid bath soap has antibacterial activity against *S. aureus*, *P. acnes*, and *P. aeruginosa* bacteria. The F1 bath soap formula which is the basis can still inhibit the three bacteria in the moderate category. The F2 bath soap formula with 3% clove bud oil content can inhibit *P. aeruginosa* bacteria in the moderate category and inhibit *S. aureus* and *P. acnes* bacteria in the strong category. While the F3 bath soap formula, which contains a combination of 3% clove bud oil and 0.5% Sappan wood ethanolic extract, can inhibit the three bacteria with a strong category.

3.4. Contact Time Test

The contact time test on the transparent solid bath soap that was made was carried out to find the most effective time needed by the bath soap to inhibit the growth of the test bacteria. The results of the contact time test of each formula against the test bacteria can be seen in Table 6, Table 7, and Table 8.

Table 6: The results of bacterial contact time test in bath soap formula F0.

Bacteria	Contact Time (second)					
	15	30	45	60	75	90
S. aureus	+	+	+	+	+	+
P. acnes	+	+	+	+	+	+
P. aeruginosa	+	+	+	+	+	+
Description: + = presence of bacteria growth; - = no bacterial growth						

Based on the results of the contact time test, it shows that the F0 soap formula cannot kill bacteria after 90 seconds of contact. Meanwhile, the F1 soap formula can kill *S. aureus*, *P. acnes,* and *P. aeruginosa* bacteria after 90 seconds of contact. The F2

Table 7: The results of bacterial contact time test in bath soap formula F1.

Bacteria	Contact Time (second)					
	15	30	45	60	75	90
S. aureus	+	+	+	+	+	-
P. acnes	+	+	+	+	+	-
P. aeruginosa	+	+	+	+	+	-
Description: + = presence of bacteria growth; - = no bacterial growth						

Table 8: The results of bacterial contact time test in bath soap formula F2.

Bacteria	Contact Time (second)					
	15	30	45	60	75	90
S. aureus	+	+	+	+	+	-
P. acnes	+	+	+	-	-	-
P. aeruginosa	+	+	+	-	-	-
Description: + = presence of bacteria growth; - = no bacterial growth						

soap formula showed that *S. aureus* bacteria died after 90 seconds of contact, while *P. acnes* and *P. aeruginosa* bacteria died after 60 seconds of contact.

4. CONCLUSION

1. The bath soap formulas F0, F1, and F2 have a good appearance and meet the soap quality requirements including pH, foam stability, free alkali testing, unsaponifiable fat, and mineral oil content, while the water content test shows that soap formulas F0, F1, and F2 have high levels of water more than 15%.
2. Solid bath soap formulas F0, F1, and F2 had antibacterial activity against *S. aureus*, *P. acnes*, and *P. aeruginosa* bacteria with moderate to strong inhibitory diameters. Meanwhile, based on the contact time test, it was shown that the F0 soap formula could not kill bacteria after 90 seconds of contact. Meanwhile, the F1 soap formula can kill *S. aureus*, *P. acnes*, and *P. aeruginosa* bacteria after 90 seconds of contact. The F2 soap formula showed that *S. aureus* bacteria died after 90 seconds of contact, while *P. acnes* and *P. aeruginosa* bacteria died after 60 seconds of contact.

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