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BINAHONG LEAVES WATER EXTRACT (Anredera cordifolia (Tenore) Steen.) AS A NATURAL FOAMING AND ANTIBACTERIAL AGENT OF ANTISEPTIC LIQUID BATH SOAP

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ABSTRACT

The research about the use of binahong leaves water extract (Anredera cordifolia (Tenore) Steen.) as a natural foaming and antibacterial agent in the production of antiseptic liquid bath soap has been conducted. The extraction of binahong leaves was done by the maceration method using water solvents, and then saponin identification was carried out using a foam test and Lieberman Burchard. Antiseptic liquid soap is made in 6 preparations with variations in the ratio of Sodium Lauryl Sulfate (SLS) and water extract of binahong leaves. Antibacterial activity test was carried out on Staphylococcus aureus and Escherichia coli using the excellent diffusion method. The results showed that binahong leaf water extract contained saponins and produced a stable foam of more than 10 minutes so that it could be used as a natural foaming agent in the production of liquid bath soap. The soap produced meets the quality requirements of SNI 06-4085-1996 concerning Liquid Bath Soap, where preparation 4 has the best results with detergency power of 40%; pH 8.87; free alkali content of 0.04%; and a specific gravity of 1,06 gr/ml. A liquid bath soap with binahong leaves water extract surfactant (preparation 6) has the highest antibacterial activity against S. aureus and E. coli with inhibition area diameter of 13.5 mm (strong) and 12.5 mm (strong) respectively.

Keywords: water extract, binahong leaves, foaming agent, antibacterial, bath soap

INTRODUCTION

Soap is an essential requirement in human life. The demand for soap is high because soap has a function to clean the body from dirt and bacteria. Some of the soap industry has started adding additives that act as foaming agents and cleaning dirt that sticks to the body. The most commonly used foaming agents are Sodium Lauryl Sulfate (SLS) and Sodium Laureth Sulfate (SLES). SLS and SLES function as surfactants in car

wash, floor cleaner, shampoo, and body wash. product. . and toothpaste [1].

The concentration of SLS and SLES in liquid bath soap is about 1% to 30% [2]. The positive impact of using SLS and SLES is that it can bind dirt that sticks to the body, so that the body becomes cleaner, while the negative impact is that it can cause mild or severe skin irritation. The concentration of SLS and SLES of 0.5% can cause mild skin irritation. In comparison, the level in bath soap products on the market currently reaches 10% - 30%, which has an impact on skin corrosion and severe irritation [3].

Previous research has shown that SLS is a soap foaming agent that is difficult to break down by water microorganisms, is toxic to aquatic animals, and reduces the quality of drinking water [4]. Also, long-term use of SLS can cause irritation and allergies to the skin because SLS removes beneficial skin fats so that the skin cannot maintain moisture [5].

Apart from problems related to foaming agents, bacterial skin problems cannot be separated from the use of soap. S. aureus bacteria usually infect the skin area, namely on the broad surface of the arms and legs. Besides, these bacteria can be transmitted through physical contact and will penetrate the injured skin, causing infection [6]. Then, there are the E. coli bacteria, which are pathogenic and harmful if they are too long in the human body. These E. coli bacteria isolates can be resistant to an antibiotic and have virulence potential [7]. Both of these bacteria can infect the human body, especially the skin, so antibacterial substances are needed, especially those contained in bath soap.

The antibacterial substance that is often used in bath soap is triclosan. However, the use of triclosan is hazardous for the body when used in high doses and the long term because it can interfere with endocrine work. Triclosan can indeed kill 99.9% of bacteria, but one bacteria that is left behind will become bacteria that are resistant (immune) to antibacterial substances. Triclosan also has properties like chlorophenol, which is carcinogenic which can cause cancer if used in the long term [8].

Natural ingredients that have properties as foaming agents and natural antibacterial substances in soap are saponins. Plants containing saponins are the binahong plant (Anredera cordifolia (Tenore) Steen.) Which belongs to the basellaceae family, which is one of the medicinal plants that have great potential in the field of phytopharmaca. Binahong leaf extract contains secondary metabolite compounds. Fraction extraction and analysis by TLC contained alkaloids, saponins and flavonoids, and terpenoids [9-10]. These compounds contribute as foaming agents and antibacterial agents. Saponins are used in a variety of pharmacological activities because of their antibacterial properties, which have been used for medicinal purposes since ancient times [11]. Saponins in binahong leaves also have an antimicrobial activity that can disrupt the stability of the bacterial cell membrane so that cells undergo lysis [12].

Binahong leaf extract can inhibit *S. aureus* bacteria in wounds where at a concentration of 10%, it has a healing effect. At a concentration of 20% and 40%, it provides a more effective healing effect seen in the percentage of wound length coverage [13]. Binahong leaf extract can also inhibit the growth of Shigella flexneri bacteria with an average inhibition zone diameter of 27.2 mm [14].

Liquid soap products contain surfactants from natural ingredients until they are hard to find on the market. This provides evidence that many liquid bath soaps still use synthetic surfactants which have the potential to cause skin irritation [15]. Several previous studies [16,17] have described the use of ethanol extracts from natural ingredients as an alternative to antiseptics. In this research, binahong leaf extraction was carried out with water solvent (water extract) and then used as a foaming agent and natural antibacterial agent in making liquid bath soap, making it safer to use as liquid soap.

METHODS

1. Tools and Materials

Plastic buckets, test tubes, test tube racks, glass stirrers, 500 ml beaker cups, 10 ml measuring cups, spatulas, dropper pipettes, glass funnels, 40 mesh sieves, water baths, watch glasses, thermometers, analytical balance, bunsen, electric stove, hot plate, magnetic stirrer, blender, rotary evaporator, oven, volume pipette, glass burette, pycnometer, measuring flask, pH meter, and a set of antibacterial test kits.

The materials used include binahong leaves, distilled water, chloroform, concentrated H₂SO₄, 2 N HCl, 0.1 N alcoholic HCl, anhydrous CH₃-COOH, aluminum foil, filter paper, PP indicator, stearic acid, KOH 40%, olive oil, CMC, BHT, SLS, perfume, acetone, ethyl ether, kaolin, perchloride, carbon black, gasoline, beef fat, 10 cm x 10 cm cloth, *S. aureus* ATCC25923, *E. coli* ATCC 25922.

2. Binahong Leaf Extraction

Extracting the simplicia powder as much as 2x250 gram maceration method with 2x1.5 L distilled water for 2x24 hours with periodic stirring. Then filter it until you get the filtrate. Furthermore, the concentrated mass is evaporated using a vacuum rotary evaporator.

3. Identification of saponins in the binahong leaf water extract as much as

0.5 grams and 10 ml of distilled water in a flask and shake vigorously for 10 seconds. The test tube is allowed to stand then observes and measures the height of the foam. Test positive if the foam height of 1-10 cm is stable for about 10 minutes and after adding 1 drop of HCl 2 N the foam remains stable [18]. Lieberman Burchard's test showed the presence of a steroid saponin type. A positive test, if a brown or violet ring is formed on the two solvent borders, it indicates the presence of triterpenoid saponins. In contrast, the green or blue color indicates the presence of steroid saponins. [10].

4. Preparation of Binahong Leaf Water Extract Liquid Soap Preparations

The formulation of liquid bath soap with SLS variations: binahong leaf water extract (gr/gr) made in this study is the following Table1 [19].

material	1	2	3	4	5	6
	(5:0)	(4:1)	(3:2)	(2:3)	(1:4)	(0:5)
Extract water	0 gr	0,5 gr	1,0 gr	1,5 gr	2,0 gr	2,5 gr
Olive oil	15 ml					
KOH 40%	8 ml					
CMC	1 gr					
SLS	2,5 gr	2,0 gr	1,5 gr	1,0 gr	0,5 gr	0 gr
Stearic acid	0,25 gr					
BHT	0,5 gr					
Perfume	2 ml					
Aquades	Ad 65 ml					

Table 1. Liquid Bath Soap Preparation Formulations

The test for the quality requirements of bath soap is carried out in accordance with the Indonesian National Standard (06-4085-1996) procedure regarding Liquid Bath Soap [20].

5. Foaming Power and Foam Stability Test

200 ml of 1% liquid bath soap solution is blended at level one for three seconds, then the volume of foam is recorded. The amount of foam was recorded after standing for 0.5 minutes and 5.5 minutes. The foaming rate is the volume of the foam after standing for 0.5 minutes. Whereas the foam stability is the combination of the foam volume when it is 5.5 minutes against the foam volume of 0.5 minutes [21].

6. Standard Manure Preparation

A total of 19.32 grams of kaolin, 600 mg of the perchloride, 80 mg of carbon black, 5 grams of gasoline, 10 grams of beef fat were weighed. Each of them was suspended with acetone into a beaker. The solution is then put into a 500 ml volumetric flask, and acetone is added to the limit mark. Then the volumetric flask is closed and shaken for 5 minutes until it is mixed [22].

7. Detergency Test

white cloth measuring 10 cm x 10 cm. A clean cloth is dried in an oven with a heating temperature of 105°C for 3 hours. Put in a desiccator for 1 hour. The dry cloth is then weighed and recorded as the dry weight of the clean cloth. The cloth is then put in a beaker glass containing standard dirt and stirred for 30 minutes until the dirt sticks to the cloth. After that, the cloth is removed and aerated for 30 minutes. The cloth that has been aerated is then heated again for 3 hours with a heating temperature of 105°C. Then the dry cloth is weighed again and recorded as the weight of the fabric and the initial dirt. The cloth is then washed using each preparation of 1% soap solution for 30 minutes at a stirrer speed of 4 rpm. After that, the cloth is removed and let stand for 30 minutes. The cloth is then heated again with a heating temperature of 105°C for 30 minutes. The cloth is then weighed and recorded as the weight of the cloth and the final dirt [22].

8. Community Antibacterial Test

The antibacterial test method was carried out by diffusion of the modified Muller Hinton Agar (MHA) media wells. Taking with a sterile loop needle, the test bacteria, namely *S. aureus* ATCC 25923 and *E. coli* ATCC 25922, then implanted it on the media so that it tilted by scratching. Subsequently incubated in an incubator at 37°C for 24 hours. The same treatment was carried out for each type of tested bacteria. Then, take the test bacteria, namely *S. aureus* and *E. coli*, on the media to tilt it with a sterile loop wire and then suspend it into a tube containing 2 ml of 0.9% NaCl solution until the turbidity is the same as the standard turbidity of the Mc solution. Farland.

After that, dripped the test solution, namely the bath soap preparation produced on the well as much as 50 µl using a micropipette, and then incubated in an incubator at 37°C for 24 hours. Do the same for the positive control (tetracycline) and negative control (sterile distilled water). Observing the drag zone formed by measuring the diameter of the resistance area around the well using vertical and horizontal calipers. The results obtained are reduced by the diameter of the well where the inhibition area is a clear area.

RESULTS AND DISCUSSION

Wash binahong leaves so that there are no stains on the sheets that can interfere with the extraction. Drying by not being exposed to direct sunlight until the leaves dry and turn blackish-green in this study aims to reduce the moisture content in the leaves, prevent enzymatic reactions, and not damage the composition of the active compounds contained therein. Binahong leaf refinement seeks to expand the sample interaction with the solvent during extraction so that the maximum extraction results are obtained [23]. The binahong leaves produced in the preparation are 500 grams.

Binahong leaf extraction is done by maceration using a water solvent. The maceration method was chosen because it is easy to work with, the equipment required is simple, and the sample used is soft [17]. The resulting macerate is dark green, which is then concentrated using a rotary evaporator at a temperature of 900C. Concentration was carried out to evaporate the distilled water solvent to obtain a water extract of the binahong leaves in the form of a gel and a green-black color of 51.93 grams so that the yield was 10.386%.

1. Saponin Identification in Binahong Leaf Water Extract

The results of the binahong leaf water extract foam test showed that the binahong leaves were positive for saponins, whereas the binahong leaf water extract produced foam with a height of 1 - 1.3 cm and was stable for more than 10 minutes. The emergence of foam indicates that the binahong leaf aqueous extract contains saponins, which can form foam when shaken in water. This foam is caused because when shaken, saponins are hydrolyzed in water to produce aglycones (hydrophobic) and glucose (hydrophilic). This aglycone can create foam because it is a sapogenin [24]. The saponin test reaction is shown in Figure 1.



Figure 1. Hydrolysis of saponins in water [18]

The formation of a brown ring in the Lieberman Burchard test shows that the saponins contained in the binahong leaf extract are triterpenoid saponins. The reaction of terpenoids with Lieberman Burchard's reagent can be seen in Figure 2.



Figure 2. Reaction of terpenoids with Lieberman Burchard reagent [18]

2. Binahong Leaf Water Extract Bath Soap

There is six-bath soap produced in this study. Control soap with a ratio of SLS foaming agent: binahong leaf water extract (gr/gr) of 5: 0 is preparation 1, while prepara-tion 2 (4: 1); 3 (3: 2); 4 (2: 3); 5 (1: 4) and 6 (0: 5). The combination of SLS and binahong leaf water extract was made because SLS and binahong leaf water extract both have properties as surfactants. Also, binahong leaf water extract also functions as a natural antibacterial agent. The increase in the concentration of

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binahong leaf water extract in bath soap was carried out to determine the amount of foam formation and its effect on its antibacterial activity against *S. aureus* and *E. coli* bacteria.

3. Chemical and Physical Properties of Liquid Bath Soap

The results of the SNI (06-4085-1996) test carried out include the physical state of liquid bath soap, pH, free alkaline content, and specific gravity which can be seen in Table 2.

Preparation		Free	Specific gravity (gr / ml)	Physical State		
(SLS: extract)	рН	alkaline content (%)		Liquid form	Smell	Color
SNI	8 – 11	Maks. 0.1	1.01 – 1.10	Homogeneous	Typical	Typical
1 (5:0)	8.40	0	1.02	Slightly liquid, homogeneous	Fragrant, soapy smell	White
2 (4:1)	8.43	0.09	1.01	Slightly liquid, homogeneous	Fragrant, soapy smell	Light green
3 (3:2)	8.60	0.08	1.02	Thick, homogeneous	Fragrant, soapy smell	Light green
4 (2:3)	8.63	0.06	1.01	Thick, homogeneous	Fragrant, soapy smell	Dark green
5 (1:4)	8.87	0.04	1.06	Thick, homogeneous	Fragrant, soapy smell	Dark green
6 (0:5)	8.90	0.02	1.10	Thick, homogeneous	Fragrant, soapy smell	Dark green

The test results of the six liquid bath soap preparations in this study are in accordance with the quality requirements of liquid bath soap based on the Indonesian National Standard (06-4085-1996). Based on the physical condition of the liquid bath soap produced, the viscosity of the resulting liquid bath soap increases along with the increasing amount of binahong leaf water extract added. In addition, the addition of the amount of binahong leaf water extract in the liquid bath soap preparation in this study also had an effect on the increase in soap pH, which ranged from pH 8.40 to 8.90. This pH test is very important to do because if the pH of the liquid bath soap produced does not match the skin's pH (pH 8.0 - 10.8) it will cause skin problems for its users[16].

The measurement of free alkaline content in liquid bath soap is intended to determine the excess base or alkali in liquid bath soap. Free alkaline levels that exceed the Indonesian National Standard can cause skin irritation. The results of the free alkaline content of the liquid bath soap produced in this study ranged from 0-0.09% according to the SNI requirements where the maximum allowed free alkaline content was 0.1%.

The results of the calculation of specific gravity in this study ranged from 1.01 to 1.10. These results are in accordance with the SNI requirements where the permissible specific gravity of liquid bath soap ranges from 1.01 to 1.10. This specific gravity value has increased and decreased due to variations in the combination of binahong leaf water extract: SLS. The greater the density of a substance, the greater its viscosity, so that it is more difficult for the substance to dissolve in water.

4. Foaming Power and Foam Stability

The data resulting from the combination of foaming power values, foam stability and detergency can be seen in Table 3.

Preparations (SLS: Water Extract)	Foaming Power (ml)	Stabilitas Busa (%)	Detergency Test (%)
Control (5: 0)	25.2	67	30.95
Preparation 2 (4: 1)	22	60	32.05
Preparation 3 (3: 2)	18.4	55	35.53
Preparation 4 (2: 3)	15.6	44	40
Preparation 5 (1: 4)	12.6	40	38.27
Preparation 6 (0: 5)	8.8	30	35.90

Table 3. Results of Foaming Power Test, Foam Stability and Detergency Power

Based on the results of the study, the foaming power and stability of the soap foam decreased along with the reduced number of SLS in the liquid bath soap made. Control liquid bath soap that uses only SLS active ingredients without binahong leaf water extract produces a greater amount of foam and is more concentrated (thick) than other liquid bath soap preparations. The more binahong leaf aqueous extract that is added to the formulation, the less the foam stability will be. This is consistent with another study [25] where the reduced SLS, the lower the foam stability. The results showed that the stability of liquid bath soap 6 preparations is smaller than other bath soap preparations, which means that the liquid bath soap foam made from binahong leaf water extract alone is very environmentally friendly because it easily decomposes in water. In addition, bath soap with an excessive amount of foam is also dangerous because it can cause skin irritation due to the large amount of foaming agents it contains [16].

5. Detergency Power

The detergency test was conducted to determine the ability of liquid bath soap to bind dirt. The results of the detergency test can be seen in Table 3. Soap of good quality is soap that has a high detergency power [26]. Liquid bath soap using binahong leaf water extract has a better detergency power compared to liquid bath soap using SLS foaming agent where the foam from binahong leaf water extract has better detergency power than the foam from SLS in liquid bath soap preparations. Liquid bath soap 4 preparations that use a combination of SLS: binahong leaf water extract with a combination of 2: 3 is a liquid bath soap that has the best detergency power compared to other liquid bath soaps with a detergency power of 40%. The detergency value in this study increased from control to preparation 4 and decreased in preparations 5 and 6. The decrease in detergency power was due to the fact that the liquid bath soap in preparations 5 and 6 was very thick. The greater the specific gravity of preparations 5 and 6, the more likely the liquid bath soap will become dirt sticking to the fabric rather than being soluble in water.

If the detergency of the liquid bath soap produced is related to its foaming power and stability, it can be seen that a bath soap that has a lot of foam and is stable does not necessarily have good detergency power. The results showed that the detergency power of liquid bath soap was not directly proportional to its foaming power and foam stability, where a good product detergency could be obtained in the presence of a combination of foaming agents at certain doses [22].

6. Antibacterial Activity

The results of the antibacterial activity test against *S. aureus* and *E. coli* can be seen in Table 4 and Figure 3. Diameter of Inhibited Zone (DDH) is the largest diameter of the clear zone formed around the well. The antibacterial strength was categorized as weak if the DDH was \leq 5 mm, moderate if the DDH was 5-10 mm, strong if the DDH was between 10-20 mm, and very strong if the DDH was> 20 mm [27].

Surfactants are surface active compounds that can dissolve fats in the bacterial cell walls, so that the bacterial cells undergo lysis which results in cell damage. In preparations 1 - 3, where the SLS concentration was higher than the water extract of binahong leaves, the antibacterial activity test on S. aureus produced a DDH value that was greater than the DDH value in E. coli. This suggests that SLS is more effective at inhibiting gram-positive bacteria than gram-negative bacteria [28-29]. Furthermore, on preparations 4 - 6, where the water extract concentration of binahong leaves was higher than SLS, the DDH value in S. aureus was as good as the DDH value in E. coli. This shows that the binahong leaf aqueous extract shows

the same inhibitory activity, both against grampositive and gram-negative bacteria. The best results were shown in preparation 6, where in *S. aureus*, together with preparation 1 had a DDH value of 13.5 mm (strong) and in *E. coli* with a DDH value of 12.5 mm (strong). The negative control showed a DDH value of 0 mm for both *S. aureus* and *E. coli*. This indicates that the control used has no effect on the antibacterial test. Meanwhile, the mechanism of action of tetracyclines (positive control) as antibacterial by inhibiting protein synthesis by preventing the binding of tRNA in ribosomes during peptide chain extension [30].

Sabun Cair	S. a	ureus	E. coli	
(SLS : Ekstrak Air)	DDH (mm)	Category	DDH (mm)	Category
Preparation 1 (5: 0)	13.5	Strong	5	Moderate
Preparation 2 (4: 1)	11.5	Strong	8	Moderate
Preparation 3 (3: 2)	9.5	Moderate	8	Moderate
Preparation 4 (2: 3)	9	Moderate	9.5	Moderate
Preparation 5 (1: 4)	11	Strong	12	Strong
Preparation 6 (0: 5)	13.5	Strong	12.5	Strong
Tetracyclines (positive control)	29	Very strong	22.5	Very strong
Sterile distilled water (negative control)	0	-	0	-



Figure 3. Inhibition zone of binahong leaf water extract liquid soap preparation against *S. aureus* and *E. coli* (1) Control, SLS: water extract (5: 0); (2) preparation I, SLS: water extract (4: 1); (3) Preparation II, SLS: water extract (3: 2); (4) Preparations III, SLS: water extract (2: 3); (5) IV, SLS preparations: water extract (1: 4); (6) Preparations V, SLS: water extract (0: 5); (7) Tetracyclines; (8) Sterile distilled water

CONCLUSION

Binahong leaf water extract can be used as a natural foaming agent in antiseptic bath soap because it contains saponins and produces a stable foam for more than 10 minutes. The resulting soap has met the quality requirements of SNI 06-4085-1996 concerning Liquid Bath Soap, where preparation 4 has the best results with a detergency power of 40%; pH 8.87; free alkaline content 0.04%; and specific gravity 1.06 gr /ml. Liquid bath soap with binahong leaf water extract surfactant (preparation 6) had the highest antibacterial activity against *S. aureus* and *E. coli* with the inhibition area diameter values of 13.5 mm (strong) and 12.5 mm (strong), respectively.

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