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Liquid Soap Formulation of Lahuna Leave Extract (*Eupatorium odoratum)* and Betel Leave Extract (*Piper betle* L.) and Activity Against *Escherichia coli*

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Abstract: Lahuna and betel are plants that have been applied traditionally as a medicine. The content of secondary metabolites in lahuna and betel leaves can be used as active compounds in manufacturing liquid soap. This study aims to evaluate liquid soap formulations of extract lahuna leave and extract betel leave and to test their activity against *Escherichia coli*. The research method applied was pH test, organoleptic test, homogeneity test, foam height test, water content test according to SNI 1996, and antibacterial activity test with agar diffusion method. The results revealed that the liquid soap formulation was in accordance with Indonesian National Standard (SNI) 1996 which was based on pH test, organoleptic test, homogeneity test, foam height test, and water content test. Also, the liquid soap formulation has very strong activity against *Escherichia coli* characterized by a clear zone that is formed more than 19 mm

Keywords*: Antibacterial, Bethel, Lahuna, Liquid soap, SNI 1996*

INTRODUCTION

Soap is one of the basic needs in maintaining the cleanliness of the body and the environment. Soap is formed from the results of saponification between oil and base (Sriwening and Susanti, 2022). In preparing soap, you can add active ingredients that are antibacterial, such as researched by Hutauruk *et al.,* (2020) adding celery herb in liquid soap formulations as a source of active compounds that can inhibit *Staphylococcus aureus bacteria*. Research using natural ingredients as a source of active compounds has been developed, one of which is white tea extract which is used as a source of active compounds for making liquid soap (Widyasanti, Winaya, Rosalinda, 2019). Other natural ingredients that can be applied as a source of active compounds are lahuna leaves and betel leaves.

Lahuna (*Eupatorium odoratum*) is a wild plant, which the rural people of Bulukumba use as a medicine that can accelerate wound healing. Lahuna leaves are the most widely used as traditional medicine. Research by Amaliah *et al.*, (2019) revealed that lahuna leaves contain flavonoids, saponins, and tannins. The content of saponins and tannins in Lahuna leaves has the potential as an antibacterial compound so that it can be used as a source of active compounds in manufacturing liquid soap.

Betel is a plant that is usually planted around the house and is widely used as a traditional medicine for itching and treating cancer sores. Research by Sarma *et al.,* (2018) reported that the ethanol extract of betel leaves has antimicrobial activity against

pathogenic bacteria such as *B. subtilis, E. coli*, *A. niger* and *S. cerevisiae* and has an antioxidant effect. Betel leaves contain metabolites such as alkaloids, flavonoids, steroids, terpenoids, saponins and tannins and research on betel leaf extracts reveals wound healing activity in test animals (Zar'ah, Syachruddin, Kusmiyati, 2021).

The formulation of extract lahuna leave and extract betel leave can be applied as a source of active compounds in manufacturing liquid soap which has antibacterial activity. The liquid soap that is made must fulfil the requirements according to the standards set by SNI. Evaluation of the quality of liquid soap can be carried out by tests such as organoleptic tests, pH tests, foam height, homogeneity tests, water content tests and antibacterial activity tests. Formulations of extract Lahuna leaves and extract betel leave are expected to be SNI-compliant liquid soaps.

RESEARCH METHODS

Materials and Tools

The equipment used in this study included standard glassware (pyrex), magnetic stirrer (XMTD-204), analytical balance (ADAM), pH meter (Mediatech Digital), porcelain cup, oven (Hot Air Oven YCO-NO1), desiccator, Microscope (OLYMPUS), *object glass*, and ruler

The materials used in this study included betel leaf extract, lahuna leaf extract, 96% ethanol, *distilled water*, coconut oil, 30% KOH, Na-CMC, citric acid, *Escherichia coli bacteria*

Procedures

Making Liquid Soap from Extract Lahuna Leave and Extract Betel Leave

Liquid soap from extract lahuna leaves and extract betel leave were made according to the formulation in Table 1. Each liquid soap formulation is only distinguished by the concentration of its active ingredients.

Table 1. Formulation of liquid soap from lahuna leaf extract and betel leaf extract

Material Name	Function	Formulation I	Formulation II	Formulation III
Lahoona leaf extract	Active substance	25 %	50%	75%
Betel leaf extract	Active substance	75%	50%	25%
Coconut oil	Fatty acid	30mL	30mL	30mL
KOH 30%	Alkali	20 g	20 g	20 g
Citric acid	pH neutralizer	11g	11g	11g
Na-CMC	Fillers and Emulsifiers	2 g	2 g	2 g
Aquades	Solvent	100mL	100mL	100mL

Testing the antibacterial activity of liquid soap preparations against Escherichia coli bacteria

The liquid soap made was tested for antibacterial activity against *Escherichia coli* using the Novaryatiin, *et al* ., (2018) method, namely the agar diffusion method using the paper disk technique.

Evaluation of SNI 1996 liquid soap preparations

Organoleptic test, is carried out to observe the physical appearance of a preparation including texture, color and smell. According to SNI, the standard set for liquid soap is that it has a liquid texture, and a distinctive smell and color. The organoleptic test was carried out by testing the preference level of 30 panelists in the form of giving questionnaires regarding the physical properties of liquid soap preparations including color, smell and texture of the preparations.

The pH test is carried out using a pH meter (which is calibrated with a pH buffer solution before each measurement), then the electrode which has been cleaned with distilled water is dipped into 1 ml of liquid soap sample which has been diluted with 10 ml of distilled water and then examined at room temperature, The pH value that appears on the pH meter scale is monitored and recorded.

The foam height test was carried out by putting 50 ml of liquid soap with a concentration of 0.1% into a 100 ml closed measuring cup and shaking it regularly for 20 seconds. foam height.

The homogeneity test was carried out by weighing 0.1 gram of liquid soap formulation on an *object glass,* then observing it under a microscope at 100x magnification.

The moisture content test was carried out by placing the cup in the drying cupboard for 1 hour, then put it in the desiccator so that the cup temperature returns to normal. Weight the empty weight of the cup and record the weight, then put 5 grams of sample in the cup and then dry it in a drying cupboard for 2 hours at 105°C. After 2 hours, removed, placed in a desiccator, and weighed the cup and the sample.

RESULTS AND DISCUSSION

Making Liquid Soap from Lahore Leaf Extract and Betel Leaf Extract

Liquid soap made from lahuna and betel extracts leaves obtained three formulations. The three formulations through observation revealed a clear yellow color, with a distinctive aroma, liquid texture, lots of foam and a rough taste after applying as shown in Figure 1.

I II III

Figure 1. Liquid Soap formulation of extract lahuna leave and extract betel leave

Examine the antibacterial activity of liquid soap preparations

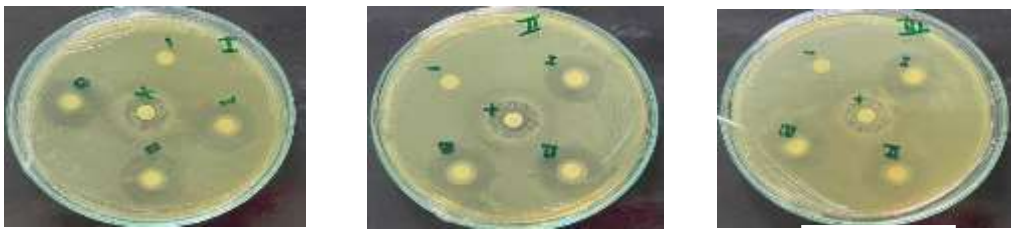
Examine the antibacterial activity of 3 liquid soap formulations applying the agar diffusion method with the paper disk technique. The test bacteria applied were *Escherichia coli bacteria.*

Table 2 . Results of Measurement of Inhibitory Power of Liquid Soap Formulations Against *Escherichia coli Bacteria*

Formulation	Cup I	Cup II	Cup III	Average(mm)	Interpretation of Inhibitory Power
I	21.4mm	20.7mm	20.6mm	20.9	*Susceptible*
II	22.1mm	23.5mm	21.4mm	22.3	*Susceptible*
III	22.5mm	22.9mm	20.8mm	22.06	*Susceptible*
(+) Ciprofloxacin	14.5mm	16.9mm	15.5mm	15.6	*resistant*
(-) Aquades	-	-	-	-	-

Description : Interpretation of Inhibitory Power
≤14 mm : *Resistant*
15-18mm : *Intermediates*
≥19 mm : *Susceptible* (CLSI, 2013)

Examine the three liquid soap formulations against *Escherichia coli bacteria* revealed results as shown in Figure 2. The interpretation of the potential inhibition of each formulation was very strong, as evidenced by the measurement of the clear zone which formed more than 19 mm. The three liquid soap formulations was far stronger than ciprofloxacin, a positive control with average inhibition 15.6 mm. Ciprofloxacin was used as a positive control because it has a broad spectrum that can inhibit Gram positive and Gram negative bacteria such as *Staphylococcus aureus* and *Escherichia coli*. Ciprofloxacin has been applied to treat a range of diseases skin and soft tissue (Shariati et al., 2022).



Cawan I Cawan II Cawan III

Figure 2. Inhibitory Power of Liquid Soap Formulation Against *Escherichia coli Bacteria*

The presence of active compounds in the extracts of lahuna and betel leaves in soap formulations is an important factor in inhibiting the activity of pathogenic bacteria. The formation of a clear zone indicates that the active compounds contained in the liquid soap formulation have antibacterial activity. According to research by Ligina and Sudarmin (2022), active compounds such as the Alkaloid group have antibacterial activity. And based on previous research, lahuna leaf extract and betel leaf extract contain alkaloid compounds. In addition, tannin compounds in betel leaves have antibacterial activity whose mechanism of action is by damaging bacterial cell membranes, and the *astringent compounds* induce

the formation of complexes bound to microbial or enzyme substrates and the formation of metal ions as well as to increase tannin toxicity (Sadiah *et al*, 2022).

Evaluation of liquid soap preparations

Organoleptic test

The organoleptic test was carried out by observing the physical appearance of the formulation in the form of color, aroma, texture, amount of foam, and rough taste. Based on the Indonesian National Standard (1996), the physical appearance of liquid soap formulations is in accordance with SNI (1996). In addition, organoleptic tests were also carried out by giving liquid soap preparations to 30 untrained panelists to assess color, aroma, texture, amount of foam, and a rough feeling when applying with really like, like, moderate like, dislike, and really dislike responses. The response of the panelists to the formulation of the soap preparations for each category of color, aroma, texture, lots of foam, and rough taste is shown in Table 3

Table 3 . Panelist Assessment

Panelist Assessment of 30 people	Formulation I					Formulation II					Formulation III				
	SS	S	CS	KS	TS	SS	S	CS	KS	TS	SS	S	CS	KS	TS
Color	12	18	0	0	0	11	19	0	0	0	9	21	0	0	0
Aroma	4	26	0	0	0	5	25	0	0	0	6	24	0	0	0
Texture	2	28	0	0	0	1	29	0	0	0	1	29	0	0	0
Lots of Foam	1	29	0	0	0	1	29	0	0	0	1	29	0	0	0
Rough	1	29	0	0	0	1	29	0	0	0	1	29	0	0	0

Description: SS (Really like), S (Like), CS (Enough Like), KS (Don't like it much), TS (Dislike)

pH test

Measurement of the pH value of liquid soap preparations was carried out using a pH meter. The pH meter applied is first calibrated in order to get accurate measurement results. Measuring the pH of liquid soap is a requirement for the quality of liquid soap, this is because the liquid soap has direct contact with the skin which if the pH is not appropriate can irritate the skin (Haque *et al*., 2022). Liquid soap formulation I has a pH value of 9.25, formulation II has a pH value of 9.34 and formulation III has a pH value of 9.84. Based on the SNI (1996) the allowable pH range for liquid body soap is 8-11. The pH value of liquid soap formulation preparations is made according to the SNI pH range.

Foam height test

The foam height test aims to see how much foam is produced and is in accordance with the standard soap foam height set by SNI (1996), namely 13-220 mm. Based on the measurement of foam height, the liquid soap formulation revealed results that were in accordance with the SNI (1996).

Table 4. Results of Measurement of Foam Height of Liquid Soap Preparations

formulation	Foam Height(mm)
I	120
II	130
III	145

Table 5. Liquid Soap Preparation of Water Content

Formulation	Empty Weight	Empty Weight + sample	Dry Weight	Water content (%)
I	1. 35.9210	1. 40.8652	1. 36.5211	1. 60
	2. 33.8884	2. 38.9132	2. 34.4740	2. 58
II	1. 34.5843	1. 39.6573	1. 35.1849	1. 60
	2. 35.6576	2. 40.7009	2. 36.3146	2. 65
III	1. 33.5218	1. 38.6081	1. 34.2148	1. 69
	2. 31.5949	2. 36.6395	2. 32. 2767	2. 68

Homogeneity test

Homogeneity, test is used to find out that all substances are evenly distributed in the preparation. The test was carried out by weighing 0.1 gram placed on an *object glass,* then observed under a microscope at 100x magnification. The results of testing formulations I, and III liquid soap of lahuna extract and betel extract showed homogeneous results marked by the absence of coarse grains while in formulation II there were small bubbles. Can be seen in Figure 3.

I II III

Figure 3. Observation Results of Liquid Soap Preparations with a Microscope

Examine the moisture content

The water content test was carried out to determine the amount of water contained in the liquid soap preparation. The water content standard set by the Indonesian National Standard (1996) is a maximum of 60%. Based on testing the water content of liquid soap formulations I and II showed data in accordance with the provisions of SNI (1996) while formulation III the results of measurements of water content exceeded 60%, this could be caused by the mixing speed and the addition of hygroscopic materials, such as Na-CMC and affected by the addition of distilled water.

CONCLUSIONS

Based on the results of the evaluation the liquid soap formulation complies with SNI 1996 stated on pH test, organoleptic test, homogeneity test, foam height test, and water content test. In addition, liquid soap formulation has activity against *Escherichia coli* which is very strong, and is characterized by a clear zone formed more than 19 mm.

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