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Formulation of Lahuna Leave (*Eupatorium odoratum*) and Sirih Leave Extract (*Piper betle L.*) as Antiseptic Liquid Soap

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Abstract

Infectious diseases caused by microorganisms are the main cause of high morbidity and mortality in the world. One of the plants that have the potential as an antiseptic is a lahuna leaf. Lahuna leaves contain active antibacterial compounds and the addition of betel leaf to the liquid soap formulations can strengthen the activity of the antiseptic produced. The purpose of this research is to test the effectiveness of liquid soap formulations of lahuna leaves and betel leaves as an antiseptic. The methods used include phytochemical screening, physical observation of liquid soap formulations, and antibacterial activity tests using agar diffusion methods. The results of the phytochemical analysis showed that lahuna leaves contain flavonoids, tannins, terpenoids, and alkaloids, while betel leaves contain flavonoids, saponins, tannins, terpenoids, and alkaloids. The liquid soap formulation of lahuna leaves and betel leaves has a clear yellow color, distinctive aroma, liquid form, rough taste, and lots of foam and has very strong inhibition against *Staphylococcus aureus* which is characterized by clear zones formed for each formulation I (22.4 mm), formulation II (21.8 mm) and formulation III (20.1 mm). These results indicate the potential of liquid soap formulations of lahuna leaves and betel leaves as antiseptic soap.

Keywords: Lahuna leave, Betel leaves, Liquid soap, Phytochemical, Antiseptic

INTRODUCTION

Today, diseases caused by infectious microorganisms such as viruses and bacteria are increasing. Infectious diseases are still the main cause of high morbidity and mortality in the world (Sachwiver, Surya, & Elianora, 2018). The types of bacteria that most often infect humans are *Staphylococcus aureus Sp* (Rizky *et al.*, 2021) and *Escherichia coli* (Rizky, Agrijanti, & Inayati, 2019).

Controlling the spread of bacterial and viral infections is very important. One effort to prevent viral and bacterial infections is by keeping the body clean. Maintaining body hygiene can be done by cleaning the limbs using soap. Types of soap that can kill bacteria and viruses are soaps that contain antiseptic active ingredients. Antiseptics are chemical substances whose mechanism of action is to destroy microorganisms or inhibit their work, to prevent infection in living tissue (Parawansah et al., 2021). The active ingredient added to soap as an antiseptic can be in the form of synthetic chemicals or active ingredients from natural ingredients. The use of synthetic active ingredients such as alcohol and triclosan has effects such as irritation, antibiotic resistance, and disruption of the hormone system. However, this happens if the use of triclosan exceeds 0.3% (Marhamah, Ujiani, & Tuntun, 2019).

Several studies have used natural ingredients as a source of antimicrobials, such as the extract of kelubi fruit pulp which has antibacterial activity against *Staphylococcus aureus* and *E. coli* (Surtina, *et al*., 2020). Other natural ingredients used as solid bath soap preparations are Gedi leaf ethanol extract (Moppangga, Yamlean, & Abdullah, 2020), and green betel extract with garlic extract as a liquid soap formulation which has antifungal activity *Candida albicans* with high inhibition (Fitriana, Estikomah, Marfu'ah, 2018). White tea extract and coconut oil according to research by Widyasanti, Winaya & Rosalinda (2019) can be formulated in the manufacture of liquid soap which has antibacterial activity. Other studies have used the ethanol extract of nutmeg leaves as a source of active compounds which have been shown to have inhibitory properties against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. (Kappele, Souhoka & Walla, 2022). Active ingredients from other natural ingredients can also be used as antiseptics such as lahuna leaf extract and betel leaf extract.

Lahuna *(Eupatorium odoratum)* is a nutritious weed plant belonging to the Asteraceae family. This

plant is not popular in Indonesia because it is a wild plant, so its utilization is not optimal among the community (Sari, Hafif, & Soesatrijo, 2016). Rural communities in Bulukumba, use lahuna leaves as a wound medicine that can accelerate wound healing. The lahuna leaves are crushed, and the leaf extract is then applied to the wound. Research (Yenti, Afrianti, & Afriani, 2011) shows that the ethanol extract of kriyuh leaves (another name for lahuna) (*Eupatorium odoratum*) in the form of cream preparations shows a faster wound healing effect than 10% *povidone-iodine.* Based on the phytochemical tests, shows that the leaves of lahuna (*Eupatorium odoratum*) are positive for flavonoids, saponins, and tannins and have the effect of lowering glucose in the blood (Amaliah, Johannes, Hassan, Tambaru, 2019).

Betel leaf (*Piper betle L.*) is a plant used by the community as medicine. Betel leaves contain essential oils and metabolite compounds that have antibacterial activity (Madhumitaa, Guhaa, & Nag, 2019). Research (Sarma, *et al*. 2018), states 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. In addition, betel leaf also contains metabolites such as alkaloids, flavonoids, steroids, terpenoids, saponins, and tannins, and research on betel leaf extract shows wound healing activity in test animals (Za'rah, Syachruddin, & Kusmiyati, 2021). Based on the GC-MS analysis of the volatile compounds contained in betel leaf extract, namely Eugene, β-caryophyllene, γ-muurolene, valencene, eucalyptol, chavicol and caryophellene oxide (Islam et al., 2020).

Formulation of liquid soap preparations from lahuna leaf extract and betel leaf extract, each of which contains potential active compounds based on phytochemical tests, is expected to have strong antiseptic activity inhibiting the growth of bacteria and viruses. Research on the utilization of lahuna and betel leaf extracts as active ingredients in soap has not been reported, so in this study, the utilization of lahuna leaf extract and betel leaf extract as active ingredients in antiseptic soap formulations was carried out.

METHODOLOGY

Instrumentals and materials

The equipment used in this study included a blender (Philips), basin, filter, standard glassware (pyrex), *rotary evaporator* (IKA RV 10 digital), analytical balance (ADAM), magnetic stirrer, and Petri dishes. The materials used in this study included betel leaf, lahuna leaf, 96% ethanol, *distilled water*, coconut oil, 30% KOH, Na-CMC, citric acid, *Staphylococcus aureus Sp bacterial culture*, *Wagner* reagent, HCl, FeCl 3%, and *libermann-burchard* reagent, Ciprofloxacin.

Methods

Sample Preparation

This study used samples of lahuna leaves and betel leaves taken in Balong Village, Kec. Ujung Loe Kab. Bulukumba. The first stage was the preparation of samples of lahuna leaves and betel leaves, each sample being cleaned and washed with running water. After cleaning, the leaf samples were drained and then air-dried for 5 days. After that, the dried samples were ground using a blender to form a powder, after which they were sieved to obtain Simplicia powder.

Extract manufacture

The process of extracting lahuna leaves and betel leaves was carried out by maceration method with 96% ethanol solvent. A total of 250 grams of lahuna leaves and 250 grams of betel leaves were macerated with 5 L of 96% ethanol for 5 days, then filtered to obtain a filtrate. The filtrate obtained was then evaporated using *a rotary evaporator* at 40℃ to obtain thick extracts from lahuna leaves and betel leaves. After that, the extract was weighed and stored in a closed container.

Phytochemical test of lahuna leaf extract and betel leaf extract

Lahuna leaf extract and betel leaf extract Phytochemical tests were carried out to determine the active ingredient content of each extract. The Flavonoid test was carried out by adding 5 ml of ethanol and a few drops of $FeCl_3$ to each extract of lahuna and betel leaves as much as 10 mg. A positive test is marked by a color change to blue, purple, green, red, to black. If up to 20 drops of $FeCl_3$ are added and there is no color change, then the flavonoid is negative.

To test alkaloid compounds, 10 mg of lahuna and betel leaf extracts were added with 10 ml of HCl and heated for 2 minutes while stirring continuously, then filtered. The filtrate was added with 5 ml of HCl and *Wagner's* reagent. Terpenoid/steroid test using *a libermann-burchard reagent*, 10 mg of lahuna leaf extract dripped *with libermann-burchard reagent* until a positive bluish-green color is formed for steroids and a positive terpenoid ring is formed.

Saponin test, lahuna leaf extract, and betel leaf extract 0.5 gram each added with 5 ml distilled water and shaken vigorously. A positive test is indicated by the formation of foam/foam. Tannin test, lahuna leaf extract, and betel leaf extract boiled each 0.5 in 20 ml of distilled water in a test tube. Filtered and added a few drops of 0.1% $FeCl_3$ until it changes color. Positive results are indicated by the formation of a brownish-

green or blackish-blue color (Kumalasari and Andiarna, 2020).

Formulation of liquid soap from lahuna leaf extract and betel leaf extract

Formulations of liquid soap preparations of lahuna leaf extract and betel leaf extract are shown in Table 1.

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

Material Name	Function	Formulation I	Formulation II	Formulation III
Lahuna 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
Aquadest	Solvent	100mL	100mL	100mL

The liquid soap formulation was prepared by reacting 20 grams of 30% KOH which was developed in 20 mL distilled water with 30 mL coconut oil. The process of making soap paste using a magnetic stirrer. The result of the reaction between 30% KOH and coconut oil forms a soap paste. The soap paste was left at room temperature for 24 hours before being dissolved in distilled water. The soap paste that had been left standing was then dissolved in 100 mL of distilled water and added to each betel leaf extract and lahuna leaf extract in the ratio as shown in table 1. In the soap solution was added citric acid as a pH stabilizer and Na-CMC as a thickener.

Testing the antibacterial activity of liquid soap preparations

The liquid soap formulation that has been made was tested for its antibacterial activity against *Staphylococcus aureus Sp.* Using the agar diffusion method with the paper disk technique (Novaryatiin, Handayani, & Chairunnisa, 2018). One Ose of *Staphylococcus aureus Sp bacteria* from the stock was inoculated into a sterile Erlenmeyer containing liquid *Nutrient broth medium* and then incubated for 18 hours at 37 ^{o}C. 50 µL of the rejuvenated bacterial suspension was rubbed onto the surface of the Petri dish containing solid media using a cotton swab. And each soap formulation and positive control (Ciprofloxacin) and negative control (Aquades) was dripped onto *a paper disk* as much as 15 µL. Then, *the paper disk* was placed on solid media, incubated at 37^{0}C for 24 hours, and observed. The clear zone formed was measured using a caliper

RESULTS AND DISCUSSION

Prepared lahuna *(Eupatorium odoratum)* leaves are brownish green and betel (*Piper betle L.*) leaves are brownish in color. 250 grams of lahuna leaf and betel leaf samples were macerated using 96% ethanol to produce 28 grams of thick extract of lahuna leaves with a yield value of 11.2%. And betel leaves obtained a thick extract of 32 grams with a yield value of 12.8%. The viscous extracts of each of the lahuna and betel leaf extracts were then subjected to phytochemical tests. Phytochemical tests of lahuna and betel leaf extracts were carried out qualitatively using several specific reagents, the results of which can be seen in Table 2.

Phytochemical tests were carried out to provide an overview of the compounds contained in lahuna leaf extract and betel leaf extract to be used as active ingredients in antiseptic liquid soap formulations. From the results of the phytochemical test, the secondary metabolites contained in the lahuna extract were terpenoids, flavonoids, tannins, and alkaloids. While the betel leaf extract contains terpenoids, flavonoids, tannins, alkaloids, and saponins. In the phytochemical test, the lahuna leaf extract was saponin negative, but previous research by (Amaliah, Johannes, Hassan, & Tambaru, 2019) showed that the lahuna leaf extract was saponin positive. The difference in the metabolites produced is due to the different places or locations for taking samples of the lahuna leaves. According to Astuti & Respatie (2022), the factors that differentiate the content of secondary metabolites in a plant are internal factors in the form of genetic traits and external factors such as abiotic stress or environmental stress and biotic stress such as microorganisms. So there may be differences in the metabolite compounds contained even with the same sample, namely lahuna leaves. Metabolites contained in lahuna leaf extract and betel leaf extract can be used as active compounds in the manufacture of antiseptic liquid soap.

Several previous studies have shown that secondary metabolites can become active compounds that have antibacterial activity. Research (Santoni, Efdi, & Suhada, 2019) revealed that active compounds such as triterpenoids, saponins, and alkaloids have antimicrobial activity.

Table 2. Phytochemical test results for Lahuna Leaf Extract and Betel Leaf Extract

Group	Lahuna leaf extract	Betel leaf extract
Flavonoids	+ Black	+ Black
Alkaloids	+ Brownish green	+ Brownish green
Terpenoids / Steroids	+ Formed a ring	+ Formed a ring
Saponins	- No bubbles	+ There are bubbles
tannins	+ Brown	+ Bluish green

Note : (+) = positive and (–) = Negative

In addition, Salimi research, *et al.* (2019) revealed that the pentacyclic lupenol triterpenoid compound in Moringa leaves has moderate antibacterial activity against *S. aureus* and *E. coli*. Flavonoid secondary metabolites isolated from the ethyl acetate fraction of jackfruit stems have antibacterial activity of *E. coli* and *S. aureus*. (Indriani & Iswan, 2020). Metabolite compounds in lahuna leaf extract can be used as active compounds in liquid soap and combining them with betel leaf extract will increase the inhibition of liquid soap because the active compounds of betel leaf can contribute the same in inhibiting pathogenic bacteria. The research by Nau'e, Yamlean & Mpila (2020) combined the ethanol extracts of cherry leaves and basil leaves as a liquid soap preparation which has a strong inhibitory effect on *S. aureus*.

The use of natural ingredients as active ingredients in liquid soap has been widely developed, one of which is the use of essential oils from citronella which exhibit antibacterial activity against *S. aureus* and *E. coli* (Rumlus *et al*, 2022). Lahuna leaf extract and betel leaf extract were prepared in liquid soap preparations with 3 formulations with different concentration variations (Figure 1). Formulation I added lahuna and betel leaf extracts with a composition ratio of 75%:25%. Formulation II with the composition of lahuna leaf extract and betel leaf 50%: 50%. While formulation III compositions of lahuna leaf extract and betel leaf 25%: 75%. Based on physical observations of liquid soap preparations can be seen in Table 3.

The results of the antibacterial activity test showed that the three liquid soap formulations had a very strong ability to inhibit *Staphylococcus aureus bacteria* as evidenced by the clear zones that formed on each petri dish (Figure 2). In 1 formulation, the average diameter of the clear zone formed on the petri dish was 22.4 mm with a very strong interpretation.

Figure 1. Liquid Soap Formulation of Lahuna Leaf Extract and Betel Leaf Extract

Table 3. Physical Observation Results of Liquid Soap Preparations (F=formulation)

F	Color	Scent	Texture	Lots of Foam	Rough
I	Clear yellow	Unique	Liquid	Lots	Rough
II	Clear yellow	Unique	Liquid	Lots	Rough
III	Clear yellow	Unique	Liquid	Lots	Rough

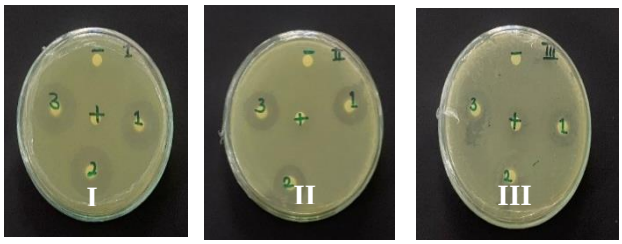

Figure 2. Results of testing the antibacterial activity of liquid soap preparations of lahuna leaf and betel leaf extracts

For liquid soap preparation, formulation II shows an average clear zone of 21.8 mm while formulation III is 20.1 mm with a very strong interpretation of inhibition. Antibacterial activity testing was carried out with three repetitions to obtain accurate data. The results of the clear zone measurements are shown in Table 4.

The inhibitory ability of the three liquid soap formulations is due to the secondary metabolites contained in the extracts of lahuna and betel leaves which are added to the liquid soap formulation. The presence of secondary metabolites is an important factor through their mechanism against bacteria.

Table 4. Results of Measurement of Inhibitory Power of Liquid Soap Formulations Against *Staphylococcus aureus Bacteria*

Formula	I(mm)	II (mm)	III (mm)	Average(mm)	Interpretation of Inhibitory Power
I	24.8	20.3	22.1	22.4	*Susceptible*
II	24.3	19.8	21.3	21.8	*Susceptible*
III	25.7	20.9	20.5	20.1	*Susceptible*
(+) Ciprofloxacin	8.6	8.9	13.9	10.46	*resistant*
(-)	-	-	-	-	-

Note: Interpretation of Inhibitory Power (Clinical Laboratory Standard Institute, 2013)
≤14 mm: *Resistant*
15-18 mm: *Intermediates*
≥19 mm: *Susceptible*

Nurhasanah, & Gultom (2020) revealed that the content of secondary metabolites such as alkaloids, phenolics, flavonoids, saponins, and steroids synergize and reinforce each other so that they can inhibit bacterial growth. The mechanism of antibacterial action of each of the secondary metabolites is different. Flavonoid compounds contained in lahuna leaf extract and betel leaf extract are antibacterial with an inhibition mechanism through 3 stages, namely inhibiting nucleic acid synthesis, inhibiting cell membrane function, and inhibiting energy metabolism (Rahman, Haniastuti, Utami. 2017).

Terpenoid compounds, one of the active compounds inhibit bacteria, by damaging the membrane. Terpenoids can react with porins (transmembrane proteins) on the outer membrane of the bacterial cell wall, form strong polymer bonds and damage the porin, reducing the permeability of the bacterial cell wall so that the bacterial cell is deficient in nutrition and causing bacterial death (Widowati, Handayani, Lasdi. 2019). Saponin compounds can increase the permeability of cell membranes so that the membrane becomes unstable and causes cell hemolysis (Mahmiah, Rama & Riwanti, 2020). Tannin compounds can inactivate microbial cell adhesin, inactivate enzymes and disrupt protein transport in the inner layer of cells (Ngajow, Abidjulu, Kamu, 2013). The mechanism of action of alkaloid compounds is to interfere with the peptidoglycan constituent components in bacterial cells so that the cell wall layer is not formed completely and causes cell death (Nurhasanah, Gultom. 2020)

CONCLUSIONS

Based on the research conducted, it can be concluded that lahuna leaf extract and betel leaf extract contain active compounds that have the potential as antibacterials. These active compounds work together to inhibit the bacteria *Staphylococcus aureus* in the form of liquid soap formulation.

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