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Formulation and Evaluation of Liquid Soap Containing Ethanolic Extract of *Eleutherine palmifolia* (L.) Merr. Leaves

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Abstract: *Eleutherine palmifolia* (L.) Merr is a native plant of South Kalimantan with considerable potential for health applications. Previous studies have reported that its leaves contain various bioactive compounds, including alkaloids, flavonoids, steroids, tannins, and saponins, which contribute to antibacterial and antioxidant activities. Liquid soap is one of the most practical and widely used dosage forms for antibacterial purposes. Therefore, this study aimed to formulate and evaluate the physical characteristics of liquid soap containing ethanol extract of *E. palmifolia* leaves and to determine the optimal formulation that meets quality standards. An experimental study was conducted using three formulations with different concentrations of Cocamide DEA (0.5%, 1%, and 1.5%). The formulations consisted of *E. palmifolia* leaf extract, virgin coconut oil (VCO), potassium hydroxide (KOH), Na-CMC, stearic acid, BHT, lemongrass oil, and distilled water. The prepared liquid soaps were evaluated for pH, viscosity, foam stability, storage stability, hedonic properties, antibacterial activity, and irritation potential. The results showed that Formula 2 containing 1% Cocamide DEA exhibited the most favorable overall characteristics, providing a balance between physical stability, user comfort, and respondent acceptance. Formula 3 produced the highest foam stability but caused a dry sensation on the skin. Furthermore, Formula 2 containing 1.5% *E. palmifolia* extract demonstrated strong inhibitory activity against *Staphylococcus aureus* and moderate inhibitory activity against *Escherichia coli*. All formulations met the required quality parameters and showed no irritation effects. In conclusion, liquid soap formulated with 1% Cocamide DEA and 1.5% *E. palmifolia* leaf extract was identified as the optimal formulation. The conclusion of the results of this product meets the requirements of SNI 06-4085-1996, exhibited good physical stability, was non-irritating, and showed potential as a natural antiseptic liquid soap.

Keywords: skin cleanser, organoleptic properties, stability assessment, antibacterial activity, dermal safety

1. INTRODUCTION

Personal hygiene refers to an individual's efforts to maintain cleanliness and health to achieve optimal physical well-being and prevent disease. Poor personal hygiene can lead to various health problems, particularly skin disorders [1]. The skin serves as the body's primary barrier to environmental exposure and microbial invasion. However, when the skin is damaged or irritated, it can act as an entry point for microorganisms, thereby increasing the risk of infection. Common pathogenic microorganisms associated with infections include *Staphylococcus aureus* and *Escherichia coli* [2]. *S. aureus* can become pathogenic when it penetrates the subcutaneous tissue and enters the bloodstream, potentially spreading to other organs and causing skin infections such as acne and boils [3]. Meanwhile, *E. coli*, a gram-negative bacterium, is commonly associated with gastrointestinal infections such as diarrhea, often transmitted through contaminated hands [4]. One of the simplest and most effective ways to improve personal hygiene is to use liquid soap containing natural antibacterial agents that can protect the skin from harmful microorganisms [5].

Soap is a sodium or potassium salt of fatty acids derived from vegetable oils or animal fats, and consists of solid soap and liquid soap. Liquid soap has several advantages over solid soap. The advantages of liquid soap are that it is easy to use, more hygienic, easy to carry and store, not easily

damaged or dirty, and is effective in removing dirt that sticks to the surface of the skin, both water-soluble and fat-soluble [6]. The use of natural active substances with antibacterial activity in liquid soap will increase its effectiveness and safety of liquid soap. One of the natural active substances that can be used is *Eleutherine palmifolia* L. Merr.

Eleutherine palmifolia L. Merr. or dayak onion is a tuber from the Iridaceae family that grows abundantly on the island of Borneo and has been used empirically for the treatment of various degenerative diseases [7]. *E. palmifolia* contains various secondary metabolites, including flavonoids, such as isoliquiritigenin. In addition, it contains naphthoquinones and their derivatives, including elecanacin, eleutherin, eleuthoside B, eleuthoside C, eleutherinoside A, eleutheronine B, and eleutheronine C. Anthraquinone compounds have also been identified, such as eleutherquinone A, and polyphenolic compounds, such as oxyresveratrol [8]. *E. Palmifolia* has antibacterial potential. Based on phytochemical screening, a 70% ethanol extract of *E. palmifolia* contains flavonoids, saponins, phenolics, and tannins[9]. These bioactive components have a mechanism of action that inhibits bacterial growth [10].

Although the antibacterial activity of *E. palmifolia* has been reported in several previous studies, information regarding its application in liquid soap formulations, particularly using leaf extract and different concentrations of Cocamide DEA as a foam-enhancing agent, remains limited. Furthermore, comprehensive evaluations involving physicochemical characteristics, storage stability, antibacterial activity, consumer acceptance, and skin irritation potential have not been extensively investigated. Therefore, this study provides novel information on the development and optimization of a natural antibacterial liquid soap containing ethanolic extract of *E. palmifolia* leaves.

Based on these potentials, *E. palmifolia* leaves can be utilized as a natural antibacterial agent in liquid soap formulations. Therefore, this study aims to formulate and evaluate the physical properties of liquid soap containing ethanolic extract of *E. palmifolia* leaves as antibacterial agents.

2. MATERIALS AND METHODS

2.1. Extraction of plant material

E. palmifolia leaves were washed, cut into small pieces, and air-dried overnight. The samples were then oven-dried at 60 °C until a constant weight was obtained. The dried leaves were pulverized into a fine powder using a blender. Powdered leaves (50 g) were macerated with 70% ethanol at a ratio of 1:10 (w/v). The maceration process was carried out for 3 × 24 h with solvent replacement every 24 h. The combined macerates were filtered using filter paper, and the filtrate was concentrated using a rotary evaporator to obtain a viscous extract. The extract was weighed and stored in an airtight container until further use [11].

2.2. Formulation of liquid soap

Three formulations of liquid soap were prepared, as shown in Table 1. Stearic acid, virgin coconut oil (VCO), and BHT were weighed and transferred into a beaker and then heated at 60–70 °C while being continuously stirred until a homogeneous mixture was obtained. Potassium hydroxide (KOH) solution was gradually added under constant stirring until saponification occurred, forming a semi-solid mass [12].

Glycerin and Na-CMC, which had been previously dispersed in distilled water to form a slurry, were then added to the mixture and stirred until uniform. Cocamide DEA was incorporated at varying concentrations (0.5%, 1%, and 1.5%) according to the formulation. Subsequently, *E. palmifolia* leaf extract and citronella oil were added and mixed thoroughly until homogeneous. Distilled water was added to adjust the final volume to 100%. The resulting liquid soap formulations were stored in tightly closed containers for further evaluation [13].

Table 1. Liquid soap formulations

Composition	Formula 1 (%)	Formula 2 (%)	Formula 3 (%)
E. palmifolia leaf extract	0,5	0,5	0,5
VCO	12	12	12
Stearic acid	3	3	3
BHT	0,02	0,02	0,02
Na-CMC	0,5	0,5	0,5
Glycerine	5	5	5
Cocomide DEA	0,5	1	1,5
Citronella oil	qs	qs	qs
Aquadest	Ad 100	Ad 100	Ad 100

2.3. Physical properties of liquid soap E. palmifolia leaf extract before and after storage

Quality evaluation of the liquid soap formulations was performed according to the Indonesian National Standard (SNI 4085:2017). The assessed parameters included organoleptic characteristics, pH, specific gravity, viscosity, foam height, and foam stability [14]. The stability of the formulations was evaluated using a freeze–thaw cycling method. Each sample was stored at 2–8 °C for 24 h, followed by storage at 40 °C for an additional 24 h. This procedure constituted one cycle and was repeated six times. Changes in the physical characteristics of the formulations were observed after each cycle [15].

2.4. Hedonic test

The hedonic test involved 30 participants. The panelists evaluated the soap shape/texture, color, aroma, clarity, and foam effectiveness [16].

2.5. Antibacterial activity test

The antibacterial activity of the selected formulation (1% Cocomide DEA) was evaluated using the disk diffusion method against *Staphylococcus aureus* and *Escherichia coli*. Nutrient agar (NA) was prepared and sterilized prior to use. Bacterial suspensions were prepared in 0.9% NaCl solution and adjusted to the turbidity of the 0.5 McFarland standard, then evenly spread onto the surface of NA plates using a sterile cotton swab to obtain a uniform lawn culture. Sterile paper discs were impregnated with liquid soap samples containing extract concentrations of 0.5%, 1%, and 1.5% and subsequently placed on the surface of the inoculated agar at equal distances. The plates were incubated at 37 °C for 24 h, after which the diameter of the inhibition zones formed around the discs was measured using a caliper. A liquid soap base without extract was used as the negative control [17].

2.6. Irritability test

Irritation testing on the volunteers' skin was conducted using a patch test. Patch testing is a skin irritation and sensitivity test conducted by applying a sample to the panelists' skin to determine the irritant properties of the soap. This study used an open patch test method, which was applied to a specific area (2.5 × 2.5 cm) on sensitive skin areas such as the nape of the neck and behind the earlobe. A positive irritation reaction is indicated by redness, itching, or swelling of the treated skin [18]. This study was reviewed and approved by the Research Ethics Committee of University of Sari Mulia under Ethical Approval Number 010/KEP-UNISM/X/2025.

2.7. Data analysis

All quantitative data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism 10.4.0 software. Differences among formulations were analyzed using one-way analysis of variance (ANOVA), followed by an appropriate post hoc test when

significant differences were observed. A p-value of less than 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Extraction and Yield of E. palmifolia Leaf Extract

The macerated extract of *E. palmifolia* leaves were concentrated using a rotary evaporator at 60 °C, resulting in a thick brown extract. The yield of the extract was 8.88%. The brown coloration of the extract indicated the presence of various secondary metabolites, such as flavonoids, phenolics, and naphthoquinone compounds, which are commonly found in *E. palmifolia*. The yield obtained in this study reflects the efficiency of the maceration method using 70% ethanol as the solvent, which is known to effectively extract both polar and semi-polar compounds. The relatively high yield suggests that the extraction process was adequate to obtain bioactive compounds that may contribute to the antibacterial activity of the formulation [19].

3.2. Physical properties of liquid soap E. palmifolia leaf extract before and after storage

The formulated liquid soap containing *E. palmifolia* extract is shown in Figure 1. Furthermore, the physical stability of the formulation was evaluated using the freeze-thaw method, and the results are presented in Table 2.

Figure 1. Liquid Soap of *E. palmifolia*

Table 2. The results of physical properties of liquid soap *E. palmifolia* leaf extract

Formula	Observation Test	Result		P Value
		Before Storage	After Storage	
1	Shape	Liquid	Liquid	-
	Colour	Golden brown	Golden brown	-
	Odor	Citronella	Citronella	-
	Homogeneity	Homogenous	Homogenous	-
	pH	9.227 ± 0.057	9.267 ± 0.015	< 0.05
	Viscosity	753.3 ± 10.07 mPas [a]	656 ± 2.0 mPas [a]	< 0.05
	Specific gravity	1.016 ± 0.0004 g/mL [a]	1.010 ± 0.0008 g/mL [a]	< 0.05
	Foam height	3.733 ± 0.058 cm [a]	3.667 ± 0.058 cm [a]	< 0.05
	Foam stability	84.36 ± 0.381% [a]	74.14 ± 0.272% [a]	< 0.05

Formula	Observation Test	Result		P Value
		Before Storage	After Storage	
2	Shape	Liquid	Liquid	-
	Colour	Golden brown	Golden brown	-
	Odor	Citronella	Citronella	-
	Homogeneity	Homogenous	Homogenous	-
	pH	9.343 ± 0.0151	9.303 ± 0.020	-
	Viscosity	860.7 ± 12.50 mPas [a]	840.7 ± 2.52 mPas [a]	< 0.05
	Specific gravity	1.018 ± 0.0001 g/mL	1.018 ± 0.0003 g/mL	-
	Foam height	4.433 ± 0,116 cm [a]	5.067 ± 0,153 cm [a]	< 0.05
	Foam stability	87.02 ± 0.707% [a]	79.09 ± 0.690% [a]	< 0.05
3	Shape	Liquid	Liquid	-
	Colour	Golden brown	Golden brown	-
	Odor	Citronella	Citronella	-
	Homogeneity	Homogenous	Homogenous	-
	pH	10.48 ± 0.020	10.40 ± 0.026	-
	Viscosity	991.3 ± 5.69 mPas [a]	928.0 ± 4.36 mPas [a]	< 0.05
	Specific gravity	1.026 ± 0.0001 g/mL [a]	1.016 ± 0.0008 g/mL [a]	< 0.05
	Foam height	3.833 ± 0.116 cm [a]	3.500 ± 0.058 cm [a]	< 0.05
	Foam stability	87.26 ± 0.575% [a]	78.29 ± 0.361% [a]	< 0.05

Note: The difference in notation between formulas 1, 2 and 3 shows a significant difference, with a p value <0.05 before and after storage.

The organoleptic evaluation showed that all formulations (F1, F2, and F3) had a liquid form, golden brown colour, and characteristic citronella odor. These results indicate that the addition of *E. palmifolia* leaf extract did not adversely affect the physical appearance of the liquid soap. According to SNI 06-4085-1996, liquid soap should have a liquid form with a characteristic colour and odor; therefore, all formulations met the required criteria [20].

The homogeneity test demonstrated that all formulations were uniform, with no visible coarse particles. This indicated that all ingredients were well dispersed within the formulation. The homogeneity of the liquid soap may be attributed to proper mixing and the optimal solubility of the formulation components [21].

The pH values of all formulations showed only slight changes after the freeze–thaw cycles. Formula 1 increased from 9.23 to 9.27, Formula 2 slightly decreased from 9.34 to 9.30, and Formula 3 decreased from 10.48 to 10.40. These minor fluctuations indicated that the formulations maintained good pH stability under extreme temperature conditions. These changes may be attributed to slight chemical interactions or redistribution of components during temperature cycling. However, all pH values remained within the acceptable SNI range (8–11), indicating that the formulations are still safe for skin application and are chemically stable [22].

The viscosity of all formulations decreased after the freeze–thaw cycles. Formula 1 showed a more pronounced decrease (from 753.3 to 656 mPa·s), while Formulas 2 and 3 exhibited smaller reductions. This decrease in viscosity may be caused by structural changes in the thickening agent (Na-CMC) due to temperature stress, which can disrupt the polymer network and reduce its thickening efficiency. Despite this decrease, all formulations remained within the acceptable SNI range (400–4000 mPas), indicating that the products retained acceptable consistency [23].

The specific gravity values of all formulations showed minimal changes after storage. Formula 1 slightly decreased from 1.016 to 1.010 g/mL, Formula 2 remained relatively constant, and Formula 3 decreased from 1.026 to 1.016 g/mL. These results indicate that the density of the formulations was not significantly affected by the freeze–thaw process. The slight decrease observed may be due to minor structural or compositional changes; however, overall, the formulations maintained good physical stability [24].

The specific gravity values of all formulations showed minimal changes after storage. Formula 1 slightly decreased from 1.016 to 1.010 g/mL, Formula 2 remained relatively constant, and Formula 3 decreased from 1.026 to 1.016 g/mL. These results indicate that the density of the formulations was not significantly affected by the freeze–thaw process. The slight decrease observed may be due to minor structural or compositional changes, but overall, the formulations maintained good physical stability [25].

Foam stability values decreased in all formulations after freeze–thaw cycles. Formula 1 decreased from 84.36% to 74.14%, Formula 2 from 87.02% to 79.09%, and Formula 3 from 87.26% to 78.29%. This reduction may be caused by structural changes in the surfactant systems due to temperature fluctuations, which can weaken the stability of the foam films. Despite this decrease, all formulations still met the minimum SNI requirement (60–70%), indicating that foam stability remained acceptable. Formulas 2 and 3 demonstrated better foam stability compared to Formula 1, likely due to higher concentrations of Cocamide DEA, which enhances foam stabilization [26].

The specific gravity values of all formulations showed minimal changes after storage. Formula 1 slightly decreased from 1.016 to 1.010 g/mL, Formula 2 remained relatively constant, and Formula 3 decreased from 1.026 to 1.016 g/mL. These results indicate that the density of the formulations was not significantly affected by the freeze–thaw process. The slight decrease observed may be due to minor structural or compositional changes; however, overall, the formulations maintained good physical stability [27].

The results of statistical analysis using GraphPad Prism 10.4.0 software. The results of the physical properties of liquid soap extract of E. palmifolia leaves are presented in (Table 3). One-way ANOVA revealed significant differences among Formula 1, Formula 2, and Formula 3 for the evaluated physicochemical parameters ($p < 0.05$). These findings indicate that variations in Cocamide DEA concentration significantly influenced the physical characteristics of the liquid soap formulations, including pH, viscosity, specific gravity, foam height, and foam stability. An increase in Cocamide DEA concentration tended to affect the physicochemical properties of the formulations, resulting in measurable differences among the tested formulations while still complying with the established quality requirements.

3.3. Hedonic Test of liquid soap E. palmifolia leaf extract

The hedonic test aims to determine panelists' preferences for the shape, color, aroma, clarity, and effectiveness of soap foam and was conducted on 30 panelists [16]. The results of the hedonic test of soap texture showed significant differences (Table 4). Formula 2 had a significant difference with formulas 1 and 3, while formulas I and III did not have a significant difference. Formula 2 had high clarity and foam effectiveness, but according to respondents, formula 2 had a softer texture and did not cause a dry feeling. The results of the hedonic test for aroma and color did not show significant differences. This shows that a concentration of 1% Cocomide DEA can produce a soft liquid soap without leaving a tight and dry feeling.

Table 4. The result of hedonic test data of liquid soap *E. palmifolia* leaf extract

Parameter	Hedonic Test Data			P Value
	Formula 1	Formula 2	Formula 3	
Shape/texture	4.17	4.63	4.00	P < 0.05
Color	3.80	3.97	3.47	P > 0.05
Odor	2.83	2.80	2.67	P > 0.05
Clarity	3.73	3.83	3.27	P > 0.05
Effectiveness of soap foam	3.83	3.88	3.90	P < 0.05

3.4. Antibacterial test

The antibacterial activity of liquid soap containing *E. palmifolia* leaf extract with concentrations of 0.5%, 1%, and 1.5% was evaluated against *S. aureus* and *E. coli* using the disc diffusion method (Table 5). The results showed that the diameter of the inhibition zone increased with increasing extract concentration in both bacterial strains [28].

Table 5. The inhibition zone of the liquid soap against *S. aureus* and *E.coli*

Concentration	Inhibition Zone Diameter (mm)	
	S. aureus	***E. coli***
Base (without extract)	7.347 ± 0.463	7.797 ± 0.864
0.5 %	8.793 ± 0.623	8.840 ± 1.028
1 %	9.667 ± 0.553	10.027 ± 0.967
1.5 %	13.653 ± 0.796	14.347 ± 0.766

The inhibition zone of the liquid soap against *S. aureus* ranged from 7.35 ± 0.46 mm (base) to 13.65 ± 0.80 mm at a concentration of 1.5%. The base formulation (without extract) already exhibited a small inhibition zone, which may be attributed to the presence of surfactants such as Cocamide DEA and fatty acid salts that possess mild antibacterial properties. However, the addition of *E. palmifolia* extract significantly enhanced antibacterial activity [29].

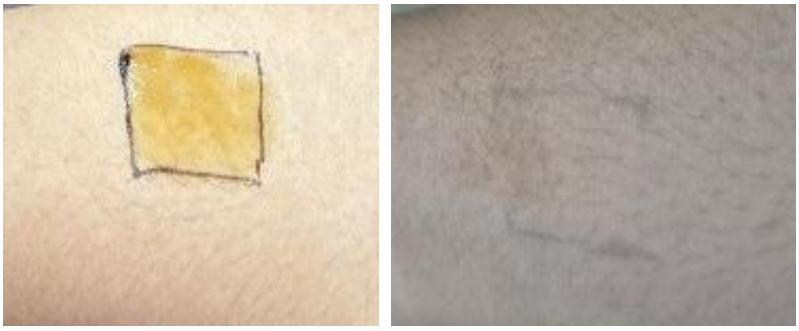
The increase in inhibition zone diameter with higher extract concentrations indicated a dose-dependent antibacterial effect. At a concentration of 1.5%, the inhibition zone reached its highest value, suggesting stronger antibacterial activity. Based on the general classification, inhibition zones in the range of 10–20 mm can be categorized as moderate to strong antibacterial activity. This activity is likely due to the presence of bioactive compounds, such as flavonoids, tannins, saponins, and phenolics, which can damage bacterial cell walls, disrupt cell membranes, and inhibit essential metabolic processes [30].

A similar trend was observed against *E. coli*, where the inhibition zone increased from 7.80 ± 0.86 mm (base) to 14.35 ± 0.77 mm at a concentration of 1.5%. The antibacterial activity against *E. coli* was generally slightly higher than that against *S. aureus* at the same concentration. This result indicates that the extract is effective against both Gram-positive and Gram-negative bacteria. The effectiveness against *E. coli* suggests that the active compounds in *E. palmifolia* are capable of penetrating the outer membrane of gram-negative bacteria. Flavonoids and phenolic compounds are known to disrupt membrane permeability, whereas tannins can precipitate proteins and interfere with bacterial enzymes [31].

Overall, both bacterial strains showed concentration-dependent responses to the extract. The highest antibacterial activity was observed at a concentration of 1.5% for both *S. aureus* and *E. coli*. The slightly larger inhibition zone in *E. coli* may be influenced by differences in the cell wall structure and susceptibility to the active compounds.

3.5. Irritability test (Patch Test)

Certain ingredients in soap can cause skin irritation, such as itching, swelling, and redness. Therefore, an irritation test was performed to determine whether these reactions occurred. The skin irritation testing is performed by applying the product to the hand and observing any changes, such as erythema and edema. Skin irritation can be caused by a soap pH that is either too high or too low. However, the results of the pH test on solid soap indicate a safe pH range for the skin [32]. The results (Figure 2) obtained in this study, no redness or rash-like appearance was found for 24 hours.



(a) (b)

Figure 2. Irritability test perfomed on the skin: a. 0 hour duration b. 24 hours duration

Based on the observations presented in Figure 2, no signs of skin irritation, including erythema, edema, itching, or rash formation, were observed in any volunteer during the 24-hour observation period. These findings indicate that the liquid soap formulation was well tolerated by the skin and did not induce adverse dermal reactions. The absence of irritation may be attributed to the appropriate physicochemical characteristics of the formulation, particularly its pH and surfactant composition, which can minimize the risk of skin barrier disruption. Similar findings have been reported in previous studies of herbal liquid soap formulations, where no redness, itching, or swelling was observed following topical application [33]. Furthermore, previous studies on *E. Palmifolia* antimicrobial herbal soap showed that the absence of erythema and allergic reactions is an indication of good dermatological compatibility and safety of the product for routine use [34]. Therefore, the incorporation of *E. palmifolia* leaf extract did not negatively affect the safety profile of the formulation and supports its potential application as a natural antibacterial ingredient in liquid soap products.

4. CONCLUSION

Liquid soap containing *E. palmifolia* leaf extract demonstrated acceptable physicochemical properties, good stability, and significant antibacterial activity against *S. aureus* and *E. coli*. The formula with 1% Cocomide DEA and 1.5% *E. palmifolia* leaf extract was considered optimal because it met the SNI 06-4085-1996 standard, showed a balance between stability and effectiveness, and was non-irritating.

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Generative AI statement

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