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FORMULATION, QUALITY TEST, AND ANTIBACTERY ACTIVITY TEST OF LIQUID SOAP OF ACALIFA (*Acalypha wilkensiana Müell. Arg*.) LEAVES on *Escherichia coli*

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ABSTRAK

Meskipun sabun antibakteri mulai dikembangkan, penggunaan daun akalifa sebagai bahan aktif antibakteri pada *liquid soap* belum banyak dieksplorasi**.** Ekstrak daun akalifa (*Acalypha wilkensiana Müell. Arg.*) merupakan salah satu tanaman yang mempunyai aktivitas antibakteri terhadap bakteri *Escherichia coli.* Bakteri *Escherichia coli* ini penyebab diare yang paling umum dengan penyebaran melalui tangan yang kotor. Salah satu cara untuk mencegah penyakit karena bakteri adalah dengan menggunakan *liquid soap* yang dibuat menggunakan proses saponifikasi dengan atau tanpa penambahan bahan lain yang tidak mengiritasi kulit tangan. **Tujuan:** Tujuan penelitian ini untuk mengembangkan dan pemanfaatan ekstrak daun akalifa (*Acalypha wilkensiana Müell. Arg.*) menjadi *liquid soap* dengan berbagai variasi konsentrasi ekstrak. Penelitian ini dilakukan secara eksperimental laboratorium. Sampel daun akalifa diekstraksi menggunakan metode maserasi dengan pelarut etanol 70%, hasil ekstrak daun akalifa yang diperoleh dilakukan uji skrining fitokimia. Jumlah zat aktif yang digunakan dengan variasi konsentrasi FI (3%), F2 (6%) dan F3 (9%). Sediaan *liquid soap* yang dihasilkan dilakukan evaluasi mutu fisik meliputi organoleptis, homogenitas, pH, tinggi busa dan viskositas. Setelah itu uji aktivitas antibakteri menggunakan metode sumuran. Hasil penelitian menunjukan ekstrak daun akalifa mengandung senyawa alkaloid, flavonoid, tanin dan saponin. Hasil evaluasi mutu fisik ketiga formulasi *liquid soap* ekstrak daun akalifa memenuhi syarat rentang yang diperbolehkan oleh SNI. Hasil uji aktivitas antibakteri menunjukan bahwa semua formulasi menghasilkan daya hambat terhadap bakteri *Escherichia coli*, dengan diameter hambat konsentrasi 3% (7,55mm±0,61) kategori sedang dan konsentrasi 6% (10,94mm±0,80) dan 9% (13,04mm±1,28) kategori kuat. Data hasil analisis menunjukan masing-masing *liquid soap* variasi konsentrasi 3%, 6% dan 9% ekstrak daun akalifa memiliki perbedaan yang signifikan (*p-value*<0,05) terhadap hasil uji mutu fisik dan aktivitas antibakteri. Berdasarkan hasil penelitian dapat disimpulkan bahwa ekstrak daun akalifa dengan konsentrasi FI (3%), F2 (6%) dan F3 (9%) dapat diformulasikan menjadi *liquid soap* yang memenuhi persyaratan mutu fisik dan memiliki aktivitas antibakteri terhadap bakteri *Escherichia coli.*

Kata Kunci: Ekstrak daun akalifa, *Escherichia coli*, *Liquid soap.*

ABSTRACT

Although antibacterial soaps have begun to be developed, using akalifa leaves as an active antibacterial ingredient in liquid soap has not been widely explored. Akalifa leaf extract (Acalypha wilkensiana Müell. Arg.) is a plant that has antibacterial activity against Escherichia coli bacteria. Escherichia coli bacteria are the most common cause of diarrhea and spread through dirty hands. One way to prevent diseases caused by bacteria is to use liquid soap made using the saponification process with or without adding other ingredients that do not irritate the skin of the hands. Objective: This study aimed to develop and utilize akalifa leaf extract (Acalypha wilkensiana Müell. Arg.) into liquid soap with various extract concentrations. This study was conducted experimentally in the laboratory. Akalifa leaf samples were extracted using the maceration method with 70% ethanol solvent, and the results of the khalifa leaf extract obtained were subjected to phytochemical screening tests. The amount of active substances used with variations in concentrations of FI (3%), F2 (6%) and F3 (9%). The resulting liquid soap preparation was evaluated physically, including organoleptic, homogeneity, pH, foam height, and viscosity. After that, the antibacterial activity test used the well method. The results of the study showed that khalifa leaf extract contains alkaloid, flavonoid,

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tannin, and saponin compounds. The results of the physical quality evaluation of the three akalifa leaf extract liquid soap formulations meet the range requirements permitted by SNI. The results of the antibacterial activity test showed that all formulations produced inhibition against Escherichia coli bacteria, with a concentration inhibition diameter of 3% (7.55mm ± 0.61) in the moderate category and a concentration of 6% (10.94mm ± 0.80) and 9% (13.04mm ± 1.28) in the strong category. The analysis data showed that each liquid soap variation in concentration of 3%, 6%, and 9% khalifa leaf extract had a significant difference (p-value <0.05) in the results of the physical quality test and antibacterial activity. Conclusion: Based on the research results, it can be concluded that khalifa leaf extract with concentrations of FI (3%), F2 (6%), and F3 (9%) can be formulated into liquid soap that meets physical quality requirements and has antibacterial activity against Escherichia coli bacteria..

Keywords: *Akalipa leaf extract, Escherichia coli, Liquid soap.*

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1. INTRODUCTION

An effective way to maintain body health is to maintain cleanliness, one of which is hand cleanliness (1). Hands are one of the important organs of the human body and a place for microorganisms to grow; dirty hands become a breeding ground for bacteria such as *Staphylococcus aureus, Streptococcus epidermis, and Escherichia coli* or other types of bacteria that can cause diseases in humans, one of which is diarrhea (2).

One of the most common microorganisms that cause diarrhea is *Escherichia coli*, or *E.coli. Escherichia coli* attaches to human intestinal cells and produces enterotoxins, which will affect secretions from the digestive tract through *cyclic adenosine monophosphate* (cAMP) or *cyclic guanosine monophosphate* (cGMP) levels (3).

According to the 2018 National Riskesdas Report data, the number of diarrhea cases in all age groups in Indonesia based on the diagnosis of health workers or symptoms experienced was around 1,017,290 cases and in East Java 151,878 cases, where East Java ranked second out of 34 provinces in Indonesia in the highest number of diarrhea cases.

Washing hands with soap and water is part of the prevention efforts through hygiene measures. This effort mechanically removes dirt from the skin surface more effectively and significantly reduces the number of disease-causing microorganisms such as viruses, bacteria, and other parasites on both hands (4). The habit of washing hands with soap can reduce the incidence of diarrhea by up to 50%, or the equivalent of saving around 1 million children worldwide from this disease each year (5).

Very few hand soaps on the market today use natural ingredients. Most use synthetic ingredients as their active ingredients. Synthetic active ingredients can negatively affect people with sensitive skin, including irritating. Synthetic active ingredients harmful to human skin health are *diethylamine* (DEA), *Sodium Lauryl Sulfate* (SLS), and triclosan, which are widely found in hand soaps on the market. Triclosan, which accumulates in fat in the human body, can cause thyroid dysfunction (6). Therefore, soap will be developed using extracts from natural ingredients as active antibacterial ingredients. The soap formulation contains olive oil and KOH, which will react through a saponification reaction. Olive oil has the potential to be used as the main ingredient in soap making because of its high oleic acid content, which has a positive effect on skin health. One of the benefits of olive oil for skin health is maintaining skin elasticity and moisture, encouraging the skin renewal process, and

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making the skin less prone to dryness and wrinkles (7) . However, this soap formulation without additional active ingredients from the extract has an inhibition zone of 0.0 mm (8) , so adding akalifa leaf extract as an active antibacterial compound is necessary. Although antibacterial soaps are starting to be developed, using akalifa leaves as an active antibacterial ingredient in *liquid soap* has not been widely explored.

Akalifa leaves (*Acalypha wilkesiana Müell. Arg.*) contain antibacterial components such as tannins, saponins, flavonoids, phenols, and alkaloids so that they have superior antibacterial properties compared to garlic; soursop leaves, betel leaves, and maja fruit (9) . Akalifa leaf extract is obtained by maceration, percolation, reflux, and soxhlet extraction using water, ethanol, methanol, ethyl acetate, n-hexane, and acetone as solvents. Ethanol is a polar solvent that is able to optimally attract saponins, flavonoids, and tannins because it is polar according to the extraction principle, namely, *like dissolves like* (10) .

et al. (2021) research showed that akalifa leaf extract has antibacterial activity against *Escherichia coli* with an inhibition zone of 30.2 mm at a concentration of 10 mg/ml, a MIC value of 20 mg/ml at a concentration of 10 mg/ml and an MBC of 5 mg/ml at a concentration of 20 mg/ml (9) . Therefore, the ethanol extract of alkalifa leaves has the potential to be developed into a soap preparation. One form of soap preparation is *liquid soap*.

The advantages of *liquid soap* are that it is more hygienic, easy to use, not easily damaged or dirty, and easy to carry and store (11) .

Based on the description above, researchers are interested in developing the potential of antibacterial compounds from aklifa leaves in *liquid soap preparations* with antibacterial activity, which are tested against *Escherichia coli bacteria.*

2. METHOD

Types and Design of Research

This research was conducted experimentally in the laboratory. The design of this study aims to develop and utilize akalifa leaf extract (*Acalypha wilkesiana Müell. Arg.*) in the antibacterial activity *of Escherichia coli* in liquid soap formulation.

Time and Place of Research

The research was conducted in February-April 2024. Pharmaceutical Technology Laboratory and Pharmaceutical Biology Laboratory, Bachelor of Pharmacy Study Program, Health Sciences, Dr. Soebandi University, Jember.

Research Tools and Materials

The tools used are an oven (*Memmert*), porcelain cup *(Iwaki)*, watch glass cup, measuring cup 50ml and 10ml *(Iwaki),* Erlenmeyer flask 250ml *(Iwaki),* beaker glass 50ml and 100ml *(Iwaki)*, 75mm glass funnel *(pyrex),* rotary evaporator, blender, analytical balance *(Ohaus)*, mortar and stamper, *incubator,* petri dish, test tube, test tube rack, dropping plate, sieve mesh no.60, vortex, LAF, BSC, *rion vt-06 viscometer*, *glass object*, *Uni pH Testa*, autoclave, micropipette, micropipette tip, 100ml spray bottle, bunsen, *hot plate* stirrer, ruler, vernier caliper, *coloni counte*, *magnetic stirrer*, 100ml plastic bottle, stirring rod, dropper pipette, horn spoon, parchment paper, L glass.

The materials used are akalifa leaves (*Acalypha wilkesiana Müell. Arg.*), 70% technical ethanol, technical distilled water, glycerin, BHT (*Butylated Hydroxy Toluene*), HPMC (*Hydroxy Propyl Methyl Cellulose*), KOH (Potassium Hydroxide), stearic acid (UD Aneka Kimia), olive oil (CV Inalab Utama), *oleum citri*, HCl 2N (CV Makmur Sejati Jember), Mayer's reagent, *Dragendorf, magnesium* powder, concentrated HCl, 10% iron (III) chloride, Nutrient Agar (NA)

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medium, *Escherichia coli bacteria* and Onemed antibacterial soap, 0.9% NaCl, spirits, MC Farland.

Data Collection and Analysis

Sampling and processing

The population in the study was old akalifa leaves without holes and red with green spots obtained in the Jember area, precisely in Baratan Village, Patrang District, Jember Regency, East Java Province. The sample in this study was akalifa leaf extract (*Acalypha Wilkesiana Müell. Arg.*) used to formulate *liquid soap* preparations.

Plant determination

Akalifa leaf samples (*Acalyhpa wilkesiana Müell. Arg.*) were determined as initial identification for physiological observations of plants conducted at the Biology Learning Laboratory of Ahmad Dahlan University, Yogyakarta. The determination aimed to ensure that the akalifa plant was truly a *wilkesiana species.*

The process of extracting akalifa leaves by maceration

The obtained samples were washed clean under running water and then dried in an oven at 50 ° C until the water content was reduced. The dried simplicia was ground using a blender until it became a fine powder, and the powder obtained was sieved.

The simplicia was weighed as much as 500 grams and put into an Erlenmeyer flask, and 1500 ml of 70% ethanol solvent liquid was added to the simplicia. The Erlenmeyer containing the sample was stored for 1x24 hours, and the resulting macerate was collected, and the residue was used for soaking or remaceration repeated 2 times. The macerate results were combined and then evaporated using *a rotary evaporator* at a temperature of 50 ° C, a pressure of 20 Psi, and a rotation of 120 rpm, then continued with an oven at a temperature of 50 ° C to obtain a thick extract of akalifa leaves.

Phytochemical Screening of Akalifa Leaf Extract

Qualitative testing of khalifa leaf extract content was carried out using chemical reactions to identify the alkaloid, flavonoid, tannin, and saponin groups. The method used was as in (12) .

Alkaloid Test

As much as 1 ml of khalifa leaf extract is added with two drops of Mayer. If it produces a white or yellow precipitate, it is positive for alkaloids. As much as 1 ml of khalifa leaf extract is added with two drops of Dragendroff reagent, producing a red-orange precipitate, then it is positive for alkaloids.

Flavonoid Test

As much as 1 ml of akalifa leaf extract is added with 0.1 grams of Mg powder and one drop of concentrated HCl. The flavonoid test is said to be positive if a red or orange color is formed.

Tannin Test

As much as 1 ml of akalifa leaf extract was added with 10 ml of distilled water, then filtered, and three drops of iron (III) chloride were added. The tannin test is said to be positive if a blackish-green or blackish-blue color is formed, indicating the presence of tannin.

Saponin Test

As much as 0.5 grams of akalifa leaf extract is added with 10 ml of distilled water on a water bath for 10 minutes, then cooled and then shaken vigorously for 10 seconds until foam and foam are formed. Add 1 drop of 2N HCl solution if the foam does not disappear, it indicates the presence of saponins.

***Liquid soap* formulation with akalifa leaf extract (*Acalypha wilkesiana Müell. Arg.*)**

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Liquid soap preparation of akalifa leaf extract was made with concentration variations of 3%, 6%, 9 %. The following is a draft formulation of a *liquid soap preparation* of akalifa leaf extract in Table 1 below.

Table 1 Formulation of *liquid soap* with akalifa leaf **extract**

Material	Formulation % w/w				Function of Materials
	F0	F1	F2	F3	
Akalifa leaf extract	-	3%	6%	9%	Active substance
Olive oil	25%	25%	25%	25%	Soap formers
KOH	6.85%	6.85%	6.85%	6.85%	Soap formers
HPMC	2 %	2%	2%	2%	Thickener
Stearic acid	5%	5%	5%	5%	Foam stabilizer
BHT	0.05%	0.05%	0.05%	0.05%	Antioxidants
Glycerin	5%	5%	5%	5%	Humectant
Oleum citri	1.5%	1.5%	1.5%	1.5%	Fragrance
Aquadest	ad 100%	ad 100%	ad 100%	ad 100%	Solvent

Making *liquid soap* from akalifa leaf extract

The prepared ingredients are weighed first for making liquid soap according to the formulation. Put 25 grams of olive oil in a beaker glass and stir using a magnetic stirrer until homogeneous; KOH 6.85 grams is added little by little while heated at a temperature of 50 ° C until a soap paste is obtained. Then, stearic acid that has been melted previously is added and stirred until homogeneous. Then, BHT 0.05 grams and HPMC 2 grams, which have been developed with hot distilled water, are added and stirred until homogeneous. After the preparation is cold, 5 grams of glycerin and akalifa leaf extract are added and stirred until homogeneous. The next process is adding 1.5 grams of oleum citri and distilled water to a volume of 100 ml. *Liquid soap* is put into a container. Add extract to *liquid soap F1 3 grams, F2 6 grams and F3 9 grams. After that, the physical quality test of liquid soap akalifa leaf extract* is continued.

Physical Quality Evaluation

Organoleptic Test

Organoleptic tests are carried out to see the physical appearance of a preparation, which includes the smell, color, and shape of the *liquid soap preparation* with akalifa leaf extract—testing based on the sensing process*.* The standard set by SNI for organoleptic testing *of liquid soap* in liquid form has a distinctive odor and color from the extract used, namely dark red (8) .

Homogeneity Test

The homogeneity test determines whether the *liquid soap preparation* is perfectly mixed and there are no particles. Then, drip the *liquid soap preparation of* akalifa leaves onto the object glass, smooth it out, and observe its homogeneity by looking at the parts that are not mixed well in the preparation. If there are no particles, the preparation can be said to be homogeneous (13) .

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pH Test

The pH test aims to determine the acidity level of a preparation; if the preparation is too acidic, it will cause irritation, and if the pH is too alkaline, it will cause dry hands. The preparation with 1 gram of *liquid soap* to be tested is diluted with 10 ml of distilled water. Then tested on a pH meter (4) . Based on (SNI 2588-2017) the pH requirement for *liquid soap* is 4-10.

Foam Height Test

The foam height test aims to see how much foam can be produced; too much foam will cause dry skin. The foam height test is carried out by inserting 1 gram of khalifa leaf extract *liquid soap* into a tube containing 10 ml of distilled water, closing it and shaking it for 20 seconds, then measuring the height of the foam produced. (14) . Based on SNI, the foam height requirement for *liquid soap* is 13-220 mm.

Viscosity Test

The viscosity test aims to determine the viscosity of *the liquid soap preparation*. The sample to be tested is placed in a container for the material, and the height of the container is adjusted so that the rotor can move and use a rotor that matches the viscosity level *of the liquid soap*. Turn on the viscometer, observe the viscometer value listed on the viscometer, and record the viscometer value listed (4). based on SNI (06-4085-1996), the requirements for good viscosity range from 400-4000 cPs.

Antibacterial Activity Testing

Well diffusion test

The good diffusion method with three repetitions was used to test the antibacterial activity of khalifa leaf extract. The *liquid soap test solution* of khalifa leaf extract with concentration variations of 3%, 6%, and 9% were dropped into each good hole as much as 50 µ l using a micropipette. The *liquid soap base solution* was used as a negative control, and onemed antibacterial soap was used as a positive control. The petri dish was incubated at 37 ° C for 24 hours until an inhibition zone was formed. The inhibition zone of the *liquid soap* preparation of akalifa leaf extract was observed and measured against *Escherichia coli bacteria* (15) .

Data Analysis Techniques

The physical quality test data of *liquid soap* extract of akalifa leaves (*Acalyhpa wilkesiana Müell. Arg.*), namely organoleptic test, homogeneity test, are descriptive data and pH test, foam height test, viscosity test and antibacterial activity are quantitative data analyzed using SPSS 25 program with a confidence level of 95%.

3. RESULTS

Table 2 Results of Ethanol Extract Yield of Akalifa **Leaves**

Yield of ethanol extract of akalifa leaves		
Weight of simple substances	Extract weight	% Yield
500 grams	156 grams	31.2%

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Table 3 Phytochemical Screening Results of Akalifa Leaf **Extract**

Identification	Reagent	Results	Information
Alkaloid	Dragendorff	Orange red sediment	+
Flavonoid	Mg powder + concentrated HCI	Orange	+
Tannin	Aquadest + $FeCl_3$ 10%	Blackish blue or ink blue	+
Saponins	Aquadest + HCI 2N	Foam is formed	+

Information:
(+) there are compounds
(-) no compounds present

Table 4of Organoleptic Test and Homogeneity *of Liquid Soap* Extract Akalifa Leaf

No.	**Formulation**	**Parameter**			
		Organoleptic Test			**Homogeneity Test**
		Form	**Smell**	**Color**	**Dispersed materials**
1.	F0	Liquid	Citrus	White	Homogeneous
2.	F1 3%	Liquid	Citrus	Light brown	Homogeneous
3.	F2 6%	A little thick	Citrus	Chocolate	Homogeneous
4.	F3 9%	Thick	Citrus	Dark brown	Homogeneous

Figure 1 Organoleptic Test **Results**

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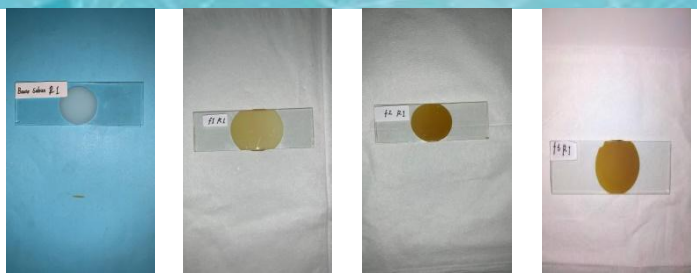


(a) (b) (c) (d)

Figure 2Homogeneity Test. (a) F0; (b) F1; (c) F2; (d) F3

Table 5 Results of pH Test, Foam Height and Viscosity *of Liquid Soap with* Akalifa Leaf **Extract**

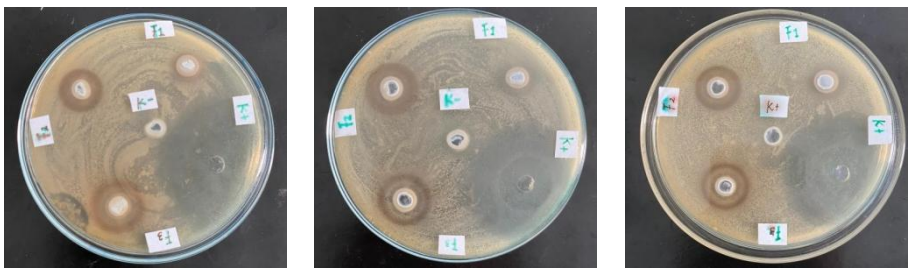
No.	Formulation	pH Test	Foam Height Test	Viscosity Test
			Mean ± SD	
1.	F1 3%	9.84 ± 0.03*	87 ± 1.00*	680 ± 10.00*
2.	F2 6%	9.49 ± 0.04*	94 ± 1.00*	766.67±25.17*
3.	F3 9%	9.21 ± 0.05*	97.33 ± 1.52*	823.33±15.28*

*Significant difference in LSD results (*Least Significant Difference*)

Table 6 Antibacterial Activity Test Results of **Akalifa** Leaf *Liquid Soap*

Sample	Replication of Inhibition Zone Diameter (mm)			Mean±SD	Information
	I	II	III		
F1 3%	6.85mm	7.97mm	7.82mm	7.55±0.61*	Currently
F2 6%	11.85mm	10.34mm	10.64mm	10.94±0.80*	Strong
F3 9%	13.20mm	14.23mm	11.69mm	13.04±1.28*	Strong
Positive Control	33.38mm	34.58mm	34.54mm	34.17±0.68*	Very strong
Negative Control	0.00mm	0.00mm	0.00mm	0.00±0.00*	There is no

*Significant difference in LSD results (*Least Significant Difference*)



Replication 1 Replication 2 Replication 3

Figure 3. Antibacterial Activity Test Results of Akalifa Leaf *Liquid Soap*

4. DISCUSSION

An important initial step in this study is to identify the plant to be used, namely the plant (*Acalyhpa wilkesiana Müell. Arg.*). Plant identification aims to avoid using the wrong plant because it can prevent the tested plant from being exchanged with other plants. Plant identification was carried out at the Biology Learning Laboratory of Ahmad Dahlan University, Yogyakarta, with letter number 011 / Lab. Bio / BI / 2024. Based on the identification results, it was concluded that the plant used in this study was an akalifa plant with the species name (*Acalyhpa wilkesiana Müell. Arg.*) in the Euphorbiacea family.

The sample in this study used old akalifa leaves with 5 to 10 red segments with green spots as the active ingredient in the *liquid soap preparation*. Aims to obtain optimal active compound content. The manufacture of akalifa leaf extract uses the maceration method. The maceration method was chosen because the process is easy, the equipment used is simple, it is suitable for compounds that are not heat resistant so that the thermolabile compounds that will be taken will not decompose or be damaged, such as flavonoids which are not heat resistant at temperatures >60 ° C and the operational costs are relatively low (16) . The results of the thick extract of khalifa leaves (*Acalypha wilkesiana Müell. Arg.*) with 1x24 hour soaking and 2 times maceration can be seen in Table 2, which shows a thick extract of 156 grams with a yield of 31.2%. The greater the yield value indicates, the greater the value of the extract produced. Based on the Indonesian Herbal Pharmacopoeia, the requirement for a good thick extract yield is a value of not less than 10% (17), so it can be said that the yield of the akalifa leaf extract meets the requirements of the Indonesian Herbal Pharmacopoeia. This is supported by the research results of Simangunsong *et al.* (2023) and Madziga *et al.* (2021) which showed that the yield of akalifa leaf extract using the maceration method using ethanol solvent without re-maceration was 7.0% lower, while the yield of akalifa leaf extract using the reflux extraction method using distilled water as a solvent was 22.85% w/w (18) (19).

The results of the akalifa leaf extract obtained were subjected to a phytochemical screening test, which aims to ensure the presence of secondary metabolite compounds that have antibacterial activity contained in the extract; the group of compounds that have antibacterial activity are alkaloids, flavonoids, tannins, and saponins. The results of the phytochemical screening test of the khalifa leaf extract can be seen in Table 3. This is in accordance with Humairoh's research (2023) that akalifa leaf extract (*Acalyhpa wilkesiana Müell. Arg.*) contains alkaloid, flavonoid, tannin and saponin compounds which are characterized by the formation of orange-red deposits (alkaloids), the formation of orange color (flavonoids), the formation of blackish blue color (tannins) and the formation of foam (saponins) (20) .

Evaluation of the physical quality of *liquid soap preparations* containing akalifa leaf extract was carried out on each formulation to determine whether the *liquid soap preparation* was in accordance with the *liquid soap standards* set by SNI (Indonesian National Standard) in 1996 and 2017. The tests carried out were organoleptic, homogeneity, pH, foam height, and viscosity tests . Organoleptic tests were carried out by observing the texture, color and aroma of the preparation using human senses . From the research that has been carried out, data regarding the results of organoleptic tests of *liquid soap preparations* containing khalifa leaf extract can be seen in Table 4 and Figure 1. Based on the formulation of *the liquid soap* produced, it meets SNI specifications, namely, each formulation has a liquid texture, is slightly thick, and has a distinctive aroma and

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color. The distinctive citrus aroma is due to the addition of *oleum citri*, *which is used as a flavoring agent to create a distinctive citrus aroma in the liquid soap* preparation. The formation of brown color in *liquid soap* is due to the use of khalifa leaf extract, which is reddish brown as an active substance so that *the liquid soap* produces a brown color. Variations in the concentration of the added extract can cause the difference in color and texture in each formulation. The higher the concentration of khalifa leaf extract, the lower the amount of distilled water used, resulting in a thick texture, and the higher the concentration of khalifa leaf extract, the thicker the color of the resulting *liquid soap preparation.*

A homogeneity test is conducted to ensure that all ingredients are evenly distributed. From the research that has been conducted, data on the results of the homogeneity test of the khalifa leaf extract *liquid soap preparation* can be seen in Table 4 and Figure 2. The evaluation of the physical quality of homogeneity shows that F1, F2, and F3 are classified as homogeneous because there are no coarse particles after the preparation is dropped on the object glass. This is in line with Rahmi's research (2023), which states that a *liquid soap formulation* is said to be homogeneous if there are no coarse particles after being applied to the object glass (21) . Based on the research results obtained, the active substances in *the liquid soap* formulations F1, F2, and F3 are dispersed in the soap base so that they have good physical quality tests in terms of homogeneity.

Testing the pH value is one of the requirements for the quality of *liquid soap.* This is because *liquid soap* comes into direct contact with the skin, so irritation can occur if the pH value is low, and a pH that is too high causes the skin to become scaly (dry). The skin can withstand and quickly adapt to a pH of 4-10 to the applied product (22) . Based on the data in Table 5, the pH test of khalifa leaf extract *liquid soap* from the three formulations has a pH value that is not much different. In general, *liquid soap products* tend to have a basic pH. This is caused by KOH, which is a strong base that is the basic ingredient of *liquid soap. The pH of the liquid soap* preparation decreased at a pH value of 9% concentration, namely pH 9.21 ± 0.05, a concentration of 6% had a pH of 9.49 ± 0.04, and at a concentration of 3% had a pH of 9.84 ± 0.03. The decrease in pH value was caused by akalifa leaf extract, which has an acidic pH of 4.41 . This was obtained from the flavonoid and tannin content. The decrease in pH value can also be influenced by changes in temperature during storage and contact of the preparation with air humidity. CO2 gas in the air can react with water in the preparation to form acid (23) . This is in line with the research of Dian *et al* (2022) that extracts that have an acidic pH value will affect the decrease in soap pH, and in the research of Marlina *et al.* (2022) stated that extracts that contain flavonoid compounds cause a decrease in pH because flavonoids themselves are acidic which contain phenolic hydroxyl groups (24) (25) . Therefore, the pH of the *liquid soap preparation* which enriched with akalifa leaf extract can lower the pH *of liquid soap* along with the increase in the concentration of akalifa leaf extract itself . Based on (SNI 2588-2017) the requirements for pH *of liquid soap* are 4-10. From the pH results, it is known that each formulation of khalifa leaf extracts *liquid soap* is within the appropriate pH limits based on SNI, so it is classified as safe for use.

The data from the pH test observations were analyzed using a one-way ANOVA test; there were significant differences in the three formulations obtained with a *p-value of* 0.000 (*p-value* < 0.05), then the LSD (*Least Significant Difference) test was carried out to determine the significant difference in pH between the two liquid soap* formulations. Based on the results of the LSD test on F1 against F2, F1 against F3, and F2 against F3, there were significant differences (Sig. 0.000 *p-value* < 0.05).

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One of the most important factors in determining the quality of cosmetic products, especially *liquid soap,* is the foam height test. The purpose of foam height is to measure the foam power produced by *liquid soap*. Foam that remains stable for a longer period is preferred because it can help clean dirt on hands (26) . Table 5 shows the results of foam height measurements on *liquid soap preparations* with akalifa leaf extract, showing that variations in the concentration of active substances affect the foam height of each formulation. Of the four formulations, F3 has the highest foam height with a height of 97.33 mm ± 1.52. However, of all the formulations tested for foam height, it has a good *liquid soap foam height* within the range of SNI 13-220 mm requirements. Based on the results obtained, it is proven that the higher the concentration, the more foam is produced. The height of the foam is influenced by the presence of saponins contained in akalifa leaf extract (27) . Foam can also be produced through saponification or the reaction of soap formation from base materials (KOH) and olive oil (28) . *Liquid soap* foam functions to remove oil or fat from the skin. If there is too much foam in the soap, it can make the skin dry. Loss of fat on the skin makes it more susceptible to irritation because fat functions as a defense for the top layer of the skin. Fat forms a skin barrier consisting of fat so that it is denser to prevent bacteria and microorganisms (29) .

The use of olive oil in this study is an alternative to SLS because of its fat content (80% unsaturated fatty acids and 20% saturated fatty acids); this olive oil will be processed into a surfactant (*surface active agent*) using the saponification method. Olive oil is an oil obtained from the pressing of olives, which has a high oleic acid content and is believed to be able to maintain skin moisture and elasticity while accelerating the skin regeneration process, thus preventing dry and wrinkled skin (30) .

The observation data of the foam height test were analyzed using a one-way ANOVA test, there were significant differences in the three formulations obtained with a *p-value of* 0.000 (*p-value* < 0.05), then the LSD (*Least Significant Difference) test was carried out to determine the significant difference in foam height between the two liquid soap* formulations. Based on the results of the LSD test on F1 against F2 and F1 against F3, there were significant differences (Sig. 0.000 *p-value* < 0.05), and F2 against F3, there were significant differences (Sig. 0.015 *p-value* <0.05).

Viscosity plays an important role in the manufacture of liquid and semisolid preparations because it determines the nature of the mixture and its flow, both when produced and put into the packaging and when used, such as consistency, spreadability, and humidity. The higher the viscosity value of a liquid, the greater the resistance to flow. Preparations that are difficult to remove from the bottle make it difficult to use. Preparations with low viscosity become easy to flow, so they easily fall from the palm of the hand when used. The physical stability of a preparation will also be affected by its viscosity (31) . Based on SNI (06-4085-1996), the requirements for good viscosity range from 400-4000 cPs.

The results of the viscosity test in Table 5 show that the highest viscosity value is F3 with a concentration of 9% with a viscosity value of 823.33 cPs ± 15.28, and the lowest viscosity value is F1 with a concentration of 3% with a value of 680 cPs ± 10. The magnitude of the viscosity value in the *liquid soap preparation* of akalifa leaf extract can be influenced by several factors, including temperature, particle size, intermolecular attraction, and the influence of the amount of distilled water, because the greater the amount of distilled water given, the lower the viscosity results and vice versa, the lower the amount of distilled water given, the higher the viscosity value produced (32) . The amount of distilled water is low because the amount of extract is high, resulting in a high viscosity value. Although the

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viscosity value in each formulation varies, the viscosity value is still within the range required by SNI.

The observation data of the viscosity test were analyzed by one-way ANOVA test, there were significant differences in the three formulations obtained with a *p-value of* 0.000 (*p-value* < 0.05), then the LSD (*Least Significant Difference*) test was carried out to determine the significant difference in viscosity between the two *liquid soap formulations.* Based on the results of the LSD test on F1 against F2 there was a significant difference (Sig. 0.001 *p-value* < 0.05), on F1 against F3 there was a significant difference (Sig. 0.000 *p-value* <0.05) and F2 against F3 there was a significant difference (Sig. 0.008 *p-value* <0.05).

The antibacterial test in this study aims to determine the antibacterial activity against *Escherichia coli bacteria* in the *liquid soap formulation* of akalifa leaf extract. The antibacterial activity test was carried out using the well method by examining the inhibition zone formed around the well hole. This method was chosen because the *liquid soap sample* is easy to insert into the hole that has been made, can inhibit bacteria more strongly, and is easier to measure the area of the inhibition zone formed because the bacteria are active not only on the upper surface of *the nutrient agar* (NA) but also down. In the antibacterial activity test, the negative control used was the F0 formulation without *liquid soap extract (base),* which aimed to ensure that the components in *the liquid soap preparation* did not have antibacterial activity against *Escherichia coli bacteria.* Onemed antibacterial soap was used as a positive control. *And this study made liquid soap* preparations with variations in the concentration of akalifa leaf extract of 3 %, 6% and 9%.

Based on Table 6 , the results of the antibacterial activity test show that all concentrations of khalifa leaf extract show active inhibition against the growth of *Escherichia coli bacteria.* However, a concentration of 9% shows very active inhibition against *Escherichia coli bacteria* with an inhibition zone diameter of 13.04 mm. The category of inhibition response is based on the inhibition zone <5 mm weak, 5-10 mm moderate, 10-20 mm strong, and >20 very strong. According to the results of measuring the diameter of the bacterial inhibition zone, the bacterial growth inhibition zone increases with the concentration of the extract, which is caused by the increase in the amount of active compounds contained in the extract. Thus, the growth of microbial cells is inhibited, or cell death increases with the increase in extract concentration. This is in line with previous studies showing that the inhibition zone against bacterial growth is larger when the extract concentration increases. Other studies also show that higher concentrations of antibacterial ingredients show stronger antibacterial activity, which is indicated by a larger diameter of the inhibition zone (33). In previous studies, utilizing akalifa leaf extract as an active ingredient in *hand sanitizer gel preparations* produced an inhibition zone of 15.23 mm (strong) at a concentration of 3% (20) .

The observation data of the antibacterial activity test were analyzed by one-way ANOVA test; there were significant differences in the three formulations obtained with a *p-value of* 0.000 (*p-value* < 0.05), then the LSD (*Least Significant Difference*) test was performed to determine the significant differences in antibacterial activity between the two *liquid soap formulations.* Based on the results of the LSD test on F1 against F2, there was a significant difference (Sig. 0.001 *p-value* < 0.05), on F1 against F3, there was a significant difference (Sig. 0.000 *p-value* <0.05) and F2 against F3 there was a significant difference (Sig. 0.019 *p-value* <0.05). There were significant differences in F1 against positive control, F2 against positive control, and F3 against positive control (Sig. 0.000 *p-value* <0.05) .

5. CONCLUSION

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Based on the research conducted, it was concluded that the extract of khalifa leaves (*Acalyhpa wilkesiana Müell. Arg.*) contains alkaloids, flavonoids, tannins, and saponins. Variations in extract levels in *liquid soap* formulations produce significant differences in physical quality, but each *liquid soap formulation* meets the range requirements permitted by SNI (Indonesian National Standard). Increasing the extract content in *liquid soap* results in a significant increase in inhibitory power. Formula 3 is the formula that produces the highest inhibitory power, namely (13.04mm ± 1.28) with a strong category.

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