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THE ANTIBACTERIAL ACTIVITY OF LIQUID SOAP SUPPLEMENTED WITH EXTRACTS COMBINATION OF *Cyperus rotundus L.* and FLOWERS OF *Plumeria acuminata*, *Michelia alba*, OR *Cananga Odorata* AGAINST *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI* BACTERIA

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ABSTRACT

Secondary metabolites found in white Frangipani flowers *(Plumeria acuminata)*, white tropical Magnolia flowers *(Michelia alba)*, Ylang flower (*Cananga Odorata*), and Nutgrass *(Cyperus rotundus L.)* are thought to be employed as antibacterial active ingredients in detergent. This research aims to create liquid antibacterial soap using an extract mixture of Nutgrass with white Frangipani flower (F1), white tropical Magnolia flower (F2), or Ylang flower (F3). Phytochemical screening tests for the ethanol extracts of Nutgrass and those three flowers, pH tests, total active ingredients tests, and antibacterial activity tests against *S. aureus* and *E. coli* bacteria were carried out during the formulation of the liquid soap preparation with the addition of these extracts. The extraction procedure was maceration with a 70% ethanol solvent, yielding extracts of 9.28 percent frangipani flower, 8.12 percent white tropical Magnolia flowers, 9.8 percent Ylang flower, and 8.88 percent Nutgrass. The liquid soap quality testing findings met the established norms (SNI 4085:2017). This research shows that the soap formulas (F0, F1, F2, and F3) can inhibit *S. aureus* and *E. coli* germs in antibacterial activity tests using the disc diffusion method. Based on the diameter of the inhibition zone, the antibacterial activity against *E. coli* of liquid soap with Nutgrass & white tropical Magnolia flower extracts (F2) is not significantly different from liquid soap without extracts (F0). The F2 formula shows to be less effective than both the two liquid soaps containing extracts of Nutgrass & white Frangipani flower (F1) and Nutgrass & Ylang flower (F3). However, the three formulas of liquid soaps (F1, F2, and F3) tend to be more effective in antibacterial activity against *S. aureus* than a liquid soap that did not contain extracts (F0).

Keywords: liquid soap, white Frangipani flower, white tropical Magnolia flower, Ylang flower, Nutgrass

INTRODUCTION

In the era of the Covid-19 pandemic, ensuring body health, especially body hygiene, is very important because many diseases are caused by bacteria and germs, such as *S. aureus* and *E. coli* bacteria. These bacteria can cause various skin problems, digestion, and cause other diseases. Soap is a way to protect the body

from bacteria, germs, dust, and dirt. Therefore, the demand for soap increases along with the increase in population and the Covid-19 pandemic. There are various soap forms; some are in solid or liquid form [1-2].

The increasing use of soap is related to increased environmental pollution caused by the waste produced. The use of antibacterial soap is used as a solution because it is believed to clean the skin; it can also prevent or treat infectious diseases caused by bacteria, especially during the pandemic era. Antibacterial soaps on the market contain active ingredients, such as diethanolamine (DEA), Sodium Lauryl Sulphate (SLS), and triclosan [3]. Sodium Lauryl Sulfate (SLS) can cause skin irritation [4]. If triclosan accumulates in the human body, it has the potential to cause thyroid dysfunction [5]. Meanwhile, suppose the use exceeds the recommended concentration. In that case, it will be carcinogenic and can irritate the epidermis and denaturation the polypeptide chain of a protein molecule so that it can change the protein structure [6].

According to the Food and Drug Association (FDA), excessive and long-term use of synthetic active ingredients can cause bacteria to become resistant to antibiotics because their chemical composition is identic to some types of antibiotics [7]. In addition, waste can hurt the environment. Some of the effects of detergent waste on the environment include aesthetic disturbances by white foam on the surface of the waters, decreased dissolved oxygen levels in the waters, changes in the physical and chemical properties of water, high eutrophication, and phosphate that can cause aquatic weeds to grow uncontrollable [8].

Several studies have been carried out to reduce the adverse effects of using synthetic surfactants on the body, and these potential hazards can be overcome by making soap using natural ingredients as active ingredients. The content of plant extracts is reported to have benefits as additional ingredients in herbal soaps and increases the ability of soap to kill bacteria. One way is to utilize secondary metabolite compounds such as phenols, saponins, flavonoids, alkaloids, essential oils, and other compounds in plants that act as antibacterial and antioxidants [8-9].

The white tropical magnolia flower contains 0.2% essential oil obtained by distillery [9]. The essential oil of white tropical magnolia flowers is very easily damaged by heating with water vapor, so isolating oil from flowers is carried out by the extraction method using volatile organic solvents such as petroleum ether [10]. White tropical magnolia flower essential oil contains phenol, cineol, eugenol, benzyl aldehyde, and phenyl ethyl alcohol. In addition to containing essential oils found in flowers, all white tropical magnolia plants (*Michelia alba*) also contain alkaloids, flavonoids, and saponins. This secondary metabolite content is spread from roots, leaves, and bark [11].

The white frangipani flowers belonging to the Plumeria genus contain various secondary metabolite compounds such as phenols, flavonoids, terpenoids, tannins, and saponins that have many

health benefits in relieving coughs, reducing fever, explain digestion. In addition, white frangipani flowers have a good level of inhibition to kill bacteria, especially *S. aureus* and *E. coli* bacteria [12-13]. Therefore, it has been used as an adjunct to health and beauty products and has shown potential as a good antioxidant and antibacterial agent.

Ylang flower (*Cananga Odorata*) contains secondary metabolites such as monoterpenes, sesquiterpene hydrocarbons, oxygen-containing sesquiterpenes, acetates, benzenoids, benzoates, and phenols [14]. Those compounds will be obtained maximally when the flowers are yellow or fully ripe [15]. Ylang flower has various medicinal properties, including medicine for malaria, asthma, antidepressants, hypertension, headaches, eye inflammation, and gout. In addition, this plant is also used in the cosmetic industry, soap, aromatherapy, and even as a food ingredient [16]. Furthermore, Ylang flower essential oil shows antimicrobial activity against gram-positive and gram-negative bacteria [17].

Nutgrass (*Cyperus rotundus* L.) is a medicinal plant belonging to the Cyperaceae family that is rarely used in daily life [18]. However, judging from the content possessed by nutgrass, it can be used as a nutritious item. Nutgrass tubers have many benefits in pharmacology as anti-inflammatory, antidiabetic, antidiarrheal, and analgesic. It also has biological benefits such as antimutagenic, cytotoxic, cytoprotective, antimicrobial, antioxidant, antibacterial, and apoptotic [19]. Nutgrass contains chemical components including alkaloids, saponins, flavonoids, terpenoids, essential oils, polyphenols, resins, starch, tannins, triterpenes, D-glucose, D-fructose, and non-reducing sugars [20]

The addition of natural extracts has been carried out for soap products with synthetic or natural surfactants. In addition, research on natural ingredients extracts in cleaning products such as soap has been also widely practised, but there is still little research that provides information about the benefits of the additional ingredients extracts for improving the quality of soap to protect the body from bacteria [21-24]. White Frangipani, Ylang, and white tropical magnolia flowers have the potential as antibacterial and are expected to increase the antibacterial quality of natural liquid soap when combined with extracts from nutgrass, which has a high potential as a natural antibacterial.

METHODS

Tools and Materials

The tools used in this study were jars, baking sheets, scissors, oven, blender, 100 mesh sieve, balance, watch glass, glass stirrer, spatula, beaker, measuring cup, Erlenmeyer, rotary evaporator, desiccator, dropper pipette, micropipette, paper. Filter, glass funnel, separating funnel, porcelain dish, petri dish, test-tube, test-tube rack, measuring flask, drip plate, hot plate, magnetic stirrer, ose needle, thermometer, caliper, pH meter, pH indicator, cotton swab, and tweezers.

The materials used were white Frangipani flowers, Ylang flowers, white tropical Magnolia flowers, Nutgrass tubers,

distillate water, ethanol (70%, 90%, 95%, 96%, 99.5%), neutral ethanol, petroleum ether, HCl (concentration 0.1M, 2M, 5M), Dragendorff's reagent, Mayer's reagent, PP indicator, Mg powder, 1% $FeCl_3$, KOH, stearic acid, anhydrous Na_2SO_4, NaOH, NaCl, olive oil, coconut oil, glycerine, MHA, pH buffer solution 7.1, cultures of *S. aureus* and *E. coli* bacteria.

Extraction

Extraction is made by the maceration method. First, samples of white Frangipani flowers, white tropical Magnolia flowers, Ylang flowers, and Nutgrass tubers were washed, cut into pieces, aired overnight, and then the samples were oven-dried at 37^oC to dry. The dried samples were then blended until smooth and weighed 50 g each of flowers and Nutgrass bulbs. Furthermore, the four samples were placed in separate containers and macerated each with 500 ml of 70% ethanol solvent, respectively.

Maceration was carried out 3x24 hours with a change of solvent every 1x24 hours. The solution obtained was filtered with filter paper, and the filtrate was evaporated with a rotary evaporator until a dry extract was obtained. The obtained extract is weighed and stored in a closed container not to be contaminated.

Phytochemical Screening

The extracts of white Frangipani flowers, Ylang flowers, white tropical Magnolia flowers, and Nutgrass were weighed as much as 0.8 grams each and dissolved in 8 ml of 96% ethanol. Then, the extract solution was tested as follows:

Test for tannins or polyphenols

A total of 2 ml of the extract solution was added with a few drops of 1% iron (III) chloride ($FeCl_3$) solution. A positive test for tannins is indicated by the formation of characteristic blue, blue-black, blue-green, and greenish-brown precipitates.

Flavonoid Test

As much as 2 ml, the extract solution was heated for ± 2 minutes, added five drops of concentrated HCl and 0.1 g of Mg powder. A positive test for flavonoids is indicated by forming a yellow orange to dark red colour.

Alkaloid test

2 ml of the extract solution was evaporated on a porcelain cup to obtain a residue, then dissolved with 5 ml of 2 N HCl. The obtained solution was then divided into 3 test tubes. The tube I was added with dilute acid, which served as a blank; tube II added three drops of Dragendorff's reagent, and the test was positive for orange precipitate. Tube III added three drops of Mayer's reagent; the positive test indicated yellow.

Saponin test

2 ml of the extract solution was put into a test tube and then shaken for 1 minute; if foam appears, then 1 N HCl is added. A positive test for saponins is indicated by the formation of foam that can last a long time.

Liquid Soap Preparations

There are three liquid soap formulations (Table 1), each formulation was supplemented with pair extracts of white Frangipani flower & Nutgrass (1%: 0.5%), Ylang flower & Nutgrass (1%: 0.5%), and

white tropical Magnolia flower & Nutgrass (1%: 0.5%).

Liquid soap is made by reacting olive oil and coconut oil in a beaker, heated, and stirring until homogeneous. Then add a solution of KOH and stearic acid melted while stirring until the solution becomes slightly lumpy. Next, add glycerine and sterile distilled water to the liquid soap formulation. Finally, add pair extracts of flower and Nutgrass until homogeneous. The liquid soap that has been obtained is then placed in a closed container and stored in the refrigerator.

Table 1. Liquid soap formulations

Composition	Control Soap (F0)	soap with white Frangipani extract & Nutgrass (F1)	soap with white tropical Magnolia flower extract & Nutgrass (F2)	soap with Ylang flower extract & Nutgrass (F3)
Olive oil	15.00 g	15.00 g	15.00 g	15.00 g
Coconut oil	10.00 g	10.00 g	10.00 g	10.00 g
KOH (Potassium Hydroxide)	5.15 g	5.15 g	5.15 g	5.15 g
Stearic acid	2.00 g	2.00 g	2.00 g	2.00 g
Glycerine	1.75 g	18.75 g	18.75 g	18.75 g
Distillate water	44.56 ml	44.56 ml	44.56 ml	44.56 ml
Nutgrass extract	-	0.50 g	0.50 g	0.50 g
frangipani flower extract	-	1.00 g	-	-
White tropical magnolia flower extract	-	-	1.00 g	-
Ylang flower extract				1.00 g

Antibacterial Test

Antibacterial tests were carried out on extracts of white Frangipani flower, Ylang flower, white tropical magnolia flower, and liquid soap formulas (F0, F1, F2, F3) using the disk diffusion method. The steps taken were to prepare the media for MHA, then prepare samples to be tested for antibacterial, cultures of *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 NaCl, Bunsen, and other necessary tools. First, make a bacterial suspension solution by taking bacteria from a bacterial culture dish with an ose needle and then dissolving it into a test tube containing NaCl with the same turbidity as the Mc standard. Farland. After that, inoculate the bacterial suspension into the MHA media until evenly distributed using a cotton swab. After the media is ready, blank discs or paper discs that have been moistened with extract and liquid soap are then placed on top of the media at a certain distance. It was then incubated for 24 hours, after which it was observed and measured the diameter of the inhibition zone with a calliper around the blank disc. Vancomycin was used as a positive control for *S. aureus* bacteria and Chloramphenicol for *E. coli* bacteria. The negative control used sterile distilled water.

Liquid Soap SNI Test

The procedures for testing the quality standard of liquid soaps are based on SNI 4085:2017 regarding liquid soap. The quality standard tests for liquid soap were carried out in this study, including the pH test and the total active ingredient test.

RESULTS AND DISCUSSION

Sample Extraction

The filtrate from maceration of white Frangipani flower extract, white tropical Magnolia flower, Ylang flower, and Nutgrass was evaporated with a rotary evaporator at a temperature of 40^{o}C. As a result, the thick

brown slurry was obtained in the extract of white Frangipani flower, Ylang flower, and white tropical Magnolia flower and a thick dark brown slurry of Nutgrass extract. The extracts yield of Frangipani flower, white tropical Magnolia flower, Ylang flower, and Nutgrass were 9.28%, 8.12%, 9.80%, and 8.88%, respectively.

Phytochemical Screening

Ylang and white tropical Magnolia flower has no precipitate against Dragendorff's or Mayer's reagents in the alkaloid test (Table 2). This result is caused by the possibility of the potassium-alkaloid complex that is formed is not to the saturation limit, so it cannot form a precipitate [25]. Alkaloids can be found in various parts of plants, but often the levels are less than 1%, so this can cause the alkaloid test to give negative results [26].

Table 2. The results of screening phytochemistry

No.	Compound	Reagent	Result				Description (+) or (-)			
			Frangipani flower	Ylang flower	white tropical Magnolia flower	Nutgrass	Frangipani flower	Ylang flower	white tropical Magnolia flower	Nut grass
1.	Tannins	$FeCl_3$	Dark chocolate precipitate	blackish green	Greenish brown	blackish green	+	+	+	+
2.	Flavonoids	Mg+HCl	Dark red	orange	Yellow	magenta red	+	+	+	+
3.	Alkaloids	Mayer	Gray precipitate	no precipitate found	no precipitate found	Orange precipitate	+	-	+	-
4.	Saponins	Dragendorf H_2O+ $C_2H_5OH \rightarrow$ (Is shaken)	Light brown Permanent foam	Deep green permanent foam (only a little)	brown Permanent foam	Brown Permanent foam	+	+	+	+

Antibacterial activity test

Antibacterial test results were carried out on the ethanolic extracts of flowers (white Frangipani, white tropical Magnolia, and Ylang), liquid soap control without extracts (F0), liquid soap containing white Frangipani flower & Nutgrass extracts (F1), liquid soap containing white Magnolia flower & Nutgrass extracts (F3), and liquid soap containing Ylang flower and Nutgrass extracts (F3) (Figure 1 and Table 3).

Based on the diameter of the inhibition zone, it was known that the four liquid soap formulas had better antibacterial activity against *S. aureus* than *E. coli*. White Frangipani flowers have greater antibacterial power than white tropical Magnolia flowers in extracts and liquid soap preparations. Antibacterial activities of two liquid soap formulas containing extracts (F1 and F3) are more effective than liquid soap control without extracts (F0) against *S. aureus* and *E. coli* bacteria. Meanwhile, the liquid soap F2 shows antibacterial activity against *S. aureus* more effective than F0, but against *E. coli* bacteria, it shows no difference. The difference in the inhibition zone can be influenced by the sensitivity level of the test organism, the culture medium and incubation conditions, the rate of diffusion of the antibacterial compound, the concentration of the antibacterial compound [27], the solubility of the antibacterial agent into the medium, and the effectiveness of

the antibacterial agent. The antibacterial activity is due to the content of several antibacterial chemical compounds, namely tannins, alkaloids, saponins, and flavonoids [28].

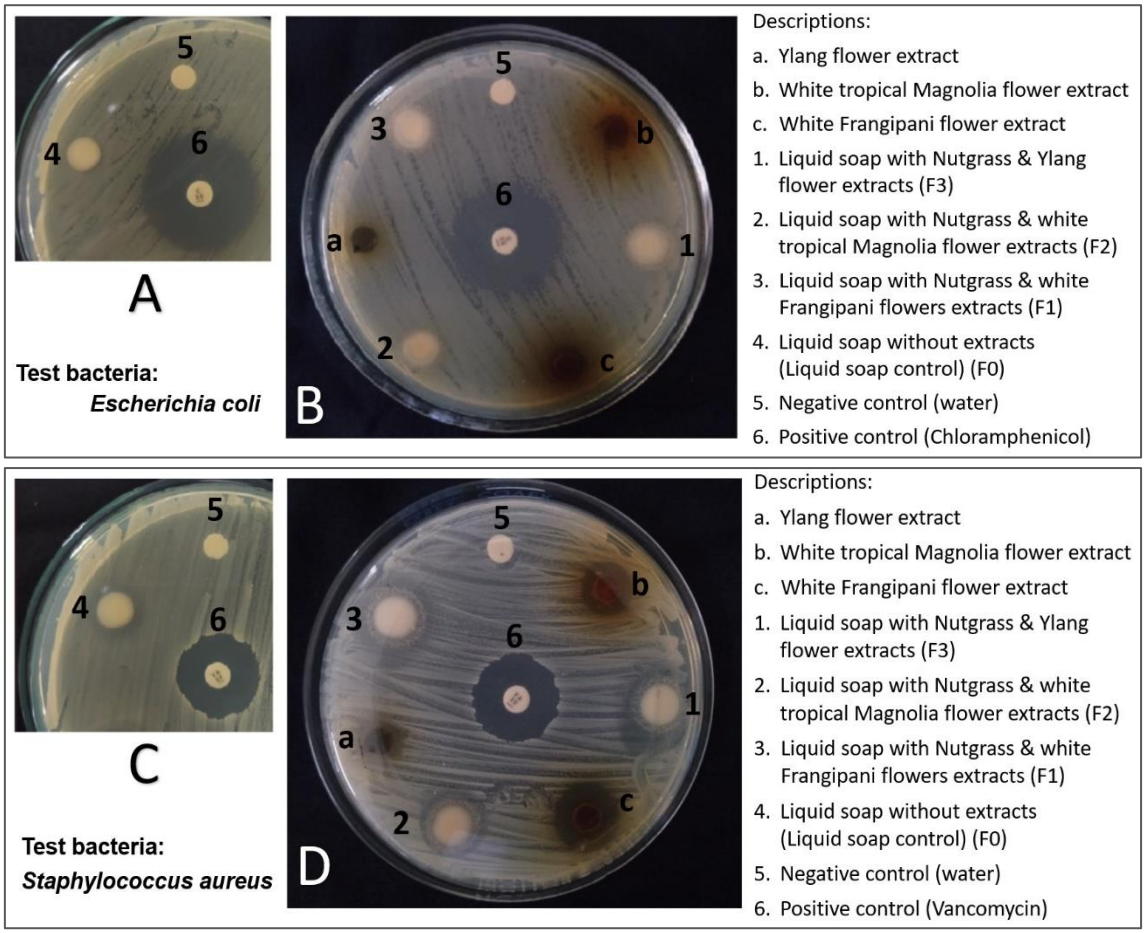

Figure 1. *E. coli*-antibacterial test of liquid soap control (A) and liquid soaps with Nutgrass & flower extracts (B). *S. aureus*-antibacterial test of liquid soap control (C) and liquid soaps with Nutgrass & flower extracts (D).

Table 3. Antibacterial activity test results of Liquid soaps against *S. aureus* and *E. coli* bacteria

No.	Samples	Bacterial inhibition zone diameter	
		S. aureus (mm)	*E. coli* (mm)
a.	Ylang flower extract	10,17 ± 0.29	7,25 ± 0.25
b.	White tropical magnolia flower extract	12,75 ± 0.25	6,92 ± 0.38
c.	White Frangipani flower extract	16,67 ± 0.14	9,83 ± 0.14
1.	Liquid soap with Nutgrass & Ylang flower extracts (F3)	17,33 ± 0.76	11,08 ± 0.38
2.	Liquid soap with Nutgrass & white tropical magnolia flowers extracts (F2)	16,42 ± 0.14	9,25 ± 0.25
3.	Liquid soap with Nutgrass & white Frangipani flower extracts (F1)	17,83 ± 0.29	10,33 ± 0.14
4.	Liquid soap without extracts (Liquid soap control) (F0)	12,75 + 0.25	9,17 + 0.14
5.	Negative control (water)*	-	-
6.	Positive control (Vancomycin/Chloramphenicol)	20.92 ± 0.14	26,50 ± 0.50

*) disk blank diameter = 6 mm

The antibacterial mechanism of phenolic compounds is by denaturing cell proteins. Protein damage is caused by hydrogen bonds between phenol and protein, which affects the permeability of cell walls and cytoplasmic membranes. The permeability of the microbial cell membrane changes because phenol interferes with the electron transport system and energy production, causing the cell to lyse due to an imbalance of macromolecules and ions [29].

The mechanism of phenol in inhibiting *E. coli* bacteria influences its dehydrogenase and oxidase enzymes. At low concentrations, phenolic compounds can damage the cytoplasmic membrane and leak the cell nucleus, while at high concentrations, they will gel with cellular proteins. This activity is very effective when bacteria are in the cleavage stage, where the phospholipid layer around the cell is thin, so phenol can easily damage the cell contents [30]. Flavonoids include phenolic compounds that can damage microbial cells by penetrating cell membranes which cause coagulation of proteins in cell membranes, thereby damaging protein structures [28]. The antibacterial activity of tannins is using protein precipitation through reactions with cell membranes, inactivation of enzymes, and inactivation of the function of genetic material. Their mechanism is by inhibiting the reverse transcriptase and DNA topoisomerase enzymes not to cause bacterial cells to form [29]. Astringent compounds present in tannins can interfere with the activity of cell walls and microbial cell membranes [28]. The mechanism of alkaloids as an antibacterial is by interfering with the peptidoglycan constituent components of bacterial cells. The cell wall layer is not completely formed and causes cell death [31]. Meanwhile, saponin compounds can interfere with cell permeability by lowering the surface tension on the cell wall. If saponins interact with bacterial cells, the bacteria will break or lyse due to leakage of proteins and enzymes from within the cells [28]

In the liquid control soap, antibacterial activity was found because the composition contained olive oil and coconut oil. Oleuropein compounds can inhibit bacterial growth by damaging bacterial cell membranes and peptidoglycan [32]. While phenolic compounds are active as antibacterial with the mechanism of forming complexes with cell proteins, they inhibit the work of enzymes in bacteria [33]. Lauric acid in coconut oil is a medium-chain saturated fatty acid that is antimicrobial (antibacterial, antiviral, and antifungal) [34].

Figure 1 shows a colony formation around the disk of the liquid soap inhibition zones. The flowers of white Frangipani, white Magnolia, and Ylang contain volatile compounds [35]. Extracts of these flowers can contain volatile or non-volatile compounds. Antibacterial effectiveness is influenced by the duration of contact between bacteria and antibacterial substances contained in the extracts. The phenomena assumption is that the volatile compounds evaporate first before the bacteria grow optimally, affecting the effectiveness of antibacterial compounds in flower extracts on the growth of *E. coli* and *S. aureus* bacteria.

The criteria for antibacterial activity are categorized based on the diameter of the inhibition zone formed according to Table 4.

Table 4. Category of antibacterial inhibition zone

Inhibition zone diameter	Growth inhibition response
<5 mm	Weak
5-10 mm	Medium
10-20 mm	Strong
>20 mm	Very strong

Antibacterial activity against *S. aureus* bacteria for the three extracts and four liquid soap formulas is strong, with a 10-20 mm inhibition zone. However, against *E. coli* tends to be medium, with a 5-10 mm inhibition zone. Therefore, the exception for liquid soap formula F1 (containing white Frangipani flower extract) and F3 (containing Ylang extract) have antibacterial activity against *E. coli* bacteria that tend to be strong.

The bacterial cell wall structure causes the sensitivity to antibacterial substances of the gram-positive and gram-negative bacteria. For example, Gram-positive bacteria tend to be more sensitive because structure of their cell wall is simpler, making it easier for antibacterial compounds to enter the cell. For example, *S. aureus* bacteria are gram-positive with cell walls composed of peptidoglycan [36]. In contrast, gram-negative bacteria have a more complex cell wall structure, lipoproteins, lipopoly-saccharides, and fats that affect the activity of antibacterial substances. The middle layer is lipopolysaccharide which acts as a barrier to the entry of antibacterial bioactive ingredients, and the inner layer is peptidoglycan with a high lipid content [29]. At the same time, the outer membrane can inhibit the diffusion of the extract inside the peptidoglycan membrane so that the extract cannot diffuse into it [37].

Liquid Soap SNI Test

pH and Total Active Compound Test

The degree of acidity (pH) is one of the essential parameters to determine whether the soap produced is acidic or basic [34]. A pH value that is too low or too high can cause an increase in the absorption of soap on the skin, which can irritate such as sores, itching, or peeling and can make the skin dry [38]. In SNI 4085:2017, the proper pH of liquid bath soap is 4.0–10.0 (SNI, 1996). In this study, the pH test was carried out three times in a row using a pH meter to ensure the pH of the liquid soap was obtained. From the tests performed (Table 5), the pH of the liquid control soap was 8.46, the liquid soap formula F1 was 7.80, the liquid soap formula F2 was 8.00, and the liquid soap formula F3 was 8,46. These results follow SNI standards, so it is safe to use. In general, liquid soap products have a pH that tends to be alkaline because the primary ingredient of liquid soap is KOH (strong base) [36].

The addition of extracts of natural ingredients can cause an increase in the pH of the soap because it is possible to add alkaline active compounds of alkaloids and saponins [34,39]. However, in the results of this study, control liquid soap (F0) and liquid soap containing Ylang flower extract (F3) have the same pH, but they have a higher pH than liquid soap containing Frangipani flower extract (F1) and white Magnolia flower extract (F2). Meanwhile, it could be due to the presence of acidic polyphenolic

compounds in the natural extract that can cause a decrease in the pH of soap [40].

Based on Table 5, the higher the total active compound, the more pH of the liquid soap. Active compound measured is the number of compounds in soap that are not saponified. Based on SNI 4085:2017, the total active ingredients in liquid soap are at least 15.0%. From this study, the total active compound in all four liquid soap formulas (F0, F1, F2, and F3) are greater than 30 %. These results follow SNI 4085:2017; they at least contain 15.0% of total active compounds. Olive oil in soap containing oleic fatty acid can increase the active compound. The oleic fatty acid helps increase skin moisture [24].

Table 5. pH value and Total active compounds of liquid soaps in this study

No.	Soap	pH	Total Active Compound
1.	Liquid soap without extracts (Liquid soap control) (F0)	8.46	46.2%
2.	Liquid soap with Nutgrass & white Frangipani flower extracts (F1)	7.80	30.6%
3.	Liquid soap with Nutgrass & white tropical magnolia flowers extracts (F2)	8.00	45.0%
4.	Liquid soap with Nutgrass & Ylang flower extracts (F3)	8.46	48.2%

CONCLUSION

Based on the result study above, the ethanol extract of white Frangipani flower, white tropical Magnolia flower, Ylang flower, and nutgrass tubers can be formulated as a liquid antibacterial soap. Liquid soaps with Nutgrass & white Frangipani flower extracts (F1), Nutgrass & white tropical Magnolia flower extracts (F2), Nutgrass & Ylang flower extracts (F3), and liquid soap without extracts (F0) meet the SNI quality standards based on the results of pH test and the total active compound test. In addition, the four liquid soap formulas (F0, F1, F2, and F3) have antibacterial activity against both *S. aureus* and *E. coli* bacteria, and their activity against *S. aureus* are higher than *E. coli* bacteria.

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