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Research Article

Formulation and Antibacterial Activity of Liquid Soap Containing Ketapang (*Terminalia catappa* L.) Leaves Extract

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Abstract

Ketapang (*Terminalia catappa* L.) is traditionally used by the community to treat the skin's infections caused by bacteria or fungi. In this study, *T. catappa* leaves extract was added to the liquid soap formula as an antibacterial. The purpose of this study was to determine the secondary metabolite compounds contained in *T. catappa* leaves extract, physical evaluation of the preparation, and antibacterial activity of liquid soap. Liquid soap formula was made with various concentrations of *T. catappa* leaves extract F0 (0%), F1 (1%), F2 (2%), and F3 (3%). The resulting soap was evaluated for organoleptic, pH, high foam, homogeneity, irritation, and its activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Escherichia coli* using the disc diffusion method. The results showed that the *T. catappa* leaves extract contained flavonoids, tannins, saponins, and triterpenoids. The liquid soap formula F0 was clear, while F1, F2, and F3 had the characteristics of brown-dark brown, homogeneous, pH between 4.6-5.2, foam stability between 67-72%, which was not significantly different and stable after five minutes of testing, and it did not irritate the skin. *Terminalia catappa* leaves extracts liquid soap has antibacterial activity at a concentration of 1%, 2%, and 3%, with the largest inhibition zone diameter produced by *S. aureus*.

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INTRODUCTION

Soap is a cleanser because it can remove dirt that sticks to parts of the body[1]. The use of liquid soap is more attractive to the public than solid soap because it is more practical, economical, not contaminated, easy to carry, and easy to store[2]. There have been many antibacterial soaps circulated in the market under various brand names.

Most of the antibacterial soap in the market still contains synthetic ingredients such as sodium lauryl sulfate (SLS) and triclosan, which have adverse effects on human skin. These side effects include sensitivity to the skin and turning off the protective layer on the skin to be more susceptible to exposure to harmful bacteria on the skin[3,4]. One of the efforts to overcome this problem is to utilize plants with antibacterial properties, one of which is ketapang (*Terminalia catappa* L.).

Terminalia catappa comes from the Combretaceae family, which is a large tree[5] that has horizontal branches with several levels, leaves of 15-25 cm long and 10-14 cm wide[6]. This plant is widely distributed in countries with tropical and sub-tropical climates, especially in coastal areas[7]. *Terminalia catappa* are often found on roadsides as decoration and shade trees[8]. *Terminalia catappa* shed their leaves every day, and most of them fall during the dry season[9].

Terminalia catappa is known as a plant with pharmacological effects and is used traditionally[6]. In Asian countries, *T. catappa* leaves are usually used to treat dermatitis, hepatitis, diarrhea, and paresis. This plant is also included in the type of vegetable in the Caribbean, where *T. catappa* leaves are used in decoction to treat ulcers and urinary tract infections[7]. In India, *T. catappa* leaves are attached to the skin to treat scabies, leprosy wounds, and other skin diseases. Besides, in Malaysia, *T. catappa* leaves are used to treat diarrhea and fever[10].

Terminalia catappa has shown biological effects such as having antibacterial and antifungal activities[7,11], antioxidants[12], antipyretic, hemostatic, hepatitis[5], anti-inflammatory, antidiabetic, antioxidant, hepatoprotective, and anticancer[13,14], antiprotozoal, antiviral, anti-diarrhea, analgesic, antimalarial, and anticancer activities[15].

Terminalia catappa leaves are known to contain chemical compounds such as tannins and flavonoids, which are thought to have antibacterial properties such as *Aeromonas hydrophila*, *Escherichia coli*, and *Staphylococcus aureus*[11]. *Terminalia catappa* also contains flavonoids, alkaloids, tannins, triterpenoids, steroids, resins, saponins, quinones, and phenolics[16-18].

According to several previous studies, giving *T. catappa* leaves extract has been shown to inhibit of several bacteria such as *Aeromonas salmonicida*, *A. hydrophila*, *E. coli*[19], *S. aureus*, *Pseudomonas aeruginosa*[5], and *Bacillus amyloliquefaciens*[11]. *Terminalia catappa* leaves extract also has antifungal activity against *Candida sp*[10]. In addition, *T. catappa* leaves extract can be used to increase the resistance of betta fish and tilapia to *A. hydrophila*[20].

In this study, the *T. catappa* leaves used were fallen leaves. Previous studies have shown that antibacterial and antifungal activity is higher in fallen *T. catappa* leaves than leaves still on trees[21]. Then the leaves are extracted with ethanol solvent and formulated into liquid soap. Subsequently, physical evaluation and antibacterial activity were carried out. This study expects that the liquid soap products produced have good physical characteristics and antibacterial activity.

MATERIALS AND METHODS

Materials

The materials used were *T. catappa* leaves, 96% ethanol, SLS, Comperland, CAB 30, NaCl, citric acid, glycerol, nipagin, Na4 EDTA, distilled water, blank disc, strains of *S. aureus*, *S. epidermidis*, and *E. coli*, antibacterial body wash soap, and Mueller-Hinton agar (MHA) media. The tools used were rotary evaporator, analytical scale, pH meter, incubator, autoclave, oven, caliper, and laminar airflow.

Methods

Sample collection

Terminalia catappa leaves were collected in Pekanbaru city and determined at the Botanical Laboratory, Universitas Riau. Samples taken were *T. catappa* leaves that had fallen around the trees with brownish leaves characteristics. The leaves of *T. catappa* were shown in **Figure 1**.

Figure 1. *Terminalia catappa* leaves

Preparation of simplicia

The collected leaves were washed under running water to remove dirt on the leaves. The leaves were then chopped into small pieces to expand the surface and speed up the drying process. The samples were dried at

room temperature until dry; then, they were sorting and ground into a powder. The powders that had been produced were macerated with 96% ethanol for three days. Afterward, they were filtered and separated between the filtrate and the residue. Maceration was repeated three times with the same type and amount of solvent; then, the macerate was collected and evaporated using a rotary evaporator.

Phytochemical screening

1. Alkaloids test: A total of 0.5 mL of the sample was inserted into three test tubes. Each tube was then added by a few drops of Wagner's, Mayer's, and Dragendorff's reagents.
2. Flavonoids test: A total of 0.5 mL of sample was heated for five minutes, then it was added with three drops of HCl concentrated and a little Mg powder.
3. Saponins test: A total of 1 mL of sample was added with 2 mL of hot water, shaken, and let stand for five minutes.
4. Tannins test: A total of 0.5 mL of sample was added with three drops of 1% $FeCl_3$.
5. Terpenoids test: A total of 0.5 ml of sample was added with three drops of Liebermann-Burchard's reagent through the test tube wall, and the results were observed[22,23].

Liquid soap formulation

As much as 40 g of Comperland was mixed with 30 g of CAB 30 and shook until it was thick. Then, 180 g of SLS was added along with 100 mL of water; then, it was stirred until blended. A total of 1 g of Na4 EDTA, 1 g of nipagin, and 400 mL of water were added and stirred until it was homogeneous. After it, 20 g of glycerin, 6 g of NaCl, 2 g of citric acid, and the remaining water were added and stirred until it was homogeneous. The mixture was stored in a tightly-closed container and waited until the shower gel's foam was gone. Furthermore, *T. catappa* leaves extract was added with a concentration of 1%, 2%, and 3%, stirred until it was homogeneous, and stored in a tightly closed container.

Physical evaluation of preparations

1. Organoleptic test: An organoleptic test was carried out by observing the physical form of liquid soap preparations using the senses. Liquid soap preparations that have been formulated were observed in terms of color, odor, as well as dosage form[24].
2. pH test: Measurement of pH values was carried out using a pH meter in a 10% sample solution, which was made by dissolving 1 g of the sample in 9 mL of water. Measurements were made by immersing the pH meter electrode, rinsed with distilled water into the solution. The pH value was determined after the numbers read on the pH meter have stabilized[25].
3. Foam stability test: The sample was weighed as much as 1 g, put into a test tube, then added with up to 10 mL of distilled water, shaken by turning the test tube back and forth, and immediately measured the level of foam produced. Then, the tube was left to stand for five minutes, then the height of the resulting foam was measured again after five minutes[26].

$$Foam\ stability = \frac{final\ foam\ height}{initial\ foam\ height} x\ 100\%$$

4. Skin irritation test: Testing was done using an open patch test. The open patch test was performed by applying the preparation to the inner forearm. A total of 1 cm of the preparation was applied and observed for 30-minute intervals for skin irritation, erythema, and redness. It was tested on the four formulations that have been made.
5. Homogeneity test: A total of 1 g of liquid soap preparation was smeared on the surface of the mica plastic, then the coarse particles were observed by being touched, and the texture of the preparation was observed.

Antibacterial activity test

All test bacteria (*S. aureus*, *S. epidermidis*, and *E. coli*) were respectively inoculated on MHA media. The 6 mm blank disc was dipped in liquid bath soap of *T. catappa* leaves extract and placed on the media's surface. The same thing was done with liquid bath soap sold in the market as a positive control and liquid soap base as a negative control. All samples were incubated at 35±2ºC for 24 hours, and the formed inhibition zone was observed[27]. The interpretation was performed by looking at the clear area around the disc, indicating no bacterial growth. Then, the diameter of the clear zone formed was measured by using a caliper.

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening was carried out to determine secondary metabolite compounds contained in a plant. Secondary metabolite compounds in one type of plant could vary, influenced by climate, soil, temperature, humidity, and others[28]. Therefore, this phytochemical screening was carried out to determine the secondary metabolite content of *T. catappa* leaves growing in the Pekanbaru area. Based on the test results, the compounds in the ethanol extract of *T. catappa* leaves were flavonoids, triterpenoids, saponins, and tannins, as shown in **Table I**.

Table I. Phytochemical screening of *T. catappa* leaves extract

No	Phytochemical test	Result	Conclusion
1	Alkaloid		
	- Mayer's	white color	-
	- Wagner's	yellow color	-
	- Dragendorff's	brown color	+
2	Flavonoid	orange color	+
3	Triterpenoid/	purple color	+
	Steroid	blue color	-
4	Tannin	green color	+
5	Saponin	stable froth or foam is formed	+

Physical evaluation of preparations

The liquid soap preparation formulation was made with four formulas, where F0 was a liquid soap base, F1 = base + 1% *T. catappa* leaves extract, F2 = base + 2% *T. catappa* leaves extract, and F3 = base + 3% *T. catappa* leaves extract. Physical evaluation of liquid soap preparations on the organoleptic test includes color, odor, and shape. Based on the color of the preparations formed on the base, F0 was clear, while F1 was brown, and F2 and F3 were dark browns. This was because the *T. catappa* leaves extract was brown. While the preparation's odor had a distinctive aroma of extracts, F0, F1, and F2 were thick, while F3 was slightly liquid. The more the addition of the extract causes the soap to become more liquid. The resulting soap preparations could be seen in **Figure 2**.

Furthermore, evaluation of the preparations was carried out, including organoleptic tests, pH, foam height, irritation, and homogeneity, as shown in **Table II**. The pH formed in liquid soap preparations ranges from 4.6 to 5.2. The SNI 4085:2017[29] stipulates that the pH quality requirements for liquid soap range from 4 to 10 so that all formulas produced have a pH value that meets the requirements as liquid soap. Furthermore, in the homogeneity test, the preparation was mixed homogeneously, and there were no coarse grains on the preparation.

Foam formation was not required and had little effect on the cleaning process, but it was more likely to patient acceptance of the product. The criteria for good foam stability, which was within 5 minutes, the foam stability obtained ranges from 60 - 70%. In this case, F0, F1, F2 had met the criteria for good foam stability, which was in the range of 67-70%, except for F3.

In the irritation test, the preparations were given to ten panelists who did not have a history of allergies. The preparation was applied to the skin of the forearm and left for 30 minutes. The test results showed no signs of skin irritation in the ten participants, such as dry skin, pain, bleeding, and cracked skin. Thus, the preparation was declared not to irritate the skin.

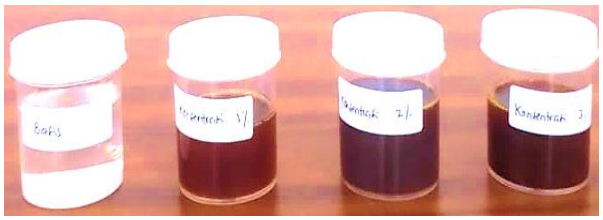

Figure 2. Liquid soap from *T. catappa* leaves

Table II. Physical evaluation of liquid soap from *T. catappa* leaves extract

Parameters	F0	F1	F2	F3
Color	Clear	Brown	Dark brown	Dark brown
Odor	Odorless	Distinctive of extracts	Distinctive of extracts	Distinctive of extracts
Shape	Thick	Thick	Thick	Slightly liquid
pH	5.2	5	4.8	4.6
Foam stability	70%	67%	70%	72%
Irritation	Nothing happened	Nothing happened	Nothing happened	Nothing happened

Antibacterial activity test

The antibacterial activity test of *T. catappa* leaves extracts liquid soap was carried out on three formulas, two controls with three times replication against the bacteria *S. aureus, S. epidermidis*, and *E. coli*. The test results showed that the preparation could inhibit bacterial growth. The higher the addition of *T. catappa* leaves extract concentration, the larger the inhibition zone's diameter, as presented in **Table III**.

The antibacterial activity test of liquid soap preparations showed a difference in the inhibition zone diameter. The higher the concentration of *T. catappa* leaves extract was added, the higher the formed inhibition zone's diameter. Thus, the higher the ability of an extract to inhibit bacterial growth. This finding was due to the presence of flavonoid compounds in the extract, which could be antibacterial[30]. This literature also supported by the results of phytochemical screening of *T. catappa* leaves extracts that were positive for flavonoid compounds.

The inhibition zone formed at each concentration of 1%; 2%; 3%; positive; and negative control on *S. aureus* bacteria was 25.1; 28.13; 30.07; 40. 67; and 6.1 mm, respectively. The inhibition zone formed on S. *epidermidis* bacteria at each concentration was 12.17; 15.13; 19.17; 25.1; and 6.17 mm. Meanwhile, the inhibition zone against *E. coli* was 6.6; 7.17; 8; 15; and 6.2 mm.

The variation of inhibition zone diameter and SD values (>1 mm) yielded from three replications, which could be seen in **Table III**, was possibly generated by several factors such as the incubation temperature, diffusion ability of the extract, and volume of the medium used. The optimal temperature for bacterial growth is 35°C; hence, the lower temperature used could produce variation in inhibition zone diameter. If three or more discs were arranged in one pile in the experimental study, the middle disc would experience incubation temperature below 35°C[31].

Table III. Antibacterial activity of liquid soap from *T. catappa* leaves extract

Sample	Inhibition zone diameter±SD (mm)		
	S. aureus	*S. epidermidis*	*E. coli*
Control (+)	40.67±1.15	25.1±1.31	15±0.85
F0	6.1±0.17	6.17±0.29	6.2±0.2
F1	25.1±0.96	12.17±0.87	6.6±0.53
F2	28.13±0.61	15.13±1.27	7.17±0.31
F3	30.07±1.01	19.17±0.76	8±1.25

Moreover, variation in inhibition zone diameter of bacterial growth also could be produced from the inconsistency of medium thickness used. The most effective medium thickness for bacterial inhibition study was approximately 4 mm thick, which thinner medium could quicken the extract solution diffusion while a thicker medium could slow it down[32]. Unfortunately, in this research, the medium thickness used was not measured; hence, it was pretty hard to make sure the medium thickness.

Based on the formed inhibition zone, it could be grouped into four groups; which were very strong (the inhibition zone >20 mm), strong (10-20 mm), moderate (5-10 mm), and weak (<5 mm)[33]. Therefore, liquid soap with a *T. catappa* leaves extract concentration of 1%, 2%, 3% in the preparation provides a very strong inhibitory power against *S. aureus*, a strong inhibitory power against *S.*

epidermidis, and moderate inhibition against *E. coli*. This result follows the literature and research objectives that the types of bacteria that could significantly infect the skin were *S. aureus* and *S. epidermidis*[34].

CONCLUSION

The physical characteristics of *T. catappa* leaves extract liquid soap meets the requirement of SNI 4085:2017 with a pH value that was safe for the skin. The addition of *T. catappa* leaves extract variations did not affect the pH value, foam stability, and irritation. However, in the organoleptic test, the higher the concentration of *T. catappa* leaves extract was added, the liquid soap's color was getting more brownish, and the shape form was slightly liquid. The addition of *T. catappa* leaves extract to liquid soap could increase the antibacterial activity. The highest antibacterial activity was shown by *S. aureus* with an inhibition zone diameter of more than 20 mm.

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