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## Formulation and Antibacterial Activity of *Averrhoa bilimbi* L. Fruits Extract in Vegetable Oil-Based Liquid Hand Soap

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### Abstract

The main method of preventing infections is good hand cleanliness. Bilimbi (*Averrhoa bilimbi*) fruits contains flavonoids, which have been tested for antibacterial activity. Polar chemicals like flavonoids are often extracted using ethanol as a solvent. Therefore, this study aimed to formulate an ethanol extract of *A. bilimbi* fruits in the form of hand soap. This formulation used vegetable oils such as olive oil, coconut oil, and castor oil, which are high in fatty acids to maintain healthy skin. Antibacterial activity was carried out against *Staphylococcus aureus* and *Escherichia coli*. The extract was prepared by maceration for 5 days using an ethanol solvent. Liquid soap contained *A. bilimbi* fruit extracts at 5% (F1) and 10% (F2). The F1 and F2 organoleptic tests showed the preparation as a thick liquid with a distinctive coconut oil aroma, light brown (F1) and brown (F2) color. pH values of 5.8 (F1) and 5.6 (F2). Foam height stability was 71.5% (F1) and 73.1% (F2). The specific gravity for the two formulas was 1.02 g/mL. The viscosity of F1 and F2 was 137.6 cP and 163.5 cP, respectively. Inhibition power against *S. aureus* using the agar diffusion method by measuring the diameter of the inhibition zone showed strong (F1) and very strong (F2) categories. The inhibition against *S. aureus* showed strong (F1) and very strong (F2) categories. While F1 has no inhibitory power against *E. coli*, F2 showed inhibition in the strong category. Based on our research, it can be concluded that *A. bilimbi* fruit extract can produce good hand soap and have bacteria-inhibiting activity. However, further formula development is still needed to obtain preparations that meet all the requirements and stability tests.



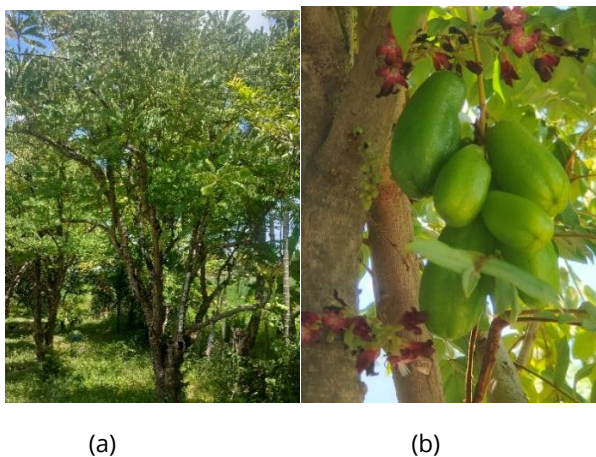
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### 1. Introduction

The recent COVID-19 pandemic has raised public awareness to maintain hand hygiene and awareness to clean hands. To prevent transmission of the virus, the Centers for Disease Control and Prevention (CDC) has recommended frequent hand washing with soap and

water for at least 20 seconds [1]. Liquid soap is a basic cosmetic used to clean the hands.

Many antiseptic liquid soaps with active herbal ingredients are currently being developed [2]. *Averrhoa bilimbi* fruit is one of the plants studied for its ability to inhibit microbial growth (Figure 1). Preliminary phytochemical studies of *A. bilimbi* fruit extract using



**Figure 1.** *Averrhoa bilimbi* L. tree (a) and fruits (b).

chemical methods and thin layer chromatography revealed the presence of carbohydrates, proteins, amino acids, flavonoids, tannins, essential oils, valepotriates, coumarins, and terpenes. The fruit is also rich in vitamin C and oxalic acid [3].

A study showed that the flavonoid content of *A. bilimbi* fruit extract also has antimicrobial activity besides being an antioxidant. The aqueous and chloroform extract of *A. bilimbi* fruit showed positive antibacterial activity against gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Corynebacterium diptheriae*, *Kocuria rhizophila*) and gram-negative (*Salmonella typhi*, *Citrobacter freundii*, *Aeromonas hydrophilla*) [4]. Another study showed the positive antimicrobial activity of *A. bilimbi* fruit extract against *E. coli*, *S. aureus*, and *B. subtilis*, which was carried out spectroscopically. The MIC results showed, respectively,  $55.6 \pm 6$ ,  $31 \pm 3$ , and  $28 \pm 2$   $\mu\text{g}/\text{mL}$  [5]. Antibacterial activity tests have been carried out on the total flavonoid content in *A. bilimbi* fruit in the range of 10-200 g/mL. The results showed antimicrobial activity against *E. coli*, *S. aureus*, and *B. subtilis* with Minimum Inhibitory Concentrations (MIC) results of  $55.6 \pm 6$ ,  $31 \pm 3$ , and  $28 \pm 2$  g/mL, respectively [5]. Another study showed the ability of ethanol extract of *A. bilimbi* fruit to inhibit Multi-Drug Resistance (MDR) bacteria. This study used the agar well diffusion method, and the resulting inhibition zone diameter was 10-14.5 mm [6]. *A. bilimbi* fruit extract is known to have more potent antimicrobial activity than leaf extract. This was indicated by more significant inhibition of gram-positive bacteria such as *S. aureus*, *Bacillus megaterium*, *Bacillus subtilis*, and *Sarcina lutea*. In addition, *A. bilimbi* fruit extract also has a more potent inhibitory against gram-negative bacteria such as *Salmonella paratyphi*, *Shigella dysenteriae*, and *Vibrio parahaemolyticus* [7].

Liquid soap contains low levels of surfactants (anionic, nonionic, and amphoteric) combined with other additives

such as viscosity modifiers, fragrances, preservatives, coloring agents, plant extracts, and antibacterial compounds. Surfactants, which are soap's main ingredients, contribute significantly to removing dirt and bacteria from the skin's surface. However, this material also can potentially disrupt the epidermis's structure, especially the stratum corneum, and can damage its lipid components. This situation causes the skin to become dry and irritated [2, 8]. Because the frequency of using this product is relatively frequent, the formula must be ensured that it is appropriate, balanced, meets the desired functional parameters, safe, and has a skin softening effect.

Vegetable oils are rich in fatty acids that are beneficial for skin health. Research results have shown that using coconut oil in liquid soap can increase skin moisture [9]. Meanwhile, castor oil contains undecylenic acid, which has germ-killing and disinfectant properties. It is useful for treating skin diseases and skin ulcers, especially those caused by bacterial or fungal infections. Castor oil is also used to protect wounds from infection and treat burns. In addition, castor oil is also used to treat acne, pigmentation, and sunburn and prevent stretch marks [10]. The content of flavonoids in olive oil, especially phenolic compounds, has antioxidant properties. Due to this activity, olive oil is commonly used in dermatology to treat skin diseases, such as acne, psoriasis, eczema, and seborrhea [11, 12].

This study aims to formulate a herbal antiseptic handwash with the active ingredient of *A. bilimbi* fruit extract. The hand soap formula contains vegetable oils that function as an oil phase and an agent to maintain skin function and health.

## 2. Materials and Methods

### 2.1. Materials

Fresh fruit from *A. bilimbi* (green, immature, fruit length 4 – 6.5 cm) was obtained from an orchard located in the Lampeuneurut area, Aceh Province, Indonesia. The fruit is washed with water to remove all unwanted materials, then rinsed with distilled water and dried by air-drying at  $\pm 25^\circ\text{C}$  for approximately 3 days. The dried *A. bilimbi* fruit was then ground into a fine powder using a mortar and stored in a sterile, airtight container for further use. For the formulation of liquid hand-soap used potassium hydroxide, stearic acid, glycerine, and Butylated hydroxytoluene (BHT) were purchased from Brataco in Jakarta, Indonesia. Hydroxypropylmethylcellulose (HPMC) was obtained from Sigma-Aldrich, USA. Castor oil, olive oil, and coconut oil used were purchased from Happy Green, an importer of essential oils in Jakarta, Indonesia.

## 2.2. Plant Extraction

Bilimbi fruit extraction was carried out according to what was done before, with modifications to the solvent and extraction time [7]. The *A. bilimbi* fruit extract was prepared using the maceration method with ethanol 96% as a solvent. Two hundred grams of *A. bilimbi* fruit powder were soaked in 1.5 L of ethanol for five days at room temperature and protected from light. After five days, the macerate was filtered with a flannel cloth. Subsequently, the solvent was changed to obtain a volume of 2 L of extract. The solid-to-solvent ratio is therefore 1/10 weight : volume. The maceration solution was concentrated under reduced pressure using a rotary evaporator at a temperature of 65 °C. The crude extract was collected and allowed to dry at room temperature.

## 2.3. Preparation of Liquid Hand Soap

In this study, two formulas were designed with the concentrations of *A. bilimbi* fruit extract at 5% (F1) and 10% (F2). The liquid hands soap base for both formulas is the same, consisting of 10% castor oil, 15% olive oil, 10% coconut oil, 5% potassium hydroxyde (10%), 3% HPMC, 2% stearic acid, 18% glycerine, 0.02% BHT, and added aquadest to 100%.

This liquid handwash was prepared using a modified Hot Process Method [13]. This method was used because it is suitable to be developed in the laboratory and on an industrial scale. For this purpose, castor oil, olive oil, and coconut oil were heated in a glass beaker at 70°C for 30 minutes. Then BHT was added and stirred until homogeneous. Glycerin was heated in a water bath separately. A lye-water solution was prepared by dissolving 10 grams of potassium hydroxide in 100 mL of distilled water (10% potassium hydroxide). After the solution was thoroughly mixed, it was slowly added to the heated oil mixture. The oil and lye-water solution mixture were poured into heated glycerin and stirred until homogeneous. The solution was stirred continuously with a mechanical stirrer until a thick mass was formed, known as soap paste. Then added stearic acid, which had previously been melted on a waterbath at 80°C, and HPMC, which had been developed with hot water. After the mass was homogeneous, *A. bilimbi* fruit extract and the remaining aquadest were added. This process was carried out with constant stirring at a temperature of 70°C until the entire mass was homogeneous.

## 2.4. Physicochemical Characterization

### 2.4.1. Organoleptic analysis

An appearance test was carried out by observing the color, texture, and smell of the liquid hand soap that is formed.

### 2.4.2. pH test

According to the established technique, pH was assessed for each batch of formulas using a pH meter (Mettler Toledo, USA) that had previously been calibrated using standard buffers of pH 4 and pH 7 [14].

### 2.4.3. Foam height stability

A total of 1 mL of liquid soap was put into a test tube, then 9 mL of water was added. Stir until dissolved and then shaken for 20 seconds; the height of the foam formed was measured. Let the foam stand for 5 minutes, and calculate the foam's height and stability [15] using the equation 1.

$$\text{Foam stability} = \frac{\text{final foam height}}{\text{initial foam height}} \times 100\% \quad (1)$$

### 2.4.4. Specific gravity

The clean, dry, empty pycnometer was weighed on an electronic balance (Mettler Toledo, United States). Then aquadest and liquid hand soap were each put into the pycnometer using a dropper until it was full. The pycnometer is closed and weighed. The specific gravity (SG) was calculated using the equation 2.

$$SG = \frac{\text{mass of liquid hand soap}}{\text{mass of water}} \quad (2)$$

### 2.4.5 Viscosity test

Liquid hand soap was measured using a Brookfield viscometer DV-1 (Ametek, United States) by inserting the sample into the container, and then the spindle (No. 2) was installed by inserting the spindle into the container until the limit mark and the safety valve was released and the rotor was turned on. Leave the tool for a while until the needle scales to a number, and then the viscosity number of the liquid hand soap is recorded.

## 2.5. Antibacterial Activity Testing

The research design used in this study was a Completely Randomized Design (CRD) consisting of 3 treatments (negative control, F1, and F2) and three replications each. The test was carried out on liquid hand soap preparations of *A. bilimbi* fruit extract by the Agar Diffusion Method using discs. Nutrient Agar (NA) media that has been sterilized, put 20 mL into a sterile petri dish 0.1 mL of the bacterial suspension (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922) was pipetted and put into a cup, then leveled and allowed to solidify. Then each



**Figure 2.** *A. bilimbi* fruit extract liquid hand soap: Hand soap contains 5% *A. bilimbi* fruit extract (F1), Hand soap contains 10% *A. bilimbi* fruit extract (F2).

**Table 1.** Results of physicochemical characterization of *A. bilimbi* fruit extract liquid hand soap.

Parameters for physicochemical characteristics	Formula	
	F1	F2
Organoleptic	Thick liquid, coconut oil aroma, light brown	Thick liquid, coconut oil aroma, brown
pH	5.8 ± 0.00	5.6 ± 0.2
Foam height stability (%)	71.5 ± 9.32	73.1 ± 6.7
Specific gravity (g/mL)	1.02 ± 0.02	1.02 ± 0.00
Viscosity (cP)	137.6 ± 1.02	163.5 ± 1.22

sterile disc paper was dipped in distilled water (a negative control), followed by liquid hand soaps F1 and F2, which were infused for 5 minutes. Then put the disc in a petri dish and let it sit for a while to allow the diffusion process. Then the plates were incubated for 48 hours at 37°C in incubator (WTC Binder, Germany). After incubation, calculate the diameter of the inhibition zone with a caliper [16].

### 3. Results and Discussions

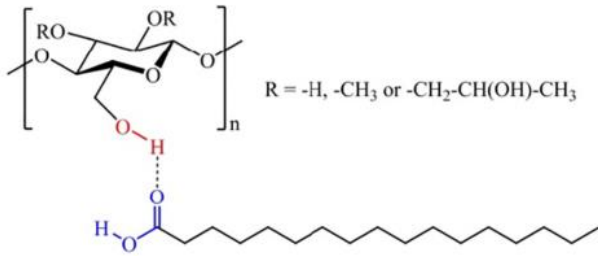
To ascertain the plants' identities, a determination was made. According to the letter of determination, the plant used was *Averrhoa bilimbi* L. fruits. The yield of ethanolic extract of *A. bilimbi* fruit was 48%. Soap is a colloidal system made by a saponification reaction. This reaction is characterized by alkaline hydrolysis of triglycerides in fats or oils [17, 18]. Therefore, potassium hydroxide was used as an alkaline agent in this liquid hand soap formula. Liquid hand soap was made using the Hot-Process Method.

In this study, three types of vegetable oil were used as precursors for liquid soap making: castor oil, olive oil, and coconut oil. In addition to functioning as a precursor, the oil content, which is rich in fatty acids, is also expected to function as a skin conditioning agent. This is important to

avoid skin irritation and dry skin, especially when the use of hand washing soap is sometimes repeated, which can pose a risk for dry skin. In addition, in this liquid soap formula, glycerine was also added. Glycerine acts as a humectant and emollient for the skin. Glycerine is hygroscopic, so it maintains the skin's moisture content by pulling water from the air and holding it from evaporating [19]. Among the vegetable oils used in this formula, coconut oil stands out the most in aroma intensity. Therefore, the resulting liquid hand soap also gives off a distinctive aroma of coconut oil (Table 1).

Their physicochemical properties determine soap preparations' quality, effectiveness, and cleaning ability. Based on the organoleptic analysis, it is known that the liquid hand soaps produced were in the form of a thick liquid (Figure 2). This consistency was influenced by the content of the extract, where it was seen that the viscosity increases with an increase in the extracted content in the formula. Besides, hydroxypropyl methylcellulose (HPMC) was also influenced viscosity as a thickening agent. Viscosity will affect the pourability of liquid soap preparations. If the viscosity is too high, the soap preparation will be difficult to remove from the container. However, if the viscosity is too low, the preparation will flow easily but run out quickly. The viscosity of liquid hand soaps measured using a Brookfield viscometer were 137.6 cP (F1) and 163.5 cP (F2). This value is lower than the viscosity of liquid soap generally, which is 3000-5000 cP [20]. This is probably because the concentration of HPMC used as a thickening agent in the formula was insufficient to produce the expected viscosity. This finding is consistent with other studies where the resulting liquid soap has a viscosity value between 118.21 cP and 201.55 cP without adding a thickening agent to the formula [21]. This finding is consistent with other studies where the resulting liquid soap has a viscosity value between 118.21 cP and 201.55 cP without adding a thickening agent to the formula [21].

The use of HPMC in this formula aims to maintain the stability of the colloid system. The mechanism of action of this stabilization was derived from HPMC's work as a precipitation inhibitor. HPMC is a cellulose ether obtained by incorporating methoxy and hydroxypropyl groups into the cellulose chain, with about 60% of the hydroxyl groups being unsubstituted. This mixture of polar and non-polar side groups shows that HPMC can form intramolecular and intermolecular hydrogen and hydrophobic bonds (Figure 3). In the dissolved state, the intramolecular bonds of HPMC are disrupted so that the polymer can form new hydrogen interactions with the dispersed droplets, thereby slowing their deposition [22]. Stearic acid is an anionic surfactant for O/W emulsion



**Figure 3.** Possible hydrogen bond between HPMC and stearic acid [23].

types. In this case, the stearic acid will coat the oil droplets to disperse them in the aquadest as the outer phase. HPMC, in this system, also works as a stabilizer by distributing stearic acid molecules evenly in the colloidal system. When the number of HPMC molecules is greater than or equal to stearic acid molecules, long chain HPMC molecules trap the stearic acid molecules and limit their movement, preventing the stearic acid molecules from contacting with water molecules so that they prevent deposition [24].

pH is an essential parameter in liquid soap preparations. Normal skin pH is slightly acidic; therefore, a very alkaline pH can damage the acid mantle, which acts as an antibacterial barrier. Highly alkaline pH can also disrupt the lipid lamellae of the epidermis, resulting in dry skin due to higher transepidermal water loss (TEWL) and allowing the entry of potential irritants and allergens. Soap preparations, when in contact with water, undergo a hydrolysis reaction and release the alkali in this product, thereby increasing the pH of the skin, which can range from 10-11 [25]. This liquid soap formula produced a pH of 5.8 (F1) and 5.6 (F2). This pH is closer to the physiological pH range of the skin, which is 4.1 – 5.8 [26]. This preparation is categorized as 'mild' soap in the cosmetic industry. This is very important, especially for babies, children, and adults with sensitive skin. A good cleaning product for babies should have a pH close to 5.5, and often a buffer is added to keep the pH close to that level. In addition, in individuals with diseases that alter the skin barrier (such as atopic dermatitis and ichthyosis), using high pH soaps can exacerbate xerosis and the function of the skin barrier, which is a gateway for pathogens to enter [27].

The pH of F2 is slightly lower than that of F1. This may be due to the higher concentration of *A. bilimbi* fruit extract in F2 (10%) than in F1 (5%). Based on research results, it is known that *A. bilimbi* fruit contains high levels of vitamin C and oxalic acid [28]. The pH values produced by the two formulas follow the provisions for liquid hand soap formulations, namely in the value range of 5.5-7[20].

Foam is one of the most critical parameters in determining the quality of soap. The purpose of foam height stability testing is to see the foam strength of liquid soap. Soap preparations are expected to produce a fairly stable foam because it can help cleanse the body [29]. The stability of the resulting foam is a reflection of the surfactant's capacity to maintain foam over a certain period [30]. In this case, stearic acid provides a reasonably good foam height stability within 5 minutes, namely 71.5% (F1) and 73.1% (F2).

The composition of the formula influences the specific gravity of soap. In this study, there was no difference in specific gravity between the two formulas (F1 and F2), so the extract concentration did not seem to affect the specific gravity. The value of the specific gravity of liquid soap should be close to the specific gravity of water. A large difference in specific gravity between the oil and water phases can result in emulsion instability, which can be observed as a whitish 'ring' around the neck of the container and 'oiling-off' or the formation of a shiny oil layer on the top of the product, which is the result of separation due to gravity, flocculation, and coalescence [31]. The results of the measurement of specific gravity obtained a value that is very close to the specific gravity of water, namely 1.02 g/mL. This already meets the requirements for emulsion stability.

In this liquid hand soap preparation, the antibacterial activity test of *A. bilimbi* fruit extract used *S. aureus* as a sample of gram-positive bacteria and *E. coli* as a sample of gram-negative bacteria. The results of this antibacterial test were shown in Table 2. The diameter of the inhibition zone is classified according to the criteria of inhibition, where the diameter of the inhibition zone is <5 mm (weak), 5-9 mm (moderate), 10-19 mm (strong), and >20 mm (very strong) [32]. The effectiveness of the preparations against *S. aureus* showed that the F1 preparation inhibited *S. aureus* bacteria with an average inhibition zone diameter of 16 mm, which can be categorized as a strong inhibitor. While F2 produces an average diameter of the inhibition zone of 20.16 mm, which is categorized as very strong. The F1 preparation did not show the ability to inhibit *E. coli*. Meanwhile, F2 showed an average inhibition zone diameter of 14.67 mm with a strong inhibition category. ANOVA analysis showed that the liquid hand soap of *A. bilimbi* fruit extract had an effect ( $P < 0.05$ ) on the growth of *S. aureus* and *E. coli* bacteria.

The ability to inhibit bacteria is thought to come from the high content of flavonoids. This is shown by a study conducted by Phat et al. (2021) where the total flavonoid content (luteolin and myricetin) was  $851 \pm 25$  mg/kg by spectrophotometric method [5]. Other studies have

**Table 2.** Antibacterial activities of liquid hand soap

Treatments	The average diameter of inhibitory zones (mm±SE)	
	<i>S. aureus</i>	<i>E. coli</i>
Aquadest (control negative)	0.00 ± 0.00	0.00 ± 0.00
F1	16.0 ± 0.86	0.00 ± 0.00
F2	20.16 ± 2.56	14.67 ± 0.57

shown that flavonoid compounds in the form of luteolin and apigenin in *A. bilimbi* fruit extract act as antimicrobial agents [7]. Flavonoids inhibit bacterial growth by disrupting the cell wall membrane [33].

Although in this study, we used vegetable oils to maintain skin homeostasis, we did not do any testing related to this. In addition, the stability test during the storage period was also not measured to prove the ability of the combination of HPMC and stearic acid to maintain the stability of the colloidal system.

#### 4. Conclusions

Based on this research, it can be concluded that the prepared liquid hand soap possessed good foam height stability, the pH of the soap matches to the skin pH, and excellent specific gravity. However, the viscosity value of the preparation is still very low. This formula still requires optimization of the thickening agent in order to obtain the appropriate viscosity. The inhibitory effect on *E. coli* was only seen at F2 (10% *A. bilimbi* fruit extract). Meanwhile, the inhibitory effect on *S. aureus* was shown by F1 (5%) and F2 (10%). Overall, the liquid hand soap formulation of *A. bilimbi* fruit extracts could potentially be used as an antibacterial preparation. However, further formula research is still required to produce preparations that fulfil all criteria and stability tests.

**Author Contributions:** Conceptualization, R.H. and A.S.; methodology, N.N.; software, F.H.; validation, N.N. and A.S.; formal analysis, R.H.; investigation, F.H.; resources, N.N.; data curation, F.H.; writing-original draft preparation, N.N.; writing-review and editing, R.H.; visualization, N.N.; supervision, E.L. and N.E.; project administration, A.S.; funding acquisition, A.S. All authors have read and agreed to the published version of the manuscript.

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**Informed Consent Statement:** Not applicable

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