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Preparation of Liquid Soap Utilising Used Cooking Oil with Aloe Vera as an Antibacterial Agent

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Abstract

Waste cooking oil has been repurposed in the preparation of liquid hand soap, enriched with *Aloe vera* extract as an antibacterial agent. To ensure the safety and efficacy of the product in bacterial inhibition, comprehensive testing of physical quality and antibacterial activity has been undertaken on the produced liquid soap. The test results affirm that the soap product complies with the Indonesian National Standard for free alkali content, acidity level (pH), foam height, foam stability, and water content. Notably, the soap exhibits a free alkali content of 0.01873%, a pH of 10, a foam height of 65 mm, and a foam stability value of 84.61%. The water content in the soap is measured at 40%. These results collectively position the soap within the criteria for high-quality soap. In the antibacterial effectiveness test against *Staphylococcus aureus* and *Escherichia coli* bacteria, the addition of *Aloe vera* has markedly expanded the inhibitory zone, demonstrating a significant enhancement in antibacterial activity. In light of the above, this study aims to address environmental pollution and health risks associated with improper disposal of waste cooking oil. Moreover, the addition of antibacterial *Aloe vera* presents an economically valuable solution by repurposing waste cooking oil into a useful product.

Keywords: antibacterial, *aloe vera*, used cooking oil, liquid soap.

1. Introduction

The rapid growth population has led to an escalating demand for food, notably cooking oil. In Indonesia, the annual consumption of cooking oil reaches approximately 290 million tons [1,2]. The repeated use of cooking oil, particularly in high-temperature frying process, can result in its degradation. Therefore, it is recommended to dispose of used cooking oil as waste after each use [3–5]. The extensive use of cooking oil contributes significantly to waste generation, with households producing around 305 thousand tons of waste cooking oil annually [6,7]. As population density continues to rise each year, there is a heightened risk of environmental pollution, posing potential threats to both health and ecosystems.

Following Regulation No. 167 of 2016 by the Governor of the Special Capital Region of Jakarta on the Processing of Used Cooking Oil Waste, proper treatment of waste from used cooking oil is imperative to mitigate potential adverse impacts on public health and the local environment [8]. Inadequate management of used cooking oil can result in detrimental consequences. Improper disposal of oil waste poses a serious threat to soil and water quality. The mineral content of clean water in the soil can be adversely affected, while aquatic ecosystems can suffer irreparable damage [9–12]. Furthermore, if oil waste is discharged improperly into water channels, it can lead to severe pipe blockages [13–16]. The consumption of used cooking oil can cause various health issues, such as damage to the small intestine, blood vessel blockages, and liver damage [17–20]. The repeated use of cooking oil produces unsaturated fatty acids that undergo oxidation, forming free radicals capable of harming organs [21,22]. Therefore, an innovative approach is required to leverage

used cooking oil, including its utilization as an ingredient in handwashing soap. This potential is justifiable given that used cooking oil contains fatty acids that can be converted into soap through the saponification reaction.

In recent times, there has been a growing public awareness and emphasis on personal hygiene, particularly in the context of hand hygiene. This trend is closely linked to the impact of the Covid-19 virus pandemic that has affected Indonesia. To enhance the effectiveness of liquid hand soap, active ingredients capable of swiftly eliminating viruses and bacteria are essential. *Aloe vera*, commonly known to the general public, is a plant acknowledged for its beneficial functions in health, one of which is inhibiting bacterial growth [23–28]. This is attributed to the antibacterial compounds present in *Aloe vera* leaves. According to research conducted by Kushwaha *et al.,* (2023) and Jha *et al.,* (2018), a phytochemical screening of *Aloe vera* leaf pulp yielded positive results for alkaloids, flavonoids, glycosides, tannins, saponins, and steroids/triterpenoids [29,30].

Quality hand soap must undergo a series of tests for both its physical properties and antibacterial effectiveness. Physical property testing is crucial as soap will directly interact with the skin, while antibacterial tests aim to assess its effectiveness in killing bacteria. Furthermore, the physical property testing of soap must adhere to the Indonesian National Standard (Standar Nasional Indonesia -SNI) to ensure product safety [31]. Consequently, this research aims to evaluate the physical properties and antibacterial effectiveness of *Aloe vera* in liquid soap. The physical parameters to be tested, following the SNI 06-3532-1994 standards, include free alkali content, acidity level (pH), foam height and foam stability, and water content. Additionally, antibacterial tests will involve *Staphylococcus aureus* and *Escherichia coli* bacteria, chosen for their common presence on the human hand surface [32–35].

2. Materials and Method

2.1 Materials

The materials enlisted for this study encompass used cooking oil, activated charcoal, Bleaching Earth powder, technical-grade potassium hydroxide (KOH), distilled water (aquades), texapon (SLES), glycerin, *Aloe vera* extract, benzalkonium chloride, 0.1 N hydrochloric acid (HCl), and phenolphthalein indicator (PP).

2.2 Methods

This experimental research aims to formulate liquid soap containing *Aloe vera* extract as an antibacterial agent. The soap samples were assessed through physical parameter tests, including free alkali content, acidity level (pH), foam height, foam stability, and water content. Additionally, the soap samples were tested for their inhibitory effects on the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria.

2.2.1 Oil purification

Initially, 500 mL of used cooking oil is poured into a pan and heated over a moderate flame. Once it reaches the optimal temperature, 10g of activated charcoal is added to the oil, and the mixture is stirred for 15 minutes while continuing to heat. Subsequently, the stove is turned off, and the mixture is left undisturbed for 5 hours.

In the next stage, the oil is reheated over a moderate flame. Upon reaching the appropriate temperature, 50g of bleaching earth is introduced into the oil, and the mixture is stirred for 15 minutes while still being heated. Afterward, the stove is turned off, and the mixture is left undisturbed for 10 hours or until the bleaching earth powder separates and settles at the bottom of the container. Following this, the mixture suspension undergoes filtration to separate the Bleaching Earth precipitate into a plastic container.

2.2.2 Soap production

A total of 6.5g of KOH solid is weighed and dissolved in 15 mL of water in a plastic container. The solution is stirred until homogeneous, beware of the heat and odor generated during the reaction. Subsequently, 25 mL of oil is poured into the plastic container and mixed with the KOH solution. The stirring process is carried out using a mixer until the mixture attains a whitish, paste-like texture, with an estimated stirring time of 30 minutes. The mixture is left undisturbed for two nights, and after this period, the texture solidifies, resembling butter. Next, 20 mL of texapon and 25 mL of hot water are added to the mixture. The stirring process continues for 30 minutes using a spoon or cake mixer for quicker results. It is crucial to pay attention while stirring texapon, as successful texapon mixing can impact the soap's viscosity and the amount of foam produced. The subsequent step involves adding 100 mL of regular water, 15 mL of glycerin, 2% *Aloe vera* extract, and 7.5 mL of fragrance to the mixture. The stirring process is continued until the mixture becomes smooth. The mixture is left for a few hours until the soap's original color is evident.

2.2.3 Physical parameter tests

Free alkali content test

A 5 g soap sample is carefully weighed and added to a 250 mL Erlenmeyer flask. Subsequently, the soap sample is dissolved using 96% ethanol, with a solvent volume of 100 mL. At this stage, a few drops of phenolphthalein indicator (PP) are added to the solution. The mixture is heated until it reaches a deep purple color. This is followed by titration using 0.1 N HCl solution, and the endpoint of the titration is observed when the purple color precisely disappears. Throughout the titration process, the volume of HCl used is recorded, providing information about the amount of acid reacting with the soap sample. The titration is conducted three times.

Acidity level (pH) test

The procedure commences with preparing a soap sample of 1g, which is then dissolved in 9 mL of distilled water. Subsequently, the pH meter is calibrated using a pH-4 buffer, and afterward, the pH meter probe is rinsed using distilled water. The pH meter electrode is then immersed in the soap sample, and the reading on the pH meter is noted. Next, the pH meter electrode is cleaned using tissue, followed by rinsing with distilled water. Finally, the electrode is dried with tissue.

Foam height and stability test

A soap sample of 1g is dissolved in 9 mL of distilled water. The reaction tube is then sealed with a stopper, and subsequently, the sample is shaken for 20 minutes. The initial foam height is recorded after the shaking process. Furthermore, the sample is left undisturbed for 5 minutes, and the final foam height recorded is noted.

Water content test

The procedure begins by preparing a soap sample, which is then weighed to 5g. The weighed sample is placed into a porcelain dish and heated in an oven for 2 hours at a temperature of 105 ℃. After the heating process is complete, the soap sample is re-weighed after cooling to obtain its final weight.

2.2.4 Antibacterial test

Nutrient agar (NA) and Nutrient Broth are prepared. Bacterial cultures of S. aureus and E. coli are rejuvenated by inoculating one bacterial loop aseptically onto NA. The bacteria are then incubated for 24 hours at their respective optimum temperatures. After the incubation process, the rejuvenated bacteria are further suspended in nutrient broth until the turbidity level matches the set standard.

The procedure commences with taking the bacterial suspension, which is then poured into a petri dish along with the addition of sterile NA. The mixture is homogenized evenly and left to solidify. The next step involves soaking filter paper discs in the sample solution to be tested. The soaked paper discs are then placed on the petri dish using forceps. Subsequently, the entire system is incubated for 24 hours to allow for bacterial growth and interaction with the tested sample.

3. Results and Discussion

3.1 Oil purification

The purification of used cooking oil aims to enhance its quality. This process comprises two stages, with the first stage involving the use of activated charcoal as an adsorbent for peroxides and colorants. Activated charcoal possesses pores capable of adsorbing impurities, and the adsorption process with activated charcoal takes place for 5 hours. A study by Samangun *et al.,* (2017) indicates that activated charcoal can adsorb 95-97% of total colorants in oil, reduce peroxide values, and eliminate undesired odors, such as rancid smells [36]. Here, activated charcoal acts as an adsorbent, adsorbing colorants and reducing the amount of free fatty acids in used cooking oil [37]. The mixture of used cooking oil and activated charcoal is stirred while heated for 15 minutes, left undisturbed for 5 hours after heating, then filtered and collected in a basin. This process not only contributes to the visual improvement of the oil but also enhances other quality parameters, such as a reduction in peroxide values, reflecting oxidative stability and resistance to oil damage due to oxidation. Figure 1 illustrates the purification of used cooking oil with the addition of activated charcoal on a heating stove.

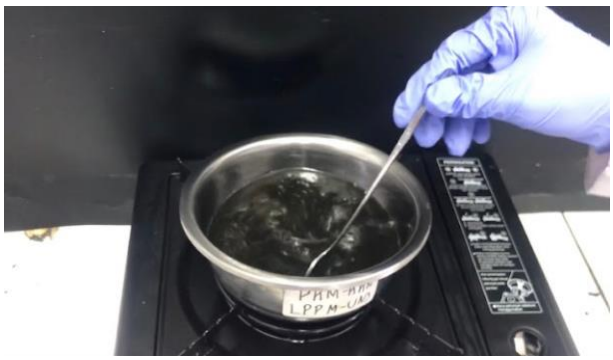

Figure 1. Used cooking oil treated with activated charcoal on a heating stove.

The second stage in the purification of used cooking oil involves the bleaching process of bleaching earth (BE). This material acts as an adsorbent, where its active side, the silanol group (Si-OH), can bind peroxide and free fatty acid

carbonyl groups [38]. The addition of BE aims to absorb impurities present in used cooking oil [39–41]. This allows peroxide compounds and free fatty acids that were not absorbed in the first stage to be adsorbed on the surface of the BE adsorbent. The adsorption process is carried out for 10 hours. Figure 2 shows that the purified oil is clearer and relatively odorless. This bleaching process essentially improves the organoleptic profile of the oil and can have a positive impact on the sustainability and consumer acceptance of the resulting used cooking oil product.

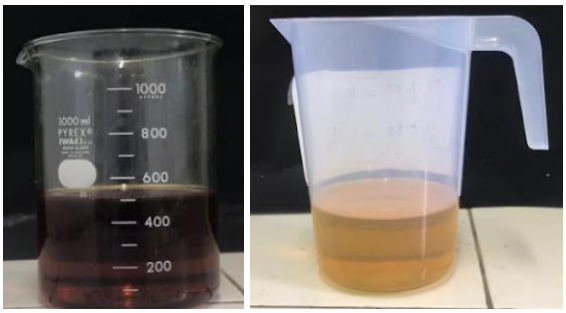

Figure 2. Bleaching of used cooking oil using activated charcoal and bleaching earth (a) before (b) after.

3.2 Soap production

The soap production begins by first preparing a potassium hydroxide (KOH) solution. This involves weighing 6.5g of solid KOH, dissolving it in 15 mL of water, and stirring it until homogeneous. The next step is pouring 25 mL of oil into a container, which is then reacted with the previously prepared KOH solution. The mixture is stirred using a mixer for 30 minutes until a soap paste is formed. The reaction that occurs is known as saponification, where the reaction between oil and KOH produces an anionic surfactant. This surfactant is responsible for lifting dirt [42]. The formed soap paste is left undisturbed for two nights to complete saponification.

Figure 3. The liquid hand soap is made from used cooking oil with *Aloe vera* as an antibacterial agent.

In the subsequent steps, 100 mL of texapon and 50 mL of water are added to the soap paste—texapon functions to create foam and remove fat and dirt [43]. Then, 100 mL of room temperature water, 15 mL of glycerin, 2% *Aloe vera* leaf extract, and 7.5 mL of fragrance are added to the mixture of soap paste and texapon. The mixture is stirred until smooth, resulting in liquid hand soap as shown in Fig. 3.

3.3 Physical parameter tests

Free alkali content test

The free alkali content test is conducted to determine the quantity of alkali not bound by fatty acids. The testing procedure involves titrating the liquid soap with HCl to ascertain the amount of alkali that did not get involved in saponification. The soap sample is preheated to facilitate the determination of the titration endpoint, assisted by the addition of some drops of PP indicator to the sample. The endpoint of the titration is marked by the color change of the sample from pink to colorless. The average volume of HCl required to reach the endpoint of titration is 0.167 mL. Subsequently, the percentage of alkali content in the sample is calculated using Equation 1 below.

$$Alkali\ conten(\%) = \frac{V \times N \times 0.0561}{W} \times 100\% \quad (1)$$

Information:

V = volume of HCl in titration (mL)
N = normality of HCl (N)
W = weight of the sample (grams)

Based on the equation, the free alkali content in the sample is determined to be 0.01873%. This content meets the standard requirements. According to SNI 06-4085-1996, the maximum allowable free alkali content in liquid soap is 0.1% [44,45].

Table 1. Comparison of the analysis of free alkali content with SNI 06-4085-1996.

Preparation	Free alkali content		Information
	SNI	Test results	
Soap sample with *Aloe vera*	Maximum 0.1%	0.01873%	Qualify

Acidity level (pH) test

The soap's acidity level, or pH test, is conducted using a calibrated digital pH meter with a pH-4 buffer solution for calibration. Calibrating the instrument aims to ensure accurate data and minimize measurement errors.

According to Dimpudus et al. (2017), the skin has resilience and can quickly adapt to products with a pH range of 8.0-10.8. Therefore, the pH test is performed to determine the soap's pH, ensuring that the soap is safe for use and direct contact with the skin [44].

Based on the test result, the soap's pH is determined to be 10.0. This outcome indicates that the soap sample meets the criteria for good soap, as per SNI 06-4085-1996, where the permissible pH range for soap is 8-11.

Table 2. Comparison of the analysis of acidity level (pH) with SNI 06-4085-1996.

Preparation	Degree of acidity (pH)		Information
	SNI	Test results	
Soap sample with *Aloe vera*	Range 8.0-11.0	10.0	Qualify

Foam height and stability test

The foam height and foam stability tests are conducted to assess the foaming ability of *Aloe vera* liquid soap. The foam height produced by the sample after stirring for 30 seconds is measured at 65 mm. Subsequently, the test sample is left undisturbed for 5 minutes to observe foam stability. Stability is achieved if the amount of foam after 5 minutes is 60-70% of the initial volume. The final foam height obtained is 55 mm. The calculation of foam stability can be performed using Equation 2 below.

$$Foam\ height = \frac{final\ foam\ height}{initial\ foam\ height} \times 100\% \quad (2)$$

According to SNI 06-4085-1996, the requirement for foam height of liquid soap is in the range of 13-220 mm [46]. The obtained foam stability value is 84.61%. Thus, the liquid soap sample meets the standard. The foam stability, with 84.61% of the foam remaining after being left undisturbed for 5 minutes, also fulfills the criteria for foam stability.

Table 3. Comparison of foam height analysis with SNI 06-4085-1996.

Preparation	Foam height			Information
	SNI	Test results		
		Before	After	
Soap sample with *Aloe vera*	13-220mm	65mm	55mm	Qualify

Table 4. Comparison of foam stability analysis with SNI 06-4085-1996.

Preparation	Foam stability		Information
	SNI	Test results	
Soap samples with *Aloe vera*	60-70%	84.61%	Qualify

Water content test

The purpose of this water content test is to determine the amount of water content present in the *Aloe vera* liquid soap. The initial weight of the sample used in this test is 5.00 grams, and the final weight after being oven-dried is 3.00 grams. Based on the obtained results, the water content can be calculated using Equation 3 below.

$$Water\ content = \frac{initial\ weight - final\ weight}{initial\ weight} \times 100\% \quad (3)$$

The water content standard set by SNI 06-4085-1996 is a maximum of 60% [18]. The water content obtained for the Aloe vera liquid soap preparation is 40%. This result indicates that the liquid soap preparation meets the standard.

Table 5. Comparison of water content analysis with SNI 06-4085-1996.

Preparation	Water content		Information
	SNI	Test results	
Soap sample with Aloe vera	60%	40%	Qualify

3.4 Antibacterial test

After the physical parameter test, which indicates that the liquid soap made from used cooking oil with the addition of *Aloe vera* as an antibacterial agent meets the SNI 06-4085-1996 standard, the inhibition zone test was conducted using the diffusion method to determine the effectiveness of *Aloe vera* antibacterial agent in the soap. This method was chosen for its simplicity and high precision. Additionally, this method is commonly used in testing the sensitivity of antibiotics, and observing bacterial responses to antibiotics, or antibacterial substances with the formation of inhibition zones (clear areas) around the wells [47,48]. The results of the antibacterial test on soap with *Aloe vera* were obtained through observations over 1 day with a 24-hour incubation

period and three repetitions for each liquid soap preparation, as shown in Table 6.

Table 6. Inhibition zone of antibacterial test of soap with Aloe vera against *Staphylococcus aureus* and *Escherichia coli* bacteria.

Active Ingredient	Inhibition Zone (mm)							
	E. coli (Gram -)			Avg	S. aureus (Gram+)			Avg
	1	2	3		1	2	3	
Aloe vera	7.68	8.22	8.40	**8,10**	9.85	9.30	10.62	**9.92**

Avg: average

The paper disc (Figure 4a) serves as the site for injecting soap samples, and then bacteria are cultured around it. The effectiveness of the antibacterial soap is observed from the formation of inhibition zones (clear areas) measured around the wells. The data analysis process for the inhibition zones test of Aloe vera liquid soap requires control sample data to determine the differences. Therefore, an inhibition zone test was conducted on the soap sample without an antibacterial agent (controls) with the same test system configuration as the soap sample with the addition of *Aloe vera*. The results of the analysis reported in Table 7 show that the addition of *Aloe vera* in liquid hand soap results in an increase in the average inhibition zone to 8.10 mm from the control sample, which has an average inhibition zone of 7.05 mm for *E. coli* bacteria. Meanwhile, in the testing against *S. aureus* bacteria, the average inhibition zone for the control sample is 9.28 mm, and the average inhibition zone for the sample with *Aloe vera* becomes 9.92 mm.

Table 7. Inhibition zone of antibacterial test of soap without antibacterial substance (control) against *Staphylococcus aureus* and *Escherichia coli* bacteria.

Active Ingredient	Inhibition Zone (mm)							
	E. coli (Gram -)			Avg	S. aureus (Gram+)			Avg
	1	2	3		1	2	3	
No anti-bacteria	6.88	7.45	6.83	**7.05**	10.23	8.66	8.95	**9.28**

Avg: average

As a comparison, another antibacterial agent was used, namely benzalkonium chloride, which is a synthetic antibacterial, with the same test system configuration as the liquid soap sample with the addition of *Aloe vera*. In the displayed test results in Table 8, with the addition of benzalkonium chloride against *E. coli* bacteria, the average inhibition zone obtained was 7.45 mm, and against *S. aureus* bacteria, it was 7.41 mm.

Table 8. Inhibition zone of antibacterial test of soap with benzalkonium chloride against Staphylococcus aureus and Escherichia coli bacteria.

Active Ingredients	Inhibition Zone (mm)							
	E. coli (Gram -)			Avg	S. aureus (Gram+)			Avg
	1	2	3		1	2	3	
Benzal-conium chloride	7.00	7.88	7.47	**7.45**	8.03	6.91	7.30	**7.41**

Avg: average

Antibacterial testing indicates a significant increase in antibacterial activity with the addition of *Aloe vera*, consistent with research conducted by Ariyani et al. (2018) on the antibacterial activity of *Escherichia coli* in *Aloe vera* gel preparations and a study by Apriani & Fathir (2021) on the inhibitory effect of *Aloe vera* latex on the growth of *Staphylococcus aureus* bacteria [49,50]. The antibacterial activity is marked by the presence of a clear zone around the disc. Antibacterial compounds will diffuse from the paper disc towards the media that has been inoculated with the test bacteria, resulting in inhibitory activity in the form of an inhibition zone, as shown in Figure 4 [51–55]. The size of the formed zone varies depending on the type and concentration of the antibacterial substance on the disc. The larger the formed zone, the greater the diameter of inhibition, indicating stronger antibacterial properties [56]. According to the David-Stout method in Table 9, the activity of *Aloe vera* as an antibacterial agent in liquid soap falls into the moderate category [57].

The difference in the inhibitory zones of *Aloe vera* activity on the growth of *E. coli* and *S. aureus* is attributed to their distinct types representing gram-negative and gram-positive bacteria, respectively. This difference is due to the number of layers in the bacterial cell wall. Gram-positive bacterial cell walls have a simple structure with a single layer and low lipid content, making it easier for active compounds to penetrate the cell. In contrast, gram-negative bacterial cell walls have a complex structure with three layers, involving an outer membrane, lipoprotein, and peptidoglycan, acting as a barrier against antibacterial bioactive substances [58]. Therefore, this research indicates that *S. aureus* bacteria have a larger inhibition zone compared to *E. coli* bacteria.

Table 9. Bacterial growth inhibition response category.

Inhibition zone diameter (mm)	Inhibition Response
≥ 20	Very strong
11-19	Strong
5-10	Moderate
< 5	Weak

Aloe vera extract can exhibit antibacterial effects on the growth of *E. coli* and *S. aureus* bacteria due to the influence of secondary metabolite compounds present in the extract. Compounds that provide antibacterial activity include alkaloids, flavonoids, tannins, saponins, and triterpenoids [29,30].

Alkaloid compounds disrupt the components of the peptidoglycan in bacterial cells, preventing the formation of the cell wall layer, and ultimately leading to bacterial cell death [59]. This mechanism is an effective way to compromise the integrity of bacterial cells, inhibiting their growth. Flavonoids are phenolic compounds that have been evaluated for their antibacterial property due to their tendency to retard the growth of pathogenic microorganisms by degrading membrane phospholipids, which leads to cytoplasmic membrane breakdown [60]. Flavonoids interact with proteins to provide benefits, but the crucial thing is that they need to keep their amphiphilic properties to penetrate bacteria and fight against them [61]. The lipophilicity level of a flavonoid influences its ability to damage the bacterial cell wall; the more lipophilic the flavonoid, the stronger its ability [62]. Tannins are a type of polyphenolic compounds that possess antibacterial properties. They can inhibit bacterial biofilm formation or inactivate enzymes, which can prevent the formation of bacterial cells [63,64]. The wide variety of phenolic compounds with different structures makes them a valuable source of potential drugs that can inhibit bacterial strains' growth. Moreover, the presence of functional groups in tannins makes them suitable to act at various levels on bacteria cells, leading to damage to cell membranes, enzyme inhibition, and DNA intercalation [65]. Saponins are amphiphilic compounds and their antibacterial activity is closely related to their surface activity [66]. According to recent studies, saponins possess detergent-like properties that can affect the bacterial cell wall by increasing the permeability of the bacterial cell membrane [67,68]. It has been speculated that the mechanism of action of saponins is related to the bacterial cell membrane system. The mechanism involves reducing surface tension, thereby increasing cell permeability or leakage, leading to the release of intracellular compounds [69].

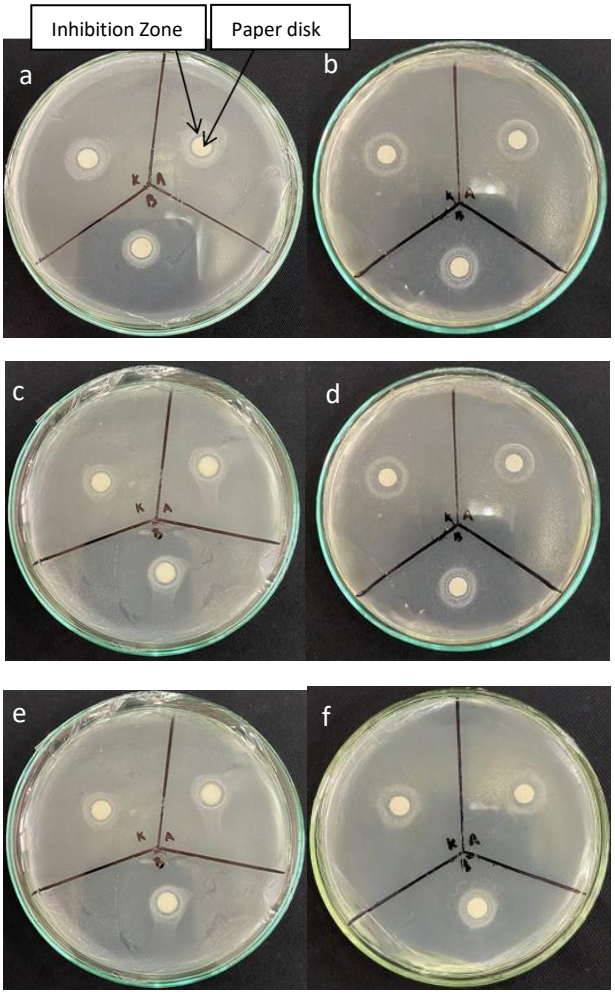

Figure 4. Inhibition zones of antibacterial test (a) soap sample without antibacterial agent (control) against *E. coli* (b) soap sample without antibacterial agent (control) against *S. aureus*. (c) soap sample with *Aloe vera* against *E. coli* (d) soap sample with *Aloe vera* against *S. aureus*. (e) soap sample with benzalkonium chloride against *E. coli* (f) soap sample with benzalkonium chloride against *S. aureus.*

4. Conclusion

The antibacterial test conducted with *E. coli* and *S. aureus* bacteria revealed optimal inhibition zones in the Aloe vera soap samples. The *Aloe vera*-infused soap samples have successfully met the SNI standards in various testing parameters, including free alkali content, acidity level (pH), foam height and stability, and water content—the soap's free alkali content measures at 0.01873%, with a pH value of 10. Notably, the soap exhibits a foam height of 65 mm and a foam stability of 84.61%. Additionally, the water content in the soap is determined to be 40%. These results collectively confirm that the soap fulfills the necessary criteria to be considered a good quality soap.

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