Comparative Study of the Antibacterial Effects of Antibacterial Liquid Soap and Moisturizing Liquid Soap on *Escherichia coli* Bacteria *in vitro*

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Abstract

Introduction: Hands play an important role in the transmission of infection, so it is very important to maintain hand hygiene. One of the best ways to do this is by washing hands with soap regularly. With so many types of soap available in the market, consumers need to be careful in choosing the right type of soap according to their needs. **Objective**: This research was conducted to test and compare the antibacterial activity of antibacterial liquid soap and moisturizing liquid soap against *Escherichia coli* bacteria. **Methods**: This study is a laboratory experimental study using the agar well diffusion method to determine the diameter of the inhibition zone formed. This study tested eight soap samples, namely four brands of antibacterial liquid soap and four brands of moisturizing liquid soap from various brands, with a positive control using trimethoprim-sulfamethoxazole and a negative control using distilled water. **Results**: The formation of inhibition zones on all soap samples, both antibacterial and moisturizing, indicated the presence of antibacterial activity on the growth of *E. coli* bacteria. The average diameter of the inhibition zones for antibacterial soap and moisturizer against *E. coli* were 20.03 mm and 18.81 mm, respectively. The p value obtained was $p \leq 0.001$, which means that each treatment had a significant difference in the diameter of the inhibition zone. **Conclusion**: This leads to the conclusion that H_1 is accepted, which indicates a significant difference in antibacterial activity between antibacterial soap and moisturizing soap against *E. coli* bacteria with the greatest antibacterial activity found in sample AB4 (24.52 mm).

Keywords: liquid soap; antibacterial; moisturizing; hand hygiene

1. Introduction

Hands are organs that interact the most with the outside world, so they have an important role in transmitting infections. The importance of hand hygiene in infection control cannot be denied. Therefore, hand hygiene is one of the global efforts to reduce infections [1].

Washing hands with soap regularly is one of the best ways to get rid of germs, avoid getting sick, and prevent spreading germs to other people [2]. The CDC recommends five steps to take when washing hands. The five steps are wet, lather, rub, rinse, and dry. In addition to the following five steps, the CDC also recommends allocating 20 seconds to wash hands to be successful in eliminating germs [3].

One important factor in washing hands is the choice of soap used. Every consumer has a preference in choosing the type of soap to use according to the needs and goals of each individual. Most individuals use soap as a medium to kill germs, the soap products they are looking for tend to have antibacterial effects which are useful for inhibiting or killing germs. One option often sought is antibacterial soap.

However, excessive use of antibacterial soap can cause the death of normal flora that protects the skin. This causes the arrival of opportunistic organisms that will cause irritation [4]. Irritation associated with

antimicrobial soap may be caused by the antimicrobial active ingredient. Health care workers often complain of dry or burning feelings, rough skin, and erythema, scaly or cracked [5]. Therefore, there is a need for an alternative soap that not only works well in killing germs but also is moisturizing and not abrasive.

Seeing the problems above, this research compares the effectiveness of antibacterial liquid soap with moisturizing liquid soap with the research object being *Escherichia coli (E. coli*). Human or animal waste is an important source of germs such as *E. coli* which can cause diarrhea and respiratory infections. This is because *E. coli* naturally resides in the intestines of humans and animals [6]. It is responsible for the deaths of around 900,000 children per year worldwide, with most deaths occurring in developing countries. *Enteropathogenic E. coli* (EPEC) is responsible for more than 81 million cases of diarrhea per year, of which 17 million occur in children [7]. *E. coli* is also an indicator of cleanliness and sanitation because the presence of *E. coli* indicates contact with feces [6].

2. Materials and Methods

This research is a laboratory experimental study, namely carrying out antibacterial tests to compare the effectiveness of commercial antibacterial liquid soap with commercial moisturizing liquid soap against *E. coli* bacteria *in vitro* using the agar well diffusion method to determine the diameter of the inhibition zone. The *E. coli* used in this experiment was a stock culture in the Microbiology Laboratory, Faculty of Medicine, Universitas Airlangga with the inclusion criteria being the sample was a homogenous colony of *E. coli* whose species has been identified and selected using simple random sampling.

The independent variable is antibacterial liquid soap and moisturizing liquid soap, while the dependent variable is *E. coli* bacteria. The materials used are antibacterial liquid soap and moisturizing liquid soap. The tools used in this research are petri dish, incubator, tube, sterile cork borer, spirit heater, MacFarland universal indicator, analytical balance, 1 ml micropipette, and lid.

The diffusion method uses eight samples, with four antibacterial liquid soap samples and four moisturizing liquid soap samples. Based on Arifin *et al*.'s formula, the experiment was replicated 4 times for 8 types of treatments, so the total sample is 32 samples [8]. This research was conducted at the Microbiology Laboratory, Faculty of Medicine, Universitas Airlangga from August 2022 to October 2023. The research procedure consisted of sterilizing the equipment, making Mueller Hinton Agar medium, preparing *E. coli* isolates, preparing the liquid soap to be used, diffusion method, and inhibition zone diameter measurement. The diffusion method uses the agar well diffusion method by dripping 1 mL of four samples of antibacterial liquid soap (AB1, AB2, AB3, AB4) and four samples of moisturizing soap (M1, M2, M3, M4) into the well, then observing the diameter of the inhibition zone. All data from observations of the diffusion method were analyzed descriptively.

3. Results

3.1. Inhibition Zone Diameter

In the diffusion method, the following observation results were obtained:

Table 1. Soap and control inhibition zone diameter of *E. coli* bacteria

Sample	1st Repetition (mm)	2nd Repetition (mm)	3rd Repetition (mm)	4th Repetition (mm)	Mean + SD (mm)
Positive Control	32.48	32.18	31.74	32.56	32.24 ± 0.37
Negative Control	0.00	0.00	0.00	0.00	0.00 ± 0.00
AB1	19.36	19.45	19.39	18.97	19.29 ± 0.22
AB2	15.22	18.55	17.44	18.07	17.32 ± 1.47
AB3	19.20	18.85	19.48	18.48	19.00 ± 0.43
AB4	24.27	25.67	24.55	23.57	24.52 ± 0.87
M1	16.47	16.39	16.89	16.50	16.56 ± 0.22
M2	23.86	23.22	24.20	24.11	23.85 ± 0.44
M3	15.50	16.03	15.35	17.47	16.09 ± 0.97
M4	18.92	18.42	18.98	18.75	18.77 ± 0.25

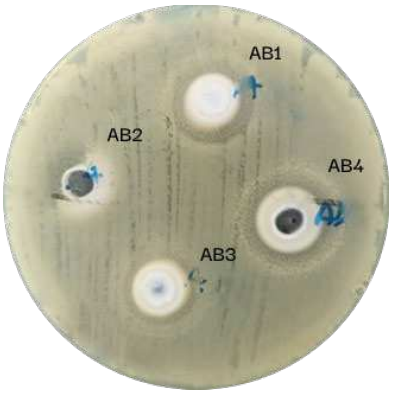

Fig. 1. Observation results of antibacterial soap antibacterial tests against *E. coli* bacteria

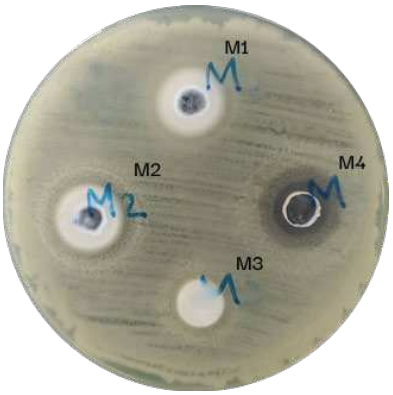

Fig. 2. Observation results of antibacterial tests of moisturizing soap against *E. coli* bacteria

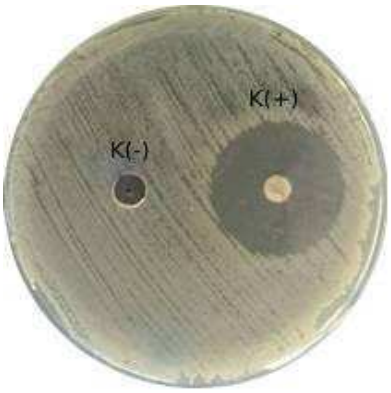

Fig. 3. Observation results of positive and negative control antibacterial tests against *E. coli* bacteria

Notes: AB = antibacterial, M = moisturizing, K (+) = trimethoprim-sulfamethoxazole, K (-) = sterile distilled water

3.2. Normality and Homogeneity Test

Normality and homogeneity tests are carried out to determine whether the data obtained may be subjected to parametric or non-parametric statistical tests [9]. Because less than 50 data were collected, the Shapiro-Wilk test was performed to determine normality [10]. Based on the results of the normality test for all variables, p value > 0.05, which indicates that all the data collected is normally distributed [11]. The next test carried out was a homogeneity test which was carried out to find out whether each treatment group had homogeneous data or not. Homogeneity was assessed using Levene's test. By comparing the data of the control group and the treatment group, the obtained p value was 0.020 (< 0.05) which means there is a difference in variance between groups of data being compared so it can be concluded that the data is taken from a heterogeneous population [12].

3.3. Comparison Test and Post Hoc Test

Based on the statistical results that have been carried out, it is found that the research data is normal but not homogenous which means that it does not meet the criteria for parametric statistical test. Because the data results did not meet the assumption of normality, the Kruskal-Wallis test and Post Hoc Games-Howell test were carried out to determine whether there were significant differences between groups of variables and to find out which groups had significant differences. The results of the Kruskal-Wallis test shows that the p value ≤ 0.001, which means that each treatment had a significant difference in antibacterial activity on the diameter of the inhibition zone produced. This gives the conclusion that there is a difference in antibacterial activity between antibacterial soap and moisturizing soap against *E. coli* bacteria [13].

Table 2. Games-Howell Post Hoc analysis test

	AB1	**AB2**	**AB3**	**AB4**	**M1**	**M2**	**M3**	**M4**	**K (+)**	**K (-)**
AB1		0.425	0.941	0.007*	<0.001*	<0.001*	0.045*	0.214	<0.001*	<0.001*
AB2	0.425		0.565	0.006*	0.968	0.016*	0.887	0.669	0.001*	0.001*
AB3	0.941	0.565		0.002*	0.004*	<0.001*	0.047*	0.985	<0.001*	<0.001*
AB4	0.007*	0.006*	0.002*		0.002*	0.895	<0.001*	0.004*	<0.001*	<0.001*
M1	<0.001*	0.968	0.004*	0.002*		<0.001*	0.978	<0.001*	<0.001*	<0.001*
M2	<0.001*	0.016*	<0.001*	0.895	<0.001*		0.001*	<0.001*	<0.001*	<0.001*
M3	0.045*	0.887	0.047*	<0.001*	0.978	0.001*		0.074	<0.001*	<0.001*
M4	0.214	0.669	0.985	0.004*	<0.001*	<0.001*	0.074		<0.001*	<0.001*
K (+)	<0.001*	0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*		<0.001*
K (-)	<0.001*	0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	

Notes: *: states there is a significant difference ($p < 0.05$)

Different from previous research, in this research, apart from the Kruskal-Wallis test, another comparison test was also carried out, namely the Post-Hoc test in the form of the Games-Howell test [14]. The Games-Howell test showed that the diameter of the inhibition zone for *E. coli* bacteria for sample AB1 did not have a significant difference with samples AB2, AB3, and M4, but there was a significant difference for samples AB4, M1, M2, M3, positive control, and negative control. For sample AB2 there is no significant difference with samples AB1, AB3, M1, M3, and M4, but there is a significant difference in samples AB4, M2, positive control, and negative control. Sample AB3 did not have significant differences with samples AB1, AB2, and M4, but there were significant differences in samples AB4, M1, M2, M3, positive control, and negative control. Sample AB4 did not have significant differences with sample M2, but there were significant differences in samples AB1, AB2, AB3, M1, M3, M4, positive control and negative control. Sample M1 did not have significant differences with samples AB2 and M3, but there were significant differences in samples AB1, AB3, AB4, M2, M4, positive control and negative control. Sample M2 did not have significant differences with sample AB4, but there were significant differences in samples AB1, AB2, AB3, M1, M3, M4, positive control and negative control. Sample M3 did not have significant differences with samples AB2, M1, and M4, but there were significant differences in samples AB1, AB3, AB4, M2, positive control, and negative control. Sample M4 did not have significant differences with samples AB1, AB2, AB3, and M3, but there were significant differences in samples AB4, M1, M2, positive control, and negative control. Meanwhile, the positive control and negative control were significantly different from all samples, both samples AB1, AB2, AB3, AB4, M1, M2, M3, M4, and all controls.

4. Discussion

The aim of this research is to determine the antibacterial activity of antibacterial liquid soap and moisturizing liquid soap against *E. coli* bacteria. The research was carried out *in vitro* using the agar well

diffusion method. The experimental results showed that all samples had an inhibitory zone diameter for both antibacterial and moisturizing liquid soap and positive controls except for the negative control. The largest inhibition zone diameter (mm) was found in the positive control trimethoprim-sulfamethoxazole (32.24 mm), followed by sample AB4 (24.52 mm), sample M2 (23.85 mm), sample AB1 (19.29 mm), sample AB3 (19.00 mm), sample M4 (18.77 mm), sample AB2 (17.32 mm), sample M1 (16.56 mm), sample M3 (16.09 mm), and negative control sterile distilled water (0.00 mm) which has the lowest diameter.

The average diameter of the inhibition zone for antibacterial soap and moisturizing soap against *E. coli* bacteria was found to be 20.03 mm and 18.81 mm respectively. Previous research showed that the diameter of the inhibition zone for liquid hand washing soap using the disc diffusion method against *Staphylococcus aureus* and *E. coli* bacteria was found to be an average of 27.7 mm and 29.3 mm [15]. Meanwhile, other research shows that the diameter of the inhibition zone of antiseptic solid soap using the disc diffusion method against *E. coli* bacteria resulted in an average of 21.9 mm for A3 (brand D), A1 soap (brand S) with an average of 21.6 mm, whereas A2 soap (brand N) with an average of 13.8 mm [16]. The differences in results obtained can be caused by differences in the diffusion methods used, differences in the type of soap preparation used, and differences in experimental conditions.

The results of the Shapiro-Wilk normality test and Levene homogeneity test show that all data is normally distributed but not homogeneous. Judging from the results of the Kruskal-Wallis test, it shows that the significance results obtained were < 0.001, which means that each treatment had a significant difference in antibacterial activity on the diameter of the inhibition zone produced. This leads to the conclusion that there is a difference in antibacterial activity between antibacterial soap and moisturizing soap against *E. coli* bacteria.

According to Morales *et al*. [17], the diameter of the inhibition zone can be categorized into four groups, namely very strong with an inhibition zone of 21-30 mm, strong with an inhibition zone of 11-20 mm, moderate with an inhibition zone of 6-11 mm, and weak with an inhibition zone of <5 mm. Therefore, samples AB4 and M2 are considered to have very effective activity in inhibiting the growth of *E. coli* bacteria, which is in the very strong category. Samples AB1, AB2, AB3, M1, M3, and M4 are considered to have effective activity in inhibiting the growth of *E. coli* bacteria, which is considered strong. All soap samples can be said to be effective in inhibiting the growth of *E. coli*.

The formation of an inhibition zone in all liquid soap samples, both antibacterial and moisturizing, indicates antibacterial activity against the growth of E. coli bacteria. This is because soap works as a surfactant. Soap is a fatty acid salt produced from the saponification process. Soap consists of two parts, namely the hydrophilic and hydrophobic parts. When soap comes into contact with water and oil, the molecules rearrange themselves into spherical structures called micelles. The polar tail ends are outside in contact with the water and the non-polar tails are protected inside. The inner tail dissolves into the oil, and the entire oil droplet is protected from water [18]. This is important because all cells are surrounded by fat, where other molecules such as proteins are embedded. Surfactants used in cleaning can kill bacteria by disrupting and breaking down cell membrane components such as lipids and proteins. The hydrophobic tail of surfactants attaches to the lipid layer surrounding cells, causing it to rupture, and can be easily washed away by water [19].

Soap on the market has different levels of effectiveness in inhibiting the growth of *E. coli* bacteria. The sensitivity of *E. coli* to soap shows that the compounds in it can inhibit bacterial growth. The composition of each product varies greatly, with active ingredients such as thymol, triclocarban, farnesol, chloroxylenol, and other active ingredients.

In addition, soap products are often combined with active ingredients made from natural ingredients. For example, sample AB4, which contains lemongrass (*Cymbopogon schoenanthus*) extract, which has a very strong inhibitory effect on *E. coli* bacteria. Lemongrass extract has the main component in the form of citral [20]. The mechanism underlying the antimicrobial effects of terpenes such as citral is interaction with the cytoplasmic membrane resulting in loss of membrane integrity [21]. Not only citral, citronellal, geraniol and citronellol contained in lemongrass (*Cymbopogon schoenanthus*) extract can also inhibit bacterial activity [22]. Another active compound found in the AB4 sample was IPMP (or by another name thymol). The main

mechanism of thymol as an antibacterial agent is disruption of membrane integrity which results in leakage of intracellular materials necessary for normal metabolism and survival [23].

Another liquid soap that has a very strong inhibition zone is M2 moisturizing liquid soap. Sample M2 has a compound composition in the form of water as a solvent, lauric acid which has antibacterial and anti-inflammatory properties [24], cocamidopropyl betaine and sodium laureth sulfate as a foaming thickener, potassium hydroxide as a pH regulator, glycerin to moisturize the skin, sodium chloride as a viscosity regulator, sodium lactate as a humectant and pH regulator, glycol distearate as an opacity agent and pearling agent, acrylates copolymer as a film-forming and thickening agent, tetrasodium EDTA which helps the product stay good and stable for a longer time, hydroxypropyl methylcellulose as a stabilizer emulsion and thickener, argania spinosa (argan oil) as an emollient, pentaerythrityl tetra-di-t-butyl hydroxyhydrocinnamate as an antioxidant and preservative, hexamidine diisethionate as an emollient and preservative, disodium distyrylbiphenyl disulfonate as a surfactant and thickener, and CI 45100 as a colorant.

Sample AB1 liquid soap has active ingredients in the form of triclocarban and farnesol. Triclocarban is thought to work by absorbing and destroying the semipermeability of the cytoplasmic membrane, which causes cell death [25]. Coupled with farnesol which also has an antibacterial effect which works by damaging bacterial cell membranes [26]. Therefore, AB1 antibacterial liquid soap has been proven to inhibit the growth of *E. coli* and is categorized as having a strong inhibition zone.

AB3 antibacterial liquid soap product has been proven to have antibacterial properties in inhibiting the growth of strong *E. coli* bacteria. This sample has active ingredients in the form of thymol and silver oxide. Thymol has potent activity inhibition capabilities of Gram positive and Gram negative bacteria. The main mechanism of action of thymol is membrane dysfunction. The hydroxyl groups present in thymol are highly reactive and form hydrogen bonds with the active sites of target enzymes, inactivating them causing dysfunction or rupture of cell membranes [27]. Previous research proved the strong antibacterial action of silver oxide on Gram-positive or Gram-negative bacteria by observing silver oxide against *E. coli* and revealing that there is the formation of “holes” in the bacterial cell wall and the accumulation of silver oxide in the cell membrane causes an increase in cell wall permeability and ultimately cell death [28].

Liquid soap which also has a strong inhibition zone is M4 moisturizing liquid soap. Sample M4 has a compound composition in the form of water as a solvent, lauric acid which has antibacterial and anti-inflammatory properties [24], potassium hydroxide as a pH regulator, myristic acid as a cleaning agent, lauryl hydroxysultaine as a surfactant and thickening agent, glycol distearate as a opacity and pearling agent, fragrance, palmitic acid as an emollient, laureth-4 as an emulsifier, carboxylic acid as an antioxidant, sodium laureth sulfate as a foaming thickener, hydroxyethylcellulose as a thickener, and etidronic acid which helps the product stay good and stable for a longer time.

Sample AB2 liquid soap has an active ingredient in the form of chloroxylenol. Chloroxylenol is an active ingredient in antiseptic soap which activates bacterial enzymes and breaks down bacterial cells, reducing Gram-negative and Gram-positive bacteria [29]. Chloroxylenol acts on the bacterial cytoplasmic membrane, causing protein denaturation. When the concentration of chloroxylenol increases, functional proteins and nucleic acids in cells clump together and stop working, ultimately leading to rapid cell death [27]. Therefore, AB2 antibacterial liquid soap can inhibit the growth of *E. coli* bacteria well and is categorized as having a strong inhibition zone.

Liquid soap which also has a strong inhibition zone is M1 moisturizing liquid soap. Sample M1 has a compound composition in the form of water as a solvent, lauric acid which has antibacterial and anti-inflammatory properties [30], potassium hydroxide, as a pH regulator, myristic acid as a cleaning agent, lauryl hydroxysultaine as a surfactant and thickening agent, glycol distearate as an opacity agent and pearling agent, fragrance, cedyl glucosside as a gentle surfactant for sensitive skin, palmitic acid as an emollient, laureth-6 carboxylic acid as a surfactant, sodium laureth sulfate as a foaming thickener, hydrocyethylcellulose as a thickener, etidronic acid which helps the product stay put. good and stable for a longer time, BHT as an

antioxidant and preservative, algin as a thickener, sodium PCA as a moisturizer, butylene glycol as a solvent and humectant, and yogurt filtrate.

Liquid soap which also has a strong inhibition zone is M3 moisturizing liquid soap. Sample M3 has a compound composition in the form of water, myristic acid, lauric acid, potassium hydroxide, potassium chloride, sodium laureth sulfate, palmitic acid, glycol distearate, fragrance, cocamidopropyl betaine, phenoxyethanol as a preservative, hydroxypropyl methylcellulose as an emulsion stabilizer and thickener, sodium chloride, tetrasodium EDTA, glycerin, BHT, etidronic acid, piroctone olamine, propylene glycol, stearic acid, citric acid, sodium benzoate, sericin, jasminum officinale flower extract, rosa gallica flower extract, PEG-40 hydrogenated castor oil, nelumbium speciosum flower oil, Prunus amygdalus dulcis oil, potassium sorbate, mentha arvensis leaf oil, cymbopogon martini oil, sodium sulfate, CI 14700, and CI 17200.

Upula *et al*. [4] investigated the impact of antiseptic soap on the normal bacterial flora of human skin. The antibacterial formulation significantly reduces the number of bacteria, thus demonstrating its effectiveness. The results showed 120 bacterial colonies, with more isolates in men. This study highlights the role of normal flora in inhibiting undesirable organisms. Although antiseptic soaps reduce Gram-positive flora, they do not cause overgrowth of Gram-negative species. Caution is advised regarding the side effects of triclosan. In conclusion, this study recommends minimizing the use of non-medical antiseptic soaps to avoid microflora disturbance and comply with regulatory limits for triclosan concentrations in cosmetics.

5. Conclusions

Based on this research, it can be concluded that there is a formation of an inhibition zone in all soap samples, both antibacterial and moisturizing, which indicates antibacterial activity against the growth of *E. coli* bacteria. The average diameter of the inhibition zone of antibacterial and moisturizing soap against *E. coli* was 20.03 mm and 18.81 mm, respectively. The p value obtained was $p \leq 0.001$, which means that each treatment had a significant difference in the diameter of the inhibition zone. This leads to the conclusion that H_1 is accepted, which indicates a significant difference in antibacterial activity between antibacterial soap and moisturizing soap against *E. coli* bacteria with the greatest antibacterial activity found in sample AB4 (24.52 mm).

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