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Original Article

Test of Combination of Essential Oils of Betel Leaf, Basil, and Lime as Active Ingredients of Liquid Soap

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Abstract

One natural ingredient with the potential to serve as the primary raw material for skincare cosmetic products is essential oils (EOs). Given the urgency of the issue, efforts must be made to develop essential oil-based cosmetic products, particularly in the form of soap. The purpose of this study is to evaluate the quality of EOs, the physical stability, and the antibacterial activity of liquid soap containing green betel leaf, basil leaf, and lime peel EOs against *Staphylococcus aureus* and *Escherichia coli* bacteria. This study follows an experimental design. The samples used in this study were EOs from green betel leaf, basil leaf, and lime peel. The physical stability test of the liquid soap includes organoleptic evaluation, viscosity, pH, and homogeneity. The antibacterial activity test was conducted using the Kirby-Bauer method on liquid soap with the formulas F1 (5%), F2 (10%), and F3 (15%). The results showed that the main components of the EOs in the betel leaf, basil leaf, and lime peel included eugenol, linalool, and limonene. All liquid soap formulas were found to be stable. In the antibacterial test, the average diameter of the inhibition zones for S. aureus was 13.5 mm, 14.5 mm, and 26.7 mm for F1 (5%), F2 (10%), and F3 (15%) formulas, respectively. For E. coli, the inhibition zones measured 6 mm, 6.5 mm, and 8.8 mm. This study concludes that liquid soap formulated with EOs can strongly inhibit the growth of *S. aureus*, while it moderately inhibits the growth of *E. coli*.

Keywords: *Bacteria; Basil; Betel; Essential Oil; Lime*

Introduction

Bacteria are microorganisms that can cause infectious diseases. Generally, bacterial infections are treated with antibiotics. However, the use of antibiotics often carries the risk of Antimicrobial Resistance (AMR) (Puvača *et al*., 2021). Bacterial resistance has led to a global health crisis. Some bacteria that are resistant to antibiotics include S*taphylococcus aureus* and Escherichia coli (Breijyeh *et al*., 2020). S. aureus is a gram-positive coccus bacterium that can cause skin infections, such as pimples or abscesses (Zheng *et al*., 2021), while *E. coli* is a gram-negative bacillus bacterium that can lead to foodborne illnesses (Liu *et al*., 2021). The results of a systematic review on antimicrobial resistance among common bacterial pathogens in Indonesia show that 18.3% of *E. coli* isolated from various hospitals are carbapenem-resistant, while 22.2% of S. aureus are methicillin-resistant (Gach *et al*., 2024).

According to the World Health Organization (WHO, 2009), one simple method recommended to reduce the cross-transmission of antibiotic-resistant pathogens is to wash hands using antibacterial agents. One of the recommended antibacterial agents is soap with active ingredients in the form of essential oils

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(Camero *et al*., 2019). Essential oils (EOs) are composed of secondary metabolite compounds, including terpenes, phenols, alcohols, ketones, and esters. They are extracted from the flowers, leaves, seeds, rhizomes, and fruits of aromatic plants (Dhifi *et al*., 2016). The advantages of using EOs include low residues, biodegradable properties, low toxicity, and low concentrations. They also have the ability to exert a synergistic effect that inhibits bacterial growth by interacting with bacterial biological membranes, affecting the mechanisms of protein synthesis, cell wall formation, and the transport of nutrients and ions in human pathogenic bacteria (Pavoni *et al*., 2020).

The use of natural ingredients as antibacterial components in soap is crucial. This is supported by the study conducted by Pérez-Garza *et al*. (2017), which reported that washing hands with antimicrobial soap (containing citric extract) was more effective in reducing the number of *E. coli* compared to non-antimicrobial soap. Antimicrobial soap was found to be 3-32 times more effective than non-antimicrobial soap (Adhikari *et al*., 2020). Given the importance of antibacterial natural ingredient extracts, it is essential to test the ability of these natural ingredients to inhibit the growth of pathogenic bacteria.

The natural ingredients to be tested in this study are betel, basil, and lime essential oils (EOs) against the growth of *E. coli* and S. aureus. The selection of betel leaf, basil leaf, and lime EOs is based on studies by Angane *et al*. (2022); Phensri *et al*. (2022); Nair *et al*. (2022), who reported that these three essential oils can inhibit various pathogenic bacteria in the moderate to strong category. Based on these findings, it is necessary to conduct a bioprospecting test of a combination of betel leaf, basil, and lime peel EOs sourced from Semarang, Indonesia.

Previous research has primarily focused on testing single doses of non-product EOs on non-resistant bacteria. Therefore, the novelty of this research lies in the integration of three essential oils with complementary antibacterial compounds (eugenol, linalool, and limonene), resulting in enhanced antibacterial performance and stable liquid soap formulations. The purpose of this study is to determine the chemical characteristics, physical stability, and antibacterial activity of a combination of liquid soap EOs at concentrations of F1 (5%), F2 (10%), and F3 (15%) against *S. aureus* and *E. coli*.

Material and Methods

Materials

The design of this study is an experiment that tests the chemical characteristics, physical stability, and antibacterial activity of essential oils (EOs) from betel leaf, basil leaf, and lime peel at concentrations of 5%, 10%, 15%, 20%, and 25%, as well as liquid soap combined with EOs in formulas F1 (5%), F2 (10%), and F3 (15%) against *E. coli* and *S. aureus*. Samples of betel leaf, basil leaf, and lime peel were collected from Semarang City, Central Java, Indonesia. *S. aureus* and *E. coli* were obtained from hospital clinical samples.

Methods

Essential oil extraction

The essential oils (EOs) of betel leaf, basil leaf, and lime peel are extracted using the steam distillation method. The distillate is separated using a separation funnel with the addition of anhydrous $NaSO_4$ to separate the water and oil phases (Alighiri *et al*., 2018). The essential oils are stored in bottles and tightly sealed. All essential oil samples are tested for organoleptic and chemical characteristics.

Essential oil antibacterial activity test

The antibacterial activity test was performed using the Kirby-Bauer method with liquid soap combined with essential oils. The test was carried out on formulas F1 (5%), F2 (10%), and F3 (15%). Concentration drops of 100 µl were applied using a micropipette (Socorex) onto a 6 mm paper disk (Oxoid) and left for 30 minutes. The dilution solvent used was Dimethyl Sulfoxide (DMSO) (Merck). The paper disk was then placed onto Muller Hinton Agar (MHA) media containing bacteria, adjusted to the McFarland standard of 0.5. The bacteria were incubated for 18 hours. The antibacterial activity of the essential oils was assessed by measuring the bacterial growth inhibition zone (mm).

Characterization of essential oils

The chemical characterization of essential oils was performed using the Thin-Layer Chromatography (TLC) method with eluents of n-hexane:ethyl acetate (5:3), n-hexane:ethyl acetate (7:3), and n-hexane:ethyl acetate:chloroform (5:1:1), as well as Gas Chromatography Mass Spectrometry (GC-MS) (Shimadzu QP2010S) with an HP5-MS column (30m x 0.32mm, thickness 0.25 µm). Helium was used as the carrying gas at a constant pressure of 100 kPa, and the injection temperature was set to 250°C. Physical parameter tests included refractive index and solubility tests. Chemical parameter tests included the ester number and acid number, following the SNI method 06-2385-2006.

Manufacturing and testing Evaluation of liquid soap

The manufacture of liquid soap is carried out using a combination of the active ingredient formula of betel leaf essential oils, basil, and lime peel in concentrations of F1 (5%), F2 (10%), and F3 (15%). The liquid soap formula is shown in Table 1.

Table 1: *Liquid Soap Formula Combination of Essential Oils*

Components	F1	F2	F3
Betel leaf EOs	1ml	2ml	3ml
Basil leaf EOs	1ml	2ml	3ml
Lime peel EOs	1ml	2ml	3ml
VCO	25ml	25ml	25ml
KOH 40%	18ml	18 ml	18 ml
Gliserin	6gr	6 gr	6 gr
HPMC	1gr	1 gr	1 gr
Propilen glikol	8gr	8 gr	8 gr
Aquadest	37ml	32 ml	27 ml

Based on Table 1, the manufacture of essential oils (EOs) liquid soap is carried out by first placing the VCO oil phase in a beaker. KOH is added little by little while constantly stirring, and the mixture is heated to a temperature of 50°C until a soap base is formed. Then, 50 mL of aquadest is added to the soap base. Stearic acid, which has been melted, is added and stirred until homogeneous, followed by the addition of BHT. HPMC material, which had previously been prepared with hot aquadest, is added to the mixture while continuing to stir until homogeneous. Next, benzyl alcohol, which had been dissolved in glycerin, is added. The stirring temperature is then lowered to 30°C, and the combination of essential oils is added and stirred until homogeneous. Finally, aquadest is added up to 200 mL, and the mixture is stirred until homogeneous. The liquid soap is then placed in an airtight, transparent container. The evaluation tests for the liquid soap include organoleptic testing, homogeneity, pH, viscosity, free alkali levels, and antibacterial activity tests.

Data Analysis

Data analysis was conducted using descriptive and comparative tests. The descriptive test involved interpreting tables and graphs.

Results

Chemical characteristics test of essential oils

The three EOs were then subjected to a characterization test, which included tests for refractive index, solubility, and the number of esters and acids, as shown in Table 2.

Table 2: *Test Results of Refractive Index, Specific Gravity, Number of Esters, and Acids*

Sample EOs	(χ ± SD)			
	Refractive index	Specific gravity (g/ml)	Ester number (mg KOH/g oil)	Acid number (mg KOH/g oil)
Betel	1.388±0.007	0.751±0.009	44.472	3.345
Basil	1.345±0.005	0.943±0.007	50.506	3.439
Lime	1.380±0.005	0.856±0.005	46.428	2.893

The refractive index is the ratio of the speed of light in a vacuum to the speed of light in a material. The refractive index value is influenced by the compound components contained in the essential oil. The research results on the refractive index for each essential oil are presented in Table 2, with the refractive index value for basil ranging from 1.340 to 1.350, for lime from 1.375 to 1.385, and for betel oil from 1.380 to 1.390. According to Aina *et al*. (2015), the refractive index is an indicator of the moisture content in essential oils. The higher the moisture content, the lower the refractive index value; conversely, the lower the moisture content, the higher the refractive index value. Rizqullah *et al*. (2018) stated that an increase in the refractive index value of essential oils indicates a higher composition of carbon chains, such as sesquiterpenes, and the length of double bonds extracted during the distillation process. The refractive index values in this study are consistent with the general standard for the refractive index of essential oils, which ranges from 1.3 to 1.7. However, they do not align with the standard value set by the Essential Oil Association (EOA), which is 1.510 to 1.5165.

The specific gravity results for betel, ocimum, and lime essential oils (EOs) are shown in Table 2: 0.75, 0.943, and 0.856, respectively. According to Erliyanti *et al*. (2020), the specific gravity value is correlated with the molecular fraction of high weight and the content of impurities in the EOs. The more molecular fractions with high weight and impurities present, the higher the specific gravity value. The specific gravity results in this study align with the Essential Oil Association (EOA) and ISO 3519:2005 standards, which set the weight values for lime EOs at 0.858 – 0.866 g/ml, basil at 0.952 – 0.973 g/ml, and betel at 0.696 – 1.188 g/ml.

Another important characteristic is the acid and ester numbers, which play a significant role in the quality control of EOs. The acid number indicates the amount of free fatty acids in the sample, while the ester number reflects the level of esters in the sample. The parameters for the ester number and acid number follow the SNI 06-2386-2006 standard. The acid number can affect the quality of essential oils, particularly basil, betel, and lime EOs. The higher the level of free fatty acids in the EOs, the higher the acid content, which influences the distinctive aroma. Based on the results in Table 2, the free fatty acid number for betel, basil, and lime was 3.345, 3.439, and 2.893, respectively, while the ester numbers were 44.47, 50.50, and 46.42. The highest number of free fatty acids and esters was found in basil. The acid and ester numbers in this study do not align with the standard for basil leaf EOs, which is less than 1, and lime peel EOs, which range from 0.82 to 2.39. The standard ester number is 18.09–33.77. According to Latifah *et al*. (2023), the higher the acid number, the greater the risk of affecting the quality of the EOs by altering their distinctive aroma. The components of essential oils will increase the acid number when oxidation occurs, especially from the aldehyde group that forms carboxylic acid. Regarding the ester number, Daryono *et al*. (2014) stated that it indicates the quality of the aroma of essential oils. Nurjanah *et al*. (2016) noted that the higher the ester number, the more components that contribute to the aroma of essential oils, making them less likely to oxidize and resulting in a strong, long-lasting fragrance.

The results of the chromatogram and identification of the dominant compounds of betel essential oil are shown in Figure 1 and Table 3

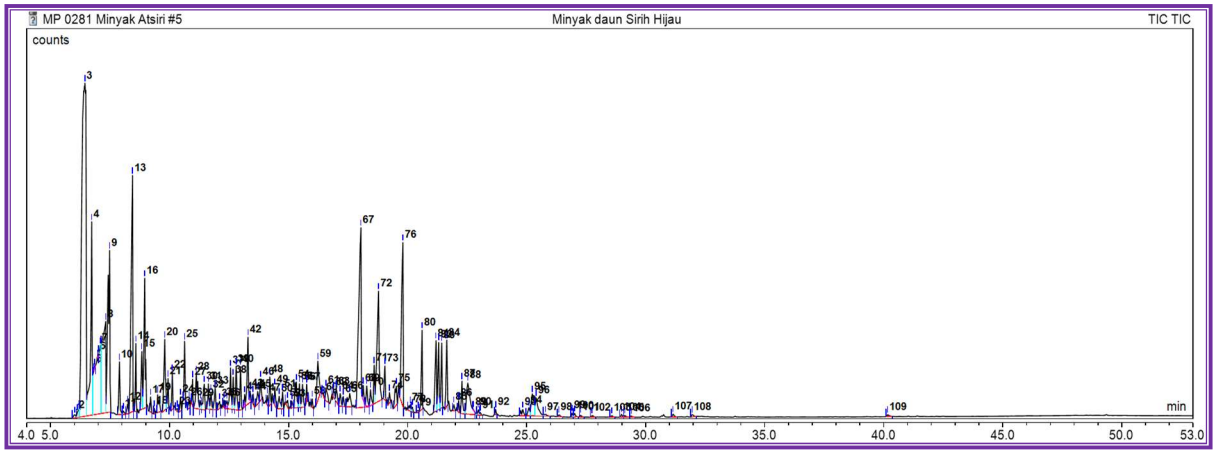

Figure 1: *Betel Oil Chromatogram*

Table 3:*Identification Result of The Predominant Components of Green Betel Leaf Essential Oil*

Compound	Retention time (minute)	Width (%area)	B.M	Compound approximation	Chemical Formula
72	18.78	3.50	164	Eugenol	C10H12O2
76	19.80	3.79	204	Caryophyllene	C15H24
82	21.30	1.19	204	Germacrene D	C15H24

Based on Figure 1 and table 3, 109 peaks have been successfully separated. The separation appeared at the beginning of the sixth minute. Eugenol compounds that appeared in TLC screening after testing using GCMS were seen at a retention time of 18 minutes and 78 seconds. This indicates that eugenol compounds are a major component contained in green betel leaf EOs. This result is by the research of Valle Jr et al. (2016), in addition to eugenol, there are caryophyllene and germacrene-D compounds. The results of the chromatogram and identification of the dominant compounds of basil leaf EOs are shown in Figure 2 and Table 4.

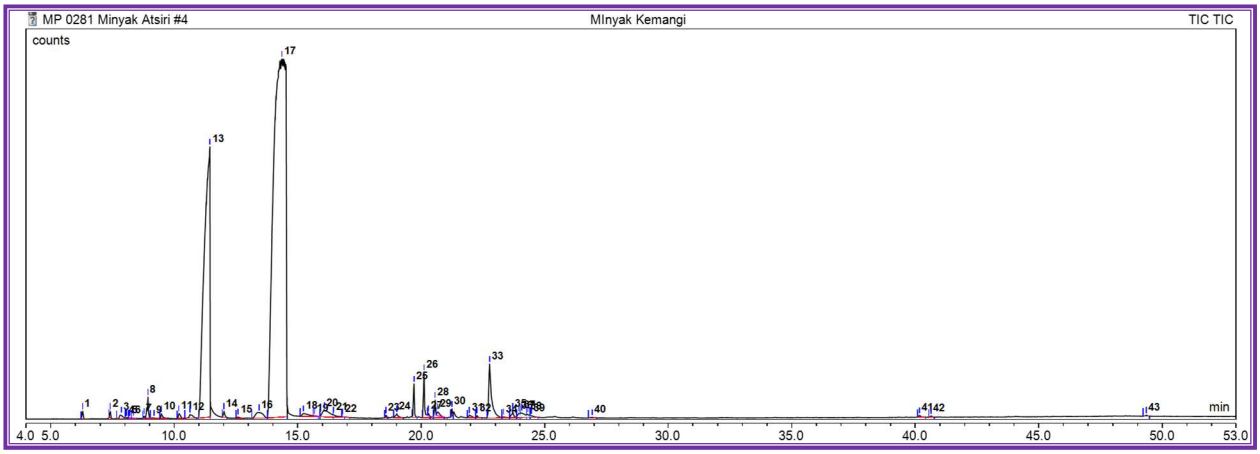

Figure 2: *B*asil leaf chromatogram

Table 4: *Identification Result of The Predominant Components of Basil Leaf Essential Oil*

Compound	Retention time (minute)	Width (%area)	B.M	Compound approximation	Chemical Formula
1	6.29	0.13	136	α-Pinene	C10H16
2	7.41	0.08	136	β-Pinene	C10H16
13	11.45	23.01	154	Linalool	C10H18O
17	14.36	67.66	148	Anethole	C10H12O

Based on Figure 2, 43 chromatogram peaks were obtained, with the highest peaks at compounds 13 and 17. According to Table 4, compound 13, with a retention time of 11 minutes and 45 seconds, is linalool, as identified in the TLC stain, where the stain corresponds to the linalool compound. The molecular mass obtained is consistent with the theoretical value of 154 m/z. This result aligns with the research of Anwar *et al*. (2023) and Purushothaman *et al*. (2018), which stated that estragole and linalool are the dominant ingredients in *O. basilicum* essential oil.

In addition to linalool, the highest chromatogram peak is at peak 17, which corresponds to anethol, with a relative percentage of 66.67%. These findings complement the research of Ezeorba *et al*. (2024), who reported that other components, such as methyl chavicol and methyl-eugenol, are found in high concentrations. Anethol and linalool are the main components in basil leaf essential oil and have pharmacological activities, including anti-inflammatory and antibacterial effects. The results of the chromatogram and the identification of the dominant compounds in lime peel essential oil are shown in Figure 3 and Table 5.

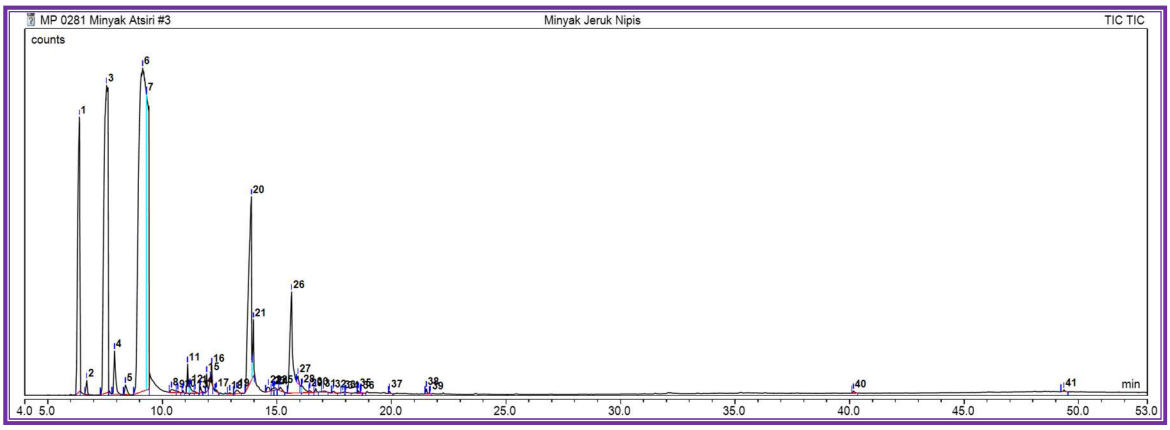

Figure 3: *Lime Oil Chromatogram*

Table 5: *Identification Result of The Predominant Components of Lime Peel Essential Oil*

Compound	Retention time (minute)	Width (% area)	B.M	Compound approximation	Chemical Formula
1	6.38	8.87	136	α-pinene	C10H16
4	7.91	1.20	136	β-myrcene	C10H16
5	8.39	0.35	136	Carene	C10H16
6	9.14	38.46	154	Limonene	C10H16

Based on Figure 3 and Table 5, 41 chromatogram peaks were obtained, with the largest content being limonene. This result is consistent with the research of Lemes *et al*. (2018), which reported that the main component of Citrus aurantifolia essential oil is limonene, at a concentration of 98.3%. Other important components found in this study include α-pinene, β-myrcene, and carene. This finding complements the research of Jain *et al*. (2020); Oliveira *et al*. (2024), which stated that linalool, β-pinene, γ-terpinene, citronellal, and citronellol are also components found in Citrus aurantifolia EOs.

Physical stability test of essential oil liquid soap

The three essential oils combined as active ingredients in making hand soap liquid concentrations of F1 5%, F210%, and F215. The results of the physical stability test of liquid soap can be seen in Table 6.

Table 6*: The Evaluation Test of Liquid Soap Combined with Essential for 28 days*

Formula	Organoleptic	Homogeneity	pH	Viscosity
F1 5%	Liquid, brownish-yellow, betel odor	Homogeneous	9.08	3.500
F2 10%	Liquid, brownish-yellow, betel odor	Homogeneous	9.09	3.583
F3 15%	Liquid, brownish-yellow, betel odor	Homogeneous	9.11	41.333

Based on Table 6, the results of the physical stability evaluation test of liquid soap containing a combination of active ingredients: betel, basil, and lime EOs, at a concentration of F1 5%, F2 10%, and F3 15%, produce a liquid with a betel odor dominant and a brownish-yellow consistency. The organoleptic results can be seen in Figure 4.

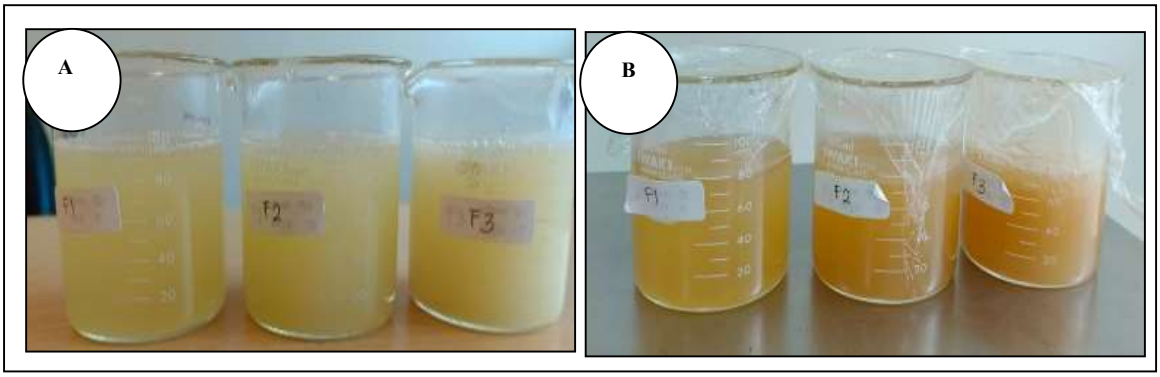

Figure 4: *Liquid Soap Organoleptics. A. Day 0 (white). B. Day 28 (brownish-yellow)*

Based on Figure 4, it can be seen that the color of the liquid soap was yellowish-white at the beginning of production but changed to brownish-yellow after 28 days of storage. This change is due to the interaction between the essential oils and the liquid soap base. Additionally, the liquid soap is considered homogeneous, as indicated by the absence of clumps of coarse particles and phase separation after 28 days of storage. The pH value ranges from 9.08 to 9.11, which meets the pH requirements for soap based on SNI 06-3734-2006, which is 8-11 (Situmorang *et al*., 2023). The viscosity values for F1, F2, and F3 are 3,500 cP, 3,583.33 cP, and 41,333.33 cP, respectively. The viscosity evaluation test of liquid soap aims to ensure it is easy to pour when applied to the skin. The viscosity of liquid soap products reflects the balance of the composition of the base and fatty acids in the soap ingredients. The more balanced the base and fatty acids, the better the viscosity of the liquid soap. The viscosity requirement for liquid soap to be easily poured is between 400-4,000 cP (Meizalin & Paramita, 2021). The viscosity of the F3 formula appears very thick from the beginning of production, and the viscosity increases after 28 days of storage. This is because the acidic essential oils interact with the HPMC base, causing the liquid soap to become thicker (Punitha *et al*., 2020).

Antibacterial activity test of essential oil liquid soap

The antibacterial activity test against *S. aureus* and *E. coli* bacteria proved the efficacy of liquid soap combined with EOs. The results are in figure 5 and 6.

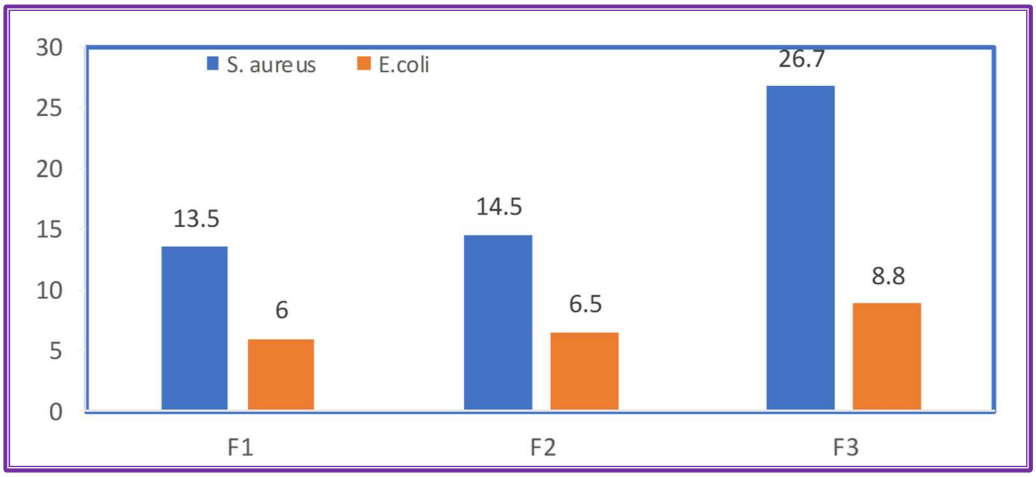

Figure 5: *Antibacterial Activity Essential Oil Combination Liquid Soap against S. aureus and E. coli*

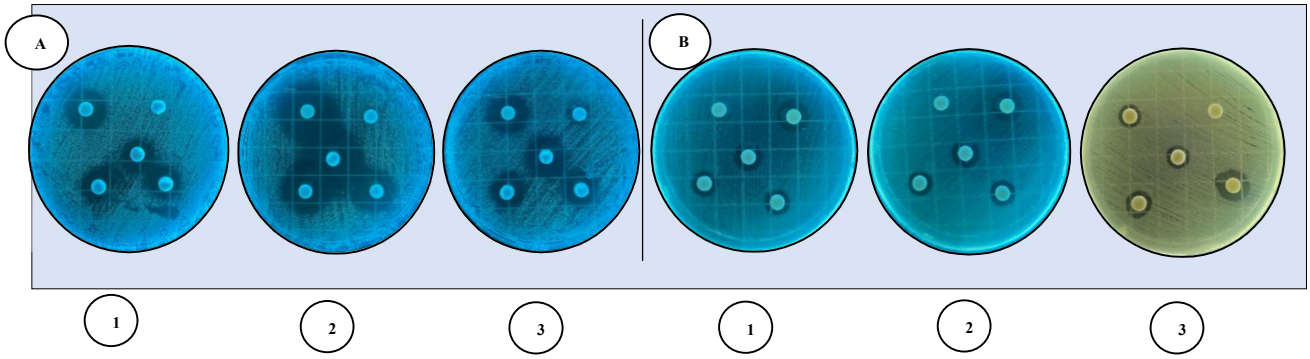

Figure 6: *Antibacterial Test Results of Liquid Soap (A). A1: F1 against S. aureus. A2 : F2 to S. aureus. A3: F3 against S. aureus. B: B1: F1 against E. coli. B2: F2 against E. coli. B3:: F3 against E coli.*

Figures 5 and 6 show the effects of base treatments, EO combinations, and liquid soaps combined with essential oils using F1 formulas at 5%, 10%, and 15%. All bases showed no inhibitory zones. The combination of 5%, 10%, and 15% essential oils against *S. aureus* resulted in average inhibitory zone diameters of 19.5 mm (strong), 20 mm (strong), and 29.5 mm (very strong), respectively, while for *E. coli*, the diameters were 9.5 mm (moderate), 11 mm (strong), and 12 mm (strong). After being formulated into

liquid soap, the average diameter of the inhibition zone against S. aureus was 13.5 mm (strong), 14.5 mm (strong), and 26.7 mm (strong), while for *E. coli*, it was 6 mm (moderate), 6.5 mm (moderate), and 8.8 mm (moderate).

Discussion

Based on Figures 5 and 6, it can be seen that among the three formulas of liquid soap products, the F3 15% formula is the best at inhibiting the growth of *S. aureus* and *E. coli*. In general, the antibacterial ability of the essential oils (EOs), after being combined and used as an active ingredient, remains stable, and there is no decrease in their ability to inhibit the growth of *S. aureus* and *E. coli*. The antibacterial ability of the liquid soap in this study is due to the combination of eugenol (betel), linalool, anethole (basil), α-pinene, and limonene (lime). According to Galovičová *et al*. (2022), limonene is the dominant component in lime peel essential oil. Han *et al*. (2020) stated that limonene and α-pinene can bind to bacterial cell membrane proteins, thereby increasing cell membrane permeability, which causes leakage of cell contents and results in bacterial cell death. The inhibitory effect is further enhanced by the presence of linalool, anethole, and eugenol, which work together to accelerate membrane damage and cause the release of cytoplasm, DNA, RNA, and bacterial cell proteins (Guo *et al*., 2021). In addition, the combination of essential oils is hydrophobic, which allows it to more easily interact with the lipophilic part of the cell membrane, thus interfering with the integrity and function of proteins, nucleic acids, energy metabolism (ATP), and bacterial enzymes (Beristain-Bauza *et al*., 2019; Putri *et al*., 2025).

According to Zhang *et al*. (2017) and Anindita *et al*. (2025), the membrane potential is the potential difference between the inside and outside of the cell, and it plays a critical role in the metabolism of bacterial cells. The treatment of liquid soap combined with essential oils (EOs) causes damage to membrane integrity and membrane depolarization, which results in a decrease in the potential of *S. aureus* and *E. coli* cell membranes. The membrane potential is more effective at inhibiting S. aureus than E. coli. The effect of the combination of EOs as the active ingredient in liquid soap on protein leakage and the disruption of protein and enzyme synthesis is explained by Wang *et al*. (2023) and Meira *et al*. (2017). The interaction of EOs with cell membranes causes damage to bacterial cell membranes, leading to the release of cytoplasmic proteins and disruption of protein and enzyme synthesis. This is characterized by a decrease in protein levels in the cell membranes of *S. aureus* and *E. coli*. According to Watson and Chiu (2016), the reduction in protein levels in cell membranes significantly affects the reduction of various energy metabolism enzymes, including adenosine triphosphatase (ATPase), alkaline phosphatase (ALPase), β-galactosidase (β-GAL), citrate synthase (CS), and isocitrate dehydrogenase (ICDH). Zhang *et al*. (2017) added that the enzymes citrate synthase (CS) and isocitrate dehydrogenase (ICDH) play a role in catalyzing the tricarboxylic acid (TCA) cycle reaction, so the decrease in these two enzymes impairs bacterial cell respiration. Therefore, a reduction in protein levels in bacterial cells is an indicator that the bacterial cell membrane has been damaged. As for the research on the effect of liquid soap combined with essential oils, it is more effective on *S. aureus* bacteria than *E. coli*. According to Perwitasari *et al*. (2023), *S. aureus* is a gram-positive bacterium that lacks a phospholipid barrier in the peptidoglycan layer, making it more easily penetrated by lipophilic EOs. In contrast, *E. coli* is a gram-negative bacterium with a phospholipid barrier in the peptidoglycan layer, which makes it more resistant to EO penetration. Furthermore, Anindita *et al*. (2022) explained that *S. aureus* is more sensitive to the treatment of EOs than *E. coli*. The sensitivity of *S. aureus* is due to the absence of an outer membrane protecting the cell wall, allowing the EOs to easily destroy the cell membranes and cause cytoplasmic leakage. On the other hand, *E. coli* has an outer membrane composed of phospholipids, acting as a barrier to the entry of various antibiotic and antibacterial compounds. Additionally, the presence of enzymes in the periplasmic space of *E. coli* may be capable of breaking down EO molecules (Gauba & Rahman, 2023; Tavares *et al*., 2020).

This study was limited to in vitro antibacterial testing using the Kirby-Bauer method, which does not fully represent real skin application conditions. The antibacterial activity was evaluated only against Staphylococcus aureus and Escherichia coli, while other relevant skin microorganisms were not assessed. Additionally, the study did not include long-term stability testing or in vivo skin irritation and safety evaluations. Future studies should include broader antimicrobial testing against additional pathogenic bacteria and fungi, as well as skin microbiome-related microorganisms. In vivo safety assessments, such

as skin irritation and sensitization tests, are necessary to confirm the product's safety for human use. Further optimization of essential oil concentration and long-term stability evaluations under various storage conditions are also recommended to support product commercialization.

Conclusion

The results of this study conclude that the dominant components of the essential oils (Eos) from betel leaf, basil leaf, and lime peel include eugenol, linalool, and limonene. The combination of these ingredients as the active component in the liquid hand soap formula can inhibit the growth of *S. aureus* in the strong category and *E. coli* in the moderate category. Based on these results, liquid soap made from this combination of Eos can be used as a pharmaceutical product for the epidemiological prevention of infectious diseases caused by *S. aureus* and *E. coli*.

Conflict of Interest

The authors declare that they have no competing interest.

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