FAKULTAS FARMASI
UNIVERSITAS MUHAMMADIYAH PURWOKERTO

PHARMACY: Jurnal Farmasi Indonesia
(Pharmaceutical Journal of Indonesia)

Journal homepage: https://jurnalnasional.ump.ac.id/index.php/PHARMACY

Received 24-11-2024 Accepted 27-06-2025 Available online 31-07-2025

Optimization of Antibacterial Efficacy of Eucalyptus and Lemon Essential Oils in Hand Sanitizer with Simplex Lattice Design

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ARTIKEL INFO

Keywords:
Antibacterial, *Citrus limon, Eucalyptus globulus*, hand sanitizer

ABSTRACT

Hand sanitizer spray is a spray kind of hand sanitizer that contains active antibacterial chemicals to eliminate germs on hands. *Eucalyptus globulus* essential oil contains antimicrobial properties, specifically Eucalyptol (50-65%). *Citrus limon* essential oil exhibits antibacterial properties due to its primary component, limonene (59.7%), which damages bacterial cell walls and inhibits bacterial enzyme activity. This study aimed to assess the impact of a hand sanitizer spray containing a combination of *Eucalyptus globulus* essential oil and *Citrus limon* essential oil on the physical properties of the product, evaluate its antibacterial activity against Staphylococcus aureus, Pseudomonas aeruginosa, and *Escherichia coli*, and identify the most effective formula using the Simplex Lattice Design method. The study employed the Simplex Lattice Design approach to compare the effects of *Eucalyptus globulus* essential oil and *Citrus limon* essential oil in hand sanitizer spray formulations with ratios of 100% : 0%, 0%: 100%, and 50%: 50%. Every formula underwent physical pH, homogeneity, and viscosity tests. Antibacterial testing was conducted on Staphylococcus aureus, Pseudomonas aeruginosa, and *Escherichia coli* microorganisms—a method for testing antibacterial activity by paper disc diffusion. The ideal formula was determined by Simplex Lattice Design calculations, resulting in a blend of 70% *Eucalyptus globulus* essential oil and 30% *Citrus limon* essential oil. The hand sanitizer spray preparations were tested for antibacterial properties on Staphylococcus aureus, Pseudomonas aeruginosa, and *Escherichia coli* bacteria. The average inhibition zones were 17.33 ± 0.67 mm, 13.33 ± 0.57 mm, and 21.2 ± 4.75 mm, respectively. The preparation exhibits pseudoplastic flow behavior and has a pH of 5.5.

1. Introduction

Hands can spread various diseases caused by viruses, bacteria, and fungi. Handwashing can decrease the germ count on hands by as much as 58%. Handwashing with soap is a hygienic practice involving using water and soap to disrupt the transmission of germs (Kurniawan & Dwiaprinia, 2021). However, nowadays, people want practical things, including cleaning their hands. People prefer to use antiseptic hand sanitizers because they are considered more accessible to carry everywhere. Hand sanitizers are alcohol-based hand sanitizers used to kill microorganisms without rinsing with water (Baizuroh et al., 2020).

The antiseptic content as an antibacterial is generally in the form of ethyl alcohol, moisturizer, and softener. The use of chemicals such as ethyl alcohol in large quantities is considered unsafe for health because alcohol is an organic solvent that dissolves layers of fat and sebum on the skin, which functions as protection against infection by microorganisms (Indrawan et al., 2015). A natural-based antiseptic that is safe when repeatedly applied to the palms of the hands is needed (Fatimah & Ardiani, 2018). One plant that can replace alcohol and has the potential to be developed as an antiseptic is *Eucalyptus globulus* and *Citrus limon.*

Eucalyptus globulus, commonly known as *Eucalyptus*, is a plant that has many benefits, starting from the leaves, roots, stems, and fruit, which are widely used in traditional medicine for various diseases such as influenza, diabetes, tuberculosis, and diarrhea (Bachir & Benali, 2012). Experimental studies of *Eucalyptus globulus* show antibiotic bacterial, antifungal, antiviral, antimalarial, and antidiabetic activity. *Eucalyptus globulus* leaves are plants that produce essential oil that can inhibit the growth of and kill *Staphylococcus aureus* bacteria (Elangovan & Mudgil, 2023). The active compounds inhibiting bacterial growth are 1,8-cineole, linalool, and pinocarveol (Ghalem & Mohamed, 2008). In previous research, *Eucalyptus globulus* essential oil had an adequate concentration of 10% as an antibacterial for *Staphylococcus aureus*. *Citrus limon*, mainly known as lemon, has been studied for its antibacterial activity. The compound that acts as an antibacterial is Limonene (Haque et al., 2022).

Additional research is required to investigate the efficacy of *Eucalyptus globulus* essential oil and *Citrus limon* essential oil as alternative active ingredients to alcohol in hand sanitizer spray against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* bacteria. Combining *Eucalyptus globulus* essential oil and *Citrus limon* essential oil can synergistically function as an antibacterial agent, resulting in a broader inhibition zone. The Simplex Lattice Design (SLD) method was used to evaluate and determine the optimal dosage formulation (Kurniawan & Dwiaprinia, 2021).

2. Research Methods

Materials

Paper disk (Oxoid®), pH meter (Mettler Toledo®), petri dish (Bottom®), sterile tube (Onemed®), incubator (Memmert®), Propipet (Glasfirn®), analytical balance (Ohaus®), oven (Memmert®), hot plate magnetic stirrer (Cimarec®), autoclave (HVE-50 Hirayama®), autoclave (All American 1925X®), viscometer (Brookfield Ametek®), microscope (Zeiss®), glassware (Iwaki®). *Eucalyptus globulus* essential oil and *Citrus limon* essential oil (PT. Lansida Herbal Technology), 96% alcohol (Brataco®), Nutrient Agar (NA) media (Brataco®), glycerin (Brataco®),

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methylparaben (Brataco®), Aquadest (Brataco), *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* bacteria (Health Laboratory and Calibration Center of the Special Region of Yogyakarta).

Methods

Producing spray hand sanitizer

Eucalyptus globulus and *Citrus limon* essential oils were obtained from PT Lansida Herbal. Based on the CoA, the results of testing *Eucalyptus globulus* oil using GC-FID showed that it contained 1.8 cineole (78.21%), limonene (11.98%), β-pinene (1.98%), α-pinene (1.42%), sabinene (0.48%), α-phellandrene (0.2%), and champor (0.04%). Meanwhile, the results of testing *Citrus limon* oil using GC-FID produced limonene (68.89%), β-pinene (11.35%), υ-terpinene (8.10%), sabinene (1.90%), α-pinene (1.68%), myrcene (1.40%), geranial (0.96%), bisabolene (0.62%), neral (0.59%), and neryl acetate (0.43%). Three formula variations were created: *Eucalyptus globulus* essential oil, Citrus lemonen essential oil, glycerin as a humectant, methylparaben as a preservative, 96% alcohol, and distilled water as a solvent. The composition of the hand sanitizer spray formula is shown in Table 1.

Three-hand sanitizer spray formulas were made with differences in each formula in the concentration of *Eucalyptus globulus* essential oil and *Citrus limon* essential oil based on the Simplex Lattice Design method with varying concentrations of 100% *Eucalyptus globulus* in F1, 100% *Citrus limon* essential oil in F2, 50% *Eucalyptus globulus* and 50% *Citrus limon* in F3. The mixing process first involves weighing each ingredient in each formula. Methylparaben 0.2 g was dissolved in 10 mL of 96% alcohol, then glycerin and 5 mL of distilled water were added. The active essential oil is added with 96% alcohol to 50 mL and then homogenized.

Test physical properties

Physical properties are tested by measuring pH, viscosity, and homogeneity. This test is essential to determine the physical stability of the hand sanitizer produced (Kurniawan & Dwiaprinia, 2021).

Antibacterial test

Tools and materials for antibacterial testing are first sterilized in an autoclave at a temperature of 121°C. Test equipment such as glass Petri dishes and test tubes are autoclaved for 20 minutes, while Nutrient Agar (NA) media already in liquid form is autoclaved for 15 minutes. Hose needles and tweezers are sterilized by burning with a Bunsen flame. For agar media, 28 g of Nutrient Agar media was used, dissolved in 1000mL of distilled water, and heated in a magnetic stirrer until everything was dissolved and sterilized in an autoclave at 121°C for 15 minutes. Antibacterial testing was carried out in Laminar Air Flow (LAF). 15mL of Nutrient Agar (NA) media was poured into a petri dish and left to solidify. After solidifying, the bacterial inoculum suspension is rubbed zigzag over the agar surface. Paper discs dipped in the test solution in various ratios, and Saniter® brand hand sanitizer spray as a positive control and placebo or base used as a negative control are placed gently on the media at a distance. The plate is left at room temperature for 10-15 minutes, then incubated at 37°C for 18-24 hours, and then the diameter of the inhibition zone around the disk is measured using a calliper or with a ruler. The test was carried out three times (triple) on each bacterium. Data analysis was done using the SLD equation (Kurniawan & Dwiaprinia, 2021).

Table 1. Hand sanitizer spray formulation

Materials	F1	F2	F3
EGEO 10%	10	-	5
CLEO 10%	-	10	5
Glycerin	0,2	0,2	0,2
Methyl paraben	0,2	0,2	0,2
Aquadest	5 mL	5 mL	5 mL
Alcohol 96%	Ad 50	Ad 50	Ad 50

EGEO: *Eucalyptus globulus* essential oil, CLEO: *Citrus limon* essential oil, F1: 100% EGEO, F2: 100% CLEO, F3: 50% EGEO + 50% CLEO

3. Result and Discussion

Physical properties of hand sanitizer gel preparations

The pH and viscosity test

The pH results of the hand sanitizer spray preparation must match the normal pH of the skin to avoid skin irritation. Adding essential oils affects the pH of the formula because essential oils have acidic and neutral properties. The *Eucalyptus globulus* essential oil used has a pH of 3.8, while the *Citrus limon* essential oil has a lower pH, namely 3.2. The viscosity test is carried out to determine the viscosity of the preparation. The test was carried out with a Brookfield Ametek viscometer at a speed of 100 rpm. Brookfield viscometers are also called cone/plate viscometers and are the most advanced and practical. A viscometer measures the twisting force of a cylindrical rotor immersed in a fluid. This fluid material is placed in a container while the shaft immersed in the fluid moves and takes viscosity measurements. Several things that affect the accuracy of this tool are sample size, cleanliness of the tool, type of material, and the time needed to stabilize the sample fluid before it can be read by the tool. The more liquid the preparation, the lower the viscosity value will be. The pH test results are shown in Table 2.

The pH test results on F1, F2, and F3 are in the value range of 5.2 – 5.9, where this value is within the specified pH standard range. The positive control has a pH of 6.8, and the negative control has a pH of 7.1. The resulting preparation meets the pH requirements for use on the skin, so it does not cause skin irritation. Based on SLD equation calculations, a relationship curve will be produced between EGEO and CLEO with the pH value shown in the graph in Figure 1. The tested preparation was a spray in suspension form due to the residue it carried. The test findings indicated that the viscosity of fluid F1 was 2.28 Pa.s, fluid F2 was 1.56 Pa.s, and fluid F3 was 2.10 Pa.s. The variation in viscosity is attributed to the specific gravity of the essential oil utilized. EGEO has a specific gravity of 0.92 g/mL at 20°C, but CLEO has a specific gravity of 0.8523 g/mL at the same temperature (Ulandari, Ningrum, and Permana, 2022). Lower specific gravity results in lower viscosity values. This preparation exhibits pseudoplastic flow behavior, meaning the liquid thins as the applied force increases.

Homogeneity test

The homogeneity test is carried out by dripping the preparation onto a clean and dry glass object and then covering it with a covered glass. The homogeneity test requires that no coarse grains be visible on the glass preparation. The aim is to ensure that the hand sanitizer spray preparation has the same uniformity. The preparation is declared homogeneous if there are no lumps on one side when observed using a microscope. Homogeneity test observations were carried out under a microscope with 40x and 100x magnification. The homogeneity test results are shown in Figure 2. Based on the homogeneity test parameters, the preparation gave poor results due to the residue left behind. Methylparaben is easily soluble in ethanol and ether and practically insoluble in essential oils. The incompatibility between methylparaben and essential oils causes methylparaben not to dissolve completely, leaving a residue in the preparation (Rowe, 2009).

Antibacterial activity of hand sanitizer spray

The antibacterial activity is categorised as very strong, strong, moderate, and weak if the diameter of the inhibition zone is ≥20, 10-20 5-10 and ≤5 mm, respectively (Masykuroh & Puspasari, 2022) (Table 3).

Table 2. *pH test results for hand sanitizer spray preparations*

Formulation	Physical properties	
	pH	Viscosity
F1	5.8±0,38	2.28 ± 0.32
F2	5.2±0,35	1.56 ± 0.29
F3	5.9±0,42	2.10 ± 0.34
Positive control	6.8±0.29	1.26 ± 0.74
Negative control	7.1±0.17	1.20 ± 0.37

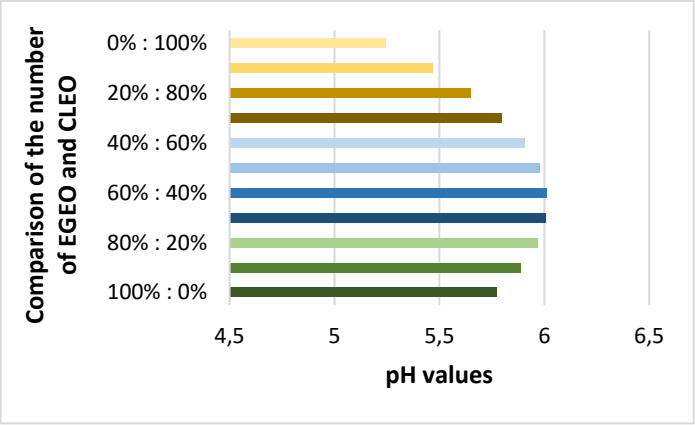

Figure 1. SLD graph on pH values

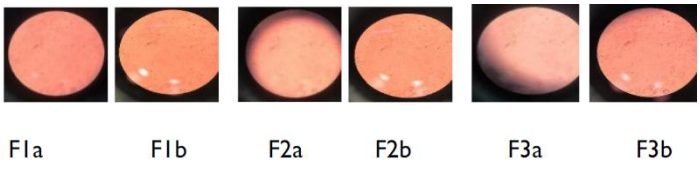

Figure 2. Hand sanitizer homogeneity test results. F1a: F1 with 40x magnification, F1b: F1 with 100x magnification, F2a: F2 with 40x magnification, F2b: F2 with 100x magnification, F3a: F3 with 40x magnification, F3b: F3 with 100x magnification

The essential oils disrupts the process of membranes or cell walls formation (Sefriyanti et al., 2020). To kill microorganisms, the test formulation material must enter the cell through the cell wall—the antibacterial substances in the formula cause cell membranes to be in a hypertonic environment. Hypertonic conditions can cause cell wall formation inhibition, so cells are only limited by a thin cell membrane (Mardiana & Handayani, 2017). The cell membrane of gram-negative bacteria is composed of a thin peptidoglycan layer, while the phospholipid layer is thicker. Antibacterial compounds can lyse cell membranes by dissolving the phospholipid layer of bacterial cell membranes (Lita et al., 2022).

The study showed that the hand sanitizer spray preparation with the active substances *Citrus limon* essential oil and *Eucalyptus globulus* essential oil had antibacterial activity against *S. aureus*, *E. coli*, and *P. aeruginosa* bacteria. In contrast, the negative control, namely placebo, had an inhibition zone for *E. coli* bacteria. Limonene, as an antibacterial, damages the cell wall structure, disrupting the work of active transport and proton strength in the cytoplasm, resulting in denaturation and inactivation of proteins such as enzymes. The antibacterial content in *Eucalyptus globulus* is 1,8-cineole, which is a terpenoid group. The mechanism of action is by reacting with porins on the outer membrane of the bacterial cell wall, forming strong polymer bonds that cause damage to the porins so that the 1,8-cineole compound can enter and reduce the permeability of the bacterial cell wall so that the bacterial cells will lack nutrition which causes bacterial growth stunted or dead (Han et al., 2020).

Table 3. Antibacterial test results for hand sanitizer spray preparations

Formulation	Inhibition zone diameter (mm)		
	Staphylococcus aureus	*Escherichia coli*	*Pseudomonas aeruginosa*
F1	10.44±1.01	20.77±4.99	19.9±6.12
F2	0±0	18.33±1	11.8±1.57
F3	15.33±5.36	19.66±7.30	11.33±2.64
Positive control	27.99±6.69	31.55±12.02	20.77±1.67
Negative control	0±0	7.77±0.19	0±0

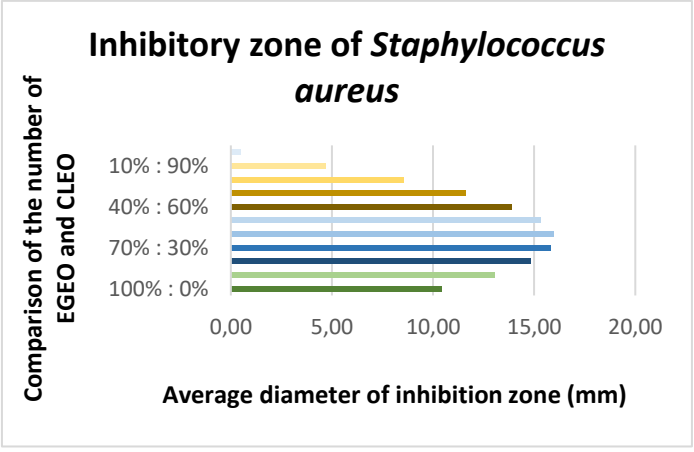

Figure 3. SLD graph inhibition zone value for *Staphylococcus aureus* bacteria

Antibacterial activity against Staphylococcus aureus

Staphylococcus aureus is a type of gram-positive bacteria. From the research results, the inhibition zone of *S. aureus* is smaller than that of *E. coli* and *P. aeruginosa*, which are gram-negative bacteria. The cell walls of gram-positive bacteria contain more peptidoglycan than those of gram-negative bacteria. Low permeability causes the active substances from essential oils to have difficulty penetrating the membrane of gram-positive bacteria, so the effect of the bacteria is less than optimal (Liu *et al.*, 2017). The testing process was carried out three times in replication; the results of the F1 test on *Staphylococcus aureus* bacteria obtained an average inhibition zone of 10.44 ± 1.01 mm, which was still included in the strong inhibition category. In Formula 2, no inhibitory power was found, which indicates that the hand sanitizer spray 100% *Citrus limon* essential oil does not have antibacterial power against *Staphylococcus aureus*. F2 did not show any inhibitory power, which indicates that the hand sanitizer spray 100% *Citrus limon* essential oil does not have antibacterial power against *Staphylococcus aureus*. Several causal factors include that *Staphylococcus aureus* bacteria can form a fibrin layer at a certain age and become more resistant to antibacterial compounds (Zeniusa *et al.*, 2019). The cell walls of gram-positive bacteria contain more peptidoglycan and polysaccharides (teichoic acids) and fewer lipids because more peptidoglycan causes low permeability, and the active antibacterial substances have difficulty penetrating the membrane of gram-positive bacteria, which causes the antibacterial effect to be less than optimal (Semeniuc et al., 2017). At F3, the average inhibition zone was obtained at 15.33 ± 5.35 mm, which is still in the strong category. This indicates that EGEO, when combined with CLEO, will have more substantial antibacterial power against *S. aureus* than given alone. Based on calculations using the SLD equation, a relationship curve will be produced between EGEO and CLEO, and the results of the antibacterial test against *Staphylococcus aureus* bacteria will be shown in the graph in Figure 3.

From the SLD calculation of the inhibition zone against S.aureus bacteria in Figure 2, it can be seen that theoretically, the EGEO: CLEO hand sanitizer with a ratio of 60%:40% will produce the highest inhibition zone against S.aureus bacteria with a value of ± 15.97 nm.

Antibacterial activity against Pseudomonas aeruginosa

P. aeruginosa bacteria are gram-negative bacteria. The test results found that the inhibition zone for gram-negative bacteria was more significant than that for gram-positive bacteria. Differences in the composition and structure of bacterial cell walls between gram-positive and gram-negative bacteria cause differences in responses. Gram-negative bacteria contain more lipids, fats, or fatty substances than gram-positive bacteria. The cell walls of gram-negative bacteria are also thinner than those of gram-positive bacteria. The outermost part of the cell wall of gram-negative bacteria is the outer membrane. The outer membrane

comprises phospholipids (inner layer) and lipopolysaccharides (outer layer). Therefore, the inhibition zone for gram-negative bacteria is more significant than for gram-positive bacteria (*Staphylococcus aureus*). Antibacterials will quickly attack gram-negative bacteria by dissolving the phospholipid layer on the outer membrane of the cell wall (Hamidah et al., 2019). In F1, which had a concentration of 100% EGEO, an average inhibition zone was found to be 19.9 ± 6.12 mm in the strong category. In F2, with a concentration of 100% CLEO, the average inhibition zone was 11.8 ± 1.56 mm, included in the strong inhibition category. In F3, with a 50% EGEO and 50% CLEO concentration, an average inhibition zone was 11.33 ± 2.64 mm in the strong inhibition category. In the positive control, the average inhibition zone was 20.77 ± 1.67 mm in the robust category. The negative control base or placebo does not have the antibacterial ability, as indicated by the absence of an inhibitory diameter in the NA medium. These results suggest that EGEO and CLEO, when formulated as hand sanitizer sprays, can inhibit *P. aeruginosa* bacteria.

Based on calculations using the SLD equation, a relationship curve will be produced between EGEO and CLEO, and the results of the antibacterial test against *Pseudomonas aeruginosa* bacteria will be shown in the graph in Figure 4. From the SLD calculation of the inhibition zone against *Pseudomonas aeruginosa* in Figure 3, it can be seen that theoretically, the EGEO: CLEO hand sanitizer with a ratio of 100%:0% will produce the highest inhibition zone against *Pseudomonas aeruginosa* bacteria with a value of ± 20.00 nm.

Antibacterial activity against *Escherichia coli*

Escherichia coli are a type of gram-negative bacteria. The test results show that *Escherichia coli* bacteria provide the most significant inhibitory power between Pseudomonas aeruginosa and Staphylococcus aureus bacteria. Factors influencing the differences in inhibitory power on E. coli may include variations in the composition of plant essential oils, structural configurations, functional groups, and the relative percentages of these components in plant essential oils that exhibit antimicrobial activity against the organism. The antibacterial activity of essential oils depends on the composition and concentration, as well as the type and concentration of the target used (Marchaban et al., 2019).

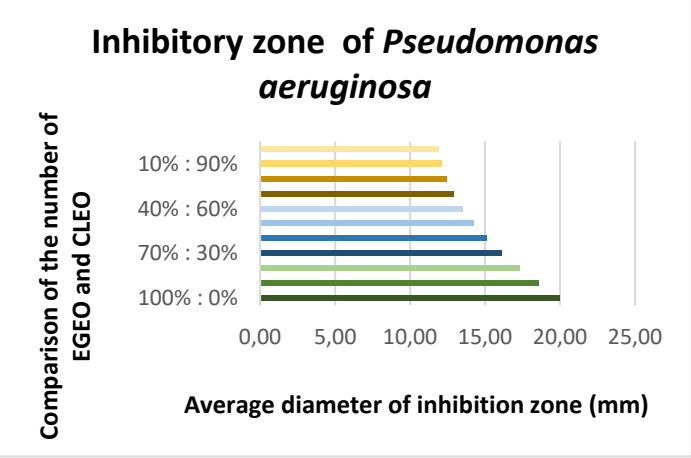

Figure 4. SLD chart of *Pseudomonas aeruginosa* inhibition zone values

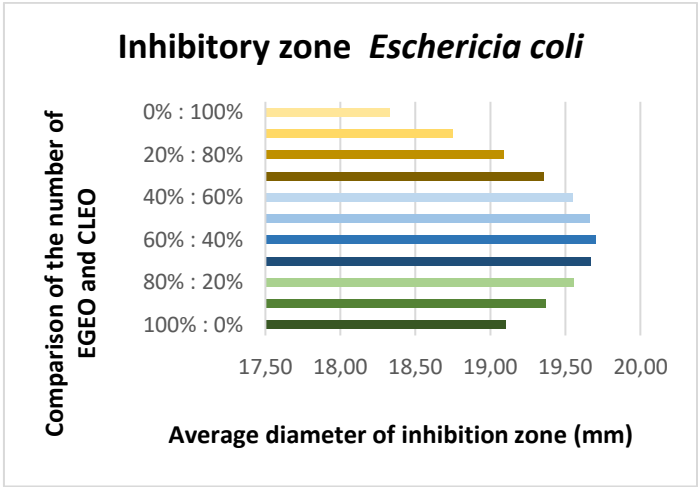

Figure 5. SLD graph inhibition zone value for *Escherichia coli*

In addition, differences in bacterial cell wall permeability are distinguished by the thickness of the peptidoglycan layer on the bacterial cell wall. Differences in cell wall permeability also affect the inhibitory power between bacteria. The cell walls of gram-negative bacteria, especially *Escherichia coli*, contain much less peptidoglycan, so the permeability of gram-positive bacteria is lower than that of gram-negative bacteria (Tavares et al., 2020).

The placebo negative control obtained an inhibition zone with an average of 7.77 ± 0.19 mm, which is in the fragile category, whereas for negative controls, ideally, no inhibition zone was found. The appearance of an inhibition zone in the negative control could occur due to the polarity factor of the 96% alcohol used as the base. Alcohol has a hydroxyl group (–OH) wh, which is hydrophilic, so it is more polar and a lipophilic group (Gunawan et al., 2020). Compounds that have optimum polar activity will have maximum antimicrobial activity. *Escherichia coli*is a bacterium with a thick polysaccharide capsule and negative hemagglutination. It is hydrophilic and has a lower adhesion ability. The interaction of an antibacterial compound with bacteria requires a balance between hydrophobic and lipophilic or hydrophilic-lipophilic balance (Alusinsing et al., 2017). Hydrophilic properties can dissolve antimicrobial compounds that dissolve in the water phase, which is where microbes live, but compounds that work on hydrophobic cell membranes require lipophilic properties so that a hydrophilic-lipophilic balance occurs so that optimum compound activity is achieved, causing the emergence of antimicrobial compounds that are soluble in water. The water phase is where microbes live—a zone of inhibition in the negative control of *Escherichia coli* media (Kurniasari et al., 2022).

Based on calculations using the SLD equation, a relationship curve will be produced between EGEO and CLEO with the antibacterial test results against *Escherichia coli* bacteria, shown in the graph in Figure 5. From the SLD calculation of the inhibition zone against *Escherichia coli* bacteria in Figure 4, it can be seen that theoretically, the EGEO: CLEO hand sanitizer with a ratio of 60%:40% will produce the highest inhibition zone against *Escherichia coli* bacteria with a value of ± 19.71 nm.

Determination of the optimum SLD formula

The SLD method can predict the optimum formula based on sample testing results. The optimum formula is determined from the highest total response value, close to 1. In this study, the optimal formulation was obtained by calculating the total response parameters of the pH test, the inhibition zone for *Staphylococcus aureus* bacteria, the inhibition zone for *Pseudomonas aeruginosa* bacteria, and the inhibition zone for *Escherichia coli.* The total response results based on the SLD calculation are shown in Figure 6.

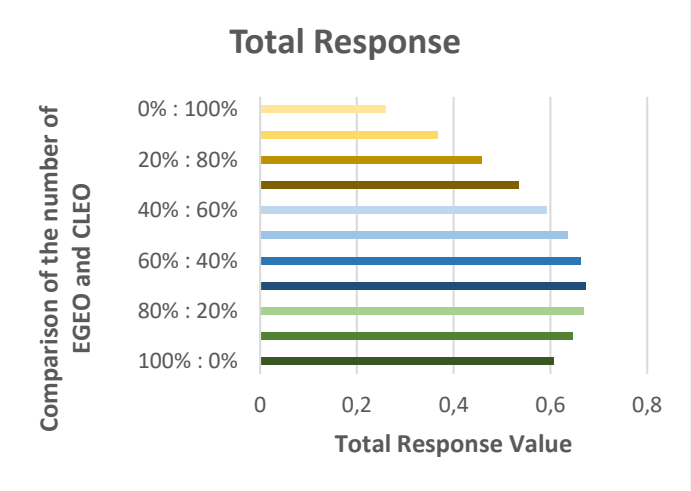

Figure 6. Total response value composition of *Eucalyptus globulus* and *Citrus limon* essential oil

Table 4. Results of the independent sample t-test validation formula with theoretical SLD

Parameter	Inhibitory zone			pH
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Escherichia coli*	
Theoretical equation results	15.80±4.49	16.12±5.44	19.67±7.99	5.66 ±0.37
Validation results	17.33±0.67	13.33±0.57	21.2±4.75	5.5
Significance (a=0.05)	0.591	0.428	0.790	0.578

Based on calculating the most significant total response, comparing EGEO and CLEO produces the most significant response value, 0.6733. Based on these results, it can be concluded that the best hand sanitizer formula composition in inhibiting *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* bacteria is a combination of 70% *Eucalyptus globulus* essential oil and 30% *Citrus limon* essential oil.

Validation of the Simplex Lattice Design method

The validity test is carried out to determine whether or not the SLD equation that has been created is valid. The test results between the SLD calculation and the actual data used Independent Sample T-Test analysis. This test used a ratio of *Eucalyptus globulus* essential oil versus *Citrus limon* essential oil at 70%: 30%. The results of the Independent Sample T-Test validation formula with theoretical SLD are shown in Table 4.

The results obtained from the SLD vs. test calculation data found no significant differences, which can be proven through the Independent Sample T-Test with a confidence level of 95%, which means that H0 can be accepted, which means the results of the experiment are the same as the predicted results. The validation test uses the Independent Sample T-test to see whether there is a significant difference. The results of the Independent Sample T-test have a 95% confidence level (α=0.05) that there is no significant difference in the physical properties and antibacterial test as seen from the significance results of p>0.005 in the pH test value, diameter of the inhibition zone for *Staphylococcus aureus* bacteria, diameter inhibition zone of *Pseudomonas aeruginosa* bacteria, and diameter of the inhibition zone of *Escherichia coli* bacteria.

4. Conclusions

The physical test resulting from the hand sanitizer spray preparation combined with *Eucalyptus globulus* essential oil and *Citrus limon* essential oil can be said to be less stable in terms of homogeneity parameters where there are still residual particles in the preparation, with pseudoplastic flow properties. The results of the pH examination showed a pH of 5.5. The optimum formula was obtained based on the highest total response value of the Simplex Lattice Design equation with a ratio of 70% *Eucalyptus globulus* essential oil versus 30% *Citrus limon* essential oil. Hand sanitizer spray, a combination of *Eucalyptus globulus* essential oil and *Citrus limon* essential oil, has antibacterial activity against Staphylococcus aureus bacteria with an average inhibition zone of 17.33 ± 0.67 mm, Pseudomonas aeruginosa bacteria with an average inhibition zone of 13.33 ± 0, 57 mm and *Escherichia coli* bacteria with an average inhibition zone of 21.2 ± 4.75 mm.

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