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EFFECTIVENESS TEST OF INHIBITORY POWER OF LIME PEEL EXTRACT (*Citrus aurantifolia*) LIQUID SOAP AGAINST *Staphylococcus epidermidis*

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ABSTRACT

Lime peel (*Citrus aurantifolia*) is a horticultural waste whose utilization has not been optimal and contributes 50-65% of the residue of the total weight so that if lime peel is not utilized it can cause environmental pollution. Lime peel contains saponins, flavonoids, alkaloids, tannins, and essential oils that have inhibitory properties so that they can be utilized as a form of liquid soap preparation from lime peel extract. This study aims to determine the effectiveness of the inhibitory power of liquid soap preparations from lime peel extract (*Citrus aurantifolia*) containing essential oils as active substances against *Staphylococcus epidermidis* bacteria. This study used an experimental research method with a disc diffusion technique method that determined the inhibition zone. Liquid soap with lime peel essential oil 6% had effective inhibitory power against *Staphylococcus epidermidis* with an average diameter of the inhibition zone of 10.21 mm which is included in the strong category. The positive control and basic control resulted in an inhibition zone formed of 14.42 mm and 7,33 mm respectively. The effectiveness of strong inhibition was because essential oils contain phenol compounds that had hydrophobic properties by splitting the fat layer on the mitochondrial cell membrane and bacteria which causes cells to become damaged and results in extensive leakage in bacterial cells so that bacteria die. Data analysis using bivariate analysis with One Way Anova test showed that essential oils did not have a significant effect on the growth of *Staphylococcus epidermidis* bacteria compared to basic control.

Keywords : Inhibitory Power, Lime Peel Extract, Essential Oil, *Staphylococcus epidermidis*

INTRODUCTION

The skin protects the inside of the body from physical or mechanical disturbances, heat or cold disturbances, radiation or ultraviolet light disturbances, germs, bacteria, fungi, or viruses. To prevent bacteria and microorganisms from entering the body through the skin of the hands, additional cleaning preparations such as soap are needed (Lestari et al., 2021). Washing hands with soap can release many germs that can cause infections at an affordable and easy price so that it can be considered as one of the effective ways to prevent disease (Zein and Newi, 2019).

One of the bacteria found on the skin of the hands that can cause skin infections is *Staphylococcus epidermidis* which is the main pathogen in living things, especially humans. *Staphylococcus epidermidis* bacteria are gram-positive bacteria that can cause opportunistic infections, especially in living things that are experiencing weakness in their immune systems (Nuryasuti, 2019). *Staphylococcus epidermidis* is one of the most common bacteria that causes nosocomial infections with an infection rate as high as

Staphylococcus aureus (Lee and Anjum, 2021). This bacteria can be removed with soap whose formulation contains ingredients with active antibacterial substances.

Early prevention to prevent infection can be done by using cleaning preparations containing active antibacterial and antiseptic compounds (Oktaviani et al., 2019). One of the ingredients that is easy to find in nature that has proven its efficacy for humans that contains antibacterial and antiseptic compounds is lime. Lime contains secondary metabolic compounds of flavonoids, tannins, polyphenols, saponins and alkaloids which have benefits as antiseptics, antioxidants, antibacterials, and can inhibit bleeding that occurs on the skin (Mu'min, 2021). The inhibitory mechanism of flavonoid compounds is thought to be able to dehydrate bacterial cell proteins and can damage cell membranes beyond repair (Parubak, 2019).

Essential oils in limes are natural substances that can inhibit the growth of harmful bacteria, one of which is *Staphylococcus epidermidis* (Mardelina, 2023). According to research by Ajeng (2021), essential oils can inhibit the growth of bacteria that are strong on the skin because the hydrophobic compound content of oxygenated hydrocarbons (phenols) can split the fat layer in the bacterial cell membrane which damages the cells so that there is extensive leakage in the cells which causes the death of bacterial cells.

So the study of making liquid soap using lime essential oil aims to identify the effectiveness of the inhibitory power of liquid soap with the formula of lime peel essential oil (*Citrus aurantifolia*) against *Staphylococcus epidermidis* bacteria. The inhibition test was carried out using the surface of Muller Hinton gel medium on a glass plate and an antibiotic disc with an antibacterial agent attached to the surface and incubated at a temperature of 35 ºC - 37 ºC for 24 hours, then the diameter of the bacterial growth inhibition zone produced around the disc was measured.

METHODS

The type of research that will be used is research using experimental methods to determine an effect or symptom that can cause a result of a certain treatment. This study was conducted to identify the effectiveness of the inhibitory power of liquid soap preparations with active ingredients of lime essential oil extract (*Citrus aurantifolia*) using the diffusion method.

The equipment used includes analytical scales, beakers (Pyrex), stirring rods, spatulas, measuring cups (Pyrex), water baths, porcelain cups, autoclaves, spirit lamps, Laminar Air Flow (Robust), incubators (Memmert), round loops, tweezers, scrapers, test tubes (Pyrex), micropipettes (Joablab), erlenmeyer flasks (Pyrex), petri dishes, stirring rods, aluminum foil, vortex (Thermo), matches, tweezers, and rulers or calipers. The materials used lime peel essential oil (*Citrus aurantifolia*), olive oil, SLS, potassium hydroxide (KOH), stearic acid, methyl paraben, NaCl, anhydrous sodium sulfate, distilled water, sodium carboxyl methyl cellulose (Na-CMC), propyl paraben, *Staphylococcus epidermidis* bacteria, nutrient gel, distilled water, paper disk, Doxycycline, and ethanol 70%.

The essential oil used as an active ingredient that provides antibacterial effects in the preparation uses lime peel essential oil from Lansida Essential Oil with CoA (Certificate Of Analysis) to prove the authenticity and quality of the ordered lime peel essential oil product. CoA contains several compounds contained in lime peel essential oil along with their concentrations. Liquid soap preparation is made using lime essential oil (*Citrus aurantifolia*) as the active ingredient with an essential oil concentration of 6%. In making liquid soap, all ingredients are weighed first according to the measurements in the formula. Dissolve each SLS and NaCl with 20 mL of distilled water, stir until homogeneous. Mix the SLS solution with the NaCl solution little by little until there are no white lumps indicating that the solution is evenly mixed and homogeneous. Add propyl paraben and

methyl paraben to the homogeneous mixture of SLS and NaCl solutions, stir until homogeneous and there are no lumps of material. Add glycerin, stir until homogeneous, then add CAPB, stir until the solution feels thick when stirred. Liquid soap is added with distilled water until the volume reaches 100 mL. Add HEC, wait for the soap to become thicker. After the preparation is finished, add lime essential oil and sufficient coloring, stir until homogeneous. Making lime peel essential oil hand soap is adjusted to each concentration that will be tested for its effectiveness. The formulation of liquid soap used in this research was presented in Table 1.

Table 1. Formulation of Liquid Soap

Material	Concentration (%)	Basic control (-)	Control (+)	Function
Lime peel essential oil (*Citrus aurantifolia*)	6	-	-	Active substance
Natrium Chloride (NaCl)	3	3	-	pH stabilizer
Cocamidopyl Betaine (CAPB)	11	11	-	Booster foam
Sodium lauril sulfat	1	1	-	Surfactant
Hydroxyethyl Cellulose (HEC)	1	1	-	Thickener
Gliserin	8	8	-	Moisturizer
Methylparaben	0,18	0,18	-	Preservative
Propil paraben	0,02	0,02	-	Preservative
Dye	qs	-	-	Colorant
Destilled water ad 100 ml	ad 100	ad 100	-	Solvent

All equipment used must be washed clean, sterilized, and dried first so that there is no contamination in the test. Tools such as measuring flasks, measuring cups, glass beakers, and micropipette tips are inserted into heat-resistant plastic or aluminum foil and then sterilized using an autoclave at a temperature of 121ºC for 15 minutes. Materials made of rubber are previously sterilized by soaking them in 70% alcohol. The ose needle used to collect bacteria is sterilized by heating the needle using a Bunsen burner flame. Tools made of glass such as Erlenmeyer flasks, beaker glasses, and test tubes are given cotton on their mouths before being sterilized. For tools such as stirring rods, tweezers, and petri dishes, they must be wrapped in paper first and then put in aluminum foil and sterilized in an oven at a temperature of 160 ºC - 170ºC for 2 hours (Setiawan et al., 2022).

NA media is made using 5 g of NA which is put into an Erlenmeyer flask then dissolved in 250 mL of aquadest and heated until boiling and all ingredients dissolve. The dissolved material is then put into an autoclave for sterilization at a temperature of 121 ºC for 15 minutes (Imansyah & Handayani, 2022). For positive controls, liquid soap is used which is available on the market. This positive control is made by mixing 1 gram of liquid soap preparation on the market with 10 mL of aquadest then stirring until smooth because only a concentration of 10% is needed (Magdalena, 2019).

The rejuvenation of test bacteria is carried out by taking bacteria using a sterile ose tip then scratching them in a zigzag manner on a petri dish that already contains slanted NA media evenly and repeatedly then incubating at a temperature of 37 ºC for 18 - 24 hours. The rejuvenated bacteria are used for activation (Supomo, 2021). To make a bacterial suspension, take several loops of bacteria that have gone through the rejuvenation process using a sterile ose tip then put them in a test tube containing 10 mL of sterile 0.9% NaCl solution then shaken using a vortex until obtained Mc turbidity 0.5 then let stand for 30 minutes. Then the bacterial suspension is taken using a pipette as much as 100 µL and then poured into the solidified NA media (Kosasi et al., 2019).

The solution for testing was made by preparing 3 test tubes and marking each tube for a concentration of 6%, negative control, and positive control. The purpose of taking

this concentration was to see the potential of the liquid hand soap preparation with the active ingredient of lime peel essential oil which was maximally able to produce an inhibition zone against the growth of Staphylococcus epidermidis bacteria. (Turnip et al., 2020).

The inhibition power test of the liquid soap preparation with lime essential oil extract was carried out using the disc diffusion method. Each petri dish containing NA media was then inoculated with a bacterial suspension taken using a micropipette. Sterile paper disks were soaked for 15 minutes in a suspension of the test solution of the lime essential oil hand soap preparation with a concentration of 6%. The positive control used a paper disk that had been soaked in a liquid soap solution on the market and the negative control used a paper disk that had been soaked in a soap base. The soaked paper disk is then placed on the NA media with a distance of no less than 24 mm from the paper disk and a distance of about 10-15 mm from the edge of the petri dish. The treatment that has been carried out is put in an incubator for 18-24 hours at a temperature of 37 ºC. How to know the presence of inhibition if a clear zone is seen free from bacteria (Sayekti, 2023)

RESULT AND DISCUSSION

The compounds contained in essential oils include Alpha-pinene, Benzene, Methyl, Beta-Pinen, Limonene, Alpha-Terpinolene, Limalool, E-Citral, Neryl acetate, and Beta-bisabolene. The content with the highest concentration is Benzene and Methyl as much as 6.33%, Beta-pinene as much as 10.81% and Limonene with a concentration of 36.32%. The evaluation was conducted on the preparation of 6% lime peel essential oil liquid soap and base, namely by organoleptic testing. The organoleptic tests conducted included testing the odor, color, and texture obtained in the soap. The results of the organoleptic test can be seen in Table 2.

Table 2. Organoleptic Test

Sample	Organoleptic			Viscosity	pH
	Odor	Color	Texture		
Basic control (-)	Odorless	Clear slightly white	Thick like gel	854 cPs	6,00
Lime peel essential oil 6%	Scent of lime	Light yellow	Thin slightly thick	205 cPs	5,77

Evaluation of liquid soap preparations was carried out using organoleptic tests, based on Table 2, the results showed that the soap base was clear, slightly white and odorless. The white color of the base was obtained from the foam that formed when mixing, after being left for a while the foam disappeared and revealed a clear, slightly cloudy soap base preparation. The texture obtained from this soap base is very thick and forms a semi-solid like a gel. The thick texture of the soap is caused by the presence of NaCl which when reacting with SLS as a surfactant can become a thickener (Mahayuni, 2023). Basically, the texture obtained from the base is very thick to the point of being like a gel.

The finished base when added with lime peel essential oil decreases in viscosity. The addition of lime essential oil causes the soap base to become thinner. The formulation of lime peel essential oil liquid soap at a concentration of 6% obtained a thin preparation texture. It showed that the more active essential oil substances added to the liquid soap formulation, the viscosity of the preparation decreases. The higher the concentration of essential oil added, the lower the viscosity of the soap. The viscosity requirements for liquid soap according to SNI standards are between 400 - 4000 cPs. Seen in the results compared to the viscosity requirements, the soap formula with a concentration of 6% essential oil does not comply with the liquid soap standard, the viscosity figure obtained is

205 cPs, according to the results of the texture test in the organoleptic test, the formula has a texture that is too thin for liquid soap.

The pH test is carried out to measure the appropriate acid-base level for liquid soap preparations in order to avoid irritation that occurs due to preparations that are too acidic or too alkaline. The pH test found that the base liquid soap preparation and 6% lime peel essential oil liquid soap were 6.00 and 5.77, respectively. The pH safety level according to SNI pH in liquid soap preparations is 4-11 (SNI 2588, 2017). In the homogeneity test, the preparation is applied to the object glass to see if there are any lumps formed. The test results indicate that there are no lumps in the preparation, which means that the preparation has been mixed homogeneously.

The effectiveness of liquid soap can be tested from the soap's ability to form an inhibition zone for the growth of Staphylococcus epidermidis bacteria. This test uses Staphylococcus epidermidis bacteria because these bacteria are commonly found on human skin. Pure cultures of Staphylococcus epidermidis bacteria were obtained from the Microbiology Laboratory of the Food and Nutrition Study Center, Gajah Mada University along with a certificate of purchase of the bacterial culture. The effectiveness test of the inhibitory power of this liquid soap preparation was carried out using the disc diffusion method (Kirby Bauer). The media used in this effectiveness test was NA (Nutrient agar) media and was carried out in LAF (Laminar Air Flow) to avoid external contamination. The results of the inhibitory power test of the preparation against Staphylococcus epidermidis can be seen in Table 3.

Tabel 3 Results of Inhibitory Power Effectiveness

Sample	Inhibition Zone Diameter			X ± SD	Result	Category
	R1	R2	R3			
Basic control (-)	8,35	6,30	7,35	7,33 ± 1,03 [a]	Moderate	<5mm (Weak) 5-10 mm (Moderate)
Control (+)	14,52	13,77	14,98	14,42 ± 0,61 [b]	Strong	10-20mm (Strong) >20mm (Very strong)
Lime peel essential oil 6%	10,29	10,10	10,25	10,21 ± 0,10 [a]	Strong	

The inhibition zone results do not include the diameter of the disc paper
Control (+) : Liquid soap on the market
-[a] : No significant difference
-[b] : There is a significant difference

Based on table 3, the results shown in the negative control treatment using a soap base without essential oil active substances formed a fairly large inhibition zone. Generally, negative controls do not show any inhibition zones formed, the factor that influences the presence of inhibition zones is because in the base formulation there is an SLS formula which is used as a surfactant which functions to clean dirt. SLS is an anionic surfactant which has an antimicrobial effect, SLS works by changing the properties of cell proteins and becoming a lyser on the bacterial cell wall by destroying cells using protease (Falk, 2019). The inhibition zone formed on the base is also influenced by methyl paraben and propyl paraben which are added to the formula with a function as a preservative. Methyl paraben and propyl paraben inhibit bacterial growth by inhibiting bacterial cell wall synthesis, oxidizing cellular components of cells, coagulation of irreversible cytoplasmic components and hydrolysis. The positive control in testing using liquid soap on the market resulted in an inhibition zone formed of 14.42 mm. The large inhibition zone formed in the positive control is because liquid soap on the market has a higher surfactant concentration and more chemicals contained in the formula that can inhibit growth and kill bacteria than the sample of the lime peel essential oil liquid soap preparation in the test.

The test carried out on the preparation was replicated three times and produced an inhibition zone diameter at an essential oil concentration of 6% with an average inhibition zone result of 10.21 mm, the diameter of the inhibition zone obtained at this concentration is also included in the strong category. The results of the inhibition zone formed indicate that lime peel essential oil added to the liquid soap preparation formulation resulted in an increase in the bacteriostatic activity of a compound contained in it, the larger the diameter of the inhibition zone formed (Puspita, 2020). The antibacterial effect or inhibitory power of lime essential oil also comes from other secondary metabolite compounds. Secondary metabolite compounds such as flavonoids, phenols, and alkaloids work in antibacterial activity by damaging bacterial cell walls. Flavonoids work by penetrating the peptidoglycan of bacterial cells so that the cell layer is formed incompletely, flavonoids also inhibit the work of bacterial DNA gyrase causing inhibition of the protein synthesis process so that bacteria cannot replicate .The effectiveness of bacterial inhibition contained in lime peel essential oil is because it contains oxygenated compounds (phenols) which have hydrophobic properties by working to split the fat layer found in the mitochondrial cell membrane and bacteria which causes cells to become damaged, this results in extensive leakage in bacterial cells so that bacteria die (Ajeng, 2021).

Normality test was conducted to determine whether the data was normally distributed. The normality test was taken from 3 treatment groups with each replication. The results of the normality test data were taken using Shapiro-Wilk. From the 3 treatment groups, the normality results showed that the data was normally distributed. The statistical data obtained were greater than 0.05 with a value on the basis of 0.973, a positive control of 0.737, and a preparation with a concentration of 6% obtained a data result of 0.384. All data tested for normality were normally distributed. The results of the homogeneity test obtained a significant value of 0.828, indicating that the value obtained was greater than and not less than 0.05, which means that the data produced was homogeneous and met the requirements of Oneway ANOVA. Then a test was carried out with parametric One Way ANOVA to determine whether or not there was a significant difference, the result was p = 0.000, that the data differed significantly between sample concentrations. Data analysis was continued with the Tukey HSD (Honestly Significant Difference) test which was used to show significant differences in the concentration and control groups. The effectiveness between each treatment group was tested using the Tukey HSD test. The results obtained from this test showed a significant difference in the control group + with a significant value of 0.000. Other treatment groups did not have significant differences between other treatment groups. Based on the results of the analysis, it indicates that there is no antibacterial effectiveness against lime peel essential oil with a concentration of 6% added to the liquid soap preparation.

CONCLUSION

Liquid soap preparation of lime peel essential oil (*Citrus aurantifolia*) with a concentration of 6% gave the greatest antibacterial effect with a diameter of the inhibition zone of 10.21 mm wider against the inhibition of the growth of *Staphylococcus epidermidis* bacteria and was included in the strong category. Based on the results of the data analysis test, there was no significant difference in the base with lime peel essential oil concentration of 6%, so this indicates that there is no effectiveness of the inhibitory power against the inhibition of the growth of *Staphylococcus epidermidis* bacteria.

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