Antibacterial Activity of Cajeput Oil (Melaleuca leucadendra) from UMKM Lamongan against Pseudomonas aeruginosa

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Abstract

Pseudomonas aeruginosa is the most common agent causing bacterial pneumonia. This bacterium is classified as a nosocomial infection-causing bacteria, thus often experiencing antibiotic resistance. This research aims to determine the antibacterial effect of cajeput oil (Melaleuca leucadendra). The study follows an experimental laboratory approach with a posttest control group design. The method used is diffusion with Mueller Hinton agar as the medium. The results obtained from the diffusion test are in the form of the diameter of the inhibition zone. The diameter of the inhibition zone of cajeput oil's antibacterial activity against Pseudomonas aeruginosa at concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% respectively are 15.73 mm; 20.82 mm; 17.91 mm; 11.83 mm; 11.25 mm; 10.92 mm; 10.46 mm; 9.93 mm; 9.57 mm. However, at 100% concentration, no inhibition zone was formed. The statistical analysis using the non-parametric Kruskal-Wallis test on the Pseudomonas aeruginosa group yielded $p < 0.05$, indicating an antibacterial effect at various concentrations of cajeput oil (Melaleuca Leucadendra) from micro, small, and medium enterprises (MSMEs) in Lamongan against Pseudomonas aeruginosa bacteria. The Man-Whitney test's analysis in this research indicates that cajeput oil (Melaleuca leucadendra) at concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% does not have a significant difference compared to the positive control ($p > 0.05$). Thus, cajeput oil (Melaleuca leucadendra) can substitute for the ability of ciprofloxacin in pneumonia treatment.

Keywords : cajeput oil (Melaleuca leucadendra), antibacterial, Pseudomonas aeruginosa

1. Introduction

Pneumonia is a disease caused by various microorganisms and results in infection of the lung parenchyma [1]. Based on data from the Ministry of Health of the Republic of Indonesia in 2014, the number of pneumonia sufferers in Indonesia in 2013 was 23% -27% and deaths from pneumonia were 1.19% [2].

The morbidity and mortality of pneumonia is mainly caused by a bacterial infection known as bacterial pneumonia. Bacterial pneumonia can be classified into 4 types, namely Community Acquired Pneumonia (CAP), Hospital Acquired Pneumonia (HAP), Healthcare Associated Pneumonia (HCAP), and Ventilator Associated Pneumonia (VAP) [3].

Pneumonia is often caused by Klebsiella pneumonia infection which is associated with the incidence of early onset Hospital Acquired Pneumonia [4]. Klebsiella pneumonia is mostly resistant to antibiotics, including ampicillin, cefuroxime, and cefazoline [5].

Apart from Klebsiella pneumonia, Pseudomonas aeruginosa is also a Gram-negative bacterium that often causes nosocomial pneumonia. The rate of Gram-negative infections continues to increase, so that there are increasingly limited therapies that can be used to treat these infection of Gram-negative bacteria[6]. The increasing number of Gram-negative infections, especially Klesbsiella pneumoniae and Pseudomonas aeruginosa accompanied by increasing bacterial resistance has now become a major problem. This shows that it is necessary to find new antibacterials that can developed one of which is traditional medicine.

The one alternative of traditional medicine is eucalyptus oil (Melaleuca leucadendra). Essential oil from eucalyptus leaves contains 32 active compounds, 7 main components of which are α-pinene (1.23%), cineol (26.28%), α-terpineol (9.77%), caryophyllene (3.38%)), α karyophyllene (2.76%), ledol (2.27%), elemol (3.14%). Whereas in dried eucalyptus leaves there are 26 components, 7 main components of which are α-pinene (1.23%), cineol (32.15%), α-terpineol (8.87%), caryophyllene (2.86%) , α-karyophyllene (2.31%), ledol (2.17%), and El emol (3.11%) [7]. The ability of the antibacterial activity of eucalyptus oil is due to the presence of the compound 1,8-cineol. This compound can act as an antibacterial with a broad spectrum that works by inhibiting bacterial growth through inhibiting the process of forming cell walls, damaging cell membranes, inhibiting enzyme activity, and destroying genetic material (DNA/RNA) present in bacteria [8].

Lamongan is one of the regions in Indonesia that produces eucalyptus oil. Many people are not too familiar with eucalyptus oil produced by Lamongan in terms of its potential or efficacy. Previously there had been research that examined the antibacterial effect of eucalyptus oil made in Lamongan on Escherichia coli bacteria. In that study, the inhibition zone began to appear at a concentration of 20% and the largest diameter at a concentration of 50%. This shows that there is an antibacterial effect of eucalyptus oil produced by Lamongan on Escherichia coli bacteria [9]. Based on previous research, researchers chose eucalyptus oil made in Lamongan which is extracted from melaleuca leucadendra so that it can be used as supporting data in developing treatments for Pseudomonas aeruginosa infections.

2. Methods

This research is a laboratory experimental type with a posttest control group design. In this study, two variables were considered to determine the antibacterial activity of eucalyptus oil (Melaleuca Leucadendra) against Pseudomonas aeruginosa bacteria, namely the independent variable and the dependent variable. The independent variables in this study were various concentrations (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%) eucalyptus oil (Melaleuca Leucadendra) from Lamongan and the result of the diameter of the inhibition zone formed through the diffusion test as the dependent variable.

2.1 Mueller Hinton Agar media preparation

Put 28 grams of Mueller Hinton Agar powder into an Erlenmeyer tube, then dissolve it in 1 liter of distilled water. Homogenize the mixture in the Erlenmeyer tube. Then, heat it on the hot plate until it boils. Cover the Erlenmeyer tube with aluminum foil. Sterilize the mixture in an Erlenmeyer tube using an autoclave at 121 ℃ for 15 minutes.

2.2 Mueller Hinton Broth media preparation

Put 21 grams of Mueller Hinton Broth powder into an Erlenmeyer tube, then dissolve it in 1 liter of distilled water. Homogenize the mixture in the Erlenmeyer tube. Then, heat it on the hot plate until it boils. Sterilize the mixture in an Erlenmeyer tube using an autoclave at 121 ℃ for 15 minutes.

2.3 Preparation of bacterial suspension (pseudomonas aeruginosa)

Pseudomonas aeruginosa bacteria from culture stocks were taken with loops and embedded in nutrient broth media. Then incubated at 37°C for 24 hours. Homogenize the bacterial suspension using a shaker at 120 rpm at room temperature. After that, the turbidity of the bacterial suspension was equalized with 0.5 Mc Farland solution (1.5 × 108 cfu/mL).

2.4 Preparation of Eucalyptus Oil (melaleuca leucadendra)

This study used eucalyptus oil (Melaleuca Leucadendra) taken from small and medium enterprises (SMEs) produced by Sendang Arum, Sambeng District, Lamongan Regency. Eucalyptus oil (Melaleuca leucadendra) was then made into a concentration of 100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10% in the following way: (1) Test tube measuring 13 x 100 mm with a total of 10 test tubes labeled number 1 to 10, (2) Test tube number 1 filled with 100% eucalyptus oil (without dilution) of 10 ml, (3) Test tube number 2 filled 9 ml of eucalyptus oil and 1 ml of solvent (ethyl acetate) and then homogenized with a vortex to get a final concentration of 90%, (4) Test tube number 3 filled with 8 ml of eucalyptus oil and 2 ml of solvent (ethyl acetate) and then homogenized with a vortex to get a final concentration of 80%, (5) Test tube number 4 filled with 7 ml of eucalyptus oil and 3 ml of solvent (ethyl acetate) and then homogenized with a vortex to get a final concentration of 70, (6) Test tube number 5 filled with 6 ml of eucalyptus oil and 4 ml of solvent (ethyl acetate) and then homogenized with a vortex to get a final concentration of 60%, (7) Test tube number 6 filled with 5 ml of eucalyptus oil and 5 ml of solvent (ethyl acetate) and then homogenized with a vortex to get a final concentration of 50%, (8) Test tube number 7 filled with 4 ml of eucalyptus oil and 6 ml of solvent (ethyl acetate) and then homogenized with a vortex to get a final concentration of 40%, (9) Test tube number 8 filled with 3 eucalyptus oil ml and 8 ml of solvent (ethyl acetate) and then homogenized with a vortex to get a final concentration of 30%, (10) Test tube number 9 filled with 2 ml of eucalyptus oil and 8 ml of solvent (ethyl acetate) and then homogenized with a vortex to get a final concentration of 20%, (11) Test tube number 2 filled with 1 ml of eucalyptus oil and 9 ml of solvent (ethyl acetate) and then homogenized with a vortex to get a final concentration of 10%.

2.5 Antibacterial effect test

The antibacterial effect test in this study using the diffusion method in the following way: (1) Prepare solid Mueller Hinton Agar in a petri dish, (2) Antibiotic ciprofloxacin disc for Pseudomonas aeruginosa bacteria with a strength of 30 µg are prepared as a positive control and ethyl acetate as a negative control, (3) Prepare a standardized suspension of Pseudomonas aeruginosa bacteria with 0.5 Mc Farland standard solution (1.5 × 108 cfu/mL), (4) Cotton swabs that have been sterilized then dipped into the bacterial liquid culture, (5) Wipe the cotton swab over the entire surface of Mueller Hinton Agar and repeat twice while rotating the plate 60˚, (6) Leave the petri dish for 3-5 minutes at room temperature, (7) Prepare wells with a diameter of 6 mm in four Mueller Hinton Agar media for Pseudomonas Aeruginosa bacteria, (8) Drop 100 µL of ethyl acetate into one wells, (9) Drop 100 µL eucalyptus oil with different concentrations (100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%) in 10 other wells , (10) Take the ciprofloxacin disc with tweezers, then the antibiotic disc is pressed on the surface of the agar medium which has been rubbed with suspension of Pseudomonas aeruginosa bacteria, (11) Incubation at 37℃ for 24 hours.

2.6 Inhibition zone diameter measurement

The petri dish is placed on a flat surface with a dark surface. Then the diameter of the inhibition zone in the control and each concentration was measured using a caliper from end to end of the clear zone (the part not overgrown with bacteria). Record the measurement results in millimeters.

3. Results

This study is a laboratory experimental test using diffusion to determine the antibacterial activity of cajeput oil (Melaleuca Leucadendra) from micro, small, and medium enterprises (MSMEs) in Lamongan at various concentrations against the growth of Pseudomonas aeruginosa bacteria. The research involved three replications. The results obtained from the diffusion test are in the form of the diameter of the inhibition zone. The measurement of the inhibition zone diameter is conducted using a digital caliper with millimeter precision (mm). The results of the inhibition zone diameter measurement in this study can be seen in Table 1.

Table 1. Inhibition Zone Diameter of Cajeput Oil (Melaleuca Leucadendra) against Pseudomonas Aeruginosa Bacteria

GROUP	1st repetition (mm)	2nd repetition (mm)	3rd repetition (mm)	Mean ± STD (mm)
(+) control	31.76	32.29	32.77	32.27 ± 0.5
(-) control	0	0	0	0
10%	15.54	15.67	15.99	15.73 ± 0.2
20%	21.88	21.22	19.37	20.82 ± 1.3
30%	18.73	18.82	16.19	17.91 ± 1.5
40%	11.63	11.94	11.93	11.83 ± 0.2
50%	10.83	11.51	11.40	11.25 ± 0.4
60%	10.36	10.92	11.48	10.92 ± 0.6
70%	10.18	10.28	10.93	10.46 ± 0.4
80%	9.94	9.93	10.11	9.93 ± 0.1
90%	9.28	10.11	9.32	9.57 ± 0.5
100%	0	0	0	0

The results of the inhibition zone diameter data were analyzed using statistical tests in the SPSS program. The first statistical tests in this research were the Shapiro-Wilk normality test and homogeneity test. The Shapiro-Wilk normality test results for Pseudomonas aeruginosa bacteria showed $p > 0.05$, indicating a normal distribution of data. However, the homogeneity test for Pseudomonas aeruginosa bacteria yielded $p < 0.05$, indicating non-homogeneous data. As both groups' data were non-homogeneous, the non-parametric Kruskal-Wallis test was conducted. The Kruskal-Wallis test results for the Pseudomonas aeruginosa group showed $p < 0.05$, indicating an antibacterial effect at various concentrations of cajeput oil (Melaleuca Leucadendra) from micro, small, and medium enterprises (MSMEs) in Lamongan against Pseudomonas aeruginosa bacteria. To identify significant differences in the mean inhibition zones among variables, a more specific Mann-Whitney test was needed. The Mann-Whitney test analysis for Pseudomonas aeruginosa bacteria revealed a significant difference in the mean inhibition zones at a concentration of 10% compared to the positive control and concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%. Similarly, a comparison between the groups of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and the negative control also showed significant differences in the mean inhibition zones.

Table 2. Mann-Whitney Test of the inhibition zone diameter formed at various concentrations of cajeput oil (Melaleuca Leucadendra) against Pseudomonas aeruginosa bacteria.

Treatment	K+	K-	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
K+	-											
K-	0,037	-										
10%	0,050	0,037*	-									
20%	0,050	0,037*	0,050	-								
30%	0,050	0,037*	0,050	0,050	-							
40%	0,050	0,037*	0,050	0,050	0,050	-						
50%	0,050	0,037*	0,050	0,050	0,050	0,050	-					
60%	0,050	0,037*	0,050	0,050	0,050	0,050	0,513	-				
70%	0,050	0,037*	0,050	0,050	0,050	0,050	0,127	0,275	-			
80%	0,050	0,037*	0,050	0,050	0,050	0,050	0,050	0,050	0,050	-		
90%	0,050	0,037*	0,050	0,050	0,050	0,050	0,050	0,050	0,050	0,376	-	
100%	0,037*	1,000	0,037*	0,037*	0,037*	0,037*	0,037*	0,037	0,037	0,037	0,037	-

4. Discussion

In this study, cajeput oil used originated from micro, small, and medium enterprises (MSMEs) in Sendang Arum, Sambeng District, Lamongan Regency. The main component of this cajeput oil is 1,8-cineole [10]. The quality of the cajeput oil used in this study was analyzed based on the Indonesian National Standard No. 3954 of 2014, indicating that cajeput oil (Melaleuca leucadendra) produced in Lamongan has superior quality with a concentration of 72.30% of 1,8-cineole content measured by gas chromatography [9]. The 1,8-cineole content present in this cajeput oil can inhibit bacterial growth by hindering cell wall formation, damaging cell membranes, inhibiting enzyme function, and destroying the genetic material (DNA/RNA) within bacteria [8]. In this study, the 1,8-cineole content in cajeput oil (Melaleuca leucadendra) produced in Lamongan demonstrated antibacterial effects against Pseudomonas aeruginosa bacteria. The antibacterial effect of cajeput oil (Melaleuca leucadendra) is evidenced by the formation of inhibition zones. The average measurement results of the inhibition zone diameter of cajeput oil (Melaleuca leucadendra) against Pseudomonas aeruginosa bacteria at concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% were 15.73 mm; 20.82 mm; 17.91 mm; 11.83 mm; 11.25 mm; 10.92 mm; 10.46 mm; 9.93 mm; 9.57 mm, respectively. However, at 100% concentration, no inhibition zone was formed. The research results indicate an inverse relationship between the concentration of cajeput oil (Melaleuca leucadendra) and the size of the resulting inhibition zone diameter. Cajeput oil (Melaleuca leucadendra) at a concentration of 20% exhibited more effective antibacterial activity compared to higher concentrations. Several possible factors might contribute to this, including the limited diffusion capacity of cajeput oil (Melaleuca leucadendra) into the media. The diffusion process of cajeput oil (Melaleuca leucadendra) into the media could be influenced by dilution factors. Additionally, the thickness of the agar media also influences the diameter of the bacterial growth inhibition zone. The effective thickness of agar media is around 4 mm. If the agar media thickness is less than 4 mm, the extract diffusion will be faster. On the contrary, if the agar media thickness exceeds 4 mm, the extract diffusion will be slower [11]. In the antibacterial activity test of cajeput oil (Melaleuca leucadendra) against Pseudomonas aeruginosa bacteria, the positive control used was ciprofloxacin antibiotic discs. The following are the interpretation categories and diameter limits of the ciprofloxacin inhibition zone.

Table 3. Diameter of inhibitory ciprofloxacin based on the Clinical & Laboratory Standards Institute (CLSI) 2021

Antimicrobial Agent	Disk Content	Interpretative Categories and Zone Diameter Breakpoints (mm)		
		Sensitive	Intermediate	Resistant
Ciprofloxacin	5 µg	≥ 25	19 - 24	≤ 18

The average measurement of the inhibition zone diameter of ciprofloxacin against Pseudomonas aeruginosa was 32.27 mm, indicating Pseudomonas aeruginosa's sensitivity to ciprofloxacin. The inhibition zone diameter by cajeput oil (Melaleuca leucadendra) against Pseudomonas aeruginosa at a 20% concentration was 20.82 mm. Therefore, the growth inhibition response of Pseudomonas aeruginosa to cajeput oil (Melaleuca leucadendra) at a 20% concentration falls into the intermediate category. However, at concentrations of 10%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, inhibition zone diameters of ≤ 18 mm were formed, indicating resistance. From the statistical analysis using the SPSS program, it was concluded that the data were normally distributed but not homogeneous. Thus, the analysis proceeded with the non-parametric Kruskal-Wallis test. The result of the non-parametric Kruskal-Wallis test for the Pseudomonas aeruginosa group was $p < 0.05$. Therefore, it can be concluded that there is an antibacterial effect at various concentrations of cajeput oil (Melaleuca Leucadendra) from micro, small, and medium enterprises (MSMEs) in Lamongan against Pseudomonas aeruginosa bacteria.

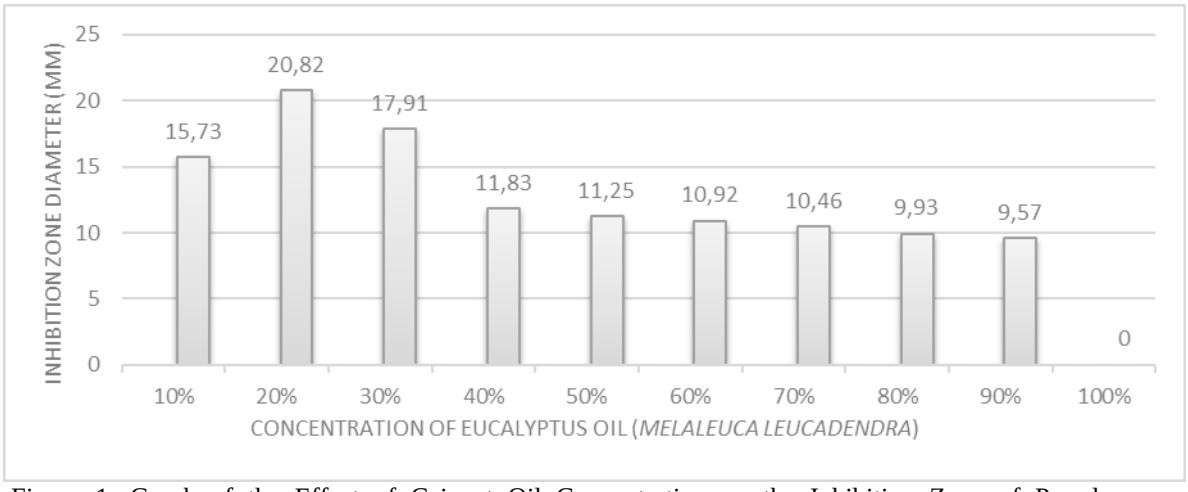

Figure 1. Graph of the Effect of Cajeput Oil Concentration on the Inhibition Zone of Pseudomonas Aeruginosa Bacteria

5. Conclusion

The cajeput oil (Melaleuca leucadendra) originating from micro, small, and medium enterprises (MSMEs) in Sendang Arum, Sambeng District, Lamongan Regency, has an antibacterial effect against Pseudomonas aeruginosa bacteria. The antibacterial effect of cajeput oil (Melaleuca leucadendra) on Pseudomonas aeruginosa reaches its peak at a concentration of 20%. According to the CLSI table (2020), the highest inhibition zone diameter formed at the 20% concentration falls into the intermediate category, hence concluding that cajeput oil (Melaleuca leucadendra) can be used as an antibiotic. Based on the results of the Mann-Whitney test in this study, it indicates that cajeput oil (Melaleuca leucadendra) at concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% does not show a significant difference compared to the positive control ($p > 0.05$). Thus, cajeput oil (Melaleuca leucadendra) can substitute for the capability of ciprofloxacin in pneumonia treatment.

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