The Potential of Cajuput Oil (Melaleuca leucadendra) Small and Medium Enterprises (SME) in Lamongan against Salmonella Typhi Bacteria

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Abstract

Typhoid fever is one of the health problems affecting communities worldwide, including in Indonesia. Typhoid fever is most commonly caused by Salmonella Typhi. The occurrence of antibiotic resistance against Salmonella Typhi is increasing, especially in South Asia and Southeast Asia, necessitating alternative treatments. Melaleuca leucadendra essential oil is one potential ingredient due to its high concentration of active antibacterial compounds. This study aims to assess the antibacterial activities of Melaleuca leucadendra essential oil from Lamongan Small and Medium Enterprises (SME) against Salmonella Typhi.

This study adopts a true experimental design with a posttest only control group design. The samples used in this study include Melaleuca leucadendra essential oil at concentrations of 10%, 20%, and 30%. These samples are being tested against Salmonella bacteria using the diffusion method. The research data obtained are in the form of the size of the inhibition zones in millimeters (mm). The data will be analyzed using the non-parametric Kruskal-Wallis and Mann-Whitney test.

The average diameter of the inhibition zone formed by Melaleuca leucadendra essential oil against Salmonella Typhi at a concentration of 10% is 28.33 mm, at 20% concentration is 33.80 mm, at 30% concentration is 36.08 mm. The statistical analysis results using the Kruskal-Wallis method for Salmonella Typhi data shows $p < 0.05$ Therefore, it can be concluded that there is an antibacterial effect of Melaleuca leucadendra essential oil from Lamongan Small and Medium Enterprises (SME) against Salmonella Typhi bacteria. The results of the Mann-Whitney test in this study, it indicates that cajuput oil at concentrations of 10%, 20%, and 30% does not have a significant difference compared to the positive control ($p > 0,05$), which is chloramphenicol. This suggests that cajuput oil may potentially serve as a substitute for chloramphenicol.

Keywords: Salmonella Typhi, Melaleuca leucadendra, antibacterial

Introduction

Typhoid fever or enteric fever is a disease caused by infection with the bacteria Salmonella typhi and Salmonella paratyphi (A, B, C) through the faecal-oral route.[1] Typhoid fever sufferers will usually experience a continuous high fever that can reach 39-40°C. Apart from that, typhoid fever can also cause other symptoms in the form of: weakness, abdominal pain, headache, diarrhea and constipation, coughing, and loss of appetite.[2] Most typhoid cases are caused by Salmonella typhi and

Salmonella paratyphi A, every year it is estimated that there are 26 million cases of typhoid and 5 million cases of paratyphoid, causing 215,000 deaths in the world.[3]

Typhoid fever requires antibiotic treatment such as chloramphenicol or cotrimoxazole as a first line, and fluoroquinolone as a second line. [4] However, between 1990-2017 Salmonella typhi and Paratyphi multi-drug resistant (MDR) were found in South Asia and Southeast Asia.[5] Salmonella typhi which is classified as MDR is no longer even affected by first-line antibiotics such as chloramphenicol, ampicillin, and co-trimoxazole, and sometimes shows resistance to second-line antibiotics such as fluoroquinolones.[6]

As cases of bacteria resistant to antibiotics increase, several studies have been conducted to test traditional medicines as an alternative for antibiotics. One example of potential traditional medicine is cajuput oil (Melaleuca leucadendra). Cajuput oil (Melaleuca leucadendra) has antibacterial active ingredients in the form of 1,8-cineole, linalool, and terpinen-4-ol which have been tested to inhibit the growth of Gram-positive and Gram-negative bacteria in certain concentrations.[7] Besides being able to inhibit bacterial growth, cajuput oil (Melaleuca leucadendra) can also be used as a respiratory tract treatment, anti-inflammatory, anti-fungal, anti-viral, anti-cancer, anti-hypertensive, antispasmodic, analgesic, and sedative.[8]

The antibacterial content of cajuput oil (Melaleuca leucadendra) also has the potential to prevent bacterial transmission. One important step to prevent the spread of disease is to wash your hands with a hand sanitizer that contains at least 60% alcohol.[9] However, the use of alcohol as a hand sanitizer can cause irritation and contact dermatitis.[10] Regarding to this, a study has proven that the use of essential oils as a hand sanitizer causes less irritation to the skin than the use of alcohol.[11] In addition, the content of 1,8-cineole in cajuput oil has been shown to strengthen the antibacterial effect of chlorhexidine gluconate as a topical antiseptic.[12] Therefore, cajuput oil has a good potential for an antibiotic alternative and to prevent disease transmission as an antiseptic.

Cajuput oil (Melaleuca leucadendra), which is naturally distributed in Indonesia, is known to have a very high content of 1,8-cineole.[13] Currently in Indonesia cajuput oil (Melaleuca leucadendra) has been produced at the Small and Medium Enterprises (SME) business level. One of the SMEs producing cajuput oil (Melaleuca leucadendra) is in Lamongan. Cajuput oil produced by SMEs in Lamongan is proven to inhibit growth of Escherichia coli at 20% to 50% concentration.[14] Based on the study above, researchers chose cajuput oil produced by SME in Lamongan to test its antibacterial activity against Salmonella typhi, so that this research can be used as additional scientific data in order to support herbal medicine scientification program.

Methods

The type of research conducted is a true experimental study aimed at testing the antibacterial effects of Cajuput oil (Melaleuca leucadendra) from Lamongan's small and medium-sized enterprises (SME) as the independent variable on the growth of Salmonella Typhi bacteria as the dependent variables using the diffusion method. The diffusion method is used to assess the potential antibacterial properties of a particular substance or compound against the targeted bacteria. The diffusion method in this research is carried out using Mueller-Hinton agar medium. Wells are then created inside the Mueller-Hinton agar plate that is been inoculated with each with Salmonella Typhi inoculum, which will be filled with cajuput oil solutions at concentrations of 10%, 20% and 30%, as well as ethyl acetate as the negative control, and chloramphenicol disks as the positive control. The results of this method can be observed from the inhibition zones on Mueller-Hinton agar, which are measured in millimeters (mm). Subsequently, the data results will be statistically analyzed using the Kruskal-Wallis and Mann-Whitney methods.

1. Preparation of Bacterial Suspension
   Salmonella Typhi bacteria from the culture stock were inoculated into Mueller-Hinton Broth and then incubated at 37°C for 24 hours. The Mueller-Hinton broth was then homogenized using a shaker at a speed of 120 rpm at room temperature. The standard concentration of the bacterial suspension in the diffusion method is 1,5 x 10^8 CFU/ml, which is equivalent to a 0.5 McFarland solution. Therefore, the bacterial suspension should be standardized to a 0.5 McFarland solution.
2. Preparation of Mueller-Hinton Media
   Mueller-Hinton agar media was prepared in the following ways: (1) Twenty-eight grams of Mueller-Hinton Agar were dissolved in 1 liter of distilled water, then homogenized. (2) The Erlenmeyer flask containing the mixture was heated on a hot plate until it boiled to dissolve the Mueller-Hinton Agar. (3) The Erlenmeyer flask was covered with aluminum foil. (4) The Erlenmeyer flask containing the mixture was placed in an autoclave at a temperature of 121°C for 15 minutes to sterilize it. (5) The mixture in the Erlenmeyer flask was poured into Petri dishes.
3. Preparation of Cajuput Oil Concentrate
   This research used 3 different concentrations of cajuput oil: 10%, 20%, and 30%. The solvent used for dilution is ethyl acetate, with the following steps: (1) Prepare 3 test tubes, label each with numbers 1-3. (2) Test tube number 1 is filled with 1 ml of cajuput oil and 9 ml of ethyl acetate as a solvent (10% concentration). (3) Test tube number 2 is filled with 2 ml of cajuput oil and 8 ml of ethyl acetate as a solvent (20% concentration). (4) Test tube number 3 is with 3 ml of cajuput oil and 7 ml of ethyl acetate as a solvent (30% concentration).
4. Test of Antibacterial Effect
   The antibacterial effect testing in this research is conducted using the diffusion method on Mueller-Hinton agar medium. The results are observed as the diameter of the inhibition zone formed on the medium after 24 hours of incubation at room temperature. The steps in the antibacterial effect testing using the diffusion method in this study are as follows: (1) Mueller Hinton Agar is prepared in Petri dishes. (2) Chloramphenicol discs with a strength of 30 µg are prepared as positive controls. (3) Ethyl acetate is prepared as a negative control. (4) Suspensions of Salmonella Typhi standardized to 0.5 McFarland (1.5 × 108 CFU/mL), are prepared. (5) Sterilized cotton swabs are dipped into the bacterial liquid culture. (6) The cotton swabs are streaked across the entire surface of the Mueller Hinton Agar, repeated twice while rotating the plate 60º. (7) Petri dishes are left undisturbed for 3-5 minutes at room temperature; they should not be left for more than 5 minutes to ensure that the bacteria are completely dry before attaching the antibiotic discs. (8) Wells with a diameter of 6 mm are prepared on the Mueller Hinton Agar. (9) Ethyl acetate (100 µL) is dropped into one of the wells. (10) Chloramphenicol discs are taken with forceps and placed in one of the wells. (11) Cajuput oil with different concentrations (10%, 20%, 30%) were added (100 µL) to 3 separate wells. (12) Incubation is carried out at 37ºC for 24 hours.
5. Measurement of Inhibition Zone
   The inhibition zone will appear as a clear area around the well on the Mueller Hinton agar, indicating that the colonies around the well have been inhibited by cajuput oil. The size of the inhibition zone formed will vary with each concentration and will be measured using calipers in millimeters. The obtained data will then be subjected to statistical analysis.

Results

This study is a true experimental research using the diffusion method to test the antibacterial activity of Cajuput oil (Melaleuca leucadendra) from Lamongan's Micro, Small, and Medium Enterprises (SME) against Salmonella Typhi bacteria. In this research, the antibacterial effect can be observed in the form of inhibition zones on the agar medium. The diameter of the inhibition zones can be measured using a caliper in millimeters (mm) as the unit of measurement. The measurement results of the inhibition zone diameters in this study can be seen in Table 1.

Table 1 Inhibition Zones of Cajuput oil (Melaleuca leucadendra) against Salmonella Typhi bacteria.

GROUP	1st repetition (mm)	2nd repetition (mm)	3rd repetition (mm)	Mean (mm)
(+) control	35,98	35,96	35,88	**35,94**
(-) control	00,00	00,00	00,00	**00,00**
10%	27,18	29,91	27,92	**28,33**
20%	29,16	42,40	29,85	**33,80**
30%	30,04	45,95	32,26	**36,08**

Based on Table 5.1, it can be observed that cajuput oil (Melaleuca Leucadendra) exhibits antibacterial activity against Salmonella Typhi. Cajuput oil shows antibacterial activity against Salmonella Typhi at a concentration of 10%, with an average inhibition zone of 28.33 mm. At a concentration of 20%, the average inhibition zone formed is 33.80 mm, and it reaches its peak at a concentration of 30% with an average inhibition zone of 36.08 mm.

Data Analysis

The data analysis was carried out through several stages, which included the normality test using the Shapiro-Wilk method, homogeneity test, non-parametric Kruskal-Wallis test, and Mann-Whitney test. The results of the Shapiro-Wilk normality test for the Salmonella Typhi data group show $p > 0.05$, indicating that the data is normally distributed. Subsequently, the homogeneity test resulted in $p < 0.05$, meaning that the set of data are not homogenous. As the data group were not homogeneous, the analysis continued with the non-parametric Kruskal-Wallis test. The results of the Kruskal-Wallis non-parametric test for the Salmonella Typhi data group show $p < 0.05$. A significance value < 0.05 in the Kruskal-Wallis non-parametric test in this study can be interpreted as an indication that different concentrations of Cajuput oil (Melaleuca leucadendra) produced by Lamongan's Micro, Small, and Medium Enterprises (SME) have an effect on Salmonella Typhi bacteria. The Mann-Whitney test is then conducted to determine whether there is a significant difference between two sets of data, both within the groups and compared to the negative and positive control. Significant differences ($p < 0.05$) were observed in the comparisons between the negative control and the positive control, as well as across all concentrations. In the comparison between the positive control, significant results were only found in the comparison with the negative control, while at concentrations of 10%, 20%, and 30%, there was no significant difference. In the case of the negative control, significant results were found in the comparisons with the positive control, as well as at concentrations of 10%, 20%, and 30%.

Table 2 Mann-Whitney Test on Inhibition Zones of Cajuput oil (Melaleuca Leucadendra) against Salmonella Typhi bacteria

Group	Control+	Control-	10%	20%	30%
Control+	-				
Control-	0,037	-			
10%	0,050	0,037	-		
20%	0,513	0,037	0,275	-	
30%	0,513	0,037	0,050	0,275	-

Discussion

The cajuput oil (Melaleuca leucadendra) used in this research was obtained from the Micro, Small, and Medium Enterprises (SME) Sendang Arum, Sambeng District, Lamongan Regency. Naturally occurring cajuput oil in Indonesia is known to have a very high content of 1,8-cineole.[13] A gas chromatography test has been conducted previously by the Perhutani Surabaya Indonesia Laboratory, which indicated that the eucalyptus oil from the "Sendang Arum" Small and Medium-sized Enterprises (SME) has a high concentration of 1,8-cineole, reaching 72.3%. According to the official website of BSILHK, cajuput oil with a 1,8-cineole compound content exceeding 60% falls under the category of super-quality of Indonesian National Standard (SNI).[14]

The content of 1,8-cineole in cajuput oil (Melaleuca leucadendra) has been shown to have broad antibacterial effects. At concentrations of 0.2–0.4%, it can inhibit the growth of Gram-positive bacteria, and at concentrations of 0.4–0.6%, it can inhibit Gram-negative bacteria.[7] Cajuput oil produced by Lamongan SME has also been proven to inhibit the growth of Escherichia coli bacteria at concentrations of 20%, 30%, 40%, and 50%.[15] According to Şimşek and Duman, the mechanism of 1,8-cineole in inhibiting bacterial growth is by damaging the structure and integrity of organelles and causing cytoplasm coagulation in bacteria due to the destruction of the bacterial cytoplasmic membrane.[12] 1,8-cineole has also been shown to kill Salmonella bacteria by destroying the cell wall and cell membrane, leading to intracellular material leakage and ultimately damaging cell integrity, resulting in bacterial cell death.[16]

This study used the diffusion method to observe the antibacterial effect of cajuput oil (Melaleuca leucadendra) on Salmonella Typhi bacteria. The antibacterial effect in the diffusion method can be assessed by measuring the inhibition zones formed on the test medium. An inhibition zone is the clear area surrounding the test bacterial colonies.[17] In general, the size of inhibition zones is measured in millimeters (mm). In this study, the average measurement of the inhibition zones of cajuput oil against Salmonella Typhi bacteria resulted in an inhibition zone of 28.33 mm at a concentration of 10%, 33.80 mm at a concentration of 20%, and 36.08 mm at a concentration of 30%. The results indicate a direct proportionality between the diameter of the inhibition zones and the concentration of cajuput oil against Salmonella Typhi. Related to the previous study by Taswin, in which the inhibition zones formed by cajuput oil against Salmonella Typhi were directly proportional to the concentration.[18] Compared to the study by Taswin, the inhibition zone formed by cajeput oil at a concentration of 10% in this study was measured to be larger, which is 28.33 mm, compared to Taswin's study which was 22 mm.[18] The differences observed in these two studies can occur due to several factors, including variations in the active content of cajuput oil, the choice of solvents used, the rate of cajuput oil diffusion through the agar, and the thickness of the agar used.[19] In this study, the content of 1,8-cineole in cajeput oil from "Sendang Arum" Small and Medium-sized Enterprises (SME) Lamongan is 72.3%, which is greater than the 1,8-cineole content in Taswin's research, which ranged from 50-65%.[18]

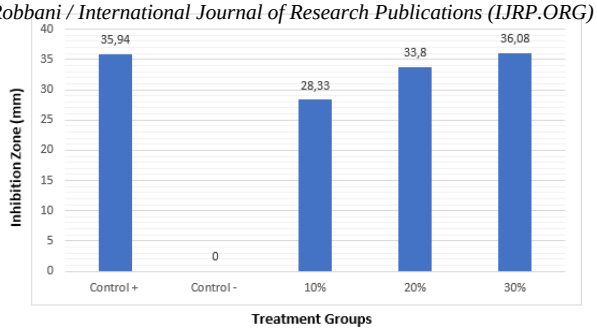

Figure 1. Graph of the Effect of Cajuput Oil Concentration on the Inhibition Zone of Salmonella Typhi Bacteria

The positive control used for comparison in this study consists of chloramphenicol antibiotic discs. Chloramphenicol was chosen as the positive control in this research because it is still a first-line treatment for Salmonella Typhi infections.[4] According to the CLSI (Clinical and Laboratory Standards Institute) table, the interpretation of chloramphenicol against Salmonella spp. is as follows: it is considered sensitive if an inhibition zone of ≥18 mm is formed, intermediate if an inhibition zone of 13-17 mm is formed, and resistant if the inhibition zone formed is ≤12 mm.[20] The average measurement of the inhibition zones of chloramphenicol against Salmonella Typhi in this study was 35.94 mm. The measurement results for the inhibition zones of chloramphenicol for Salmonella Typhi was ≥18 mm, which means that Salmonella Typhi bacterial samples used in this study are sensitive to chloramphenicol. The diameter of the inhibition zones produced by cajuput oil against Salmonella Typhi at concentrations of 30% was 36.08 mm, which is larger than the inhibition zone produced by chloramphenicol, measuring 35.94 mm. This suggests that cajuput oil may have a higher antibacterial effect than chloramphenicol at concentrations of 30% against Salmonella Typhi bacteria.

The results of the non-parametric Kruskal-Wallis test for the Salmonella Typhi data group show a p-value of < 0.05. This indicates that there is an effect of various concentrations of cajuput oil on the diameter of the inhibition zone in the bacteria. The results of the Mann-Whitney test in this study indicate that cajuput oil at concentrations of 10%, 20%, and 30% does not have a significant difference compared to the positive control, which is chloramphenicol. Therefore, there is a possibility that cajuput oil at these concentrations can be used as an alternative to chloramphenicol.

Conclusion

There is an antibacterial effect of cajuput oil (Melaleuca leucadendra) on Salmonella Typhi bacteria. The antibacterial effect can be observed from the inhibition zones formed by cajuput oil at concentrations of 10%, 20% and 30% against Salmonella Typhi. The diameter of the inhibition zones produced by cajuput oil against Salmonella Typhi at concentrations of 30% was 36.08 mm, which is larger than the inhibition zone produced by chloramphenicol, which was 35.94 mm. This suggests that cajuput oil may have a higher antibacterial effect than chloramphenicol at concentrations of 30% against Salmonella Typhi. Furthermore, based on the results of the Mann-Whitney test in this study, it indicates that cajuput oil at concentrations of 10%, 20%, and 30% does not have a significant difference compared to the positive control, which is chloramphenicol. This suggests that cajuput oil may potentially serve as a substitute for chloramphenicol in the treatment of typhoid fever and help reduce antibiotic resistance cases.

Acknowledgement

The author would like to thank Dr. Wiwin Retnowati, S.Si., M.Kes. (wiwin-r@fk.unair.ac.id) as the first supervisor, Nurina Hasanatuludhhiyah, dr., M.Si. (nurina-h@fk.unair.ac.id) as the second supervisor, and Dr. Agung Dwi Wahyu Widodo, dr., M. Si., M.Ked. Klin., Sp. MK(K) (agung-d-w-w@fk.unair.ac.id) as the examiner.

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