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In Vitro Inhibitory Activity of Dayak Onion (Eleutherine bulbosa Mill.) Essential Oil Against Klebsiella pneumoniae

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ABSTRACT

Background: The cases of Klebsiella pneumoniae infection in West Java province is still high. Klebsiella pneumoniae bacterium can produce the Extended Spectrum Beta Lactamase enzyme leading to antibiotics resistant, so it is important to look for natural and inexpensive antibiotic alternatives; one of which is Dayak onion plant (Eleutherine bulbosa Mill). The content found in Dayak onion is believed to inhibit the growth of Klebsiella pneumoniae bacteria. This study aims to determine the effectiveness of Dayak onion essential oil on the growth of Klebsiella pneumoniae bacteria.

Methods: This study was an experimental study with a post-test control group design using the culture of Klebsiella pneumoniae on the media Muller Hinton Agar in 7 groups. Groups 1 to 5 (K1 to K5) were given sequentially Dayak Onion essential oil solution in 10% DMSO solvent with concentrations of 50%, 25%, 12.5%, 6.25%, and 3.125%. Group 6 (K6) was positive control (+) with 5 µg Levofloxacin, and Group 7 (K7) was Negative (-) with 10% DMSO. The treatment results were measured by the diameter of the host zone and the collected data were analyzed using One-way ANOVA with appropriate Post hoc analysis.

Results: The results showed that that there are differences in each group to inhibit the growth of bacteria ($p < 0.005$). K1 are higher than K2, K3, K4, K5, and K7 with increasing concentrations showed greater inhibition. Levofloxacin (K6) showed the highest inhibitory power.

Conclusion: The results showed that that there are differences in each group to inhibit the growth of bacteria ($p < 0.005$). K1 are higher than K2, K3, K4, K5, and K7 with increasing concentrations showed greater inhibition. Levofloxacin (K6) showed the highest inhibitory power.

Keywords: Klebsiella pneumoniae, Dayak onion essential oil, bacterial inhibition
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1. Introduction

Indonesia is a country that has an increasing number of infectious diseases, one of which is a disease caused by the bacterium Klebsiella pneumoniae. The latest estimate in 2014 in the West Java province, the case of Klebsiella pneumoniae infection was 48.1%, increasing by 3.1% compared to 2013 coverage of 45%. Especially in Cirebon, there are 95.62% of children under five diagnosed with Klebsiella pneumonia infection (Dinas Kesehatan Provinsi Jawa Barat, 2015). Populations which are

susceptible to Klebsiella pneumoniae infection are children less than 2 years old, adult over 65 years old and those who have health problems (malnutrition and immunological disorders) (Budijanto and Sutardjo, 2018; Marcdante and Kliegman, 2014). The Klebsiella pneumoniae bacterium is a gram of negative bacterium that can cause nosocomial infections, infections of the respiratory system, and infections of the tract (Budijanto and Sutardjo, 2018). Bacterial virulence factors that affect pathogenesis in the human body are polysaccharide capsules, endotoxin, cell wall receptors. The capsule structure functi (Brooks et al., 2013). Ons to protect bacteria from phagocytosis by polymorphonuclear granulocytes and prevent bacterial death by bactericidal serum. The presence of antigens in the capsule can increase bacterial pathogenicity Treatment of klebsiella pneumoniae infection includes levofloxacin which belongs to the fluoroquinolone group which has a broad spectrum anti-bacterial effect. The action mechanism of levofloxacin is by inhibiting the DNA-gyrase enzyme resulting in DNA chain damage and causing bacteria to die. However, continuous antibiotics can resistance to bacteria because klebsiella pneumoniae is capable of producing Extended Spectrum Beta Lactamase (ESBL) which can paralyze the work of various types of antibiotics (Katzung et al., 2002; Perhimpunan Dokter Paru Indonesia, 2003). Therefore, to reduce the occurrence of resistance to antibiotics, researchers use Dayak onion essential oil as an alternative treatment Dayak onion (Eleutherine bulbosa Mill.) is a plant originating from Kalimantan which has an anti-bacterial effect (Firdaus, 2014). The content of onion essential oil which is an important role in inhibiting bacterial growth is alkaloid that works by disrupting the peptidoglycan component (Karou et al., 2006). Flavonoids work in 3 ways: inhibiting nucleic acid synthesis, inhibiting cell membrane function and inhibiting energy metabolis (Rijayanti, 2014). Tannins work to inhibit the reverse transcriptase enzyme (Firdaus, 2014). All compounds contained in Dayak onion essential oils have their respective duties in inhibiting bacterial growth. Previous research shows that Dayak onion was able to inhibit the growth of staphylococcus aureus bacteria. Therefore, this study aims to determine the effectiveness of Dayak onion essential oil on the growth of klebsiella pneumoniae bacteria.

2. Method

This study was an experimental study with a post-test control group design. This experimental study was conducted in the microbiology laboratory of the Faculty of Medicine, Swadaya Gunung Jati University, from January to February 2019. The material used was Dayak onion (Eleutherine bulbosa Mill.) obtained from Central Kalimantan province. Pure Klebsiella pneumoniae bacteria were obtained from the Microbiology laboratory of the Faculty of Medicine, University of Indonesia. Cultured bacteria were in Mueller Hinton media. As a solvent, DMSO 10% and aquadest were used. For comparison, levofloxacin antibiotics were used. The tools used were vortex, ose, autoclave, incubator, test tube, micropipette, blue tip, test tube rack, burner and matches, and sterilized petri dishes. Muller Hinton Agar (MHA) media was made by mixing 38 grams of Mueller Hinton Agar (MHA) dissolved in 1 liter of distilled water, heated, stirred until homogeneous and sterilized using an autoclave at 1210 C, for 15 minutes. The Klebsiella pneumonia suspension was cultured by incubating for 2-6 hours with a temperature of 37oC and turbidity was likened to the standard Mc Farland 0.5. Essential oils of Dayak onion (Eleutherine bulbosa Mill.) are obtained from the Lansida Herbal Laboratory in Yogyakarta, and the concentration of essential oils of Dayak onion were 50%, 25%, 12.5%, 6.25%, and 3.125%, respectively, by adding 10% DMSO solvent up to the volume expected. The concentration was chosen because previous research shows that the increasing concentration will make the inhibition zone bigger. However, the previous studies have not examined up to a concentration of 50%. The researchers used Dayak onion essential oil of 50% compared to its half concentration, starting from 50%, 25%, 12.5%, 6.25% and 3.125%. As a comparison, the control (K6) was used in the form of a Levofloxacin solution of crushed tablets of 1 mg into 5 ml of distilled water, while negative control (K7) has 10% DMSO. The experimental procedure was carried out by culturing the suspended standard Klebsiella pneumonia bacteria Mc Farland 0.5 on the homogeneous Muller Hinton Agar (MHA)

medium. The research subjects were grouped into 7 groups and made sumps in order to use a cork borer in the petri dish, then filled the well with K1 with Essential Oil Solution with a concentration of 50%, K2 with a concentration of 25%, K3 with a concentration of 12.5%, K4 with a concentration of 6.25% , K5 with a concentration of 3.125%. As a control, K6 was given levofloxacin of 5 µg, and K7 was given 10% DMSO. Then incubated at 37°C for 1 X 24 hours before the inhibition was observed. The potential for anti-bacterial was marked by the presence of a clear zone around and measured in millimeters in diameter. The data were obtained by using a statistical data processing computer program, i.e. One-way ANOVA test, to test differences between groups. The data were examined for the normality of the distribution using the Shapiro Willik test, because the test results showed that the data were normally distributed. Thus, the requirement for One-way ANOVA was fulfilled. Furthermore, the Bonferroni post hoc test was used to see the difference in inhibitory power in each treatment group.

3. Results

Measurements of inhibitory zones in bacteria were carried out after bacteria were incubated at 37°C for 1 X 24 hours. Differences in inhibition zones in all groups were analyzed using descriptive analysis. Figure 1 shows the average difference in each treatment group.

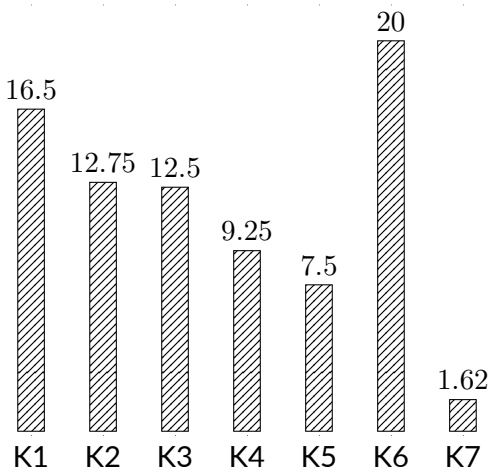

Figure 1. Diameter inhibition zone

Figure 1 shows the differences in mean Inhibition zones in each group. K1 of onion essential oil with a concentration of 50%, K2 concentration of 25%, K3 concentration of 12.5%, K4 concentration of 6.25%, K5 concentration of 3.125%, K6 positive control, K7 of negative control.

The inhibition of bacterial growth in each group was analyzed using the One-Way ANOVA test to see the difference in inhibition of bacterial growth between treatment groups. The One-Way ANOVA test results show that there are differences in inhibition zones with p-values of <0.05. At K1, administering essential oils of Dayak onion with a concentration of 50% shows that there was a inhibition zone on bacterial growth with an average diameter of 16.50 mm. At K2, administering essential oils of Dayak onion with a concentration of 25% obtained an average diameter of inhibition zone of 12.75 mm. At K3, administering essential oils of Dayak onion with a concentration of 12.5% obtained the average diameter of the inhibition zone of 12.50 mm. At K4, administering essential oil of Dayak onion with a concentration of 6.25% obtained an average inhibition zone diameter of 9.25 mm. At K5, administering Dayak onion essential oil with a concentration of 3.125% obtained an average zone diameter the inhibition was 7.50 mm. At K6, administering levofloxacin of 5 µg obtained a mean diameter of the inhibition zone of 20 mm. Meanwhile, at K7, the negative control of 10% DMSO obtained an average diameter of the inhibition zone of 1.62 mm. The strongest inhibition was in positive control K7 with a

diameter inhibition zone of about 20 mm, followed by K1 using essential oil of Dayak onion 50% with a diameter of inhibition zone 16.50 mm, followed by K2 using essential oil of Dayak onions 25% with a diameter of 12.75 mm, and followed by K3 using essential oils of Dayak onion 12.5% with a diameter of inhibition zone of 12.50 mm. Inhibition in the medium category is found in K4 using essential oils of Dayak onion 6.25% with diameter inhibition zones of 9.25 mm, followed by K5 using essential oils of Dayak onion 3.125% with diameter inhibition zones of 7.50 mm, and the lowest inhibitory power was found in negative controls (K -) with the inhibitor zone diameter of 1.62 mm. All data in each group show that the data were normally distributed and homogeneous data. Therefore, the data was carried out by Post hoc Bonferroni analysis to see differences between groups in inhibiting bacterial growth.

Table 1. The Bonferroni Post hoc test analyzes differences in inhibition zones

	K1	K2	K3	K4	K5	K6	K7
K1	#						
K2	0.004	#					
K3	0.002	1.000	#				
K4	0.000	0.090	0.018	#			
K5	0.000	0.000	0.000	1.000	#		
K6	0.009	0.000	0.000	0.000	0.000	#	
K7	0.000	0.000	0.000	0.000	0.000	0.000	#

Table 1 shows that K1 compared with K2, K3, K4, K5, K6 and K7 are different in inhibiting bacterial growth ($p < 0.005$). There was no significant difference in K3 compared to K2 in inhibiting bacterial growth ($p = 1$). There was no significant difference in K4 compared to K2 in inhibiting bacterial growth ($p = 0.090$) while K4 compared to K3 was different in inhibiting bacterial growth ($p < 0.005$). K5 compared with K1, K2, K3 was different in inhibiting bacterial growth ($p < 0.005$) while K5 compared to K4 had no significant difference in inhibiting bacterial growth ($p = 1$). K6 compared with K1, K2, K3, K4, K5 was different in inhibiting bacterial growth ($p < 0.005$). K7 compared with K1, K2, K3, K4, K5, K6 was different in inhibiting bacterial growth ($p < 0.005$).

4. Discussion

This study was carried out in 24 hours of treatment showing that the administration of positive control (K6) using levofloxacin of 5 µg had the strongest and most effective inhibitory effect with a diameter of 20 mm. Previous research said levofloxacin inhibits important enzymes, topoisomerase, which is needed by bacterial cells for cell division DNA. In addition, levofloxacin has bactericidal and bacteriostatic effects even with low doses which cause death in bacteria and inhibitory forces in petri dishes are formed (Grillon et al., 2016; Riahifard et al., 2017). The mechanism of action of the Levofloxacin medicine is by inhibiting the DNA-gyrase enzyme, resulting in damage to the DNA chain and causing bacterial death (Ganiswarna, 1995). However, the continuous use of Levofloxacin can lead to antibiotic resistance because Klebsiella pneumoniae is capable of producing Extended Spectrum Beta Lactamase (ESBL) and Klebsiella pneumoniae Carbapenemase (KPC) which can paralyze the work of various types of antibiotics. The mechanism of action of ESBL is by attacking penicillin and cephalosporin β-lactam ring bonds and producing penicillanic acid and chalcosporic acid so that antibacterial compounds are inactive while Klebsiella pneumoniae Carbapenemase (KPC) works as a self-protection cell wall from external threats (Riahifard et al., 2017; Ganiswarna, 1995). The treatment group K1 shows effective results with inhibition zone diameters of 16.50 mm when compared with K2 group with inhibition zone diameter of only 12.7 mm, K3 group with inhibition zone diameter of only

12.5 mm, K4 group with inhibition zone diameter of only 9.25 mm, group K5 with inhibition zone diameter of only 7.5 mm, and group K7 with inhibition zone diameter of only 1.62 mm. These results are in accordance with previous studies stating that ethanol extract of Dayak onions can inhibit microbes with minimal inhibitory concentrations and an inhibitory diameter of 14.49 mm (Puspadewi et al., 2013). At a concentration of 15%, it can inhibit by 18.6 mm (Novaryatiin et al., 2018). In this case, the higher the concentration, the wider the inhibitory zone. Compounds that are thought to be contained in onion essential oil include flavonoids, alkaloids, tannins that have different working mechanisms. Flavonoids have antibacterial properties in which the activity of flavonoids against bacteria is done by damaging bacterial cell walls consisting of lipids and amino acids. As a result, flavonoids are able to enter the cell nucleus bacteria and will react with DNA and cause damage to the structure of the lipid DNA, so that the bacteria will lysis and the cell will die (Sari et al., 2015). Besides, flavonoids also have immunomodulatory abilities that can increase the production of IL-2 (interleukin 2) stimulating the proliferation and differentiation of T cells then Th1 cells secrete IFN-γ (interferon gamma) which has the potential to activate macrophages (Indrisari et al., 2017). The mechanism of action of alkaloids inhibits the formation of peptidoglycan so that the cell wall is not fully formed and causes cell death. While the Tannin function inhibits the reverse transcriptase and DNA topoisomerase enzymes. Inhibition of the reverse transcriptase enzyme can cause no reverse transcription process to occur so that DNA is not formed and the Klebsiella pneumoniae bacterium will die (Amanah et al., 2019). The characteristics of Dayak onion essential oil are also known to have two solubility properties, namely hydrophilic and lipophilic, which are expected to have a significant effect on inhibiting bacteria. It is because their bioactive compounds affect bacterial cell walls making them more permeable and causing extensive leak from bacterial cells or the release of molecules and critical ions causing death in bacteria (Ganiswarna, 1995; Sari et al., 2015). Gram negative bacteria easily absorb solutions, making it easier for solutes to enter the bacterial cell wall. The closer the bacteria are to the solute from the well which diffuses into the agar, and the more solute the substance becomes, the easier the bacteria will be killed by the substance (Rustama et al., 2005). Although, peptidoglycan in the cell wall of Gram negative bacteria is not easily destroyed by onion essential oil. However, if the concentration of the substance is sufficiently concentrated, the bacteria will be killed. This research has been carried out and tried in accordance with the appropriate procedure, however it still has limitations, one of which is that the researchers do not know which active substance has the most anti-bacterial role in this Dayak onion essential oil.

5. Conclusion

This study shows that the antibiotic levofloxacin has a wider inhibition zone diameter compared to the administration of Dayak onion essential oil. Dayak onion essential oil shows a smaller inhibition zone, however, the potential to inhibit it appears and cannot be ignored. For further research, it is recommended to isolate the active ingredient contained in Dayak onion which is estimated to be equivalent to administer antibiotics.

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Conflict of Interest

There is no conflict of interest.

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