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Research Article

The Effect of Sembukan Leaf Extract (Paederia Foetida) on the Growth of Klebsiella Pneumoniae Bacteria with the Disc Method and the Contact Method

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ABSTRACT

Sembukan leaf (Paederia foetida) is a wild plant which is known to have many benefits and can be used as a medicinal plant. These plants contain bioactive compounds that function as antibacterial. This study aims to determine the ability of sembukan leaf extract to inhibit the growth of Klebsiella pneumoniae. The methods used in this study were disc diffusion and contact methods, with variations in the concentration of curd leaf extract 0%, 25%, 50%, 75% and 100%. The research data obtained were analyzed by One Way ANOVA with 95% confidence level ($p<0.05$). The best concentration with the disc diffusion method at a concentration of 75% with an inhibitory diameter of 15.25 mm with a strong category and the best percentage of inhibition method on sembukan leaf extract at 69.42% with a bacteristatic category at a concentration of 75%, while the results of the One Way ANOVA test that has been done obtained a significant value of $p=0.000$ ($p<0.05$) indicates a significant effect on the administration of sembukan leaf extract against Klebsiella pneumoniae bacteria. The results showed that sembukan leaf extract was able to inhibit the growth of Klebsiella pneumoniae.

***Keywords:** Contact method, disc method, Klebsiella pneumoniae, sembukan leaf (Paederia foetida)*

INTRODUCTION

The Indonesian state consists of various tribes, each tribe has a wealth of knowledge about traditional medicine which is one of the interesting aspects to be studied more deeply as an effort to prove scientifically through laboratory tests. The efforts that have been made will later add to the wealth of knowledge that can maximize the use of local traditional plants to support the herbal medicine industry in Indonesia(Ismawati et al., 2020). Utilization of the development of natural materials as traditional medicine today is more in demand and widespread in the community so that its use is increasing(Ningsih et al., 2017).

Today, traditional medicine has existed for a long time and is carried out from generation to generation. Therefore, to maintain this culture, natural plants can be used as other alternatives to replace antibiotics and serve as antibacterials. One of the plants that is known to contain secondary metabolites so that it has many benefits and can be used as a medicinal plant, namely the sembukan plant (Paederia foetida) (Badrunasar, 2017).

Sembukan plant (Paederia foetida) belongs to the Rubiaceae family. Usually this plant grows vines on fences or gardens and yards. Hemp has soft stems half a meter in diameter and up to 10 meters in length (Abriyanto et al., 2012). The parts of healing that can be used as medicine are the stems and leaves. How to use it is pounded and added water or boiled and then drunk Ismawati et al., 2020).

Sembukan leaf extract is proven to contain chemical compounds such as iridoid glycosides, paederolone, paederone, paederine and paedenine. In addition, it also contains active compounds such as alkaloids, friedelan 3-1, beta-sitosterol and epifriedelino, iridoid glycosides, asperuloside, paederoside and scandoside, sitosterol, stigmasterol, campesterol, ursolic acid, palmitic acid and methyl mercaptan which cause a foul odor in the smell.(Chauhan et al., 2010).

Klebsiella pneumoniae bacteria are gram-negative bacteria in the form of bacilli, non-motile, and classified as facultative anaerobic bacteria (Kuswiyanto, 2018). These bacteria cause urinary tract infections (UTIs), respiratory tract infections and cause bacteremia in individuals with low immune disorders.(Schroll et al., 2010).

The methods used to test the antibacterial include the diffusion method, the dilution method and the contact method. The diffusion method is a method that is often used to determine the activity of antimicrobial agents by placing a plate that has been given an antimicrobial substance on agar media that has been planted with microorganisms so that it will diffuse into the media (Pratiwi, 2008). The contact method is an antibacterial test method that aims to evaluate the activity of microbial growth or death by counting the number of microbes after being given a number of antibacterial substances that are contacted at a certain time (Hidayat et al., 2020).

Based on research that has been done byHidayat et al (2020)showed the presence of antibacterial against Vibrio cholerae bacteria. From this study, the extract of sembukan leaf (Paederia foetida) at a concentration of 100% formed a bacteristatic effect of 15.36%, at a concentration of 80% was the concentration that could form the highest bacteristatic effect of 64.86% compared to a 60% concentration of 52.02. %, at a concentration of 40% was 34.23%, while at a concentration of 20% it was 18.91%.

According to research Vitalia (2021) showed that crude extract of sembukan (Paederia foetida) leaves against Pseudomonas aeruginosa bacteria using the disc diffusion method was able to inhibit the growth of Pseudomonas aeruginosa bacteria with an optimum dose of 1250 ppm of 11.72 mm compared to a dose of 1000 ppm of sembukan leaf extract of 11.72 mm. 9.99 mm, at a dose of 750 ppm sembukan leaf extract 9.81 mm, at a concentration of 500 ppm 9.40 mm, and at a dose of 250 ppm sembukan leaf extract 9.12 mm.

From the studies mentioned above, so far the antibacterial inhibition of sembukan leaves has not been investigated against the bacterium Klebsiella pneumoniae. Therefore, in this study, testing was carried out on one of the bacteria that can cause infectious disease, namely Klebsiella pneumoniae using the disc diffusion method and the contact method. The purpose of this study was to determine the ability of sembukan leaf extract and the best concentration in inhibiting the growth of Klebsiella pneumoniae bacteria using the disc diffusion method and the contact method.

MATERIAL AND METHODS

This research was conducted at the Bacteriology Laboratory, D-IV Study Program of Medical Laboratory Technology, University of Muhammadiyah Sidoarjo. This research was carried out from April to May 2022. The research materials used were sembukan leaf (Paederia foetida), Klebsiella pneumoniae bacteria, aquades, 96% ethanol, spirtus, NA (Nutrient Agar), MHA (Muller

Hinton Agar), Pz sterile, MacConkey, H2O2, antibiotic ciprofloxacin, Mc.Farland 0.5. The tools used in this study were handscoon, needle loop, autoclave, loop loop, analytical balance, microscope, blender, evaporator, test tube, petri dish, beaker glass, tube rack, label paper, cover glass, incubator, dropper, spatula. , funnel, aluminum foil, Bunsen, Erlenmeyer, sterile cotton, measuring cup, funnel, filter cloth, black marker and filter paper.

This research was conducted by experimental method using the concentration treatment of sembukan leaf extract (Paederia foetida), namely 0%, 25%, 50%, 75%, and 100%. This treatment was repeated 4 times. The results of data analysis were analyzed using One way ANOVA. The research phase includes several stages, namely sample preparation, extract making, confirmation test and antibacterial activity test.

1. Sample preparation stage

Cure leaves (Paederia foetida) used in this study were dark green leaves that had been picked as much as 1 kg then sorted wet and cleaned of dirt attached to running water until clean and drained, after draining cut into small pieces and then weighed 500 grams. Then air-dried first until the color changes for about 5 days (Rose Simanungkalit et al., 2020). The dried sembukan (Paederia foetida) leaves were then weighed with a weight of 250 grams and then mashed with a blender to a fine powder and then weighed to determine the final weight.

2. Extract manufacture

The manufacture of sembukan leaf extract (Paederia foetida) was carried out by maceration. Sembukan leaf powder (Paederia foetida) was taken as much as 100 grams for extraction and then soaked in 1000 mL of 96% ethanol (1:10) and allowed to stand for 3 × 24 hours, stirring occasionally and covered with aluminum foil. After that, it is filtered to take the filtrate using a funnel and gauze or a filter. The filtrate is then evaporated at a temperature of 40°C to obtain a thick extract of the leaves of sembukan (Paederia foetida).(Ramadhan et al., 2020 modified)

3. Klebsiella pneumoniae confirmatory test

The fresh Klebsiella pneumoniae isolates were then inoculated on Mac. Conkey Agar media and incubated for 24 hours at 37°C. Then one single colony was taken and put into sterile Pz and then incubated for 24 hours at 37°C. After incubation, gram staining was performed to see the shape of the cells through a microscope with 1000 times magnification. Furthermore, to observe the formation of gas, a catalase test was carried out by giving 2 - 3 drops of H2O2 (Himawan, 2021).

4. Antibacterial activity test Disc Diffusion Method

The inoculated culture of Klebsiella pneumoniae was taken as much as 1 ose and put in sterile Pz then compared for turbidity using standard Mc.Farland 0.5 solution. Then it was inoculated into MHA (Muller Hinton Agar) media by means of a swab and waited 15 minutes. Soak the disc paper into each leaf extract of sembukan (Paederia foetida) for approximately 2 hours, while for the negative control the disc paper was immersed in 10% DMSO in a petri dish and the positive control was immersed in the antibiotic ciprofloxacin. Then the paper discs containing various concentrations of sembukan leaf extract, positive control and negative control were placed on MHA media that had been inoculated with Klebsiella pneumoniae bacteria and incubated for 24 hours at 37°C.

5. Test of antibacterial activity Contact Method

The results of bacterial inoculation which were marked by the presence of turbidity in sterile Pz were pipetted as much as 0.1 ml into a test tube, then 0.1 ml of extract was added. Then each test tube was incubated for 24 hours. After the incubation time is reached, 0.1 ml is planted in NA media (Nutrient Agar) by the scatter method and then incubated for 24 hours (Hidayat et al., 2020 which

has been modified). Analysis of the results of the inhibition was carried out by calculating the total number of microbes that grew.

RESULTS AND DISCUSSION

Klebsiella pneumoniae confirmatory test

The confirmatory test results showed that the Klebsiella pneumoniae bacteria grown on Mac.Conkey media grew with pink colonies but could not ferment lactose completely. If taken with the ose, it will be attracted because the colony has a capsule. This is in accordance with the statement of Kusuma (2013) which states that Mac. Conkey is a selective medium containing a special dye that can ferment lactose so that bacteria that ferment lactose will grow as red colonies while bacteria that do not ferment lactose will grow as colorless bacteria. Observation of the color of Klebsiella pneumoniae colonies on Mac media. Conkey can be seen in Figure 1.

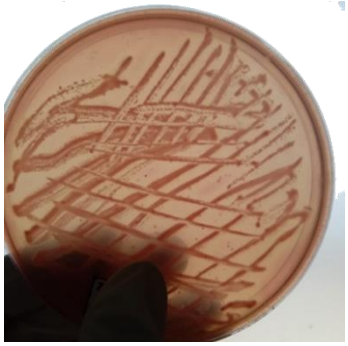

Figure 1. Colony shape and color *Klebsiella pneumoniae on Mac media. Conkey*

From the results of microscopic observations carried out, it was shown that Klebsiella pneumoniae was a gram-negative bacterium with a rod or bacillus shape. Gram negative bacteria will lose crystal violet when dropped with alcohol, while gram positive bacteria retain crystal violet dye so that the cells turn purple. This is due to the difference in the thickness of the peptidoglycan layer between gram-positive and gram-negative bacteria. The results of observing bacteria with a microscope at 1000x magnification can be seen in Figure 2.

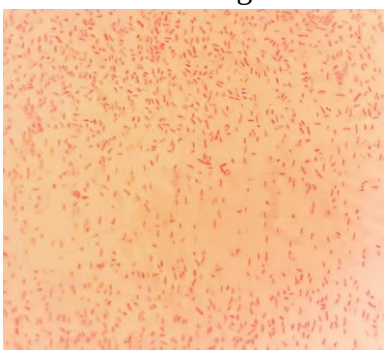

Figure 2. Morphological from *Klebsiella pneumoniae at 1000x magnification*

Results of Inhibitory Test Results of Sembukan Leaf (*Paederia foetida*) Against the Growth of Bacteria *Klebsiella pneumoniae* with Disc Diffusion Method

The average diameter of the inhibition zone can be seen in Table 1. that the inhibition test of sembukan leaf extract against Klebsiella pneumoniae using the disc diffusion method showed that both sembukan leaf extract treatment and control were able to inhibit the growth of Klebsiella pneumoniae. The formation of the inhibition zone appeared after incubation for 1 × 24 hours. The inhibition zone formed was measured using a caliper with an accuracy of millimeters (mm).

Table1. Inhibition of healing leaf extract against *Klebsiella pneumoniae*

Concentration	Average inhibition diameter (mm)
0%	0
25%	5.5
50%	8.5
75%	15.25
100%	7.75
K. Negative(-)	0
K. Positive (+)	26.2

Based on Table 1, it can be seen that the highest average inhibition diameter was 15.25 mm at 75% extract concentration in the strong category and the lowest inhibition was at 25% extract concentration with an inhibitory power of 5.5 mm in the medium category. At a concentration of 50% it has an inhibitory power of 8.5 mm and at a concentration of 100% it has an inhibitory power of 7.75%. Meanwhile, there is no inhibition in the negative control. In this study, the positive control used was ciprofloxacin which had an inhibitory result of 22.3 mm, meaning that it had sensitivity to Klebsiella pneumoniae bacteria.Ciprofloxacin is said to be sensitive if the clear zone formed is 21 mm, intermediate if an inhibition zone of 16-20 mm is formed and is said to be resistant if an inhibition zone is formed 15 mm (CLSI, 2018).

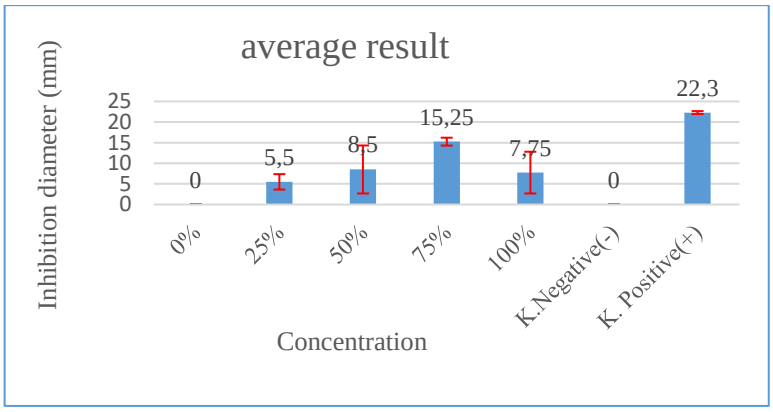

Figure 3. Graph of the average inhibition of sembukan leaf extract

Ciprofloxacin was used as a positive control to prove whether Klebsiella pneumoniae bacteria were resistant to these antibiotics (Misella et al., 2019). In addition, ciprofloxacin antibiotics are broad-spectrum antibiotics, which have many types of antibacterial activity, viruses, fungi and protozoa (Puasa, 2019). While the negative control used was DMSO 10%, there was no inhibition zone. Use of DMSO 10% as a negative control because DMSO is able to dissolve in polar and non-polar compounds and has bactericidal properties so that antibacterial activity can be ascertained without being influenced by the solvent (Huda et al., 2019). Utomo (2018) stated that the negative control was used as a comparison that the solvent used as a diluent did not affect the antibacterial test results of the compound to be tested.

Inhibition zones are formed from a concentration of 25% to a concentration of 100%. The concentration used affects the size of the resulting clear zone. The larger the diameter produced, the stronger the bioactive compounds from the sembukan leaves in inhibiting bacterial growth (Hidayat,

2020). The diameter of inhibition starts from 5.5 mm-15.25 mm in the medium to strong category. The diameter of inhibition increased from a concentration of 25%-75%, but decreased at a concentration of 100%. This decrease occurred because the extract that was too concentrated was not able to diffuse well into the medium containing the inoculum. Therefore, at a concentration of 100% there was no increase in the diameter of inhibition (Maleki et al., 2008).

The results of the observation of the antibacterial activity test showed that sembukanleaf extract (Paederia foetida) was able to inhibit the growth of Klebsiella pneumoniae bacteria. Antibacterial activity is caused by the presence of antibacterial compounds that can inhibit bacterial growth or cause bacterial death by several mechanisms, namely inhibition of cell wall synthesis, inhibition of cell membrane function, inhibition of protein synthesis, inhibition of nucleic acid synthesis and inhibition of metabolite synthesis on catalytic enzyme reactions. (Mardjono et al., 2012).

The data obtained were followed by data analysis using the One Way ANOVA test with a 95% confidence level ($p < 0.05$). Based on the one way ANOVA test, it is known that the significant value is $p = 0.000$ (<0.005), which means that there is a significant difference or there is an effect of giving sembukan leaf extract (Paederia foetida) to Klebsiella pneumoniae bacteria.

In general, the diameter of the inhibition zone tends to increase with increasing extract concentration. But there is a decrease in the area of the inhibition zone at some higher concentrations, such as in gram-negative bacteria at a concentration of 100%. This is similar to research Maleki et al (2008)which causes the diameter of the inhibition zone is not always directly proportional to the increase in antibacterial concentration, this occurs due to differences in the rate of diffusion of antibacterial compounds on agar media and different types and concentrations of antibacterial compounds.

The presence of inhibition is caused because the extract of sembukan leaves contains compounds that are positive in phytochemical testing so that there are antibacterial substances such as alkaloids, saponins, tannins, flavonoids, phenolics. Osman et al (2009) stated that there was a higher content of phenolic compounds and antioxidant activity in sembukan leaves compared to the standard antioxidant quercetin, a standard antioxidant compound. The effect caused by the presence of phenolic content is that phenol compounds are protein coagulation so that the clotted proteins can no longer function and interfere with the formation of bacterial cell walls, in the end the bacteria lose the ability to form colonies and cause cell death.

The presence of Klebsiella pneumoniae bacterial growth activity cannot be separated from the antibacterial substances possessed by sembukan. According to Dewi (2020) has active compounds contained in sembukan leaves as antimicrobial substances including tannins, saponins, flavonoids, phenols, terpenoids, and alkaloids. These compounds have different antimicrobial mechanisms of action.

Results of Inhibitory Test Results of Leaf Hemp (Paederia foetida) Against the Growth of Klebsiella pneumoniae Bacteria with the Contact Method

Based on the inhibition test of sembukan leaf extract on the growth of *Klebsiella pneumoniae* using the contact method, Table 2 shows that the sembukan leaf extract was able to inhibit the growth of *Klebsiella pneumoniae* bacteria. The total of *Klebsiella pneumoniae* bacteria used in this study was 1.57×108 CFU/mL and after contact with the concentration according to the treatment, *Klebsiella pneumoniae* bacteria decreased. In contact with 0% concentration there is no decrease. This is because at a concentration of 0% no extract was given. In contact with a concentration of

25% the number of bacteria decreased by 0.41×108 CFU/mL with the death of the test bacteria by 26.11%. On contact with a concentration of 50% the number of bacteria decreased by 0.82×108 CFU/mL with the death of the test bacteria by 52.11%. In contact with 75% concentration the number of bacteria decreased by 1.09×108 CFU/mL with the death of the test bacteria by 69.42%. On contact with 100% concentration the number of bacteria decreased by 0.52×108 CFU/mL with the death of the test bacteria by 33.12%.

Table 2. Inhibition of Healing Leaf Extract on Growth *Klebsiella Pneumoniae By Contact Method*

Concentration	Number of bacteria before contact (Cfu/mL)	Number of bacteria after contact (Cfu/mL)	Decrease in the number of Klebsiella pneumoniae (Cfu/mL)	Percentage of deaths (%)
0%	1.57×10^8	0	0	0
25%	1.57×10^8	1.16×10^8	0.41×10^8	26.11
50%	1.57×10^8	0.75×10^8	0.82×10^8	52.22
75%	1.57×10^8	0.48×10^8	1.09×10^8	69.42
100%	1.57×10^8	1.05×10^8	0.52×10^8	33.12
K. Positive (+)	1.57×10^8	0	0	0
K. Negative (-)	1.57×10^8	0	0	0

In the negative control using DMSO 10%, there was no decrease in bacteria. This is because the negative control was not given extract or antibiotic treatment. According to Huda et al (2019), 10% DMSO is able to dissolve in polar and non-polar compounds and has bactericidal properties so that antibacterial activity can be ascertained without being influenced by the solvent. While the positive control using the antibiotic ciprofloxacin did not decrease the bacteria. This shows that ciprofloxacin has the power to kill the bacteria *Klebsiella pneumoniae*. The presence of this killing power was determined by observing the presence or absence of Klebsiella pneumoniae colonies growing on NA (Nutrient Agar) media after 24 hours of incubation (May, 2016).

At a concentration of 100% there was a decrease in Klebsiella pneumoniae. This is because there are active compounds contained in sembukan leaf extract that do not dissolve completely at a concentration that is too high, causing the extract to be difficult to diffuse completely (Hidayat et al., 2020). In positive control and negative control, antibacterial activity was influenced by several factors, namely the content of antibacterial compounds, the diffusion power of the extract, the type of bacteria inhibited, and the concentration of the extract (Brooks et al., 2013).

The antibacterial mechanism of sembukan leaves causes interference with the permeability of the cell membrane as well as by causing the denaturation of cell membrane proteins. Compounds that play a role in this are alkaloids and phenols. Protein denaturation is damage to the tertiary structure of the protein that causes damage to the protein and causes the protein to not function. Protein functions in bacterial cell metabolism, protein damage can cause disruption of bacterial metabolic processes, causing disruption of bacterial cell life and causing bacterial cell death (Osman et al., 2009).

According to Nurcahyanti & Wandra (2012) that the active compounds found in sembukan plants have biological activities that can provide health effects in the mechanism of healing diseases. One of these compounds is essential oil. The essential oil is obtained from fresh leaves that have an unpleasant smell which contain the compound paederin and dimethyl disulfide. These compounds

are found in high amounts in the leaves. These compounds are thought to be compounds that play a role in inhibiting the growth of *Klebsiella pneumoniae* bacteria.

The data obtained were followed by data analysis using the One Way ANOVA test with a 95% confidence level ($p < 0.05$). Based on the one way ANOVA test, it is known that the significant value is $p = 0.000$ (<0.005), which means that there is a significant difference or there is an effect of giving sembukan leaf extract (*Paederia foetida*) to *Klebsiella pneumoniae* bacteria.

This antibacterial activity often inhibits protein synthesis or binds to ribosomes (Agfadila, 2017). According to Hidayat et al (2020) if the death of the test bacteria is at least 99.99% then at that concentration it is said to be bactericidal (kills bacteria). On the other hand, if the death of the tested bacteria is less than 99.99%, then the concentration is said to be bacteristatic (inhibits bacterial growth. The difference between bacteristatic and bactericidal is in the speed at which bacteria die. Long-term use of antibacterials can cause bacteria to grow back).(Agfadila et al., 2017)

CONCLUSION AND SUGGESTION

Sembukan leaf extract (Paederia foetida) at a concentration of 25% - 100% was able to inhibit the growth of Klebsiella pneumoniae ATCC 1706 bacteria by disc diffusion and contact methods. The best concentration of sembukan leaf extract (Paederia foetida) which is able to inhibit the growth of Klebsiella pneumoniae ATCC 1706 bacteria is the disc diffusion method at a concentration of 75% with an inhibition diameter of 15.25 mm (strong category) and in the contact method at a concentration of 75% it formed a bacteristatic effect with a decrease in the number of bacteria by 1.09 × 108 and the number of deaths of test bacteria by 69.42% .

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