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THE EFFECTIVENESS TEST OF ALOE VERA EXTRACT INHIBITING THE GROWTH OF STAPHYLOCOCCUS AUREUS BACTERIA

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Abstract

Staphylococcus aureus bacteria are resistant to several kinds of antibiotics, so there is a need for natural antibiotics derived from plants, namely aloe vera. The objective of this study was to determine the effect of aloe vera leaf extract in inhibiting the growth of Staphylococcus aureus bacteria. The type of research used was experimental using the disc diffusion method with a total of 25 samples with five concentrations of 20%, 40%, 60%, 80% and 100% then the data were analyzed using the One Way ANOVA test. The result is that aloe vera extract can inhibit the growth of Staphylococcus aureus bacteria, it is proven by the presence of a disc zone at 100% concentration with a diameter of 11.5 mm. The results of statistical analysis with the One Way ANOVA test, in a value of 0.000, meaning that there was a significant difference. The conclusion obtained is that aloe vera extract can slow down the growth of Staphylococcus aureus bacteria with the highest concentration it cannot be used as an antibiotic because it is less than the standard value of 14 mm.

Keywords: Aloe vera; Effectiveness; Staphylococcus aureus

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INTRODUCTION

Staphylococcus aureus is a grampositive bacterium that has a round morphology and can live in colonies like and can grapes secrete pigment substances. Staphylococcus aureus bacteria are usually found in the air, dust, waste, and can live in food and produce enterotoxins but have no effect on the outside of food, and signs of poisoning will appear, such as diarrhea, vomiting, hypothermia, nausea, weakness, and helpless. In addition, other diseases that can arise are infections in the hair follicles, infected wounds, meningitis and pneumonia (Adriani & Lasti, 2014; Sunarti & Paninsari, 2019).

Staphylococcus aureus bacteria are resistant to several kinds of antibiotics due to the wrong choice of the right antibiotic, inappropriate dosage, and undisciplined use of the drug. *Staphylococcus aureus* resistance to antibiotics, namely clavulanic acid, ampicillin, penicillin G, amoxicillin, ciprofloxacin, sulbenicillin, also chloramphenicol (Diyantika *et al.*, 2017).

It will make the infection difficult to treat with these antibiotics, another way that can be used to control resistance to bacteria is to use antibiotics properly so there is the development of alternative drugs from natural ingredients that can inhibit the growth of *Staphylococcus* bacteria which is expected to be safer, more efficient, and effective by creating natural antibiotics from the body-plants that have the potential as antibiotics. One of the plants that is cheap and easily available to be used as natural antibiotics is *Aloe vera* (Country, 2014).

Aloe vera is one of a variety of medicinal plants. Aloe vera leaf extract contains many active substances including Saponins, Anthraqumonealonin, Barbaloin, Isobarbaloin, Anthrax Zero, Aloeemodin, Anthracenesiamat, chrysophanic acid, and Eteraloin resistant to *Aloe vera* can be used as antibacterial, antiseptic, and antibiotic. *Aloe vera* extract has some very good antibacterial activity, including against *Staphylococcus aureus* bacteria which is one of the causes of infection (Natsir, 2013).

As an antibacterial substance, Aloe vera has active substances such as tannins, saponins and flavonoids. Saponins are alkaloid substances that can destroy DNA and RNA in bacteria. Tannins as antibacterial substances are able to inactivate adhesins so that the bacteria are no longer attached to the epithelial cells of the host. The content of flavonoids in aloe vera is able to make bacterial cell walls lyse. The above mechanism can inhibit the growth of bacteria(Survati et al., 2018)

The objetive of this research was to determine the effect of aloe vera extract in forming the inhibition zone of *Staphylococcus aureus* bacteria. The significance of this research is to inform the public of the benefits of aloe vera as an inhibitor of the growth of *Staphylococcus aureus* bacteria which is one of the causes of infectious diseases.

MATERIALS AND METHODS

The type of research used was experimental which was carried out at the microbiology laboratory of the Palembang Health Laboratory Center (BBLK) on 23 – 24 April 2021 with a total of 25 samples with 5 concentrations, namely 20%, 40%, 60%, 80% and 100%.

This research began with sterilizing the tools to be used and then making the media *Muller Hinton Agar* (MHA) as well as extracting aloe vera leaf peel which then the extract was filtered and evaporated using hotplate to get a thick *Aloe vera* leaf extract then the extract was made with five predetermined concentrations using the Concentration formula $E = \frac{e}{e+a} \ge 100\%$ (Yusitta, 2018).

Before carrying out the extract sensitivity test, you must do a bacterial strain test because to find out whether the bacteria used was *Staphylococcus aureus* with the ATCC 25923 strain, a catalase test, coagulase test and gram staining re carried out. If the results obtained are appropriate then proceed to make a bacterial suspension according to the standard Mc Farland's turbidity was 0.50 – 0.62 and directly inoculated *Staphylococcus aureus* ATCC 25923 into an MHA medium dish and then leveled, after the bacteria were inoculated, input paper discs from *Aloe vera* leaf extract at concentrations of 20%, 40%, 60%, 80 %, 100%, and 1 negative and positive control which will be tested for bacterial incubation with an incubator at 37oC for 24 hours after completion of incubation. Bacterial growth was observed to see whether there was an inhibition zone that occurred (Made, 2015).

Data analysis was carried out by using a computer statistical test using the SPSS 16 program and using statistical analysis that was adjusted to the distribution of existing data. Existing data was then tested using one way ANOVA. If the data obtained had a significant difference, then it was continued using the Posthoc test using the Tukey HSD test to see how big the difference between concentrations was.

RESULTS AND DISCUSSION

Testing of *Staphylococcus aureus* strains was carried out to ascertain the type of bacteria. The test results data are presented in table 1.

Table 1. Bacterial Strain Test Results

Test Test	Results			
Catalase	Bubble +			
Coagulase	Agglutination +			
Gram stain	Gram + Coccus +			

Based on table 1 above, the catalase test showed that the bacteria produced positive bubbles which meant that it was *Staphylococcus aureus* bacteria. Meanwhile, the results of the coagulase test show that agglutination occurs, which means it is *Staphylococcus aureus* because it is pathogenic.

The results of gram staining showed that gram positive bacteria were in the form of cocci that emit a purple color in gram staining, which can be seen in Figure



Figure 1. The Map of Research Location

Figure 1 above shows the purple color caused by the bacteria trying to stay with the first color, namely Crystal violet. This result shows that the bacteria was *Staphylococcus aureus*. (Hayati *et al.*, 2019).

The results of the test of the effectiveness of aloe vera extract as an inhibitor of the growth of *Staphylococcus aureus* bacteria using various concentrations obtained the following results:

Test	Control + (mm)	Inhibition Zone (mm)					
		Controls -	20%	40%	60%	80%	100%
P1	33	0	0	4	5	8	9
P2	33	0	0	3	6	7	10
Р3	33	0	0	5	5	7	9
P4	33	0	2	4	6	6	8
P5	33	0	0	3	4	8	11.5
Average	33	0	0.4	3.8	5.2	7.2	9.2

Table 2. Results of Inhibition Zone of Aloe vera Extract

In the results of table 2 above, it is clear that the positive control (as a comparison control) using the antibiotic novobiocin formed an inhibition zone of 33 mm and in the negative control using aquadest, no inhibition zone was formed at all. In the five types of concentrations used in this study, it can be seen that the inhibition zones formed were very varied, namely the larger the inhibition zone was formed along with the high concentration used (Rahardjo *et al.*, 2017).

The greatest inhibition zone value was 11.5 mm at 100% aloe vera concentration. The sensitivity of bacteria to antibiotics was influenced by the diameter of the inhibition zoneat that was formed. The zone of inhibition in question is that there is no visible bacterial growth around the disc (Nurhidayanti, 2021).

Bacteria are declared sensitive if the inhibition zone on the disc and bacteria is 14 mm or more, it can be considered that the bacteria are sensitive to the suspension used, if the distance between the disc and the colony is 11 mm, it can be said that the suspension is less effective in inhibiting bacterial growth (Mardiah, 2017). The inhibitory power of *Aloe vera* extract belongs to the medium category. The categorization of the inhibition zone was divided into 4 groups, namely: weak activity (less than 5 mm), moderate activity (5 to 10 mm), strong activity (less than 10 to 20 mm), very strong activity (less than 20 to 30 mm). The zone of inhibition around the disc is said to be the resistance to antimicrobials (Datta *et al.*, 2019).

The results of statistical test data analysis using One Way ANOVA on aloe vera extract obtained a significant value of 0.000 meaning p = < 0.05 because the probability value is $p \Rightarrow 0.05$ meaning there is a significant difference. The difference in question is the difference between the concentration used and the inhibition zone formed from the influence of the concentration because the p value = <0.05. The results of this study are supported by previous research conducted by Tree et al., (2012) which obtained sig. <0.05, which means that there is a difference between the concentration of aloe vera leaf peel extract against Staphylococcus aureus and Escherichia coli bacteria (Tree et al., 2014).

The posthoc test data used the Tukey HSD test to determine the difference between concentrations based on the data obtained showing a significant difference with a p value <0.05. There was a significant difference between the average bacterial growth at each treatment concentration. It proves that aloe vera leaf skin has effectiveness in inhibiting the growth of bacteria *Staphylococcus aureus* with active substances in *Aloe vera* such as phenols, saponins, tannins, anthraquinones. These compounds play an important role in the ability as an antibacterial. The phenolic compounds in aloe vera have a mechanism of inhibiting bacterial growth (Kadek *et al.*, 2013).

Proteins in bacteria are able to bind to the above compounds through non-specific bonds to form a complex phenol protein, if the concentration is low, the complex phenol protein will decompose and the cytoplasmic membrane is damaged and causes leakage of the cell contents, as a result the growth of these bacteria can be inhibited. If the concentration is high, the substance will coagulate with cellular resulting proteins in lvsis of the cytoplasmic membrane (Kadek et al., 2013). Saponins have antibacterial activity through decreasing cell surface tension, causing cell leakage and compounds in cells will come out (Gunawan, 2018).

The mechanism of action of tannins is to attack the cell wall in the polypeptide section to cause the cell wall to be imperfectly formed, it makes the cell wall in bacteria destroyed due to osmotic pressure and physical pressure makes bacterial cells die (Armiati, 2018).

Anthraquinone compounds are antimicrobial compounds with a broad spectrum. *Aloe vera* contains several anthraquinone glycosides (barbaloin, aloeemodin and aloin). The antibacterial properties of Aloe-emodin work by slowing the transfer of electrons in the mitochondrial respiratory system, where phenolics are compounds derived from phenol and can change the nature of bacterial proteins, by increasing the permeability of cells which causes cells to become blocked and damaged (Armiati, 2018).

CONCLUSION

The conclusion from the results of the research above is that the inhibition zone in each concentration has various diameters and the concentration that has the highest inhibition zone value is a concentration of 100% but based on the standard sensitivity of a bacterium, namely 14 mm of aloe vera leaf extract, it cannot be said to be effective as an antibiotics because the inhibition zone formed by 11.5 mm has not reached the standard. sensitivity The results of statistical tests there are significant differences in the inhibition zone formed from the effect of the given concentration. The higher the concentration of aloe vera extract, the larger the inhibition zone will be.

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