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EFFECT OF ETHANOL EXTRACT OF *WIJAYA KUSUMA* LEAVES (*Epiphyllum oxypetalum*) AS AN INHIBITOR OF *Salmonella typhi* BACTERIA

PENGARUH EKSTRAK ETANOL DAUN WIJAYA KUSUMA (*Epiphyllum oxypetalum*) SEBAGAI INHIBITOR BAKTERI *Salmonella typhi*

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Abstract

This study aims to determine the effect of ethanol extract of *wijaya kusuma* leaves (*Epiphyllum oxypetalum*) as an inhibitor and determine the Minimum Inhibitory Concentration (MIC) against *Salmonella typhi* bacteria. Quantitative research method with an experimental research design using a Completely Randomized Design (CRD) and the results were analyzed using One Way ANOVA test and continued with the Tukey test. Test method with disc diffusion method (Kirby-Bauer) in antibacterial test with extract concentration 0; 0.1; 0.2; 0.3; 0.4; 0.5; and 0.6% (g/v) and liquid dilution method in Minimum Inhibitory Concentration (MIC) test with extract concentration 0; 0.02; 0.04; 0.06; 0.08; and 0.1% (g/v). The results showed that ethanol extract of *wijaya kusuma* leaves (*Epiphyllum oxypetalum*) had a significant effect on *Salmonella typhi* bacteria with the best concentration of 0.6% which had a mean inhibition zone area of 45.05 ± 4.80 mm^2 and the Minimum Inhibitory Concentration (MIC) was in the concentration range of 0 to 0.02% (g/v). The ethanol extract of *wijaya kusuma* leaves (*Epiphyllum oxypetalum*) has an effect as a growth inhibitor of Salmonella typhi bacteria.

Keywords: Antibacterial; *Epiphyllum oxypetalum*; *Salmonella typhi*

Abstrak

Penelitian ini bertujuan untuk mengetahui pengaruh ekstrak etanol daun wijaya kusuma (Epiphyllum oxypetalum) sebagai inhibitor dan menentukan Minimum Inhibitory Concentration (MIC) terhadap bakteri Salmonella typhi. Metode penelitian kuantitatif dengan desain penelitian eksperimen menggunakan Rancangan Acak Lengkap (RAL) dan hasil dianalisis menggunakan Uji One Way ANOVA dan dilanjutkan dengan uji Tukey. Metode pengujian dengan metode difusi cakram (Kirby-Bauer) pada uji antibakteri dengan konsentrasi ekstrak 0; 0,1; 0,2; 0,3; 0,4; 0,5; dan 0,6% (g/v) dan metode dilusi cair pada uji Minimum Inhibitory Concentration (MIC) dengan konsentrasi ekstrak 0; 0,02; 0,04; 0,06; 0,08; dan 0,1% (g/v). Hasil menunjukkan ekstrak etanol daun wijaya kusuma (Epiphyllum oxypetalum) berpengaruh secara signifikan terhadap bakteri Salmonella typhi dengan konsentrasi terbaik 0,6% yang memiliki rerata luas zona hambat 45,05 ± 4,80 mm^2 dan Minimum Inhibitory Concentration (MIC) terletak berada pada rentang konsentrasi 0 hingga 0,02% (g/v). Ekstrak etanol daun wijaya kusuma (Epiphyllum oxypetalum) memiliki pengaruh sebagai inhibitor pertumbuhan bakteri Salmonella typhi.

Kata Kunci*: Antibakteri; Epiphyllum oxypetalum; Salmonella typhi*

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INTRODUCTION

Indonesia is a developing country that still faces serious health problems with a high mortality rate from bacterial infections (Najib, 2018). One of the related bacterial infections is typhoid fever which is an infection of the gastrointestinal tract generally caused by *Salmonella typhi* bacteria with fecal-oral spread through food or drink contaminated with bacteria (Malau & Budiyono, 2015). World Health Organization (WHO) reports typhoid fever cases in the world reaching 16–33 million with 500–600 thousand deaths per year (Ochiai et al., 2008). Typhoid fever is characterized by symptoms including prolonged nausea and vomiting, accompanied by diarrhea, fever, abdominal cramps, and headache (Nurul, 2017).

Bacterial infections are usually treated with antibiotics. However, in recent years, it has been reported that treatment of *Salmonella typhi* bacterial infections with synthetic drugs such as antibiotics has become ineffective due to cases of bacterial resistance, the presence of resistance can cause antibiotics to be ineffective against bacteria, which can aggravate the infection (Trisharyanti, 2017). The first report of *Salmonella typhi* bacterial resistance cases in 2016–2018 in Pakistan recorded 8.188 cases of typhoid fever with the resistance of *Salmonella typhi* bacteria to antibiotics reported causing morbidity and mortality rates to increase in typhoid fever cases so that treatment and therapy do not run smoothly in the healing process of infection (Akram et al., 2020). A 2016 Global Review report using a world population simulation model predicted that bacterial resistance will become the world's first cause of death by 2050, predicting at least 10 million deaths per year, especially in Asia as various problems arise due to bacterial resistance, including infections caused by bacteria resistant to various antibiotics (Meriyani et al., 2023). In addition, some people have difficulty in purchasing antibiotics due to the high price, especially when the dosage gets higher. This encourages efforts to find sources of antibacterial drugs from natural ingredients, in the hope that it can be a safer and more economical alternative treatment for bacterial infections.

According to Atmojo (2013), Indonesian people understand the use of plants as medicine, which is based on empirical knowledge alone which is a form of community interaction with the environment. Among the plants utilized by the Indonesian people is *wijaya kusuma* (*Epiphyllum oxypetalum*). The *wijaya kusuma* plant (*Epiphyllum oxypetalum*) in Lahat Regency in South Sumatra Province is generally used as an ornamental plant in the yard because it has beautiful flowers with white color, fragrant aroma, and includes flowers with a fairly large size, unfortunately, the beauty of the *wijaya kusuma* plant can only be seen at night. *Wijaya kusuma* is a plant that is safe for consumption because it does not have toxic or harmful properties. Some people consume *wijaya kusuma* brew, which is dried, boiled, and water drunk, which can help reduce headaches and relieve inflammation suffering from diseases such as cough or asthma (Artini & Aryasa, 2018).

Previous studies have shown that *wijaya kusuma* leaves have antibacterial activity against several pathogenic bacteria as reported by Dandekar et al. (2015) by testing using three different types of solvents namely ethanol, acetone, and petroleum ether; found that *wijaya kusuma* leaves extract contains phytochemical compounds that are beneficial in fighting various diseases and infections and have potential as biotherapeutics. In addition, according to Upendra and Khandelwal (2012), *wijaya kusuma* leaves extract is proven to be able to inhibit the growth of bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, and *Bacillus subtilis*. However, there has been no specific research examining the antibacterial effect of *wijaya kusuma* leaves against *Salmonella typhi* bacteria that cause typhoid fever. Therefore, it is important to conduct further research related to the use of this natural material as an alternative to reducing cases of bacterial resistance.

Previous studies on antibacterial tests generally used concentrations with high percentages, but according to opinions expressed by experts, lower concentrations are considered more effective as antibacterials because the use of antibacterial substances in high concentrations is at risk of causing side effects on the human physiological system. For example, studies have shown that celery (*Apium graveolens*) and cucumber (*Cucumis sativus*) leaves are effective in lowering blood pressure in hypertension. However, their use should be done with caution as overuse can lead to a drastic drop in blood pressure (Katno & Pramono, 2010). Based on this, it is important to study as an alternative treatment to overcome bacterial resistance by using lower concentrations. Therefore, this study aims to determine the effect of *wijaya kusuma* leaf extract (*Epiphyllum oxypetalum*) as an antibacterial against *Salmonella typhi* bacteria and look for Minimum Inhibitory Concentration (MIC) which has a lower concentration which is expected in the use of lower concentrations but has the same or greater effect than high concentrations in inhibiting the growth of *Salmonella typhi* bacteria can be safer for long-term consumption and the use of low concentrations can save more material for making *wijaya kusuma* leaves extract so that it becomes more economical and efficient.

MATERIALS AND METHODS

Place and Materials

The place of research was at the Biology Education Laboratory, Indralaya Campus, Sriwijaya University in Indralaya Regency, Ogan Ilir, South Sumatra. *Wijaya kusuma* leaves with *Epiphyllum oxypetalum* species obtained in Lahat Regency, South Sumatra Province. The bacterial isolate tested was *Salmonella typhi*. The solvent used in the extraction process is 96% ethanol. The agar medium used is *Salmonella Shigella Agar* (SSA).

Research Procedure

Quantitative research method with laboratory experiment design. This research includes several stages starting with the preparation of tools and materials. The implementation stage includes the preparation of ethanol extract from *wijaya kusuma* leaves, sterilization of tools and materials using an autoclave, making *Salmonella Shigella Agar* (SSA) media, and testing bacterial suspensions. Then the final stage involves treatment with antibacterial tests, measurement of the inhibition zone area, determination of the Minimum Inhibitory Concentration (MIC) test concentration, Minimum Inhibitory Concentration (MIC) test, and analysis of the data obtained. The parameters measured were the area of the inhibition zone of bacterial growth and the Minimum Inhibitory Concentration (MIC).

The treatment design used in the study was a Completely Randomized Design (CRD). In the antibacterial test, each extract concentration was six treatments with distilled water as the control and amoxicillin antibiotic concentration 0.01% as the comparison control. Repetition of four replicates. In the Minimum Inhibitory Concentration (MIC) test, each extract concentration was six treatments with three replicates.

Preparation of *Wijaya Kusuma* Leaves Extract (*Epiphyllum oxypetalum*)

Fresh dark green leaves of *wijaya kusuma* were taken as much as 5 kg and washed thoroughly with running water, then dried by wind-angling for a month. The results of drying by aerating obtained simplistic as much as 170 g. Extraction of *wijaya kusuma* leaves by maceration method with a ratio of simplistic and solvent 1:10 dissolved with 96% ethanol as much as 1.7 L. The use of 96% ethanol in the maceration process is because ethanol has a high polarity so it can extract more compounds than 70% ethanol (Amini et al., 2019). The longer the maceration process, the more active compounds will be dissolved. Doing maceration 3 times for 3 × 24 hours can maximize the extraction of active compounds (Indarto et al., 2019). Every 24 hours, the filtrate and pulp are separated and re-macerated. A vacuum rotary evaporator was used to collect the filtrate and evaporate the ethanol solvent at 50 °C and 500 rpm. Next, the weight of the condensed extract was weighed and the percentage yield was calculated against the weight of the extracted leaves powder. The total yield of evaporation results (thick extract) obtained as much as 6 g which will be used in research on antibacterial tests and MIC tests to be diluted.

Preparation of *Salmonella typhi* Test Bacteria Suspension

Pure culture of *Salmonella typhi* bacteria was rejuvenated on *Salmonella Shigella Agar* (SSA) media incubated for 1 × 24 hours at 37 °C in an incubator before being made into a bacterial suspension. Preparation of the test bacterial suspension by adding bacterial culture with 0.9% NaCl in a sterile test tube and homogenized, then the turbidity and absorbance values were compared with a standard solution of 0.5 Mc. Farland standard solution. The Mc. Farland standard 0.5 is equivalent to a bacterial suspension with a concentration of $1–1.5 \times 10^8$ CFU/mL by checking the absorbance on a spectrophotometer (Sutton, 2011).

Preparation of Test Concentration of *Wijaya Kusuma* Leaves Extract

Making the concentration of the extract was tested by making the main solution of 100% concentration first by dissolving the thick extract with sterile distilled water in a ratio of 1 g of thick extract to 1 mL of distilled water, then the main solution is diluted using the dilution formula, namely M1 × V1= M2 × V2. The dilution of the main solution used in the antibacterial activity test was 10 mL for each concentration by diluting the main solution of 100% concentration using the dilution formula in 10 mL of distilled water to make the tested extract concentrations from 0.1% to 0.6%. In MIC testing, the dilution at each concentration uses 100 mL of distilled water to make extract concentrations of 0.02% to 0.1%. Take the main solution and distilled water using a micropipette due to the small volume of solution taken.

Antibacterial Test

The test method uses disc diffusion (Kirby-Bauer) by pour plate to see the inhibitory effect of *wijaya kusuma* leaves extract on bacteria. The concentrations tested were 0.1; 0.2; 0.3; 0.4; 0.5; and 0.6% (g/v). Measurement of the diameter of the inhibition zone is indicated by a clear area, which is an area that does not grow bacteria in millimeters (mm). The inhibition zone area is measured by measuring the total area minus the disc paper area (Afifi, 2018). The disc paper used is Macherey Nagel Germany disc paper with a diameter of 6 mm made of cellulose material so that the inhibition effect that occurs during testing can be seen.

The results of the antibacterial test can determine the concentration in the Minimum Inhibitory Concentration (MIC) test taken from the lowest concentration that actively inhibits *Salmonella typhi* bacteria. The concentration of the extract used starts from the lowest concentration, and then decreases until there is no inhibition against bacteria (0%). The concentrations tested in the MIC test were 0; 0.02; 0.04; 0.06; 0.08; and 0.1% (g/v).

Minimum Inhibitory Concentration (MIC) Test

The Minimum Inhibitory Concentration (MIC) test method uses liquid dilution to determine the Minimum Inhibitory Concentration (MIC) by measuring the absorbance value and turbidity level using a spectrophotometer. The Minimum Inhibitory Concentration (MIC) test is carried out by comparing the absorbance value of the treatment after incubation with the value before incubation. If there is the lowest concentration of extract shows results with clearer or less turbid media and lower absorbance values than before incubation, the Minimum Inhibitory Concentration (MIC) is obtained (Wiharningtias, 2016).

Data Analysis

The data obtained in the form of the measurement results of the diameter of the inhibition zone of the antibacterial test results were carried out using the One-Way Analysis of Variance (ANOVA) test based on the sig. Value, f-count, and f-table. The ANOVA test aims to see if there is a difference and significance between the effect of *wijaya kusuma* leaves extract on *Salmonella typhi* bacteria at each concentration tested. After that, if it is known that the ANOVA test results show significant results, further testing is carried out based on the coefficient of variation obtained from the ANOVA test to determine the best concentration and the differences between each treatment tested.

RESULTS

Antibacterial Test

Based on Figure 1, there is an inhibitory effect on *Salmonella typhi* bacteria in each treatment using *wijaya kusuma* leaves extract which shows differences in the inhibition zone area against *Salmonella typhi* bacteria by forming a clear area around the disc paper. Based on the testing of ethanol extract of *wijaya kusuma* leaves against *Salmonella typhi* bacteria, after incubation for 24 hours, the diameter of the inhibition zone was measured and calculated by measuring the clear area around the disc paper. The results are presented in Figure 2.

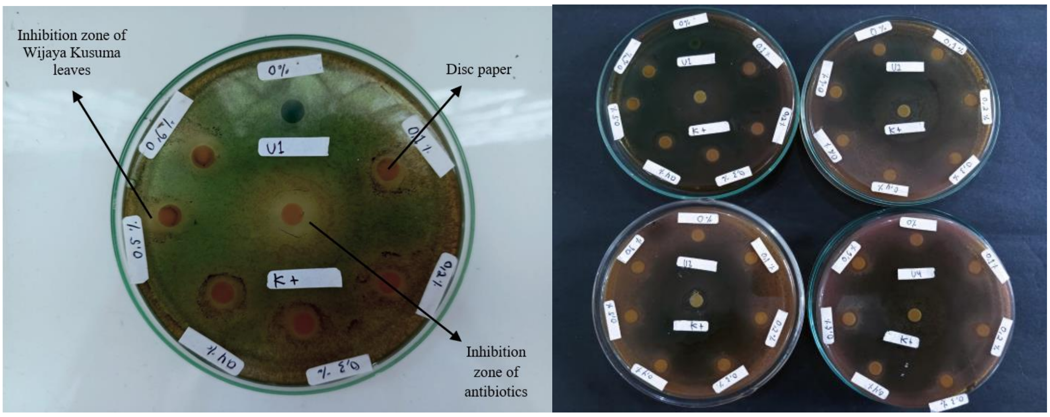

Figure 1. Zone of inhibition formed by ethanol extract of *wijaya kusuma* leaves against *Salmonella typhi* bacteria

Figure 2 shows that each concentration of *wijaya kusuma* leaf extract has an inhibitory effect on *Salmonella typhi* bacteria and shows differences in the antibacterial inhibition zone between each concentration in forming the inhibition zone area. At 0% concentration, no inhibition zone was formed, while concentrations of 0.1; 0.2; 0.3; 0.4; 0.5; and 0.6% formed inhibition zones. The treatment that produced the lowest mean inhibition zone area was 0.2% concentration with a mean of 30.96 ± 4.83 mm^2, while the highest mean inhibition zone area was 0.6% concentration with a mean of 45.05 ± 4.80 mm^2. While the comparative control antibiotic amoxicillin concentration of 0.01% has a mean inhibition zone area of 100.85 ± 20.95 mm^2 with a ratio of extract and antibiotic of 1:134.

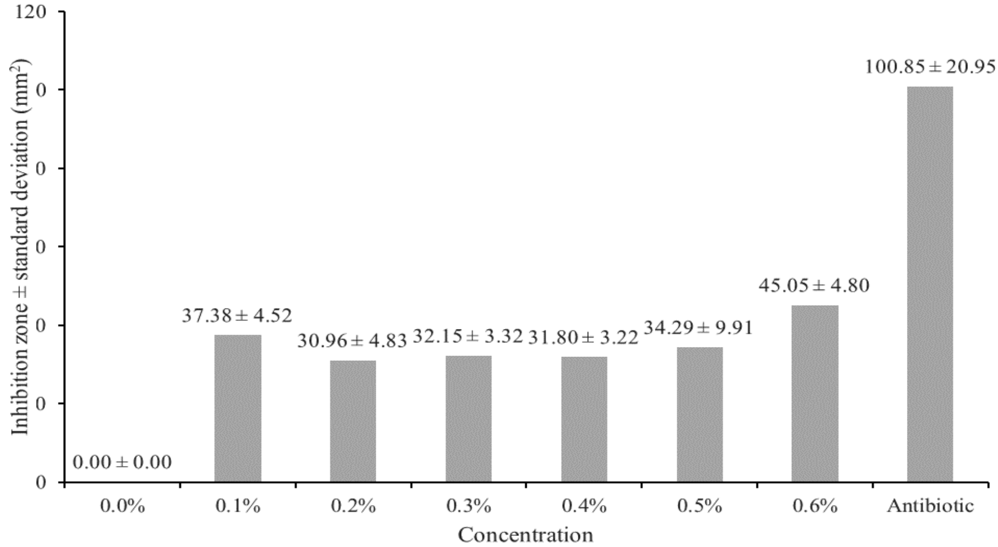

Figure 2. Graph of mean area of inhibition zone of ethanol extract of *wijaya kusuma* leaves against *Salmonella typhi* bacteria

Data Analysis

Based on the results of One-way ANOVA testing, the sig. Value was obtained 0.00 at the 0.05 test level which shows that the ethanol extract of *wijaya kusuma* leaves (*Epiphyllum oxypetalum*) has a significant effect in inhibiting the growth of *Salmonella typhi* bacteria. In addition, a larger f-count value of 30.241 was obtained compared to the f-table value of 2.572 so that at the 0.05 test level it showed that it was significantly different, so it was continued with the Tukey test to determine the best concentration and differences between each treatment tested. Previous testing has produced a Coefficient of Variance that meets the requirements for the Tukey test with a Coefficient of Variance value of 2.44% which is lower than 5%.

Table 1. Tukey test results mean zone of inhibition of ethanol extract of *wijaya kusuma* leaves against *Salmonella typhi* bacteria

Concentration (%)	Means ± standard deviation	Notation on Tukey test (5%)
0	0.00 ± 0.00	A
0.1	37.38 ± 4.52	B
0.2	30.96 ± 4.83	BC
0.3	32.15 ± 3.32	CD
0.4	31.80 ± 3.22	DE
0.5	34.29 ± 9.91	E
0.6	45.05 ± 4.80	EF

The results of the Tukey post-hoc test can be interpreted based on the letter notation that appears for each concentration (Table 1). If concentrations share the same letter notation, whether at the beginning or the end, it indicates that there is no significant difference between the treatments. Conversely, if the letter notation differs, the treatments are considered significantly different. This analysis is essential to determine the effectiveness of the applied treatments and to assess whether an increase in concentration truly affects the research outcomes. Therefore, a proper interpretation of the letter notation will help in drawing more accurate conclusions.

Minimum Inhibitory Concentration (MIC) Test

After the antibacterial test has been carried out and the results are analyzed to determine the best concentration based on the test results, followed by the MIC test to find the Minimum Inhibitory Concentration using *wijaya kusuma* leaves extract against *Salmonella typhi* bacteria using the liquid dilution method (Figure 3). The concentration tested in the MIC test starts based on the lowest concentration in the previous test which is active in inhibiting bacteria in the antibacterial test, namely at a concentration of 0.1%, which indicates that the concentration of the extract in the MIC test will start from a concentration of 0.1% to a concentration where bacterial growth cannot be inhibited, namely at 0%, so that the concentrations tested in the MIC test are 0.1%, 0.08%, 0.06%, 0.04%, 0.02%, and 0% (g/v).

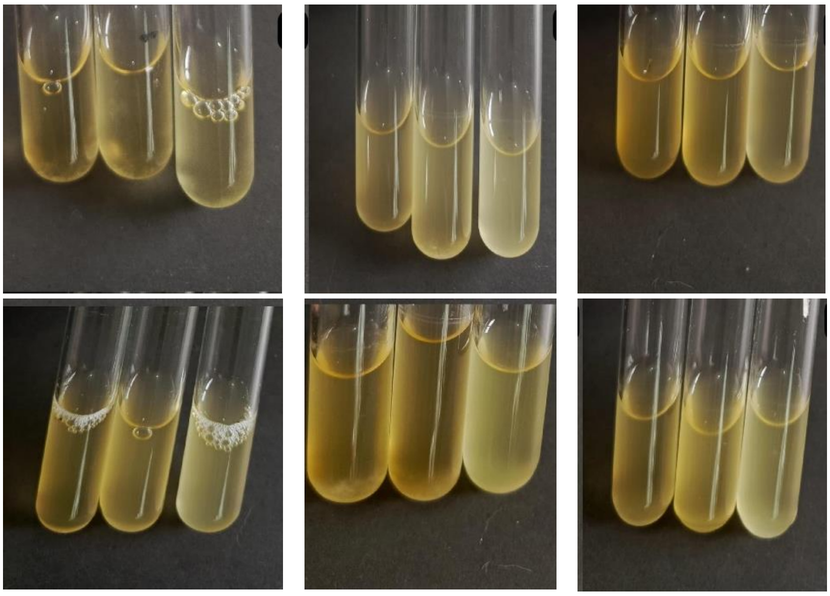

Figure 3. Observation of the turbidity level of the liquid dilution test results in the Minimum Inhibitory Concentration (MIC) test

The results of the MIC test research using the liquid dilution method with a spectrophotometer tool to see the absorbance value between after and before incubation and find the difference in absorbance values to determine the decrease in absorbance values affected by ethanol extract of *wijaya kusuma* leaves by looking at the light absorbed by the spectrophotometer, and the test results can be displayed in Table 2 below.

Table 2. The difference in absorbance value before and after incubation of ethanol extract of *wijaya kusuma* leaves against *Salmonella typhi* bacteria

Treatments	Concentration (%)	Before Incubation X ± SD	After Incubation X ± SD	Difference (X)
P0	0.00	1.98 ± 0.08	2.01 ± 0,10	0.03
P1	0.02	1.96 ± 0.11	1.65 ± 0,02	- 0.31
P2	0.04	2.01 ± 0.11	1.66 ± 0,01	- 0.35
P3	0.06	2.02 ± 0.06	1.64 ± 0,01	- 0.38
P4	0.08	1.95 ± 0.03	1.64 ± 0,02	- 0.31
P5	0.10	2.58 ± 0.11	1.61 ± 0,02	- 0.97

Note: X= means; SD= standard deviation

Table 2 shows that the absorbance value containing ethanol extract of *wijaya kusuma* 0.02; 0.04; 0.06; 0.08; and 0.1% plus bacteria decreased after incubation, but at 0% concentration (control) the absorbance increased after incubation. This is because there are compounds of *wijaya kusuma* flower leaf extract that actively inhibit bacteria at concentrations of 0.02; 0.04; 0.06; 0.08; and 0.1%, so that bacteria are inhibited and the medium becomes clearer with the difference in absorbance decrease worth -0.31 to -0.97. While at 0% concentration, there are no compounds that inhibit bacteria, so that when after incubation the absorbance increases by 0.03 due to bacterial growth which causes the medium to become cloudier. Based on the results of the MIC test after incubating for 24 hours, and then measuring the level of turbidity using a spectrophotometer, the Minimum Inhibitory Concentration (MIC) of ethanol extract of *wijaya kusuma* leaves (*Epiphyllum oxypetalum*) against *Salmonella typhi* bacteria is in the concentration range of 0–0.02%. In the MIC test, no ANOVA test or further tests were carried out because the MIC test was carried out using liquid dilution which was only to find where the minimum inhibitory concentration range was against *Salmonella typhi* bacteria.

DISCUSSION

Based on the results of the study, there are differences in the average inhibition zone area in Figure 2 in each treatment of ethanol extract of *wijaya kusuma* leaves against *Salmonella typhi* bacteria. Some of the factors that cause large and small differences in the inhibition zone formed are the high and low concentration of extracts, temperature and incubation time, length of contact time, type of solvent used, and the thickness of the bacterial cell wall structure (Oktaviani et al., 2020). The zone of inhibition formed around the disc paper occurs because bacteria cannot grow due to the inhibition of microbial growth by antibacterial compounds that diffuse into the media (Yusmaniar & Nida, 2017). Antibacterial substances can inhibit bacterial growth with a mechanism consisting of five ways, namely inhibition of the synthesis process in the cell wall, changing cell permeability, changing nucleic acid molecules, inhibiting enzyme activity, and inhibiting proteins and nucleic acids in the synthesis process (Wilapangga & Syaputra, 2018).

Based on Figure 2, shows that the ethanol extract of *wijaya kusuma* leaves with concentrations of 0; 0.1; 0.2; 0.3; 0.4; 0.5; and 0.6% get the results of the average inhibition zone area which is smaller than the average inhibition zone area of amoxicillin antibiotics against *Salmonella typhi* bacteria with a ratio of 1:134 which indicates the effect of bacterial inhibition caused by amoxicillin antibiotics is greater than the effect of ethanol extract of *wijaya kusuma* leaves. This happens because research on *wijaya kusuma* leaves as antibacterial is an initial research that is sustainable and can be developed, in contrast to amoxicillin antibiotics which have gone through a long research process for a long time.

Based on the results of the One Way ANOVA test, a significance value of 0.00 was obtained at a 0.05 significance level, indicating that all concentrations of ethanol extract from *Epiphyllum oxypetalum* leaves had a significant effect on inhibiting the growth of *Salmonella typhi* bacteria. Given these results, a follow-up analysis using the Tukey test was conducted to further examine the differences among the tested concentrations. This analysis also aimed to determine the concentration that provided the most optimal inhibitory effect on bacterial growth.

The results of the Tukey test, as presented in Table 1, indicate that the 0.6% ethanol extract concentration exhibited the highest antibacterial activity, with the largest average inhibition zone measuring 45.05 ± 4.80 mm². Furthermore, statistical analysis showed that the 0.6% concentration did not significantly differ from the 0.1% and 0.5% concentrations but demonstrated a significant difference when compared to the 0; 0.2; 0.3; and 0.4% concentrations against *Salmonella typhi* bacteria.

Based on the graph of the average results of the inhibition zone area (Figure 2) in each concentration treatment, the average inhibition zone area shows that the graph goes up at certain concentrations and down at certain concentrations and this graph is not consistent with the assumption that the higher the concentration of the extract, the greater the inhibition area formed. This happens because according to Omojate et al. (2014) stated that plant extracts contain various compounds with different antimicrobial mechanisms because some antimicrobial compounds have a certain dose in inhibiting bacteria whose inhibitory effect on bacteria increases at low concentrations, reaching a peak at a certain concentration. At low concentrations, one compound may dominate and cause a strong inhibitory effect. However, at high concentrations, the compound may become saturated or inactive, allowing other compounds with weaker inhibitory effects to dominate, ultimately resulting in lower inhibition. So that is what results in the rise and fall of bacterial inhibition between each concentration treatment.

The inhibitory effect on the growth of *Salmonella typhi* bacteria is due to the presence of secondary metabolite compounds that are soluble in ethanol extract of Wijaya leaves which have an active effect in inhibiting bacteria. Phytochemical analysis showed that *wijaya kusuma* (*Epiphyllum oxypetalum*) leaves contain several compounds such as saponins, phenolics, steroids, glycosides, tannins, terpenoids, alkaloids, flavonoids, sterols, resins, and phlobatanin (Upendra & Khandelwal, 2012). One of the mechanisms of action of antibacterial compounds, for example, alkaloids whose ability as antibacterials disrupts the peptidoglycan component of bacterial cells, resulting in the cell wall layer not forming perfectly (Lesmana et al., 2022). In addition, saponins as antibacterial by denaturing proteins. The similarity of saponin's surface active substances with detergents allows it to lower the surface tension of bacterial cell walls. This causes damage to the permeability of the bacterial membrane, which disrupts the survival of bacteria due to damage to the cell membrane (Sani et al., 2014). The mechanism of flavonoids as antibacterial is by inhibiting nucleic acid synthesis, inhibiting cell membrane function, and inhibiting energy metabolism (Pendit et al., 2016). The mechanism of action of phenol compounds in killing bacterial cells, namely by denaturing bacterial cell proteins so that the denaturation of bacterial cell proteins results in all metabolic activities of bacterial cells stopping because all bacterial cell metabolic activities are catalyzed by enzymes which are proteins (Marfuah et al., 2018). As a result, bacterial cells around the disc paper are damaged, which ultimately forms an inhibition zone around the disc paper (Ruhimat, 2015). The mechanism of action of steroids as antibacterial is related to lipid membranes whose sensitivity to steroids can cause lysosomes to leak and membrane integrity to decrease and the morphology of the cell membrane changes so that cells become brittle and lysed (Sari et al., 2017). Therefore, it can be interpreted that the inhibitory effect on *Salmonella typhi* bacteria in SSA media is caused by antibacterial compounds in *wijaya kusuma* leaves that have different mechanisms of action as inhibitors of bacterial growth.

According to Wiharningtias (2016), the determination of MIC is based on the difference between absorbance values before and after incubation, if the final absorbance value after incubation is higher than the absorbance value before incubation, it indicates that the bacteria are still growing. Conversely, if there is no change in absorbance value before and after incubation, or if the absorbance value after incubation is lower than the absorbance value before incubation, this indicates that bacterial growth has been inhibited.

Based on Figure 3, which presents the results of the Minimum Inhibitory Concentration (MIC) test, it was observed that at ethanol extract concentrations of *Epiphyllum oxypetalum* leaves of 0.02; 0.04; 0.06; 0.08; and 0.1%, with the addition of bacteria, there was a decrease in absorbance values after incubation, ranging from -0.31 to -0.97. This indicates the presence of active compounds in the extract that inhibit bacterial growth so that the medium becomes clearer. On the other hand, at 0% concentration (control), the absorbance increased after incubation with the addition of absorbance in the control was 0.03. This is due to the absence of compounds that inhibit bacteria at this concentration, resulting in bacterial growth that makes the medium cloudy. So the concentration of *wijaya kusuma* leaves extract from 0.1–0.6% percentage is an inhibitor against *Salmonella typhi* up to the MIC with a concentration range between 0–0.02%.

According to Kowalska and Dudek (2021) regarding drug sensitivity tests, including MIC tests, based on the direct interaction of pathogens isolated from patients with antibacterial substances, MIC is considered to have the greatest importance in the optimization of targeted antibiotic therapy and is the best parameter available to reflect the effectiveness of antibiotics against bacterial strains. The inefficiency of medical therapies used to cure patients with bacterial infections requires not only the active search for new therapeutic strategies, but also the careful selection of antibacterials based on various parameters, including MIC which defines the degree of susceptibility or resistance of a particular bacterial strain *in vitro* to a given antibiotic, so the use of antibacterials with low MIC values during the treatment of infections can improve the efficacy or efficiency of therapy. So the concentration of *wijaya kusuma* leaf extract from the percentage of 0.1–0.6% which is a low concentration inhibitor against *Salmonella typhi* up to the MIC with a concentration range between 0–0.02% which based on the results of this study, is expected that the use of low concentrations is safer for long-term use by humans. The use of antibacterial substances in low concentrations is expected to provide the same or even greater effect in inhibiting bacterial growth, without the concern of side effects that may arise from the use of high concentrations. In addition, the use of low concentrations can save materials for the manufacture of *wijaya kusuma* leaves extract so that it is more efficient and relatively cheaper.

CONCLUSION

Ethanol extract of *wijaya kusuma* leaves (*Epiphyllum oxypetalum*) has an effect as a growth inhibitor of *Salmonella typhi* bacteria. The results of testing the ethanol extract of *wijaya kusuma* leaves in the antibacterial test had a significant effect on *Salmonella typhi* bacteria with the best concentration of 0.6% which had a mean inhibition zone area of 45.05 ± 4.80 mm^2 and in the Minimum Inhibitory Concentration (MIC) test in inhibiting *Salmonella typhi* bacteria in the concentration range of 0–0.02%.

Suggestions for further research to identify compounds in *wijaya kusuma* leaves to find out more about compounds with effective inhibitory effects against *Salmonella typhi* bacteria and it is advisable to conduct further research on the Minimum Kill Concentration (MKC) test against *Salmonella typhi* bacteria using *wijaya kusuma* leaves.

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