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Potential of Fig Fruit Ethanol Extract (*Ficus carica* L.) Against *Staphylococcus aureus* MRSA and *Klebsiella pneumoniae* ESBL Bacteria

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Abstract

Ficus carica L. commonly known as figs originate from West Asia and have bioactive components with antibacterial properties. This study aimed to determine the antibacterial activity of fig ethanol extract in inhibiting the growth of MDR *Staphylococcus aureus* MRSA and *Klebsiella pneumoniae* ESBL bacteria, to determine the MIC and MBC values, and the types of compounds contained in fig ethanol extract. The method began with fig extraction using concentrations of 20%, 40%, 60%, 80%, and 100% with 4 repetitions, testing MIC and MBC using the liquid dilution method, TLC-Bioautography test with ethyl acetate: methanol: aquades (7: 2: 1) eluent. The results showed that fig fruit extract had an inhibition zone with an average value of 3.30 mm at a concentration of 100% which was still in the weak category, the MIC value of *Staphylococcus aureus* MRSA bacteria was a concentration of 100% and *Klebsiella pneumoniae* ESBL bacteria was a concentration of 80%. The MBC value of MDR bacteria was not obtained because there was still bacterial growth. The results of the TLC-bioautography test of ethanol extract of fig fruit (*Ficus carica* L.) can inhibit bacterial growth indicated by the presence of a clear zone with an Rf value of 0.5 cm (unidentified), 0.31 cm (Flavonoid), 0.2 cm (Phenol). The compounds that act as antibacterials against *Staphylococcus aureu*s MRSA and *Klebsiella pneumoniae* ESBL bacteria are the flavonoid and phenol groups.

Keywords: Fig Fruit (*Ficus carica* L.); Antibacterial Activity; MIC; MBC; TLC-Bioautography.

INTRODUCTION

Infectious diseases are diseases caused by pathogenic microorganisms such as bacteria. Infectious diseases can also be caused by various microorganisms such as bacteria, viruses, and fungi are organisms that can attack the body (darsono *et al*., 2020).

Currently, many pathogenic bacteria are resistant to several antibiotics, known as MDR (*Multi Drug Resistant*) bacteria. Indonesia is ranked 8th out of 27 countries with the highest *multi-drug resistant* predicate in the world (Estiningsih *et al.,* 2020). One of the *Staphylococcus aureus* MRSA bacteria is a gram-positive bacterium that is pathogenic and is often found in contaminated milk (Ramadhan et al., 2020). Another bacteria that can cause infectious diseases is *Klebsiella pneumoniae* ESBL bacteria, which is a gram-negative bacterium from the Enterobacteriaceae family that often causes lung infections (pneumonia), urinary tract infections (UTIs), and gastrointestinal infections (Kurama, et al., 2020).

MDR (*Multi Drug Resistant*) bacteria is a condition where bacteria are resistant to more than one antibiotic. MDR (*Multi Drug Resistant*) bacteria can be caused by several things, namely the use of antibiotics with inappropriate doses and diagnostics. The types of bacteria detected include *Staphylococcus aureus*, *Streptococcus pneumoniae, Neisseria* sp., *Escherichia coli, Shigella* sp., *Salmonella* sp., *Enterobacter Aerogenes, Pseudomonas aerogenosa*, and *Klebsiella pneumoniae* (Kurniawan et al., 2024).

One type of medicinal plant that has many benefits is figs (*Ficus carica* L). Figs have a high nutritional content and have the potential as a medicinal ingredient (Nugraha, 2020). Figs (*Ficus carica* L.) are plants that have antibacterial activity against gram-positive and gram-negative bacteria, and have been shown to have health benefits. The benefits of figs in the scientific scope and pharmacological activity are used as antibacterials, antioxidants, antitumors, while for traditional medicine they are used to treat ulcers, digestive disorders, and diarrhea (Ramadhanti, 2023). Figs are an important source of bioactive components such as phenols, benzaldehyde, terpenoids, flavonoids, and alkaloids which have antioxidant properties (Ramadhan et al., 2020).

Research on figs (*Ficus carica* L) as an antibacterial is still minimal and there is still a lot of bacterial resistance to antibiotics, the author needs to develop an antibacterial compound as a basis for conducting

research on the antibacterial activity test of ethanol extract of figs (*Ficus carica* L) against MDR bacteria using the TLC bioautography method.

MATERIALS AND METHODS

Place and time of research

The research was conducted at the Microbiology Laboratory and Chemistry Laboratory of the Faculty of Mathematics and Natural Sciences (FMIPA) State University of Medan, The research was conducted in December 2023 - March 2024.

Extraction of Fruit

The figs (*Ficus carica* L.) used in this study were fresh and not rotten. The collected figs were cleaned with running water. The sampling technique used was random, namely simple random sampling method.

Figure 1. Fig fruit (*Ficus carica* L.).

The study conducted to obtain fig extract using the squeezing method. The squeezing method was done by blending the figs. Fig extraction was done by the maceration method. Maceration was done 2 times. 2,500 grams of finely ground figs are put into a glass jar, then soaked in 2,500 ml of ethanol solvent p.a with a solvent ratio of 1: 1 (w / v). The first extraction process was carried out for 5 days and stirred every 24 hours. The extract was filtered using filter paper to separate it from the pulp. The second maceration is carried out with a ratio of 1: 0.5. Maceration was carried out twice. The extract results were combined and concentrated using a rotary vacuum evaporator at a temperature of 40 °C until a thick extract is obtained.

Bacterial Rejuvenation

Two grams of nutrient agar (NA) media was taken, dissolved in 100 ml of distilled water and put into an Erlenmeyer flask, heated on a hotplate until dissolved and homogeneous, then the dissolved NA media was put into a sterile test tube. The NA media that had been put into the test tube was sterilized by autoclaving at a temperature of 121°C for approximately 15 minutes. The media was left at room temperature until it solidified in a tilted position, when scratching the bacteria, the tube was brought close to the Bunsen, covered with cotton and incubated for 24 hours at a temperature of 37°C all done aseptically in laminar airflow.

Antibacterial Activity of Ethanol Extract of Fig Fruit

This antibacterial activity test was carried out using the Kirby-Bauer method or disc diffusion method. A total of 10 mL of Mueller Hinton Agar (MHA) media was inserted into a petri dish, scratching the bacteria evenly into the petri dish using a sterilized cotton bud, placing a 6 mm diameter disc paper that had been soaked with fig extract with variations of 20%, 40%, 60%, 80%, 100%. In this study, the negative control used was the solvent used as a diluent for the extract used in the study, namely ethanol, the positive control used was chloramphenicol. The petri dish was incubated at 37°C for 24 hours.

Minimum Inhibitory Concentration (MIC)

MIC was conducted using the modified Kirby and Bauer liquid dilution method (Fatisa, 2013). A total of 4 ml of sterile NB media was put into each test tube, and 0.5 ml of extract was added with each extract having 5 concentrations (20%, 40%, 60%, 80%, 100%), then the tubes were incubated for 24 hours at 37°C in an incubator. After incubation, the bacterial absorbance was measured again using a spectrophotometer.

Minimum Bacterial Concentration (MBC)

MBC was further tested, namely on all tubes used in MIC, by taking 0.2 ml of the suspension that showed the MIC, then put into a test tube containing 5 ml of sterile NB media. The test tube was incubated for 24 hours at 37°C in an incubator, then the absorbance value was measured using a spectrophotometer.

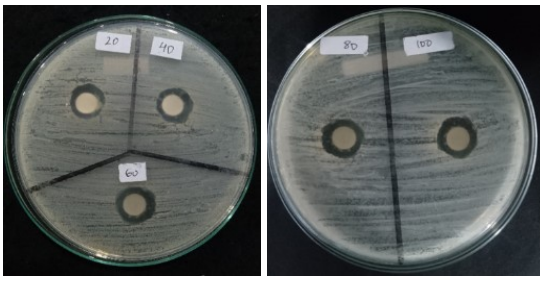
Thin Layer Chromatography

Separation of fractions will occur when a solvent was added as a mobile phase, three solvents used are ethyl acetate, methanol and aquades with a ratio of (7: 2: 1). The mobile phase was put into a glass bottle (chamber). The chromatogram is made by cutting the TLC plate at a size of 8 x 2 cm, the upper and lower boundaries are marked using a pencil (0.5 cm upper limit and 1 cm lower limit).

Ethanol extract of figs was spotted using a capillary pipette on the TLC plate, spotted using a capillary pipette. The TLC plate that had been spotted was inserted into the chamber and eluted, after the solvent had reached the upper limit, the TLC plate was removed and dried, the spots that emerged were observed with UV light at a wavelength of 366nm (Hidayat et al., 2017).

TLC-Bioautography Testing

The solidified MHA media was swabbed with *Staphylococcus aureus* MRSA and *Klebsiella pneumoniae* ESBL bacteria using a sterile ose needle, then the media was left for 10 minutes so that the bacterial suspension could be absorbed, after solidifying and absorbing, the eluted TLC plate was placed and pressed slightly on the surface of the media, after 30 minutes the TLC plate was lifted then the MHA media was incubated at 37°C for 24 hours, the inhibition zone formed on the surface of the media was observed.



a b

Figure 3. Antibacterial activity test of ethanol extract of figs against *Staphylococcus aureus* MRSA bacteria.

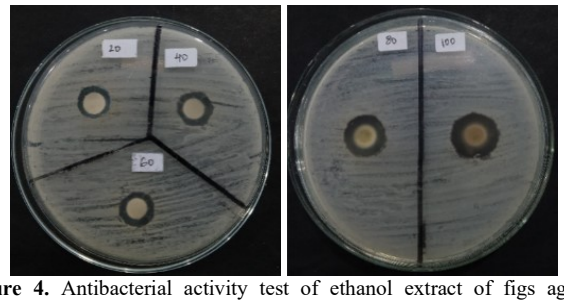


Figure 4. Antibacterial activity test of ethanol extract of figs against *Klebsiella pneumoniae* ESBL bacteria.

RESULTS AND DISCUSSION

Antibacterial Activity Test

The study revealed that fig fruit extract (*Ficus carica* L.) has antibacterial activity against *Staphylococcus aureus* MRSA and *Klebsiella pneumoniae* ESBL bacteria. The resulting thick extract is brown in color with a weight of 149.81 gr. The results of the observations can be seen in Figure 2.

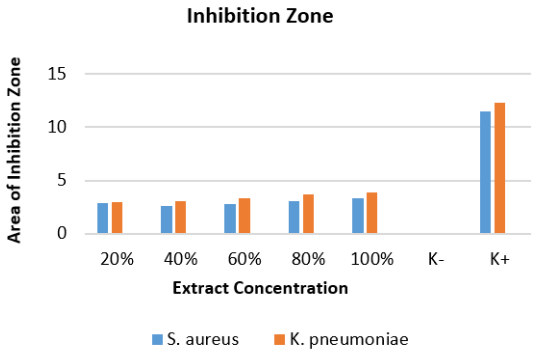


Figure 2 Comparison of Inhibition Zone Results Formed from Ethanol Extract of Fig Fruit (*Ficus Carica* L.)
Description: Positive Control: Chloramphenicol Antibiotic, Negative Control: Ethanol Solvent p.a.

Based on the data obtained, it can be said that each ethanol extract of fig fruit (*Ficus carica* L.) has the ability as an antibacterial against *Staphylococcus aureus* MRSA and *Klebsiella pneumoniae* ESBL bacteria, indicated by the presence of an inhibition zone formed around the disc paper in the inhibition test that has been carried out, with an incubation time of 24 hours with an incubation temperature of 37°C. Based on the data, the extract of fresh fruit juice has the potential to inhibit the growth of gram-positive and gram-negative bacteria which is still in the weak category.

The diameter of the inhibition zone formed does not always increase with the increase in the concentration made, this is due to differences in the diffusion rate of active antibacterial compounds in agar media (Wahlanto *et al*., 2020). Higher concentrations do not always provide a greater inhibitory effect but have a smaller inhibitory ability compared to other concentrations (Zeniusa *et al.,* 2019). Several possibilities that cause this to happen, such as the lack of diffusion power of the extract into the media. The higher the concentration of the extract, the lower the solubility (thickens like a gel) so that it can slow down the diffusion of the active ingredients of the extract in the media so that it can reduce the ability of the extract.

Inhibition zones with small diameters have low antibacterial activity, while inhibition zones with large diameters have high antibacterial activity. The components of substances, quality and quantity contained in medicinal plants that can weaken, strengthen, improve, or change the inhibition zone cause a large increase and decrease (Afriani *et al*., 2024).

Minimum Inhibitory Concentration (MIC)

MIC value testing was carried out using a UV-Vis spectrophotometer at a wavelength of 625 nm to measure the absorbance value (turbidity value) before and after incubation in the test tube. The results of the observations are seen in Figure 5

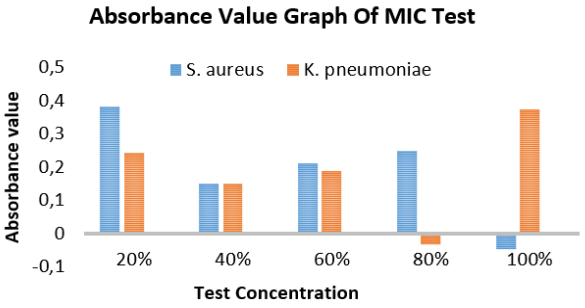


Figure 5. Graph of Absorbance Value of MIC Test.

Based on Figure 5, the MIC value in *Staphylococcus aureus* MRSA bacteria is at a concentration of 100% with an average of -0.0476 while in *Klebsiella pneumoniae* ESBL bacteria at a concentration of 80% with an average of -0.0315. The amount of absorbance or absorption depends on the content of the substance in it. The more the content of the substance in the sample, the molecules that will absorb light with a certain wavelength which causes the resulting absorbance value to be greater, in other words the absorbance value is equivalent to the concentration (Yanlinastuti, 2016).

In the table, negative absorbance values indicate a decrease in the absorbance value, indicating a decrease in the number of cells that have been incubated, while positive absorbance values indicate no decrease in the absorbance value, indicating that the number of bacterial cells is still increasing after incubation.

Minimum Bactericidal Concentration (MBC)

Minimum Bactericidal Concentration (MBC) was carried out by taking the tube from the MIC test results. Based on the results of the research that has been carried out, the MBC value was not obtained because the absorption value after incubation showed a significant increase that there was still bacterial growth.

The increase in absorbance value depends on the level of substance contained in the solution, the greater the level of substance contained, the more molecules will absorb light at a wavelength. At a concentration that experiences a decrease in the average absorbance value, because the molecules are in small quantities to absorb light coming at a certain wavelength (Putri *et al,* 2023).

Determination of MIC and MBC in this antibacterial test was carried out using the liquid dilution method. The advantage of using this method is the use of more efficient media. The parameters used are turbidity (there is bacterial growth) and clarity (no bacterial growth), which can be seen after incubation for 24 hours. However, this tends to be subjective, so the absorbance values before and after incubation are used to help determine the presence or absence of bacterial growth using a UV-Vis Spectrophotometer.

The advantage of the spectrophotometric method is that this method provides a simple way to determine the quantity of a very small substance, and the results obtained are quite accurate, where the numbers read are directly recorded by the detector and printed in the form of digital numbers or graphs that have been regressed. The weakness of the UV-Vis spectrophotometer is that it cannot distinguish samples from other particles that absorb light at the same wavelength, it can also be caused by the amount of light absorbed by the solution in the tube is not completely absorbed by the extract compound, but is also absorbed by dead bacterial cells (Sekeon *et al*., 2018). The concentration of the sample affects the absorption of its turbidity so that the higher the concentration, the higher the absorption.

Thin Layer Chromatography (TLC)

From the results of TLC-Bioautography on ethanol extract of figs (*Ficus carica* L.) there are secondary metabolite compounds that have the potential to be antibacterial in S*taphylococcus aureus* MRSA and *Klebsiella pneumoniae* ESBL bacteria, namely the flavonoid and phenol compound groups. According to Gultom 2020, compounds that act as antibacterials against *Klebsiella pneumoniae* ESBL bacteria are alkaloid, flavonoid, and phenol compounds. Flavonoids are polyphenol compounds that function as antibacterial compounds by disrupting the integrity of bacterial cell membranes. Phenol compounds are one of the compounds that are antibacterial, with their performance that can damage bacterial cell walls so that they have the potential to be natural antibacterials in pathogenic bacteria (Anwariyah, 2011).

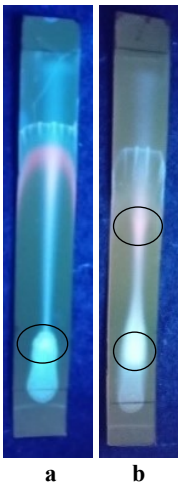


Figure 6. Results of spots on UV light 366 after spraying reagents: a. After spraying $AlCl_3$ b. After spraying $FeCl_3$

According to research conducted by Irwan (2017), spraying with the reagent $AlCl_3$ is marked by the presence of a yellow stain color, indicating that the compound is positive for flavonoids. Flavonoids are polyphenol compounds produced by plants, various studies have been conducted that flavonoid compounds have been proven to have antibacterial activity. The activity of flavonoid compounds as antibacterials is stimulated through molecular mechanisms, namely they

can damage the sheath of gram-negative and gram-positive bacteria. Flavonoid compounds are also thought to be able to damage bacterial cell components (Cahyani *et al*., 2023).

Detection of phenol content in solution with color changes in the solution after being dripped into green, red, jet black, blue, or purple. Spray reagent $FeCl_3$ will produce blue, red purple, dark brown, green and strong black to determine phenol compounds. Spray reagent $FeCl_3$ will produce blue-black or green-blue to determine tannin compounds (Mariana *et al.,* 2013).

TLC-Bioautography

The results of the TLC bioautography test of ethanol extract of figs (*Ficus carica* L.) against *Staphylococcus aureus* MRSA and *Klebsiella pneumoniae* ESBL bacteria can be seen in Figure 7 and Figure 8.

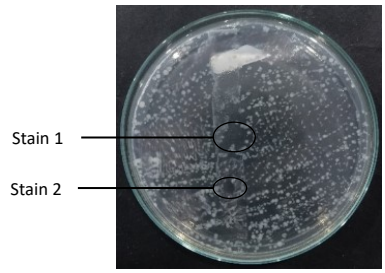


Figure 7. Results of TLC Bioautography Test of ethanol extract of figs (Ficus carica L.) on *Staphylococcus aureus* MRSA bacteria.

Testing the antibacterial activity of fig fruit ethanol extract using the TLC bioautography method showed positive results against *Staphylococcus aureus* MRSA bacteria. Figure 7 shows spot 1 which has the potential to be an antibacterial with an Rf value of 0.53 cm whose chemical compound was not identified and spot 2 has the potential to be an antibacterial with an Rf value of 0.31 cm which is suspected of containing secondary flavonoid metabolite compounds.

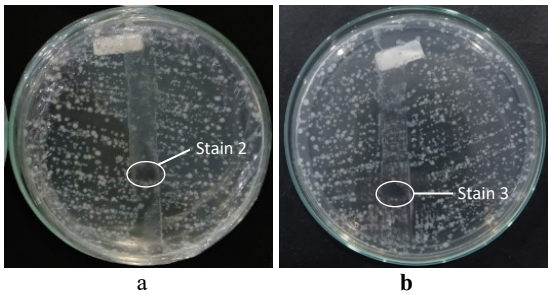


Figure 8. Results of TLC Bioautography Test of Ethanol Extract of Fig Fruit (Ficus Carica L.) on *Klebsiella Pneumoniae* ESBL Bacteria (a) $AlCl_3$ (b) $FeCl_3$

TLC bioautography showed positive results against *Klebsiella pneumoniae* ESBL bacteria. Figure 8 shows that spots that have the potential to be antibacterial are spots 2 with an Rf value of 0.31 cm which is suspected of containing secondary metabolite compounds of flavonoids, then sprayed with the reagent $AlCl_3$ showed positive results and the appearance of reddish yellow fluorescence spots under UV 366 nm, and spots 3 with an Rf value of 0.2 cm are suspected of containing secondary metabolite compounds of phenol, sprayed with the reagent $FeCl_3$ and showed positive results with the formation of a green color.

CONCLUSION

The ethanol extract of Fig Fruit (*Ficus carica* L.) has antibacterial activity against *Staphylococcus aureus* MRSA and *Klebsiella pneumoniae* ESBL bacteria with a weak category. The Minimum Inhibitory Concentration (MIC) in *Staphylococcus aureus* MRSA bacteria is -0.0476 at a concentration of 100%, while the value in *Klebsiella pneumoniae* ESBL bacteria is -0.0315 at a concentration of 80%. The MBC value in *Staphylococcus aureus* MRSA and *Klebsiella pneumoniae* ESBL bacteria cannot be found because it increases after incubation, which indicates that there is still bacterial growth so that the bacteria cannot be killed. Secondary metabolite compounds that have the potential to be antibacterial found in Fig Fruit (*Ficus carica* L.) are flavonoid and phenol compounds.

Competing Interests: The authors state that there are no competing interests.

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