

Antibacterial Activity Test of Ethanol Extract of Spoon Leaves (*Plantago major*. L) Against *Salmonella typhi*

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ABSTRACT

Typhoid is a bacterial infection disease of *Salmonella typhi*. Conventionally, typhoid treatment is carried out with antibiotics. The irrationality of antibiotics against *S. typhi* is reported to trigger bacterial resistance, thus requiring an alternative antibacterial agent against typhoid. The diversity of Indonesian medicinal plants is still promising as a potential source of antibacterial agent, including sendok leaf. This study was aimed to determine antibacterial activity of ethanol extract of kacapiring leaves and its fractions against *S. typhi*. Subsequent fractionation was carried out using liquid-liquid partition method to obtain n-hexane, ethyl acetate, and ethanol-water fractions. Antibacterial test was carried out using disk diffusion method to determine the diameter of inhibitory zone at concentrations of 20%, 25%, 30%, and 40%. The best antibacterial activity was revealed by residue fraction of 30% and 40% with inhibition zone diameter of $8,5 \pm 0.051$ mm and $8,7 \pm 0.081$ mm, respectively. Sendok have moderate antibacterial activity against *S. typhi*.

Keywords : *S. typhi*, Sendok leaf, Extract

INTRODUCTION

Infection is one of the causes of death (mortality) in the world with a death toll of 15 million in 2010 and two-thirds of these deaths were caused by bacteria and viruses. Infection is a disease disorder caused by bacteria, parasites, viruses, microbes and pathogens from outside that invade the body and cause infection. Infection needs special attention in the treatment and prevention efforts, especially in typhoid. The World Health Organization (WHO) globally estimates that typhoid reaches 11-20 million cases each year, resulting in around 128,000-161,000 deaths each year. Currently, antibacterial treatment is generally carried out by administering antibiotic drugs such as amoxicillin, ampicillin, and chloramphenicol and other antibiotics used as antibacterial therapy. Irrational use of antibiotics causes many cases of bacterial resistance, and the emergence of side effects and other adverse reactions. Therefore, to reduce resistance and adverse side effects, other alternatives are needed through the discovery of new antibacterial innovations that are expected to be more effective in treatment and safer in use. Typhoid infection is caused by *Salmonella typhi* bacteria. (*S. typhi*) through food or drink that enters the body. Antibacterial is an agent or substance that functions to inhibit the growth and proliferation of bacteria and can kill certain bacterial cells. This study began with the extraction process, then antibacterial tests were carried out to determine its activity and continued with fractionation and testing of antibacterial activity.

METHODS

A. Tools and Materials

The tools used are Analytical balance. (Sartorius CP224S), oven (Mettler), Rotary evaporator (Heidolph), Micropipette 10-1-100 μ l (Socorex), Micropipette 100-1000 μ l (Eppendorf), Hotplate, Microtip, microtube, vortex (Labnet), Aluminum foil (Klin-pak), Autoclave (ALP), Laminar Air Flow (Thermo SCIENTIFIC 13000 SERIES A2) and Incubator (Clifton). The materials used in this study include: Gardenia augusta leaves taken randomly in Umbulsari District, Jember Regency, Salmonella thypi obtained from the Microbiology Laboratory, Faculty of Medicine, Brawijaya University. Sodium Agar (NA) and Mueller Hinton Agar (MHA), 96% ethanol, n-hexane, ethyl acetate, physiological NaCl 0.9%, 10% DMSO (dimethyl sulfoxide), sterile distilled water, blank disks and 30 μ g chloramphenicol disks. This research was conducted at the Microbiology and Biotechnology Laboratory, Department of Pharmaceutical Biology, Faculty of Pharmacy, University of Jember in April 2019 to August 2019.

B. Extraction

Ethanol extract of kacapiring leaves is made by weighing 200 grams of powdered simplicia then put into a macerator and added 2000 mL of 96% ethanol (1:10) to carry out maceration extraction and stirred every 24 hours. The macerate is then filtered using a Buchner funnel to separate the residue. The filtrate obtained is concentrated using a rotary evaporator to separate the chemical compounds attached to the solvent. The remaining residue is then re-macerated using the remaining solvent from the concentration. The results of repeated maceration are then filtered, evaporated and dried until a thick extract is formed. The thick extract is then weighed and its yield is calculated.

C. Making Bacterial Media

Media Na

Na media is used as a culture medium or bacterial rejuvenation. Bacterial culture media is made by weighing 0.69 g of Na powder then dissolving it with 30 mL of distilled water in a 50 mL Erlenmeyer flask and heating it until it is homogeneous and completely dissolved. Furthermore, 5 mL of Na media is poured into 5 test tubes and sterilized using an autoclave at a temperature of 121°C and for $\pm$ 15 minutes. After that, the sterile Na media is put into a refrigerator with a 45°C tilt position until a slanted agar medium is formed. The Na media that has been made is then rejuvenated. The bacterial rejuvenation process is carried out by taking pure bacterial colonies and then scratching them on the NA media using an ose needle in a test tube. The process is carried out aseptically under LAF and incubated at a temperature of 37 °C for 18-24 hours.

D. Media MHA

MHA media is used as a medium for bacterial testing. The media is made by weighing 20.9 g then dissolving it with 550 mL of distilled water and heating it until it is homogeneous and completely dissolved. 15 ml of MHA media is then poured into 36 test tubes and sterilized by

autoclaving at 121°C for ± 15 minutes. Then the sterile MHA media is put into the refrigerator until a solid agar medium is formed.

E. Preparation of Test Solution and Control Group

Ethanol extract test solution of kacapiring leaves and its fractions were made with four series of concentrations, namely 20%, 25%, 30%, and 40%. Preparation of the extract test solution was carried out by making a stock solution, namely weighing 0.4 g of extract then dissolving it with 1 mL of 10% DMSO to obtain a concentration of 40%. The 40% test solution was then diluted to obtain concentrations of 30%, 25% and 20%. Preparation of the test solution on the fraction used the same concentration, namely 20%, 25%, 30%, and 40%. Preparation of the test solution on the fraction was carried out with the same process as the preparation of the extract test solution. The negative control used was 10% DMSO made by adding 1 ml of 100% DMSO then adding 9 ml of distilled water and vortexing until homogeneous. The positive control used was a 30 μ g Chloramphenicol disc.

F. Preparation of *S. typhi* Suspension

Bacterial suspension is made by inserting 10 mL of 0.9% NaCl solution into a test tube, then the bacterial culture that has been made in Na media is inoculated into a test tube that has been filled with 0.9% NaCl solution aseptically and vortexed until the suspension is homogeneous. The bacterial suspension is then measured for absorbance using spectrophotometry to be equivalent to Mc Farland 0.5, which is between 0.08-0.13 (3×10^8 CFU/mL). If the bacterial suspension has met the absorbance range according to the Mc Farland standard, the bacterial suspension is ready to be used for antibacterial testing.

G. Antibacterial Test

Antibacterial tests were conducted to determine the potential of compounds contained in the ethanol extract of glass plate leaves and fractions. The antibacterial testing process on the extract was carried out by dripping test solutions of 20%, 25%, 30%, and 40% concentrations and 20 μ L of 10% DMSO negative control onto the available sterile discs and left for 18-24 hours. The bacterial suspension that had been made previously was poured 50 μ L into a petri dish that had been filled with MHA solid media and leveled using a cotton bud. The extract test solution, 10% DMSO negative control, and 30 μ g chloramphenicol disc positive control were then placed into the petri dish that had been filled with test bacteria. Then incubated at 37 °C for 18 hours.

RESULT

The results of the antibacterial activity test can show that of all the experimental samples that have antibacterial activity are in the ethanol water fraction samples at concentrations of 30% and 40%. The results of the antibacterial activity test of the ethanol extract of kacapiring leaves and its fractions against *S. typhi* can be shown in Figure 1.

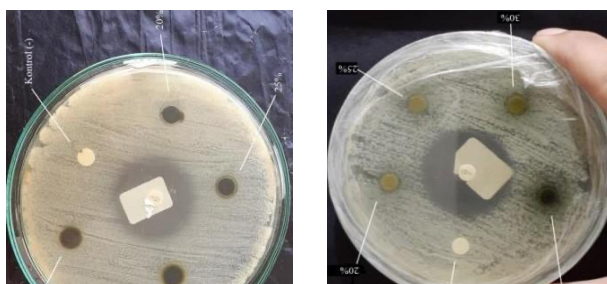


Figure 1. The result of antibacterial test

DISCUSSION

Extraction of spoon leaf *simplicia* powder was carried out using the maceration method. The maceration method was chosen because it was considered easier and simpler, did not require a heating process, and was safe for active substances that were degraded due to heating. The use of ethanol was based on its ability to extract most of the secondary metabolites contained in the *simplicia* powder. The liquid-liquid partition method was used in fractionation. This method was chosen because it was relatively easier and simpler, and did not require special processing techniques.

Based on the results of the yield obtained, it showed that the ethanol-water fraction produced the highest yield followed by the hexane fraction and the ethyl acetate fraction with the lowest yield. The ethanol-water fraction is a polar fraction where this fraction can be suspected of containing compounds consisting of flavonoids, glycosides, tannins, polyphenols, saponins, and several alkaloid compounds. The hexane fraction is a non-polar fraction which can be suspected of containing fatty acid, steroid, and terpenoid compounds. The ethyl acetate fraction is a semipolar fraction and can be suspected of containing compounds consisting of alkaloids and flavonoids.

The large yield obtained is due to differences in compound distribution that depend on the polarity of the solvent where according to the principle of like dissolve like that compounds will be attracted to each other based on the similarity of their polarity properties. Based on the results of statistical analysis obtained extract samples, n-hexane fractions, ethyl acetate fractions at all concentrations and ethanol-water fractions at concentrations of 20% and 25% did not have significant differences against the negative control group ($p > 0.05$). While the ethanol-water samples with concentrations of 30% and 40% showed differences against the samples with concentrations of 20% and 25% and the negative control group ($p < 0.05$). The results of this analysis can be concluded that the extract samples, n-hexane fractions, ethyl acetate fractions at all concentrations and ethanol-water fractions at concentrations of 20% and 25% do not have antibacterial activity because they do not have significant differences against the negative control group. While the ethanol-water fraction samples with concentrations of 30% and 40% showed significant differences against the negative control group so that the significant difference indicates antibacterial activity against *S. typhi*. However, the statistical results of the 30% versus 40% ethanol-water fraction samples did not show any significant differences so that the results were considered to have the same inhibitory activity value against the growth of *S. typhi*.

The presence of antibacterial activity is caused by the presence of secondary metabolite compounds in kacapiring leaves. AlFaruq et al. (2017). in their study reported that *Gardenia coronaria* leaves contain compounds consisting of alkaloids, glycosides, steroids, tannins, flavonoids, saponins, gums, and amides. in other studies it was also reported that kacapiring leaves contain flavonoids, steroids, saponins, polyphenols, and alkaloids. based on the principle of chemotaxonomy in the same genus, it shows that this study is in accordance with the results of previous studies. the presence of antibacterial activity in this study can be suspected to be due to the presence of secondary metabolite compounds contained in the

ethanol-water fraction or polar compounds. The presence of antibacterial activity in the ethanol-water fractions of 30% and 40% concentrations is due to the presence of polar compounds. Secondary metabolite compounds included in polar compounds consist of flavonoids, glycosides, polyphenols, tannins, saponins, and several alkaloid compounds. In addition to having antibacterial activity against *S. typhi*, kacapiring leaves also have activity against other bacteria. In reports of several other studies, it was proven that 50% kacapiring leaves have antibacterial activity against *S. aureus* 8.5 mm. Nuralifah et al. (2019)). also reported that the antibacterial activity of kacapiring leaves has antibacterial activity against *S. aureus* reported that 40% leaves have antibacterial activity against 8.7 mm

CONCLUSION

Antibacterial activity is found at concentrations of 30% and 40% respectively at 7,564 and 8,529 mm. However, between the two concentrations, it cannot be determined which is the most active because statistically they have almost the same inhibitory activity value against the growth of *S. typhi* bacteria. added containing recommendations on research or input that can be used directly by readers.

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