



ANTIBACTERIAL ACTIVITY TEST OF BINAHONG LEAF (*Anredera cordifolia* (Ten) Steenis) AGAINST *Staphylococcus Aureus* and *Pseudomonas Aeruginosa*

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ABSTRACT

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Binahong (*Anredera cordifolia*(Ten.)Steenis) is one of the plants that is thought to be an alternative as an antibacterial. The purpose of this study was to determine the antibacterial activity of extracts and fractions of Binahong leaves (*Anredera cordifolia* (Ten.) Steenis), as well as to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericida Concentration (MBC) of extracts and fractions against *Staphylococcus Aureus* and *Pseudomonas Aeruginosa* was carried out in vitro using the microdilution method and the parameter was turbidity. The results showed that the MIC produced on the *Staphylococcus Aureus* bacteria was 512µg/mLwith ethyl acetate extract and fraction while the MIC produced on the *Pseudomonas Aeruginosa* was 512 µg / mL for extract, the methanol- water fraction and the best MIC was produced by the ethyl acetate fraction. With a concentration of 256 µg / mL. Binahong (*Anrederacordifolia*(Ten.)Steenis) leaves have a bacteriostatic effect on *Staphylococcus Aureus* and *Pseudomonas Aeruginosa*.

INTRODUCTION

The prevalence of infectious diseases continues to increase, one of which is due to the factor of resistance, where antimicrobial resistance has become a public health problem in both developed and developing countries. This problem is a serious threat to public health, including in Indonesia. this problem arises from the unwise use of antimicrobials which results in ineffective antimicrobial therapy. Therefore, antimicrobial resistance must be controlled (WHO, 2017) Indonesia is a tropical country that has a high prevalence of infectious diseases.This bacterial disease is usually treated with antibiotics such as chloramphenicol, dicacyclin and methicillin, but it is important to note that excessive use of antibiotics.Danger of resistance of bacteria and medical costs are quite high, raising awareness to find alternatives to antibiotics by using

Traditional medicines derived from plants as alternative medicines against bacterial infections (Wise, 2003). The binahong plant is one of the medicinal plants that have great potential in the future to be researched. Various community experiences, binahong can be used to help the healing process of disease, because it has an effect as an antioxidant (Susanti, 2019), antibiotic, antibacterial, antiviral, and anti-inflammatory (Kurniawan & Aryana, 2015). Research conducted by (Nuryanti et al., 2014) shows that the ethanol extract of Binahong leaves (*AnrederaCordifolia* (Ten.) Steenis) has antibacterial activity against several bacteria, especially for infections caused by *B. cereus*, *B. Subtillis*, *MSSA*, *MRCNS*, *E. coli* and *P. aeruginosa* with minimum inhibitory concentrations (MIC) of 256, 256, 512, 512, 256 and 256 µg / mL. It

was reported again that in research conducted by (Ayu et al., 2018) that the N-hexane extract of Binahong Leaves (*Anredera Cordifolia* (Ten.) Steenis) was combined with anti-tuberculosis drugs. It shows that Binahong Leaf n-hexane extract (*Anredera Cordifolia* (Ten.) Steenis) has the best activity when compared to other extracts. In this study, tests were carried out on the extract and fraction of Binahong leaves so that it was known that the compounds had antibacterial activity based on polarity.

MATERIALS AND METHODS

Tools and Materials: The tools were used in this study were petri dish, mikro pipet, erlenmeyer, sterile scissors, incubators, ose needle, ruler, sterile knife, centrifugator, shaker, test tube, tube rack, spiritus burners, analytical balance, pipette, spatula, glass beaker, measuring cup, stirring bar, bath, autoclave, fatty cotton, yarn mattress, micropipette, LAF, tip, heat-resistant plastic, sterile tissue. The materials used in this study were 70% alcohol, comparative antimicrobials (Tetracycline), distilled water sterile, Binahong leaves (*Anredera Cordifolia* (Ten.) Steenis), MHA, MHB Bacteria samples were used in this study were *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Method: This research was experimental, which was done by testing the Binahong leaves extract and fraction on the bacteria *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The antibacterial activity of Binahong leaves is determined through several stages, from collecting materials in the Bandung, West Java. Then the material is determined, Binahong leaves processing starts from wet sorting, dry sorting to extracting. Furthermore, sterilization of tools and materials using an autoclave. Then rejuvenation of the test bacteria and making a bacterial suspension with the concentration is needed in the study. Binahong extract and fraction testing was carried out singly on the bacteria *Staphylococcus aureus*, and *Pseudomonas aeruginosa* with the method used was microdilution to determine the value of Minimum Inhibitory Concentration (MIC) and Minimum Kill Concentration (MBC). The bioautography test is then performed.

RESULTS AND DISCUSSION: This research begins with the process of finding

Binahong leaves samples obtained in Bandung, West Java. Making simplicia was done by collecting 750 g of simplicia. The part that was leave was taken as a sample. After getting wet mold, wet sorting, washing, changing the shape are made to reduce the surface area, followed by drying process using oven, dry sorting and storage. Tests of water soluble extracts and soluble extracts in ethanol aim to determine the amount of active compounds extracted in the solvent from a number of simplisia powders. From the data above, 49% based on data consist of water soluble extracts and 27% consist ethanol soluble extracts indicate that the compounds contained in the binahong leaves simplicia are more soluble in water compared to etanol. Test for total ash content and test for insoluble ash content in acid aim to determine minerals and inorganic contamination (Sudarmadji, 1986). The amount of total ash in the binahong leaves extract indicates that an extract containing much or at least minerals is obtained. Based on table 1, the total ash content is 14%, showing the presence of insoluble ash content in the acid and the presence of sand or other impurities in low levels. Phytochemical screening is carried out to determine the class of compounds contained in the simplicia. Phytochemical screening includes: examination of Flavonoids, Alkaloids, Saponins, Tannins, Quinons, Steroids and Triterpenoids. The results obtained from the leaves of Binahong (*Anrederacardiofolia* (Ten) stennis) contain flavonoids, saponins, tannins, steroids / triterpenoids. Based on table 2 shows The results obtained from the leaves of Binahong (*Anrederacardiofolia* (Ten) stennis) contain flavonoids, saponins, tannins, steroids / triterpenoids. After the extraction process is carried out, the fractionation process is carried out using the liquid-liquid extraction method (ECC). Fractionation using more than one solvent, the solvent used is methanol water with a ratio (2: 8) as a polar solvent, N-hexane 100 ml as a non-polar solvent and ethyl acetate 100 ml as a semi-polar solvent, the treatment was carried out three times. The testing antibacterial activity of the binahong leaves extract and fraksi were conducted using the Microdilution method. This method can find out the MIC.

Table 1. Simplicia Characterization

| No | Test | Results (%) |
|----|------------------------|-------------|
| 1 | Water- soluble content | 49 |
| 2 | Etanol-soluble content | 27 |
| 3 | Total ash content | 14 |

Table 2. Phytochemical Binahongsimplicia screening result

| No | Test | Results |
|----|-----------|---------|
| 1 | Alkaloid | - |
| 2 | Flavonoid | + |
| 3 | Saponin | + |
| 4 | Tanin | + |

Information: + = Detected; - = not detected

Tabel3. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Test Bacteria

| Sample | <i>Staphylococcus aureus</i> | | <i>Pseudomonas aeruginosa</i> | |
|----------------------------|------------------------------|------|-------------------------------|------|
| | MIC | MBC | MIC | MBC |
| Extract tes | 512 | >512 | 512 | >512 |
| Fraction of water methanol | >512 | >512 | 512 | >512 |
| Fraction of n he | > 512 | >512 | >512 | >512 |
| Fraction of ethil acetate | 512 | >512 | 256 | >512 |
| Tetracyclin | 32 | >512 | 1 | >512 |

Information: MIC = Minimum Inhibitory Concentration

MBC= Minimum Bactericidal Concentration

This is defined as the lowest concentration of a compound that can decrease 80% or more growth compared to negative controls (NCCLS, 2009) and the Minimum Kill Concentration (KBM) which is the lowest concentration that shows no growth (Holetz, 2002). The MIC value of Binahong leaves extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa* were 512 ppm. The results of the MIC values of tetracycline compared to all antibiotics in the test bacteria were classified as strong where the MIC value was 1 ppm. KBM test results showed that all samples still contained bacterial growth, which means the value of KBM against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Tetracyclin* were at a concentration of >512 ppm.

The best MIC (minimum inhibitory concentration) from the antibacterial test was the ethyl acetate fraction, which was monitored using Thin Layer Chromatography (TLC) where the ethyl acetate fraction of binahong

leaves was placed on the TLC plate. The eluent used was chlorophome: methanol in a ratio (9: 1). Furthermore, it was observed at uv 254 nm and uv 365 nm

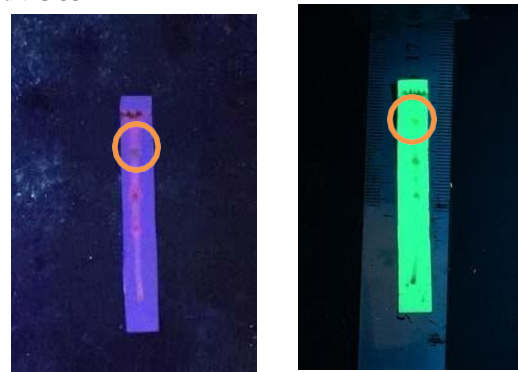


Figure 1. The results of the Thin Layer chromatography

Bioautographic testing is carried out to determine which active compounds are thought to have antibacterial activity (Papatungan et al., 2019). The bioautography method used is contact bioautography. This method is based on the diffusion of the compounds that have been separated by thin layer chromatography (TLC).

The ethyl acetate chromatography plate was then placed on the surface of the agar media which had been inoculated with the *Pseudomonas aeruginosa* bacteria, left for 30 minutes, then the chromatography plate was removed and removed from the surface of the medium. After making observations there is a clear zone on the media so that it indicates the presence of inhibition against the tested bacteria. In this monitoring, the Rf is 0.81. The spots are proven to inhibit the growth of the tested bacteria. The compounds that play a role in antibacterial activity are thought to be tannin.



Figure 2. The results of the Bioautography of fraction ethilacetat to the *Pseudomonas aeruginosa*

CONCLUSION

From the results of research on testing the antibacterial activity of Binahong leaves it can be concluded that:

- Binahong leaves extract has antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria.
- Binahong leaves extract with the best MIC value that is 256 ppm with KBM value > 512 ppm shows the antibacterial activity of *Pseudomonas aeruginosa*.
- Bioautography results obtained a clear zone at the point with an Rf of 0.81. The spots are proven to inhibit the growth of the tested bacteria. The compounds that play a role in antibacterial activity are thought to be tannins.

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