

Antibiofilm Activity of Ethanol Extract of Binahong Leaves (*Anredera cordifolia*) Against *Escherichia coli* and *Staphylococcus aureus*

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ABSTRACT

Background: Binahong leaves (*Anredera cordifolia*) exhibit antibiofilm activity against *Escherichia coli* and *Staphylococcus aureus* due to their active compounds, such as flavonoid, saponin, tannin, and alkaloid. These compounds work by inhibiting bacterial adhesion, damaging extracellular polymers, and disrupting quorum sensing. **Aims:** This study aims to test the antibacterial and antibiofilm activity of binahong leaf extract (*Anredera cordifolia*) against *Escherichia coli* and *Staphylococcus aureus*. **Methods:** This study used the disc diffusion method for antibacterial testing and the Microtiter Plate Assay method for antibiofilm activity, with each test performed in triplicate. **Results:** In the antibacterial test, *Escherichia coli* produced a weak inhibition zone with an average of 4.7 mm at a concentration of 125 mg/mL, while *Staphylococcus aureus* produced a strong inhibition zone with an average of 11.0 mm at the same concentration. In the antibiofilm test, an extract concentration of 170 mg/mL showed an MBIC50 value against *Escherichia coli* ATCC 25922, with a biofilm inhibition percentage of 53.41%, while a concentration of 165 mg/mL showed an MBIC50 value against *Staphylococcus aureus* ATCC 25923, with a biofilm inhibition percentage of 52.54%. **Conclusion:** The ethanol extract of binahong leaves has both antibacterial and antibiofilm activity against *Escherichia coli* and *Staphylococcus aureus*.

KEYWORDS: Binahong leaves, phytochemical screening, antibacterial, antibiofilm, *Escherichia coli*

INTRODUCTION

Biofilms are collections of microorganisms that form a community and are protected by an extracellular matrix. The presence of this matrix can inhibit the entry of antibiotics, making it more difficult for the bacteria to be eliminated by antimicrobial agents. This condition can

cause infections to persist longer and contribute to antibiotic resistance. Bacterial resistance to antibiotics is the resistance of bacteria that can cause infections to antibiotics that were initially effective against them. Some resistant bacteria include *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*,

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Streptococcus pneumoniae, non-typhoidal *Salmonella*, *Shigella*, and *Neisseria gonorrhoeae*. Biofilm formation by bacteria is closely linked to the development of antibiotic resistance (Yuliani, Prasetyo, and Liberitera, 2017). As bacterial resistance increases in the healthcare sector, the discovery and development of new drugs, particularly antibacterial drugs, is necessary (Guntur et al. 2021).

Worldwide, the prevalence of antibiotic use increased by 65% between 2000 and 2015 in 76 countries, with an average consumption increase of around 39% per 1,000 patients. Antibiotic use increased by about 65% between 2000 and 2015 in 76 countries, with an average consumption increase of around 39% per 1,000 patients. Some types of antibiotics, including ampicillin, tetracycline, sulfonamides, ciprofloxacin, clindamycin, and erythromycin, have resistance rates of at least 50% of the total amount used (Rahmadi, 2024). Based on data from the Antimicrobial Resistance Control Committee for the year 2013, by 2019, the percentage of resistant bacteria increased from 40% to 60%, and then to 60.4%. Research conducted in Palangkaraya shows that the general public's knowledge about

the use of antibiotics is still quite low, with only 25.71% having good knowledge, 37.14% having sufficient knowledge, and 37.14% having poor knowledge (Marsudi, 2021).

Biofilm is one of the important factors in determining the pathogenicity level of a bacterium. The two bacteria most frequently found in biofilm-related diseases are *Staphylococcus aureus* and *Escherichia coli*, especially in urinary tract infections, which can lead to recurrent or chronic infections (Ballen, Ratia, and Soto 2022). In biofilm formation, *Staphylococcus aureus* can cause changes in gene transcription from planktonic cells to bacterial cells that are resistant to the host's immune system because antibodies are not optimal in the process of phagocytosis and elimination of cell growth, so that host cells become a good place for *Staphylococcus aureus* to live and grow. Infections caused by biofilm formation ultimately lead to antibiotic resistance, making the infections increasingly difficult to cure (Dewi, Darmawi, and Helmi, 2020). *Escherichia coli* can form biofilms during its growth as a defense mechanism against unfavorable environmental conditions, such as nutrients, oxygen, and antibiotics. By forming a polysaccharide extracellular matrix (EPS), *Escherichia coli* can form biofilms that are more resistant to antibiotics and disinfectants than those that

live planktonically (Triana, 2018).

One of the plants in Indonesia that has antibacterial properties is the binahong leaf (*Anredera cordifolia* (Ten.) Stennis). Binahong leaves contain tannin, flavonoid, alkaloid, and saponin. The binahong plant has many benefits in curing various diseases and also as an antibacterial agent. The part of the binahong plant that can be used as herbal medicine is the leaves (Halim, 2022).

METHODS

Chemicals and Reagents

The main materials used in this study were *Escherichia coli* ATCC 25922 [manufacturer/catalog no.], *Staphylococcus aureus* ATCC 25923 [manufacturer/catalog no.], binahong leaf extract, 70% and 96% ethanol (Bratachem), ciprofloxacin (Kimia Farma), dimethyl sulfoxide, crystal violet, sodium chloride by Merck, Nutrient Agar (NA), and Trypticase Soy Broth (TSB) by Sigma-Aldrich.

Sample Preparation

The sample used was binahong leaves (*Anredera cordifolia*) that were randomly picked, choosing leaves that weren't too young or too old, were still fresh, and came from Makassar (Tamalanrea District), South Sulawesi.

Binahong leaves (*Anredera cordifolia*) are washed thoroughly, then chopped and dried by airing them in a room protected

Antibiofilm Activity of Binahong Leaves from sunlight or in a drying cabinet (Raharjo, 2023).

Extraction

Binahong leaf powder is extracted using the maceration method with 70% ethanol solvent until all the simplisia is moistened and left for approximately 10 minutes to allow the simplisia to be thoroughly moistened. Then, ethanol solvent is added until the simplisia powder is completely submerged. This process was carried out for 3x24 hours, in maceration with solvent replacement every 24 hours. Then it was evaporated using a rotary evaporator until a thick extract was obtained and the yield was calculated (Fadilah, 2020).

Phytochemical Screening

Phytochemical screening was conducted to identify the compounds contained in the binahong leaf extract, including flavonoid, saponin, tannin, and alkaloid (Halim, 2022).

Flavonoid

A quantity of two grams of binahong leaf ethanol extract was placed into a test tube and heated for 5 minutes. Next, 0.2 gram of magnesium powder and 5 drops of concentrated HCl were added. A positive result was indicated by the formation of a yellow color (Achmad dkk, 2024).

Saponin

A quantity of two grams of ethanol extract from binahong leaves was placed in a test tube, distilled water was added, and

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and the mixture was shaken for 30 seconds. A positive result was indicated by the formation of foam after shaking (Prantika et al., 2024).

Tannin

A quantity of two grams of ethanol extract of binahong leaves was placed in a test tube, and 2 to 3 drops of 1% FeCl₃ were added. A positive result was indicated by the formation of a blackish-green color (Achmad dkk., 2024).

Alkaloid

Two grams of ethanol extract of binahong leaves were placed in a test tube, HCl was added, and then several drops of Wagner's reagent were added. The brown precipitate indicated a positive alkaloid test (Susanty and Yudhistirani 2018).

Antibacterial Asssay

Media Preparation

A total of 4 grams of Nutrient Agar (NA) was weighed and dissolved in 100 mL of distilled water using an Erlenmeyer flask. The solution was then homogenized with a stirrer over a water bath until boiling. The medium was then sterilized using an autoclave at 121°C for 15 minutes and left at room temperature until the medium solidified (Iif Hanifa Nurrosyidah et al. 2021).

Preparation of Bacterial Suspensions

A bacterial suspension of *Escherichia coli* was prepared by taking 1–3 loops of bacterial culture, then placing it into a test

tube with 10 ml of 0.9% NaCl, and shaking until well mixed. The turbidity was adjusted to McFarland 0.5, which is equivalent to 1.5×10^3 CFU/mL (Restyana, Ihtiramidina, and Ida Kristianingsih, 2019). *Staphylococcus aureus* was prepared using the same procedure.

McFarland Preparation

A volume of 0.05 mL of 1% BaCl₂ solution was pipetted into a screw-cap test tube, followed by 9.95 mL of 1% H₂SO₄ solution. The two solutions were then combined in the screw-cap test tube and vortexed until evenly mixed (Rosmania and Yanti, 2020).

Preparation of Test Solutions

The extracts were prepared in 5 ml at concentrations of 75 mg/ml, 100 mg/ml, and 125 mg/ml, and the negative control was 5% dimethyl sulfoxide. The extracts were weighed and then dissolved in 5% dimethyl sulfoxide.

Antibacterial Activity Test

About 10 mL of Nutrient Agar (NA) was poured into a Petri dish, and the medium was then mixed until it was homogeneous and ready for use. Sterile cotton was then used to inoculate the medium with the bacterial suspension. Sterile paper discs were then soaked in 30 µL of extract solutions at various concentrations, dimethyl sulfoxide was used as a negative control, and ciprofloxacin as a positive control. The treatment was repeated three

times. The Petri dishes were incubated at 37°C for 24 hours. Antibacterial activity was determined by measuring the diameter of the inhibition zone formed over a certain period of time (Fajrina dkk., 2018).

Antibiofilm Assay

Biofilm inhibition activity testing was carried out using the Microtiter Plate Assay method with three repetitions. Binahong leaf extract was used at serial concentrations of 180 mg/ml, 175 mg/ml, 170 mg/ml, 165 mg/ml, 160 mg/ml, 155 mg/ml, 150 mg/ml, 145 mg/ml, 140 mg/ml, 135 mg/ml, 130 mg/ml, and 125 mg/ml. The medium was added to the test wells and control wells. Add the binahong leaf extract to the test wells, and add 200 µL of ciprofloxacin antibiotic to the positive control wells. Then add the bacterial suspension to the test wells and negative control wells. Cover the microplate and incubate in an incubator at 37°C for 24 hours. After incubation, the contents of the microplate are removed and washed three times with sterile distilled water and dried. After the microplate is dry, 200 µL of 1% crystal violet dye is added to the test wells to stain the biofilm biomass, then incubated at room temperature for 30 minutes, after which the contents of the microplate are removed. Then, 200 µL of 96% ethanol solution was added to all wells and incubated for 15 minutes. The microplate was then measured using an ELISA reader

at a wavelength of 595 nm. (Kining et al, 2016). Calculated using the formula:

$$\frac{\text{OD Negative control} - \text{OD Experimental sample}}{\text{OD Negative control}} \times 100$$

RESULTS AND DISCUSSION

The results of the extraction of 500 grams of binahong (*Anredera cordifolia*) leaves, 145.3 grams of thick extract was produced with a yield percentage of 29.06%. The requirement for thick extract yield is that the value must not be less than 10%. Based on the table above, a yield percentage of 29.6% was obtained, which means it meets the standard requirements (Kementarian Kesehatan RI, 2017).

Alkaloid testing using Wagner's reagent produces a brown precipitate, indicating the presence of alkaloids. Furthermore, flavonoid testing with Mg powder and HCl produces a yellow color, indicating the presence of flavonoids that act as antioxidants and anti-inflammatories. Tannin testing with reagent is indicated by the formation of a black color. Hydrolyzed tannin will show a blue-black color, while condensed tannin will show a green-black color when added with FeCl₃. The saponin test using distilled water produces foam when shaken. The foam formed contains saponins, some of which are soluble in polar or hydrophilic solvents, while other compounds are soluble in non-polar or hydrophobic solvents (Prantika et al., 2024). See Table 1 as the result.

Table 1. Phytochemical screening test results

Compound	Result	Description
Alkaloid	(+)	Brown precipitate
Flavonoid	(+)	Yellow
Tannin	(+)	Blackish green
Saponin	(+)	Foam

In antibacterial testing with 3 replicates on binahong leaf extract samples against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 bacteria. Antibacterial activity has several categories of inhibition zones, namely weak, moderate, strong, and very strong. If the inhibition zone is less than 5 mm (<5 mm), it is classified as weak; 5-10 mm is classified as moderate; 10-20 mm is classified as strong; and more than 20 mm (>20 mm) is classified as very strong (Rahmawati et al., 2026). Based on the Table 2 showed that, the antibacterial activity testing against *Escherichia coli* with binahong leaf extract at various concentrations exhibited relatively low inhibitory power against bacterial growth. At a concentration of 125 mg/ml, the average diameter of the inhibition zone reached 4.7 mm, which is categorized as weak. Concentrations of 100

mg/ml and 75 mg/ml produced lower inhibition zones, 1.65 mm and 0.61 mm, respectively, which were also classified as weak. The results of this study indicate that as the concentration of the extract decreases, its antibacterial activity against *Escherichia coli* also decreases. As a comparison, a positive control of ciprofloxacin was used, which serves as a standard because it has been proven effective in inhibiting the growth of Gram-negative bacteria, including *Escherichia coli*. The results of the positive control showed an average inhibition zone of 33.5 mm, which falls into the very strong category. Meanwhile, the negative control of DMSO showed no inhibition zone (0.00 mm), so it can be concluded that the solvent does not have antibacterial activity against *Escherichia coli*. The antibacterial activity against *Staphylococcus aureus* showed differences in inhibitory power for each extract concentration. The average diameter of the inhibition zone at a concentration of 125 mg/mL was 11.0 mm, which falls into the strong category. This indicates that at a high extract concentration, it can significantly inhibit

Table 2. Antibacterial activity of binahong leaf extract

Concentration	Inhibition Zone (mm)	
	<i>E. coli</i>	<i>S. aureus</i>
125 mg/ml	4.78±6.77	11.07±3.63
100 mg/ml	0.83±0.49	4.40±4.60
75 mg/ml	0.62±0.06	2.25±1.39
Ciprofloxacin 0,01%	33.5±1.74	29.93±1.83
Dimethyl sulfoxide 5%	0	0

the growth of *Staphylococcus aureus*. In contrast, for concentrations of 100 mg/mL and 75 mg/mL, the average inhibition diameters were only 4.4 mm and 2.25 mm, respectively, which are considered weak. This suggests that antibacterial activity decreases at lower extract concentrations. As a comparison, the positive control using ciprofloxacin showed an average inhibition zone of 29.9 mm, categorized as very strong, in line with its properties as a standard antibiotic. Meanwhile, in the negative control (dimethyl sulfoxide), no inhibition zone was formed, confirming that the inhibitory effect originated from the test extract, not the solvent. These results indicate that the extract has antibacterial potential against *Staphylococcus aureus*.

Research results show that the inhibition zone diameter of *Staphylococcus aureus* is larger than that of *Escherichia coli*. This difference in sensitivity is due to the structure of each bacterium. Because of their different structures, gram positive bacteria often exhibit higher antibacterial activity than gram negative bacteria. As a result, antibacterial agents more easily bind to and penetrate gram positive bacteria (Sari et al., 2017). In the positive control (ciprofloxacin), both had strong inhibition zones because ciprofloxacin is a fluoroquinolone drug that functions to inhibit bacterial DNA synthesis, thereby inhibiting microbial resistance, and is a

broad-spectrum antimicrobial (Daud et al., 2023). Meanwhile, the negative control (DMSO) did not produce an inhibition zone because it has no antibacterial effect. DMSO only acts as a solvent that dissolves organic materials in the extract and does not damage the bacterial cell wall, thus not affecting bacterial growth. It can be concluded that the inhibition that occurred was truly derived from the active compounds in the extract, not from the solvent (Putri, 2022).

In this study, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as test bacteria. *Escherichia coli* ATCC 25922 is a Gram-negative bacterium that can form biofilms and produce endotoxins that can suppress the immune response. Meanwhile, *Staphylococcus aureus* ATCC 25923 is a Gram-positive bacterium that is an opportunistic pathogen and has a relatively complex biofilm formation mechanism (Lihawa et al., 2023).

In this study, a biofilm inhibition test was conducted to determine the extract's ability to inhibit biofilm formation by *Escherichia coli* and *Staphylococcus aureus* bacteria at various concentrations. Optical Density (OD) values were used as an indicator of the level of biofilm formation, with higher OD values indicating more abundant or thicker biofilms (Rollando, 2019).

The biofilm inhibition test of *Escherichia coli* ATCC 25922 bacteria showed that the extract concentration affected the ability to inhibit biofilm (Table 3). In the treatment group with the highest volume, namely 180 mg/ml, the average value was 0.231 with a biofilm inhibition percentage of 67.36%. This value is the highest inhibition compared to other treatment groups. As the extract concentration decreases, the inhibition percentage also decreases. In the control group, the positive control showed an average OD value of 0.415 with a biofilm inhibition percentage of 43.31%. These results confirm that the positive control is able to inhibit biofilm formation, although its inhibitory power is still lower than binahong extract at high concentrations. Meanwhile, the negative control had an average OD value of 0.708, indicating high biofilm formation because no treatment was given, so that the bacteria could grow and form biofilms optimally. The media control had an average OD value of 0.164, which serves to ensure that the media does not affect biofilm formation, while the blank control showed an average OD value of 0.052 as a baseline value without biofilm. These results prove that binahong leaf extract has strong potential in inhibiting the formation of *Escherichia coli* ATCC 25922 bacterial biofilms. Thus, the higher the percentage of inhibition of the extract, the greater its inhibitory ability. The antibiofilm

concentration of 170 mg/mL in *Escherichia coli* is higher than the antibacterial concentration of 125 mg/mL. This is due to the presence of an extracellular matrix in the biofilm, which can limit the penetration of active compounds, making bacteria in the biofilm more difficult to inhibit and requiring higher extract concentrations.

The increase in the inhibitory activity of *Escherichia coli* biofilm along with increasing extract concentration indicates a relationship between the concentration of the test material and antibiofilm activity. The higher the concentration of the extract used, the more active compounds that have the potential to play a role in inhibiting biofilm formation. This result is in line with the research of Wang et al. (2025) which reported that natural compounds are able to inhibit the formation of *Escherichia coli* biofilm in a concentration-dependent manner. In addition, natural compounds can inhibit the production of *Extracellular Polymeric Substances* (EPS) which play a role in the formation and stability of biofilms. Thus, the high percentage of inhibition at higher concentrations of binahong leaf extract indicates the potential of the extract as an antibiofilm agent against *Escherichia coli*. This is presented in the Table 3.

The inhibition test of *Staphylococcus aureus* ATCC 25923 bacterial biofilm at the Table 4, showed that the concentration of

Table 3. Biofilm inhibition test of binahong leaf extract

Treatment	Extract concentration (mg/ml)							Control			
	180	175	165	155	145	135	125	PC	BC	MD	BL
Average	0.231	0.289	0.404	0.469	0.539	0.602	0.665	0.415	0.708	0.164	0.052

Note: PC (Ciprofloxacin (0.01%)); BC (*Escherichia coli*); MD (*Tryptic Soy Broth Media*); BL (DMSO 5%)

Table 4. Biofilm inhibition test of binahong leaf extract

Treatment	Extract Concentration (mg/ml)							Control			
	180	175	165	155	145	135	125	PC	BC	MD	BL
Average	0.263	0.289	0.331	0.416	0.497	0.573	0.625	0.308	0.697	0.186	0.050

Note: PC (Ciprofloxacin 0.01%); BC (*Staphylococcus aureus*); MD (*Tryptic Soy Broth*); BL (DMSO 5%)

the extract had a significant effect on the ability to inhibit biofilm. In the treatment group with the highest extract volume of 180 mg/ml, the average OD value was 0.263 with a biofilm inhibition percentage of 62.25%. This shows that the higher the concentration of binahong leaf extract, the greater its inhibitory effect on biofilm formation. However, as the extract concentration decreased, the percentage of biofilm inhibition also decreased. At concentrations of 175 mg/ml to 155 mg/ml, the inhibition percentage gradually decreased from 58.56% to 40.29%, while at concentrations of 150 mg/ml to 125 mg/ml, the inhibition continued to decrease significantly, reaching a lowest value of 10.29% at 125 mg/ml. In the control group, positive control, negative control, media control, and blank control were used as comparators. The positive control showed an average OD of 0.308 with a biofilm inhibition rate of 55.84%, indicating that the positive control was effective in inhibiting biofilm formation. Meanwhile, the negative control showed an average OD

value of 0.697, indicating that *Staphylococcus aureus* ATCC 25923 bacteria were able to form a strong biofilm. The control medium with an average OD value of 0.186 serves to ensure that the medium does not affect biofilm formation, while the blank control with an OD of 0.050 shows the baseline value without biofilm growth. The results indicate that binahong leaf extract can inhibit the growth of *Staphylococcus aureus* ATCC 25923 biofilm, with the highest effectiveness at a concentration of 180 mg/mL. A decrease in the inhibition percentage and lower concentrations suggests that the active compounds in the extract play an important role in its antibiofilm activity; thus, the higher the extract's inhibition percentage, the greater its inhibitory capability.

The test results showed that binahong leaf extract has the ability to inhibit the formation of *Staphylococcus aureus* ATCC 25923 biofilms. Higher inhibitory activity at increasing concentrations indicates that the concentration of the extract plays a role in the resulting antibiofilm effect. This is in

line with the research of Barbarossa et al. (2025) that plant extracts have the potential to inhibit *Staphylococcus aureus* biofilms. In addition, Kim and Kim (2025) showed that the antibiofilm activity of natural ingredients can increase along with the increase in the concentration used. Therefore, the highest inhibitory results at a concentration of 180 mg/mL indicate the potential of binahong leaf extract as an antibiofilm agent against *Staphylococcus aureus*.

The percentage of inhibition of *Escherichia coli* ATCC 25922 biofilm showed an increasing pattern as the concentration of the extract administered increased. At low concentrations (125 mg/ml–165 mg/ml), the inhibition activity was still relatively small, showing a percentage value that did not reach 50%. At concentrations of 170–180 mg/ml, the inhibition value reached $\geq 50\%$, namely 53.41% to 67.36%. This condition indicates that binahong leaf extract has a strong antibiofilm effect in this concentration range. Thus, the concentration capable of inhibiting biofilm formation by 50% (MBIC₅₀) can be determined to be in the range of 170–180 mg/ml. These results indicate that the extract's effectiveness in inhibiting the formation of *Escherichia coli* ATCC 25922 biofilm is dose-dependent, and the achievement of values above the MBIC₅₀ threshold strengthens the evidence that the

extract has significant potential as an antibiofilm agent.

The percentage of inhibition of *Staphylococcus aureus* ATCC 25923 biofilm showed an increase as the concentration of the extract administered increased. At low concentrations (125–160 mg/ml), the inhibition activity was still relatively small, showing a percentage value that did not reach 50%. At concentrations of 165–180 mg/ml, the inhibition reached $\geq 50\%$, namely 52.54% to 62.25%. This condition shows that the extract provides a stronger antibiofilm effect with increasing doses. Based on the concentration threshold capable of producing 50% inhibition (MBIC₅₀), it can be determined to be in the range of 165–180 mg/ml. These results indicate that the extract's effectiveness in inhibiting the formation of *Staphylococcus aureus* ATCC 25923 biofilm is dose-dependent, and achieving values above the MBIC₅₀ threshold strengthens the evidence that the extract has significant potential as an antibiofilm agent.

CONCLUSION

Based on the research results, it can be concluded that ethanol extract of binahong leaves has antibiofilm activity against *Escherichia coli* and *Staphylococcus aureus* bacteria. From this study, ethanol extract of binahong leaves can inhibit biofilm formation of *Escherichia coli* with an MBIC₅₀ value of 53.41% at a concentration

of 170 mg/mL, while *Staphylococcus aureus* has an MBIC50 value of 52.54% at a concentration of 165 mg/mL.

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