Analysis of Anti-Bacterial Acetivity Test of Binahong Leaves Ethanol Extract Agains Bacteria

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Abstract

Antibacterial is a compound that can be used for the treatment of infections caused by bacteria. Traditionally Binahong plants (Anredera cordifolia) are plants known to the public to handle various diseases. Binahong leaf that has antibacterial natural compounds such as oleanolic acid, ursolic acid and apigenin. This study aims to examine the antibacterial activity of ethanol extract of binahong leaves against staphylococcus aureus bacteria. This research is using experimental method. Leaves binahong obtained from Sawang Aceh Utara. The maceration stage is done by using ethanol 96% for 3 days and then in doing phytochemical test to find out secondary metabolic content. Antibacterial testing using discs. The results of phytochemical tests found secondary metabolic compounds class of alkaloids, polyphenols, saponins, quinones, and triterpenoids. Antibacterial test using 25%, 50%, 75% concentration, negative control (Aquadest), and Positive control (Amoxicillin) obtained the results are 6mm, 6mm, 6.67mm, 0mm, 30,67 mm. Based on the research of ethanol extract of binahong leaf has an inhibitory power to the growth of Staphylococcus Aureus bacteria at 75% concentration. At concentrations of 25% and 50% there was no inhibition zone of Staphylococcus aureus bacteria growth.

Keywords: *Ektrak Etanol, Antibacterial, Daun Binahong, Staphylococcus Aureus*

1. Introduction

Infection is the process of invasion and reproduction of microorganisms that occur in human body tissues that may not be clinically visible or may cause local cellular injury due to metabolic competition, toxins, intracellular replication or antigen-antibody response (Dorland, 2002). Infection is the main cause of disease in the world, especially in tropical areas, such as Indonesia. Infectious diseases or those caused by microorganisms are diseases that are often found in the community (Setiaji, 2009).

According to the World Health Organization (WHO) report, infectious diseases are the biggest cause of death in children and adults with more than 13 million deaths every year, and it ranks second (25%) after death caused by cardiovascular (31%) of 53.9 million cases of death in the world and is the main cause of death in children under 4 years of age (Maradona, 2013). Infectious diseases in Indonesia are a cause of death with a high death rate and are still a major health problem around the world. One of the causative agents of infection is the bacteria Staphylococcus aureus and Escherichia coli (Ramadhaniati, 2006).

Staphylococcus Aureus is a Gram-positive bacteria with a round shape and a diameter of 0.7-1.2 µm. The bacteria are composed of irregular groups like grapes, do not form spores and do not move. This bacteria grows at an optimum temperature of 37oC

(Jawetz et al., 2010). Substances that can inhibit the growth of bacteria are called antibacterial, antibacterial can be divided into two, namely bacteriocide and bacteriostatic (Placzar, 1996).

The process of infection can be through 2 ways, namely exogenous and endogenous. Exogenous infection of bacteria can occur through parts of the body's external tissues that are infected, while endogenously can occur when bacteria colonize an area in the body and enter another area in the body (Placzar, 1996).

Staphylococcus Aureus can also infect acne, impertigo, wound infection, conjunctivitis in the eyes. Treatment of Staphylococcus Aureus bacteria can also be done using antibiotics such as ampicillin, penicillin, tetracycline, cloxacillin, cephalosporin, vancomycin and methicillin. Bacterial resistance to antibiotics occurs a lot, for that, traditional medicine is needed, plants that are used as traditional medicine, one of which is Anredera cordifoia (Ten) steenis binahong leaves (Paju et al., 2013).

Binahong plant (Anredera cordifolia) is a plant that grows in Indonesia, China, Brazil, Australia, Paraguay, North Argentina, and America. This plant grows easily in lowlands and highlands. Binahong leaf plant can also grow in Sawang North Aceh and has been widely cultivated as an ornamental plant in tropical regions of the world. The binahong plant is a native plant from Brazil and is commonly called the Madeira grape tree or mignonette grape tree (Sri, 2011).

Binahong Anredera cordifoia (Ten) steenis leaves have activity as an antioxidant that is useful as a medicine for healing wounds from surgery, typhoid, intestinal inflammation, gout, dysentery and hemorrhoids (Mahyuni, 2015).

2. Method

This research was conducted from February to May 2018 in the laboratory of Abulyatama University. The tools used in this research are laboratory glass, analytical balance, evaporator, stirring rod, aluminum foil, needle, cotton caliper, micropipette, bunsen, pipette, water bath, autoclave, caliper. The ingredients used in this research are Binahong leaves (Anredera cordifolia), Nutrient brpth (NB), aquadest, ethanol 96% HCL, Methanol, FeCl3 NaaCl, H2SO4. This research uses the type of experimental research, the design of this research is to test the concentration variation on the growth of Staphylococcus Aureus bacteria.

Sample preparation

The Binahong leaves that have been obtained, washed clean, drained and chopped, then dried by airing for about 5 days and continued drying in the oven at a temperature of 400C until dry. Then the dried Binahong leaves are put in a blender until smooth.

Phytochemical test

Binahong leaf ethanol extract was tested for alkaloid and polyphenol, tannin test, saponin test, steroid test, and triterpenoid test. Testing was done at the microbiology laboratory of the Faculty of Medicine, Abulyatama University.

a.Alkaloid Test

Each as much as 1 gram of ethanol extract and binahong leaves added 0.5 HCl 2%. The solution is divided into 2 tubes. Tube 1 added 2-3 drops of dragendroff reagent, tube 2 added 2-3 drops of mayer. The formation of orange sediment in tube 1 and white sediment in tube 2 shows the presence of alkaloids.

b. Alkaloid Test

Each as much as 1 gram of ethanol extract and binahong leaves added 0.5 HCl 2%. The solution is divided into 2 tubes. Tube 1 added 2-3 drops of dragendroff reagent, tube 2 added 2-3 drops of mayer. The formation of orange deposits in tube 1 and sediment

Each as much as 1 gram of ethanol extract and binahong leaves added 0.5 HCl 2%. The solution is divided into 2 tubes. Tube 1 added 2-3 drops of dragendroff reagent, tube 2 added 2-3 drops of mayer. It forms sediment

c.Flavonoid test

Binahong leaf plant extract was put into a reaction tube and then dissolved in 1-2 mL of methanol. After that, Mg metal and 4-5 drops of concentrated HCl were added to form a red or orange colored solution indicating the presence of flavonoids.

d. Polyphenol test

Two hundred Mg of extract is dissolved in 10ml, water then heated for 10 minutes and filtered, the filtrate is dripped with FeCl3 as much as 3 drops, then the color change is observed, the positive thing about polyphenols is the formation of a blackish green or dark blue colored solution, then the material contains polyphenols.

e.Tannin test

1 g each of ethanol extract and binahong leaves was added to 20 mL aquadest, then cooled for 30 minutes. Then added 5 drops of 10% NaCl solution, then cooled and filtered, the filtrate was divided into 2, filtrate 1 (as a control), then the remaining filtrate was tested by adding 3 drops of FeCl3. Then compared to the color of the control solution, the black blue color shows the presence of hydrolyzed tannin and the brownish green color shows the presence of condensed tannin.

f. Saponin test

Each extract of ethanol and binahong leaves as much as 0.5 g was added to 10 mL of hot water and cooled after cooling immediately shaken vigorously for 10 seconds, if a stable foam was formed for 10 minutes at a height of 1-10 cm and after adding 1 drop of HCl 2N the foam does not disappear, it indicates the presence of saponin compounds.

g. Test of steroids and triterpenoids

Each extract of ethanol and binahong leaves as much as 1 g was extracted with n-hexane until it became colorless, then the residue of the extract was added to 10 mL of chloroform and stirred for 5 minutes. Take the chloroform layer using a pipette and add anhydrous sodium sulfate, filter and divide into 2 parts. The first filtrate (as a control) if there is any remaining filtrate, add 3 drops of acetic anhydride in 1 drop of concentrated H2SO4, and observe the color change that occurs with the control. If a blue-green or purple-red color is formed, it indicates a steroid or triterponoid compound.

Antibacterial activity

a. Sterilization of Tools

Sterilization of tools is used before all equipment is used, which is by wrapping all equipment using brown paper and then putting it in an autoclave at a temperature of 121oC with a pressure of 15 pai (per square inch) for 15 minutes. Tools that do not withstand high heat are sterilized with 96% alcohol.

b. Formation of Nutrient Broth Media (NB)

The formation of nutrient broth (NB) liquid media by preparing the ingredients is to weigh 6.5 grams of NB media and then dissolve it with 500 mL aquadest and erlenmeyer then cover it with aluminum foil. The suspension is heated to boiling and then put into a reaction tube, each 5 mL and then covered with cotton. This process is done aseptically, then sterilized in an autoclave at a temperature of 121oC with a pressure of 15 pai (per square inch) for 15 minutes. Then placed in a tilted position for 1x24 hours at room temperature.

c. Bacterial Culture Rejuvenation

The pure culture of bacteria is rejuvenated on solid agar media by taking 1 ounce of bacteria and then a ose needle containing Staphylococcus aureus bacteria is scratched aseptically on the nutrient agar medium in the dish by bringing the dish closer to the flame when scratching the ose carum then the petri dish is closed again and incubated for 24 hours at a temperature of 27oC in an incubator, then one colony is taken and grown on NB media, then voted to be homogeneous, then incubated for 24 hours at a temperature of 20oC in an incubator, there is bacterial growth, if the media is cloudy then compared to NB media without bacteria.

d. Preparation of Bacterial Suspension

1 mL of pure Staphylococcus aureus bacteria rejuvenating results were taken and put into a reaction tube containing 5 ml of physiological 0.9% and then vortexed to make it homogenous, then compared to the Mc Farland standard with a bacterial density of 108 cells/ml and then diluted 100 times in physiological NaCl media 0.9 % and NB media. Obtained bacterial suspension as much as 106 bacteria cells/mL, bacteria ready to be tested.

e. Antibacterial Activity Test

The antibacterial activity test is done by using the diffusion method, which is by using the disc method. Antibacterial substances are saturated into the disc paper and planted on a solid agar seeding medium that has been mixed with the tested bacteria, then incubated at a temperature of 37oC for 18-24 hours. Next, it was observed that there is a clear area (zone) around the disc paper that shows the absence of bacterial growth.

Data Analysis

The research results were described based on morales classification and analyzed using Analysis of Variance (ANOVA) non-parametric statistics to find out the effect of ethanol extract of binahong leaves (Anredera cordifolia) on Staphylococcus Aureus isolates and followed by Mann Whitney test.

3. Results and Discussion

Phytochemical Test Results

Phytochemical test results of ethanol extract of binahong leaves (Anredera cordifolia) showed that there are secondary metabolic compounds, alkaloids, quinones, polyphenols, saponins, triterpenoids, as shown in table 1.

Table 1. Phytochemical test results of ethanol extract of binahong leaves (Anredera cordifolia)

Treatment	Test for Secondary Metabolite Compounds	Repeat:		Amount	Average	Results	SD	Morales
		I	II	III				
	Flavanoid	6	6	6	18	6	-	
P_1	Kuinon	6	6	6	18	6	+	0 Soil
P_2	Polifenol	6	6	6	18	6	+	0 Soil
P_3	Tannin	6	7	7	20	6,666667	+	0,57735 Moderate
P_4	Saponin	30,5	31,6	30,2	92,3	30,76667	+	0,737111 Very Strong
P_0	Steroid	6	6	6	18	6	-	0 Soil
	Triterpenoid						+	
	Alkaloid						+	

Antibacterial Test Results

The results of the inhibitory power of ethanol extract of binahong leaves against the growth of Staphylococcus aureus bacteria with concentrations of 25%, 50%, 75%, and P0 negative control (Aquadest), P4 positive control (Amoxicilin) obtained the antibacterial inhibitory power of Staphylococcus aureus in a row is 6 mm , 6mm, 6.67mm, 0mm, 30.67mm.

The diameter of the inhibition zone of the ethanol extract of binahong leaves can be seen in Figure 1 and Table 2.

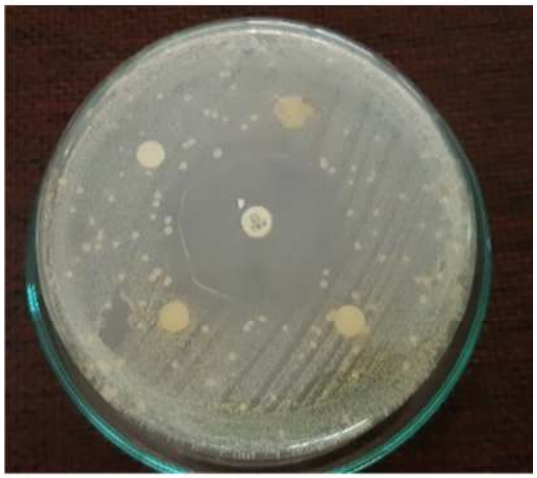

Figure 1. Inhibition zone of binahong leaf ethanol extract against Staphylococcus Aureus

Table 2. Results of inhibition zones of Staphylococcus Aureus bacteria

Information:

P_0 : Negative Control (Aquadest)

P_1 : Ektrak etanol daun binahong konsentrasi 25%

P_2 : Ektrak etanol daun binahong konsentrasi 50%

P_3 : Ektrak etanol daun binahong konsentrasi 75%

P_4 : Positive Control (*Amoxicilin*)

SPSS output results can be seen in the Ranks table (appendix 6) that the mean rank value shows the average rank in each treatment. In the case of Amoxicillin administration, the highest average was 14.00 compared to other administrations. While the administration of binahong leaf extract obtained the highest average at a concentration of 75% compared to concentrations of 25%, 50%, and aquadest. Overall, from each treatment and concentration of binahong leaf extract, only the positive control (Amoxicilin) was very influential. An analysis of the test table was conducted using statistical analysis to find out the differences in all the treatments that are statistically significant.

Table 3. Statistical test results table

	Inhibition Zone	Repitition
chhi-square	12,152	0,00
df	4	4
asymp sign.	,016	1,000

From the table of statistical test results, a chi-square value of Fhitung > Ftabel (12, 152 > 5, 692) was obtained, so it can be concluded that Ho is rejected and H1 is accepted. Another way to know the influence can be seen from the significant asymptotic value 0.016 < 0.05 in this hypothesis Ho is rejected and H1 is accepted. It means that there is a significant influence of both binahong leaf extract, Amoxicilin, and aquadest. Kruskal wallis test results have a significant impact on each treatment so further tests are needed, namely the Mann Whitney test to find out which treatment is significantly different

Table 4. Comparison between treatments

Treatment	Significant
Between 1 and 2	0,00
Between 1 and 3	0,00
Between 1 and 4	0,00
Between 1 and 5	0,00
Between 2 and 3	4,500
Between 2 and 4	4,500
Between 2 and 5	1,500
Between 3 and 4	4,500
Between 3 and 5	1,500
Between 4 and 5	1,500

Description:
1. (1) Amoxicillin positive control
2. (2) Aquadest negative control
3. (3) Concentration 25%
4. (4) 50% concentration
5. (5) Concentration 75%

Based on table 4 above, it can be seen that the significant value is < 0.05, which means that it is significantly different. While a significant value > 0.05 means that it is not significantly different.

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Discussion

Phytochemical Test

A total of 180 grams of binahong (Anredera cordifolia) was dried and 170.607 grams were obtained. Maceration was then performed with 900 ml of 96% ethanol solvent for 3 x 24 hours at a temperature of 27oC to prevent solvent evaporation. The maceration solution is then separated from the leaves and the solution. The solution is concentrated by using a vacuum rotary evaporator at a temperature of 66oC, at that temperature ethanol can evaporate and 7g of blackish green binahong leaves are obtained.

Based on Table 1 above, it can be seen that the ethanol extract of binahong leaves contains secondary metabolic compounds, among others: alkaloids, quinones, polyphenols, saponins, triterpenoids. This research is in accordance with the results of previous research which stated that binahong leaves have secondary metabolic compounds (Kurniawan et. al. & Selawa et al., 2013). This research obtained secondary metabolic compounds of alkaloids, saponins, quinones, triterpenoids and polyphenols. The results of this research are in accordance with the research conducted by Paju in 2013.

The content of flavonoids in binahong leaves in this study was not found. The results of this research are not in accordance with the research conducted by Widya Selawa and Kurniawan who stated that there are flavonoid compounds in binahong leaves (Retnowati et al., 2011 & Ainurrochmah et al., 2013). This is due to the difference in geographical location that can affect the secondary metabolic compounds in binahong leaves. The binahong leaves used in this study live in the highlands and the soil and climate conditions are also very influential in the growth of secondary metabolic compounds in the binahong leaves.

Anti-bacterial test

Based on the results of the study, Table 2 shows that the ethanol extract of binahong leaves (Anredera cordifolia) against Staphylococcus Aureus bacteria with concentrations P0 negative control (Aquadest), P1, P2, P3, P4 positive control (Amoxicilin) in a row of 0 mm, 6 mm , 6mm, 6.67mm, 30.67mm. Only a small amount of inhibitory effect occurred on Staphylococcus Aureus bacteria, this is influenced by the absence of flavonoids in the extract of binahong leaves (Anredera cordifolia). Flavonoid compounds are chemical compounds that are bacteriostatic, by inhibiting the growth of bacterial cells and damaging the cytoplasmic membrane (Retnowati, 2011). Other compounds contained in binahong leaves (Anredera cordifolia) that can inhibit antibacterial are saponins, alkaloids and tannins.

Compounds of the saponin group can damage the cytoplasm membrane, so that the growth of bacteria becomes inhibited and causes cell death. While alkaloids have the ability to be antibacterial, by disrupting the peptidoglycan component so that the cell wall layer is not formed perfectly. Tannin can shrink the bacterial wall, the destruction of the bacterial wall will cause inhibition of bacterial cell growth and eventually the bacteria will die (Retnowati et al, 2011). Other compounds contained in binahong leaves are polyphenols and triterpenoids.

Polyphenols are secondary metabolic compounds that can inhibit and damage the growth of bacterial cell walls, making it easier for other compounds to interact with other cell components. Triterpenoids can reduce the premability of bacterial cell walls so that bacterial cell walls lack nutrients and bacterial growth can be inhibited, in triterpenoid compounds there are acetic acid and terminolic acid which can also inhibit the growth of Staphylococcus Aureus bacteria (Haryati et al., 2016).

Based on the results of research, the ethanol extract of binahong leaves has inhibitory power against the growth of Staphylococcus Aureus bacteria at a concentration of 75%. At the concentration of 25% and 50% there is no growth inhibition zone of

Staphylococcus Aureus bacteria. The results of this research are in accordance with the results of previous research conducted by Lucia 2011 that Staphylococcus Aureus bacteria has an inhibition zone but in the weak category. At a concentration of 25%, there is no inhibition zone of Staphylococcus Aureus bacteria (Souza, 2014).

4. Conclusion and Suggestions

Conclusion

Based on the research results, it can be concluded that:

1) Phytochemical results of binahong leaves (Anredera cordifolia) contain secondary metabolic compounds of alkaloids, quinones, saponins, polyfrnols and triterpenoids.
2) Binahong leaf ethanol extract (Anredera cordifolia) against Staphylococcus Aureus bacteria with repetitions of 25%, 50%, 75%, aquadest negative control and Amoxicilin positive control and has an inhibition zone of 6 mm, 6 mm, and 6.67 mm, 0 mm , and 30.67 mm.
3) The use of ethanol extract of binahong leaves has the ability to inhibit the growth of Staphylococcus Aureus bacteria at a concentration of 75%. At the concentration of 25% and 50% there is no growth inhibition zone of Staphylococcus Aureus bacteria.

Suggestions

It is necessary to conduct further research related to the ethanol extract of binahong leaves at a higher concentration and more repetitions to obtain the maximum inhibitory power against the growth of staphylococcus aures bacteria.

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