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Inhibitory test of binahong (Anredera cordifolia (Ten.) steenis) n-hexane fraction against bacillus subtillis bacteria by diffusion method

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ABSTRACT

Binahong plants (Anredera cordifolia (Ten.) Steenis) contain alkaloids,flavonoids,saponins, and terpenoids, the chemical compounds of binahong leaves have many antibacterial properties. This study aims to determine the antibacterial inhibition of n-hexane (Anredera cofdifolia Ten.) Steenis) against Bacillus Subtillis. This type of research is experimental descriptive, binahong leaf n-hexane fraction was previously obtained by extraction using maceration method with 70% ethanol solvent obtained by using a 10% DMSO dissolved and tested for antibacterial inhibition with the method of solid well diffusion against Bacillus Subtillis in the series of concentrations of 20%,40%,60% dan 80% positive controls used were chloramphenicol 10% and negative control DMSO 20%. The results of the study showed that n-hexane fraction of binahong leaves (Anreders cordifolia (Ten.) Steenis) was able to inhibit the growth of the bacterium Bacillus subtillis. The greater the concentration used, the greater the results obtained.

1. Introduction

Binahong (Anredera cordifolia (Ten.) Steenis (lat. Steenis) is a potential medicinal plant that can cope with various diseases. In Europe and America, this plant can be quite known, but the experts there have not been interested in serious and in-depth research, even though its various medicinal properties have been recognized. Part of the binahong hampr

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plant can all be utilized from roots, stems, flowers to leaves, but the most commonly used for health or as a herbaceous medicine is binahong leaves [1].

Bacillus Subtillis is a gram-positive, rod-shaped, bacterium that grows in *aerobic* and anaerobic conditions. The spores are resistant to heat (high temperatures), able to degrify carbohydrates. This bacterium and its endospores are widespread in soil, vegetation, water [2] and carried away by dust particles in the air. Its endospores, due to their high resistance to heat, can survive for a long time. The cell morphology is rod, unless one species has spherical cells and in the form of a package, motile due to *flagella* or nonmotile.

Based on research conducted by Virgianti [3] on "Inhibitory Power of Binahong Leaf Ethanol Extract (Anredera cordifolia (Ten.) Steenis) against Streptococcus pyogenes bacteria in Vitro" with the result that in vitro assays bland power binahong leaf extract (Anredera cordifolia (Ten) Steenis) with concentric variants 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% against Streptococcus pyogenes bacteria in In Vitro formed a clear zone that Binahong Leaf Ethanol Extract is able to inhibit the growth of Streptococcus pyogenes bacteria with a clear zone and an inhibitory zone of 15.15 mm.

Based on the second study conducted by Friska Makalungsenge 2015 [4], on "Test of Antibacterial Activity of Binahong Leaf Extract against *Staphylococcus aureus* bacteria by the n-hexane Fraction has a higher inhibitory power with an inhibitory power diameter of 14.25 mm at a concentration of 75% 13.31 mm at a concentration of 25% 13.31 and at a concentration of 50% 10.125 mm at 100% concentration and the n-hexane fraction is able to inhibit bacterial growth *Staphyloccus aureus.*

2. Method

The implementation stage of this study includes the preparation of tools and materials, sample preparation, determination, making simplicia, making extracts, fractionation, identifying flavonoids, and antibacterial inhibitory tests.

2.1. Tools and Materials

The tools used in this study include: autoclave, stirring rod, Bunsen, petri dish, glass funnel, split funnel, enkas, erlenmayer, beaker, measuring cup, incubator, ose needle, watch glass, micropipette, water bath, drip pipette, test tube, well puncher.

The ingredients used in this study were aquaades, n-hexane, aqua pro injection, *bacteria Bacillus subtillis*, DMSO 10%, binahong leaves, ethanol 70%, flannel cloth.media NA, NB media, filter paper, chloramphenicol.

2.2. Sample setup

The sample used was a binahong plant obtained from the soko senep sukoharjo area of Central Java.The part of the sample used was a green leaf with a red bar that was used as much as 5 kg.

2.3. Determination

Determination is carried out in advance to obtain certainty that the plants used are derived from the intended plants, so that they are likely to avoid errors in the collection of research materials.

2.4. Creation of Simplisia

The prepared sample is then cleaned using running water to remove the dirt that sticks to the binahong leaves in the sample and then drained the leaves which will be done by paving then dried using an oven at 60 °C until the leaves are dry marked with brown leaves and can be kneaded. Then the dried leaves are mashed using a blender then sift using sieve number 40 and calculated LOD (*Loss on Drying) on* simplisia.

2.5. Extract Creation

500 grams of binahong leaf powder was extracted by maceration using 5L of 70% ethanol for 5 days, while stirring once, storing in a cool place and protected from sunlight. Then the extraction results were separated between the filtrate and the powder by using sarting paper with the help of a split funnel. The macerated filtrate that has been mixed is then concentrated with a vaccum rotary evaporator until no filter drips on the tool. The concentrated fitrates are collected in porcelain dishes and evaporated on waterbath to obtain a thick extract. The viscous extract is then weighed with a fixed weight to ensure the solvent is completely lost. Then the extract was stored in a temperature of 700C. (The Extract Manufacturing Scheme can be seen in Figure 1).

2.6. Test Ethanol-Free Extract

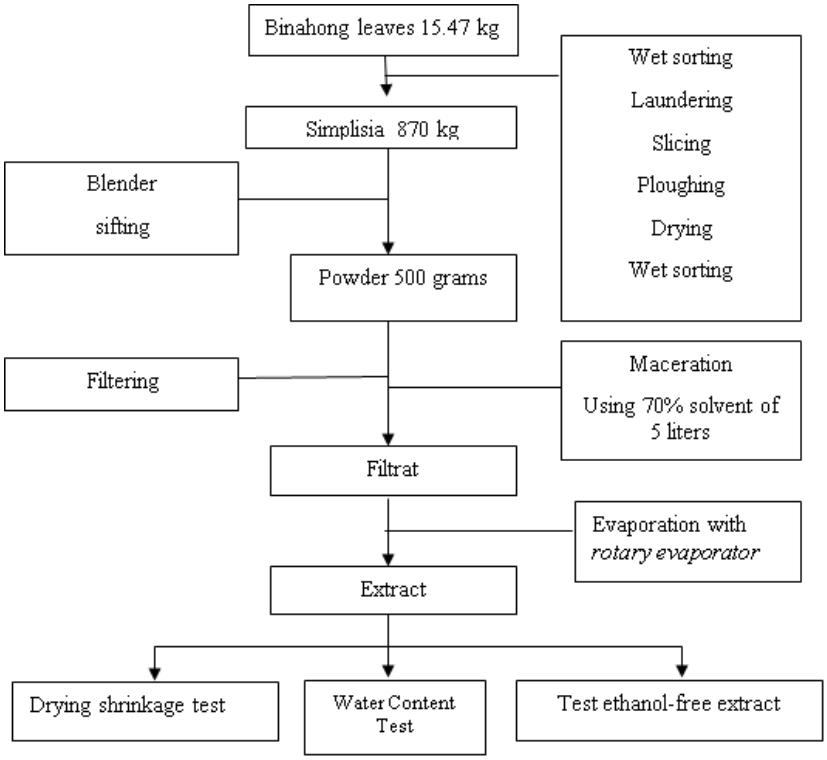
The ethanol test of binahong leaf extract was carried out to find out whether binahong leaf extract is completely free of ethanol. Ethanol-free examination is carried out by adding concentrated sulfuric acid and acetic acid to binahong leaf extract which is then heated. The results of the ethanol-free test in binahong leaf extract are characterized by no characteristic smell of ether or fragrance.

2.7. Simplician Drying Shrinkage Test

Drying shrinkage is the reduction in the weight of the material after it has been dried in a predetermined way. Unless otherwise stated in each of the monographs, simplicia shall be in powder form with fine degree number 8, a drying temperature of 105 °C and a shallow shrinkage of the lid that has previously been heated at the setting temperature and in the tare. Flatten the material in the weighing bottle by shaking the bottle, until a layer ±5-10mm thick forms, put it in a drying chamber, open the lid, dry at the determination temperature until the weight is fixed. Before each drying leave the bottle in a closed state of mendigin in a desiccator to room temperature.

2.8. Extract Water Content Test

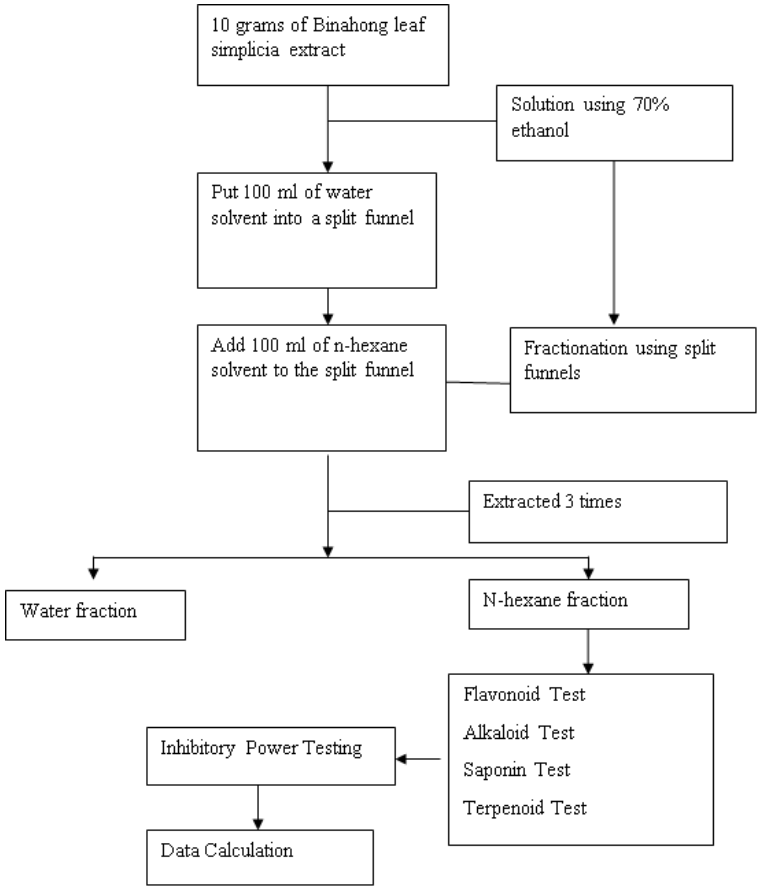
The water content test is carried out to measure the water content in the sample. The moisture content test was carried out by weighing binahong leaf extract as much as 1-2 grams using krus which were known to weigh the extract dried in the oven with a temperature of 105 °C for 5 hours and weighed continue drying and weighing at a distance of 1 hour until the difference between 2 consecutive weighing is not more than 0.25% [5].



Scheme 3.1. Extract Creation

2.8. Fractionation

Fractionation of the viscous extract of binahong leaves (*Anredera cordifolia (* Ten.) Steenis)is made by weighing a thick extract of 10 grams. The weighed extract is saturated with water by 100 ml. Then add a 100 ml n-hexane solvent and then shake it inside the split funnel and occasionally the split funnel shutoff valve is opened to remove the gases produced in the shaking process. The solution mixture is allowed to stand until there are two layers. The n-hexane layer is removed and accommodated in a container, the process of adding the fractionation result obtained is evaporated using *watterbatch* and then weighed. To see the fraction creation scheme can be seen in schema 2 of the following pages:



Scheme 3.2. Fraction Creation

2.9. Phytochemical Screening

Flavanoid Identification. Binahong leaf extract of 10 mg dissolved in 10 ml of ethanol, heated, The solution is taken as much as 2ml in a test tube. Magnesium tape (Mg) 1 cm is added and in the concentrated HCL is added 5 drops. The formation of a pale yellow, greenish-yellow to brick-red solution indicates the presence of flavonoids.

Alkaloid Identification.1 ml of binahong leaf extract is put into a test tube then added 2 ml of mayer reagent. Positive results are shown by the formation of white deposits.

Saponin Identification. 0.5 g of binahong leaf extract is put into a test tube. Then 19 ml of akuades are added and shaken for 10 seconds. If a foam 1-10 cm high is formed for not less than 10 minutes, if the foam is not lost, HCL 2N is added. If there is still constant foam, it shows a positive result containing saponins.

Terpenoid Identification. Binahong leaf extract of 1 ml was added anhydrous plus concentrated H_2SO_4 drop by drop as much as 0.2 ml to the base of the tube and observed the occurrence of purple color.

2.10. Inhibitory Power Test

The tool used for the inhibitory power test is sterilized by oven at 180°C for 1 hour. Previously thoroughly washed, dried and wrapped in paper. The material (except the extract) to be used is sterilized inside the autoclave at 121°C for 15 minutes.

In the inhibitory power test, it takes *Oblique Nutrient Agar* (NA) Media and Seed Media. MediaNA 0.4 grams is dissolved in aqueous to 20 ml, and heated to dissolve, poured into a sterile test tube of 5 ml, and covered with a cotton swab with aluminum foil. NA media was sterilized using an autoclave for 15 minutes at 121°C, let it sit at room temperature, tilted until the media solidified. Thin media is used for bacterial inoculation. 2.8 gram NA medium is dissolved in aqueous to a volume of 140 ml, and heated to dissolve. NA media was sterilized using an autoclave for 15 minutes at 121°C, poured into a petri dish, let stand until solidified. The manufacture of seed media is carried out aseptis.

After that, aBacterial Suspension is carried out. Bacteria grown in NA (oblique) media are taken with ose, and suspended into a 0.9% NaCl solution of 10 ml, waited until cloudy. Comparison of bacterial suspension with the turbidity standard of Mc. Farland solution. Making the Binahong Leaf Extract Concentration Series by means of ethanolkenikir leaf extract, 4 concentration series are made, namely: 20mg / ml, 40mg / ml, 60mg / ml, and 80 mg / ml using a 10% DMSO solution of 5 ml.

The bacterial suspension is taken 50 µm, put into a petri dish that has contained the medium and flattened using a spreading rod. Wells are made 4 that contain 1 equal concentration. It was then incubated at 37°C for 24 hours. The bright zone formed around the perimeter of the well is measured using a ruler. The antibiotic used in the positive control was chloramphenicol, as much as 1 gram of chloramphenicol dissolved in 10 ml of 10% DMSO. Negative control in this study using a 10% DMSO solution.

3. Results and Discussion

3.1. Results of Determination and Identification of Binahong Plants

The purpose of determination is to obtain a clear identity truth of the plants studied and avoid mistakes, the results of the identification of binahong plants show that the binahong plants used in this study are really binahong plants (Anredera cordifolia (Ten.) Steenis).

3.2. *Loss On drying* (LOD) Binahong Leaves

Binahong leaves that have been drained at room temperature then ventilated at 60 °C aim to reduce the moisture content of binahong leaves. The water contained in binahong leaves can cause simplicia to be easily overgrown with mold so that it cannot be stored for a long time. The results of drying can be seen in Table 1.

Table 1. Drying of Binahong Leaf Simplisia

Wet Weight (grams)	Drying time	Simplisia Weight(grams)	LOD(*Loss On Drying)*
15.585	48 jam	870	94

Simplisia leaves binahong drying results obtained an amendment of 94%. Then the amount of the lost compound at the time of drying is 94%. Terms of shrinkage drying of binahong leaf simplicia not more than 10%.

3.3. Shrinkage Results of Drying of Binahong Leaf Simplisia Powder

Drying shrinkage is the rate of the evaporated part of a substance. The method of shrinkage drying is that the powder is weighed as much as 1 gram in a closed shallow crucible that has previously been heated at 105 °C for 30 minutes and is compared. The result of the drying shrinkage test was obtained by 8.

3.4. Binahong Leaf Extract

Binahong leaf extract 500 grams of simplicial powder and 70% ethanol solvent as much as 4 liters. The binahong leaf samples used for maceration are binahong leaves that are green, not hollow, not wilted or rotten, and still fresh. The obtained binahong leaf samples are washed with running water until clean with the aim of providing samples from the remnants of dirt that stick, after which the binahong leaves are drained at room temperature to remove residual water after washing.

Binahong leaves are dried using an oven at 60 °C which aims to reduce the moisture content that can cause mold so that it can make it easy to rot. The dried binahong leaves are then mashed using a blender before sifting. The purpose of smoothing simplicia into a splice is to enlarge the surface area of the sample so that the interaction of the solvent with the compounds to be taken is more effective and the compounds can be extracted completely.

In this study, samples were extracted using the maceration method with ethanol solvent 70% ethanol used part of the filtering liquid because it is more selective, germs are difficult to grow in ethanol 20% and above, non-toxic, neutral, good absorption can mix with water in all ratios, and the heat required for concentration is less[6]. Maceration of binahong leaf powder is carried out for 5 days with stirring 3x in 24 hours, then filtered with flannel cloth and filter paper. The yield of extract amendments obtained was 15,744. The thick extract of binahong leaves from maceration is blackish brown, odorless, and slightly chelate. Hasil perhitungan ekstrak dapat dilihat pada Tabel 2.

Table 2. Amendment Results of Binahong Leaf Extract

Simplician Powder(grams)	Extract (grams)	Amendment (grams)
500	44.588	15,744

3.5. Ethanol-Free Test

The ethanol-free test is carried out to free the extract from ethanol in the maceration process, so that the extract obtained is a pure extract without any contaminants. The ethanol-free test is performed with the addition of concentrated sulfuric acid in a test tube and closed using a cotton swab at the mouth of the tube which is then heated to a boil. The result of the identification of cotton odor, namely no ester odor, means that in the sudaah extract there is no ethanol.

3.6. Extract Water Content Test

Test the moisture content of the extract using the distillation method, the purpose of the extract moisture content test is to provide maximum limits on the magnitude of the missing compounds in the drying process [5]. The extract moisture content test procedure is carried out by weighing as much as 1 gram of extract on empty crucs that have been heated at 105 °C for 1 hour and have been tested. The extract-filled crutches are then heated for 5 hours by opening the lid to a constant weight [5]. The water content test of binahong leaf extract is 6.6%, then the water content test results of the extract meet the requirements, while the water content requirement of binahong leaf extract is not more than 8.85%.

3.7. N-hexane fraction of Binahong Leaves

Fractionation is the process of separation between liquid and liquid substances. Fractionation is generally done using a separate funnel that is conical in shape and covered

with half a ball, has a blockage above it and a tap underneath. The purpose of fractionation is to separate compounds that have non-polar properties will dissolve in non-polar solvents, semi-polar ones will dissolve in semi-polar solvents and polar ones will dissolve in polar solvents.

Fractionation is carried out by weighing 10 grams of binahong leaf extract partitioned with 100ml of aqueous, then the solution is partitioned by adding 100 ml of n-hexane (1:1) and shaken in a separate funnel and occasionally a separator flask valve is opened to remove the gases from the shaking. Fractionation shaking produces two layers, namely the top of the n-hexane layer and the bottom of the water layer. This happens because the density of n-hexane is 0.655 grams/ml which is smaller than the density of water, which is 1,000 grams/ml. The results of the water and n-hexane fractions are separated, the n-hexane layer is accommodated in the container for strengthening, the process of adding solvent is carried out until the n-hexane layer becomes clear. The fraction results can be seen in Table 3.

Table 3. Results of n-Hexane Fraction of Binahong Leaves

Maceration Extract (g)	n-Hexane fraction (g)
50	570.12

3.8. Phytochemical Screening Results of n-Hexane Fraction

The results of phytochemical skiring content of compounds of n-hexane fraction of binahong Flavonoid leaves, alkaloids, saponins, and terpenoids can be seen in Table 4.

Table 4. Phytochemical Screening Results

No	Testing	Result	Information
1	Flavonoid	Jinga red	+
2	Alkaloid	White precipitate	+
3	Saponin	There is foam	+

Description: (+)= contains a class of compounds
(-)= does not contain a class of compounds

Skirining phytochemicals on the n-hexane fraction of binahong leaves (*Anredera cordifolia* (Ten.) Steenis) contains flavonoid compounds,alkaloids,saponins.

The n-hexane fraction of binahong leaves positively containing flavonoids is characterized by the attraction of red or orange compounds in the alcohol layer. The addition of HCL 2N is useful as a hydrolyzer of O-glycosyl, a hydrolyzed glycosyl compound replaced by hydrogen ions from acids due to its electrophilic nature. Magnesium powder binds to flavonoids to form complex compounds that are red or orange.

The n-hexane fraction of binahong positive leaves contains alkaloid compounds characterized by the formation of a white precipitate, this sediment appears due to the formation of potassium-alkaloid compounds. The addition of HCI so that there is no hydrolysis reaction of the contained potassium-alkaloid compounds. Nitrogen atoms contained in alkaloid compounds will form coordinate covalents with potassium ions which are metal ions.

The identification of saponin compounds is characterized by the formation of a stable foam not lost 10 minutes from the scouring. Saponins have compounds that have hydrophilic and hydrophobic groups. Saponins when cornered form foam due to the presence of a hydrophilic group that binds to water while hydrophobic will bind to air so that it forms froth.

3.9. Antibacterial Inhibitory Power Test of Binahong Leaf n-Hexane Fraction

Antibacterial inhibitory power testing using the well diffusion method. According to Elya et al, the 2017 well diffusion method in this study is based on the diffusion ability of antimicrobial substances in agar plates that have been inoculated with micro-tests. The n-hexane fraction of binahong leaves was tested on the bacterium *Bacillus Subtillis* with a concentration of 20%,40%,60% and 80% of the comparison control used as a positive control used pure chloramphenicol and a negative control of 10% DMSO.

The reason for using chloramphenicol as a positive control is that chloramphenicol is a group of aminoglycosides that have broad spectrum properties against gram-positive bacteria and gram-negative bacteria, so that the antibiotics used in this study can inhibit *Bacillus Subtillis* bacteria which are gram-positive bacteria. Chloramphenicol can inhibit the protein synthesis needed for the formation of bacterial cells, so chloramphenicol inhibits the RNA function of bacteria. The reason for using DMSO 10% as a negative control is because DMSO solvents that have no biological activity and DMSO is an aprotic, colorless solvent, and can dissolve polar and non-polar compounds.

The suspension of test bacteria uses NB (Nutrient Broth) media which has been added to one bacterial ose culture from NA (oblique) media. The antibacterial testing step uses the well diffusion method, namely the media is given 50 microns of bacterial suspense from NB media which is then leveled using a spreading rod. NA media is treated to make well holes using a sterilized cork punching device. The addition of the reaction n hexane of binahong leaves is placed on the perforated medium, the incubation period is 24 hours at a temperature of 37 °C. The observation table of well diffusion test measurements can be seen in Table 5.

Table 5. Observation of well diffusion test measurements

Replication	Control + Chloramphenicol	Control-Dmso10%	Fraction concentration				Media controls
			20%	40%	60%	80%	
1	29,55 mm	0	7 mm	10,mm	6 mm	16,5mm	-
2	26,85 mm	0	7,5mm	10,5mm	8,5 mm	10,5mm	-
3	31,85 mm	0	7,5mm	10 mm	10,5mm	12,5mm	-
Average	29,40 mm	0	7,33mm	10,16mm	18,5mm	13,1mm	-

Table 5 explains that the n-hexane fraction of binahong leaves has an inhibitory power against *bacillus subtillis* bacteria which is intended by the presence of inhibitory power (clear zone) around the well hole. The increase in the concentration of the n-hexane fraction of binahong leaves is equal to the diameter of the inhibitory power obtained. This

happens because the greater the concentration used, the higher the amount of active substances contained in the n-hexane fraction such as alkaloids, flavonoids, saponins, and terpenoids so that the inhibitory power produced is greater. The n-hexane fraction contains alkaloid compounds, flavonoids, saponins and terpenoids with the mechanism of action of bacterial inhibition.
The mechanism of action of flavonoids as antibacterials is to form complex compounds with extra-cellular and dissolved proteins so that they can damage bacterial cell membranes and followed by the release of intracellular compounds.

4. Conclusion

Based on research that has been carried out from the inhibitory power test of binahong leaf n-hexane fraction against Bacillus Subtillis bacteria , it can be concluded that 80% of binahong leaf n-hexane fraction has a strong inhibitory power in inhibiting the growth of Bacillus Subtillis bacteria with an inhibitory power diameter (31.1mm), but still lower compared to the positive control of chloramphenicol 29.4. In this study, the higher the concentration of the n-hexane fraction of binahong leaves (20%,40%,60%, and 80%) the higher the antibacterial inhibitory power against Bacillus Subtillis.

REFERENCES

[1] D. Susetya, *Khasiat dan Pemanfaatan Daun Ajaib Binahong*. Yogyakarta: Pustaka Baru Press, 2015.

[2] G. F. Brooks, J. S. Bute, and S. A. Morse, *Jawetz, Melnick,& Adelberg Mikrobiologi Kedokteran*. Jakarta: Penerbit Buku Kedokteran EGC, 2010.

[3] D. P. Virgianti, "Daya hambat ekstrak etanol daun binahong (anredera cordifolia (ten.) Steenis) terhadap pertumbuhan bakteri streptococcus pyogenes secara in vitro," *J. Kesehat. Bakti Tunas Husada J. Ilmu-ilmu Keperawatan, Anal. Kesehat. dan Farm.*, vol. 13, no. 1, 2015.

[4] F. Makalunsenge, Y. K. Salimi, and S. Duengo, "UJI AKTIVITAS ANTIBAKTERI EKSTRAK DAUN BINAHONG TERHADAP BAKTERI STAPHYLOCOCCUS AUREUS," 2015.

[5] D. Depkes RI, "Parameter Standar Umum Ekstrak Tumbuhan Obat," Jakarta, 2000.

[6] D. Depkes RI, "Sediaan Galentik," Jakarta, 1986.