

## ANTIBACTERIAL ACTIVITY OF BINAHONG ROOT EXTRACT (*Anredera cordifolia* (Ten.) Steenis) IN TREATMENT OF BOILS

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### ABSTRACT

Infectious diseases caused by *Staphylococcus aureus* can be cured with traditional medicine, One example of a plant that has antibacterial power is binahong root. This study aims to determine the minimum inhibitory concentration (MIC) and minimum killing concentration (MBC) of binahong root extract against the growth of *Staphylococcus aureus* bacteria. Binahong root extract was obtained from maceration using absolute ethanol solvent. Furthermore, the extract obtained was made with a concentration dilution of 5%, 10%, 15% and 20%. The method used is disc diffusion. The results showed that each concentration of binahong root extract had an inhibitory effect on the growth of *Staphylococcus aureus* bacteria. This is influenced by the active compounds contained in the binahong root extract such as flavonoids, saponins, tannins, and alkaloids. Binahong root extract concentrations of 5%, 10%, 15%, and 20% were found to have zones of inhibition against the growth of *S. aureus* bacteria and the minimum inhibitory level (MIC) of Binahong root extract against the growth of *S. aureus* bacteria at a concentration of 5%.

**Keywords :** *infection, antibacterial, staphylococcus aureus, binahong.*

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### INTRODUCTION

Infectious diseases still top the list of causes of illness and death in developing countries, including Indonesia. For sufferers, apart from causing physical suffering, infection also causes a decrease in performance and productivity (Wahyono, 2007). (Gibson, 1991) explains that bacterial infections still dominate the potential for severe infections, sepsis, septic shock and multiorgan dysfunction. (Nasronuddin, 2007) states that deaths due to gram-positive bacteria are 40% and by gram-negative bacteria are 60%. Nasronuddin (2007) added that to treat infections caused by bacteria, antibiotics have an important role, antibiotics are expected to be able to eliminate the bacteria that cause infection. Infectious diseases caused by bacteria, therapy has been carried out using antibiotics. The problem that arises is that there are many cases of bacteria that are resistant to antibiotics (Kuswandi et al., 2001). The emergence of resistance, even multi-resistance, causes many problems in the treatment of infectious diseases. Therefore, multiresistance to antibiotics is a serious problem (Sudarmono, 1993). So efforts are needed to develop traditional

medicines derived from plants that can kill antibiotic-resistant bacteria. One of the plants that is empirically used as an antibacterial drug is binahong (*Anredera cordifolia* (Ten.) Steenis).

Herbal plants in Indonesia have been widely used as traditional medicine ingredients. One of the natural ingredients used as a medicinal ingredient is the binahong plant (*Anredera cordifolia* (Ten.) Steenis). (Saputra, 2008) Binahong has roots, tubers, stems, flowers and leaves which contain active compounds, namely flavonoids, alkanoids, terpenoids and saponins. (Prayudi, 2009) (Sulsisyani, 2013) Active flavonoid compounds can act directly as antibiotics by interfering with the function of microorganisms such as bacteria and viruses. (Manoy, 2009) (Ying, 2011) Binahong (*Anredera cordifolia* (Ten.) Steenis) also contains active antimicrobials so it can be used to prevent the growth of bacteria, one of which is *Staphylococcus aureus* bacteria. *Staphylococcus aureus* is a bacteria that causes various types of epidermal and subcutaneous infections such as pyogenic, boils, pneumonia infections, suppurative lesions, and wounds (Otieno, et al. 2008). According to WHO data, in 2008 infectious diseases claimed the lives of more than 9,500,000 people every year (Mathers, et al. 2008). *Staphylococcus aureus* produces coagulase, an enzyme-like protein that can coagulate plasma containing oxalate or citrate.

Producing coagulase is considered to be the same as having the potential to become an invasive pathogen. According to Anggun Anggraini (2012), there is antibacterial activity of the ethanol extract of Binahong leaves (*Anredera cordifolia* (Ten.) Steenis) against *Staphylococcus aureus* bacteria in vitro, the higher the concentration of the ethanol extract of Binahong leaves, the greater the inhibitory power against *Staphylococcus aureus* bacteria. According to Agus, R.M (2013), the test results obtained from the triterpenoid isolate from Binahong Leaf Extract (*Anredera cordifolia* (Ten.) Steenis) were able to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria at a minimum inhibitory concentration of 100-2000 ppm with weak inhibitory power. Noorhamdani AS (2010) stated that Binahong leaf extract (*Anredera cordifolia* (Ten.) Steenis) has an antimicrobial effect against *Staphylococcus aureus* with a minimum kill level of 12.5%.

Mufid Khunaifi (2010) stated that the MIC (Minimum Inhibitory Level) of Binahong (*Anredera cordifolia* (Ten.) Steenis) leaf extract against *Staphylococcus aureus* bacteria was at a concentration of 25% and the KBM (Minimum Kill Level) against *Staphylococcus aureus* was 50%. Ani Umar, et al (2012) found that the use of ethanol extract of Binahong leaves on mice wounds caused wound healing for 7 days and the results of using ethanol extract of Binahong leaves (*Anredera cordifolia* (Ten.) Steenis) were the same as the positive control used, namely anti-microbial acid. fusidate. Based on the description above, previous research on the binahong plant only affected the leaves and stems but not the roots of the binahong plant (*Anredera cordifolia* (Ten.) Steenis). This study aims to determine the antibacterial activity of binahong root (*Anredera cordifolia* (Ten.) Steenis) against *Staphylococcus aureus* bacteria. What is the minimum inhibitory concentration (MIC) and minimum kill concentration (KBM) of binahong root extract (*Anredera cordifolia* (Ten.) Steenis) against growth of *Staphylococcus aureus* bacteria.

## METHOD

This research took place at the Fisheries Products Technology Laboratory, Pattimura University, Ambon. From 02 to 19 July 2021. The samples in this study were 800 grams of binahong roots (*Anredera cordifolia* (Ten.) Steenis) and *Staphylococcus aureus* FNCC 0047.

### Tools and materials

Autoclave; Sterilizes tools and media, Incubator; Growing *Staphylococcus aureus* bacteria, Bunsen lamp; Sterilization of tube needles, Culture petri dish and antibacterial activity test site, Test tube Cultivation of *Staphylococcus aureus* bacteria, Whatman filter paper Separates suspension particles from liquid, Paper disk Testing the inhibitory power of binahong root extract, Micro pipettes Move small amounts of liquid accurately, Aluminum foil for binahong root extract to dry, Label paper Sample marker, Measuring cup Measures the solution, Beaker glass for binahong root simplicia powder, Erlenmeyer Place for maceration of binahong root extract, Rotary evaporator Evaporation of binahong root extract resulting from maceration, Analytical balance: Weigh the binahong root extract powder, Blender: Smooth the binahong roots, Colony counter Counts bacterial colonies, Ose needle Scratching bacteria on the media, Cotton Cover the test tube, Brown paper Wrapping equipment during sterilization, Camera for research

documentation, Shaker Stirring a mixture of substance solutions to form a homogeneous solution by vibration, Laminar air flow To work aseptically, Test tube rack Places test tubes, Vernier caliper Measure the diameter of the clear zone, Marker Marks the clear zone, Hot plate Heats the media.

Binahong root powder Produces pure binahong root extract, Pure culture of *Staphylococcus aureus* FNCC 0047, Research object Aquades Wash the binahong roots, Ethanol absolute Maceration solution for binahong root extract, 90% Alcohol For sterilization of tools that are not heat resistant, Nutrient Agar (NA), Media As a medium for bacterial growth to test inhibitory power, Nutrient Broth (NB) Media As a medium for bacterial growth to test inhibitory power.

### **Work procedures**

#### **1. Making simplicia**

The binahong roots are washed and drained, cut into pieces and air-dried. After drying, the simplicia is sorted again and ground into powder (Materia Medika Indonesia, 1995).

#### **2. Extraction of binahong roots**

The ground binahong root powder was put into an Erlenmeyer and 500 ml of ethanol solvent was added, then shaken for one hour to reach a homogeneous condition in a water bath shaker at a speed of 120 rpm (rotation per minute) for 1 hour. Next, the solution is macerated for 24 hours at room temperature, after 24 hours, the solution is filtered or separated using a Bucher filter. Then the filtered residue was air-dried and re-macerated for 24 hours, the maceration was repeated 3 times. The results of filters 1-3 are mixed and concentrated using a rotary vacuum evaporator at a temperature of 50°C until a concentrated extract is obtained.

To obtain binahong root extract concentrations of 5%, 10%, 15%, 20%. Binahong root extract was weighed as 0.5 grams, 0.10 grams, 0.15 grams and 0.20 grams then dissolved in distilled water.

### **Sterilization of tools**

Sterilization of equipment is carried out before all equipment is used, namely by wrapping all equipment in brown paper then placing it in an autoclave at a temperature of 121°C with a pressure of 15 Psi (Per Square Inch) for 15 minutes. Tools that cannot withstand high heat are sterilized with 90% alcohol (Tille, 2017).

### **Making nutrient broth (NB) media**

Making liquid nutrient broth (NB) media by preparing the ingredients, namely weighing 4 grams of NB media then dissolving it with 500 ml of distilled water using an Erlenmeyer then covering it with aluminum foil. The suspension was heated until it boiled and then put into test tubes, 8 ml each and then covered with cotton. This process is carried out aseptically, then sterilized in an autoclave at 121°C with a pressure of 15 psi for 15 minutes. Then stored for 1x24 hours at room temperature (Oxoid, 2006).

### **Making nutrient agar (NA) media**

This is done by preparing the ingredients for the medium, namely by weighing 14 grams of Nutrient Agar (NA) media then dissolving it with 500 ml of distilled water with an Erlenmeyer then covering it with aluminum foil. The suspension was heated until it boiled and then put into test tubes, 10 ml and 5 ml each, then covered with cotton. This process is carried out aseptically, then sterilized in an autoclave at 121°C with a pressure of 15 psi for 15 minutes. Then it is placed in an inclined position for 1x24 hours at room temperature (Oxoid, 2006).

### **Preparation of a comparison solution of 50 µg/ml tetracycline**

Tetracycline was weighed as 50 mg, then distilled water was added to make 200 ml, so that the level obtained was 0.25 mg/ml. To carry out the test, 1 ml of the above solution is pipetted and then distilled water is added to make 5 ml, so that a concentration of 50 µg/ml is obtained. This concentration is obtained based on the Standard Interpretive Antibiotic (Zimbro, et al., 2009).

### **Regeneration of bacteria**

Take one eye ose bacteria from the stock of bacteria to be used. Then inoculation was carried out in Nutrient broth (NB) media and incubated for 24 hours at 37°C. Prepare 2 Nutrient Broth (NB) media with 1 media as a negative control (without bacterial inoculation) as a comparison for bacterial growth in the inoculated media. The treatment was repeated 3 times for the first regeneration which was then used for testing (Pratama, 2005).

### Preparation of McFarland Standard Solution

McFarland 0.5 solution is usually used as a comparison for the turbidity of bacterial cultures in liquid medium with a density between  $1 \times 10^7$  cells/ml -  $1 \times 10^8$  cells/ml (Lagrange, 2007). Then store it in a place protected from direct sunlight.

### Antibacterial Testing

The disc is dipped into the sample solution until it is evenly distributed over the entire surface of the disc with various concentrations that have been prepared. Pour the sterilized nutrient agar (NA) media into the petridish. The nutrient agar (NA) media that has cooled and solidified is then planted with bacteria. The planted bacteria are spread evenly over the entire surface of the nutrient agar (NA) using a spreader. Then the disc is placed in nutrient agar (NA) media that has been planted with bacteria. The next step was carried out by incubation for 24 hours at 37°C. The greatest antibacterial activity is indicated by the largest diameter of the clear zone formed from this concentration. The smallest concentration of the sample that is able to inhibit the inoculated bacteria by forming a clear zone is the Minimum Inhibitory Concentration (MIC) value of the sample. (Pratama, 2005).

### Observation (How to Measure the Diameter of the Inhibition Zone)

The inhibition zone was formed twice, namely measurements based on the vertical and horizontal center lines, then the results were averaged. The tool for measuring the inhibition zone is a caliper (Jamilah, 2010).

### Analysis data

The analysis used is single anova (One Way Anova). Before carrying out the Anova Test, a Normality Test was first carried out using the Kolmogorov-Sminorv Test and a Homogeneity Test using the Levene Test. If the Anova test results show significant results ( $H_0$  is rejected) then proceed with the post-hock test using Duncan with a confidence level of 95%.

## DISCUSSION RESULT

The results of the antibacterial activity test of binahong root extract (*Anredera cordifolia* (Ten.) Steenis) against the growth of *Staphylococcus aureus* FNCC 0047 bacteria can be seen in the following table.

Table 1. Results of antibacterial activity test of binahong root extract (*Anredera cordifolia* (Ten.) Steenis)

Concentration	treatment			mean	Inhibitory Force Response
	1	2	3		
5%	12,1 mm	13,1 mm	12,1 mm	12,43 mm	Strong
10%	19,2 mm	19,2 mm	20,1 mm	19,50 mm	Strong
15%	25,1 mm	22,3 mm	21,5 mm	22,96 mm	Very strong
20%	24,3 mm	24,7 mm	23,8 mm	24,26 mm	Very strong
Control positif	29,3 mm	29,3 mm	29,3 mm	44,93 mm	Very strong
Control negatif	0 mm	0 mm	0 mm	0 mm	No

The Anova test results showed that binahong root extract had a significant effect at a significant level ( $\alpha < 0.05$ ) on the minimum inhibitory concentration (MIC) against *S. aureus*. The Anava test results were then carried out by the Duncan test to see the differences in each treatment and can be seen in the following table.

Table 2. Average after administering binahong root extract against *S.aureus*

Treatment	mean±SD
Control Positif (+)	44.934±5.1394 <sup>d</sup>
Concentration 5%	12.433±5774 <sup>a</sup>
Concentration 10%	19.500±5196 <sup>bc</sup>
Concentration 15%	22.967±1.8903 <sup>b</sup>
Concentration 20%	24.267±4509 <sup>c</sup>

Duncan's results showed that the minimum inhibitory concentration (MIC) in the positive control group given tetracycline was significantly different from the treatment group given binahong root extract on *S. aureus* with a concentration of 5%, concentration 10%, concentration 15% and concentration 20%. The results of the minimum inhibitory concentration (MIC) in the treatment group given binahong root extract with a concentration of 5% were significantly different from those in the group treated with binahong root extract with a concentration of 10%, a concentration of 15% and a concentration of 20%. The results of the minimum inhibitory concentration (MIC) in the treatment group given binahong root extract with a concentration of 10% were significantly different from the treatment group given binahong root extract with a concentration of 15% and a concentration of 20%. The results of the minimum inhibitory concentration (MIC) in the treatment group given binahong root extract with a concentration of 15% were significantly different from the treatment group given binahong root extract with a concentration of 20%. Davis and Stout (1971) explained that the classification of bacterial growth inhibition responses based on the diameter of the clear zone consists of 4 groups, namely weak response (diameter  $\leq 5$  mm), medium (diameter 5-10 mm), strong (diameter 10-20 mm), and very strong (diameter  $\geq 20$  mm). Based on the results of the inhibitory power test of binahong root ethanol extract. A 5% concentration provides a strong response to *S. aureus* bacteria with an inhibitory zone diameter of 12.1 mm. A concentration of 10% provides a strong response to *S. aureus* bacteria with an inhibitory zone diameter of 19.2 mm. A concentration of 15% provides a very strong response to *S. aureus* bacteria with an inhibitory zone diameter of 22.3 mm. A concentration of 20% provides a very strong response to *S. aureus* bacteria with an inhibition zone diameter of 24.7 mm.

This shows that the higher the concentration of binahong root ethanol extract, the more concentrated the solution and the greater the amount of antimicrobial substances contained in it. If the antimicrobial substances in the binahong roots are greater, the more *S. aureus* bacteria will be damaged, both the body structure and the metabolic system, so that the bacteria affected by the antimicrobial substances will die or have their growth inhibited. The results of this research show that extracts from a part of the plant, namely binahong roots, are efficacious in inhibiting the growth of *S. aureus* bacteria. This is in accordance with the opinion expressed by Mandava et al that plant extracts and their components have a significant influence on antibacterial activity on oral bacteria (Fatisa, 2013). The active compound components contained in binahong roots include flavonoids, alkaloids, terpenoids, polyphenols, saponins and essential oils (Danan et al., 2019; Rimporok et al., 2015). Flavonoids are natural pigment compounds that are yellow to colorless, soluble in water and resistant to heat. Flavonoids are often synthesized by plants in response to microbial infections. The antibacterial mechanism is to form a complex with cellular and dissolved extract proteins and with microbial walls. Another possibility is that flavonoids play a direct role by interfering with the function of microorganism cells and inhibiting the microbial cell cycle (Ginting et al, 2020). Rimporok et al stated that flavonoid active compounds are the largest substances that can play a direct role as antioxidants and antibacterials. Flavonoids are phenolic compounds that work by denaturing proteins which can cause cell metabolic activity to be catalyzed by an enzyme which is a protein. Because flavonoids have the ability to form complexes with dissolved extracellular proteins and cell walls, so that microorganisms cannot attach to and invade cells (Susanti, 2016). Flavonoids are also able to release transduction energy to the bacterial cytoplasmic membrane and inhibit bacterial motility (Manik et al., 2016). Apart from that, flavonoids can also cause damage to bacterial cell walls through inhibition which results in the merging of uncross-linked glycan chains into the peptidoglycan of the cell membrane so that it becomes a weak structure (Sulastrianah et al., 2014).

Binahong roots also contain a chemical, namely saponin, which is a compound that acts as a strong surfactant agent like soap, because it can reduce surface tension between cells. Saponins that are absorbed on the cell surface will cause damage by increasing membrane permeability, so that essential materials needed by bacteria to live are lost and can cause cell death (Ginting et al, 2020). Saponins act as a chemical barrier in the plant's defense system against pathogens. Saponins can cause leakage of certain proteins and enzymes in bacterial cells (Ravi et al., 2016). Saponin is a compound that functions as an antibacterial, accelerates the growth of new cells, stimulates the formation of fibroblasts, inhibits bacterial growth, and also has antifungal properties (Yuliana et al., 2015). The mechanism of saponin as an antibacterial is by

damaging the bacterial cell membrane due to increased membrane permeability due to saponin interacting with the bacterial cell wall (Sulastrianah et al., 2014). Alkaloids have the ability to act as antibacterials by disrupting the peptidoglycan components in bacterial cells, so that the cell wall layer does not form completely and causes cell death. Polyphenols help fight the formation of free radicals in the body so they can slow down premature aging. In general, polyphenols have antibacterial properties with their mechanism of action by damaging bacterial cell membranes and can induce the formation of complex compound bonds to enzymes or microbial substrates which can increase toxicity (Rachman et al, 2018). Tannin compounds are also known to have antibacterial properties. Tannin works as an antibacterial by interfering with bacterial surface receptors by binding to adhesin proteins on bacteria which will cause inhibition of protein synthesis for cell wall formation and a decrease in the adhesion of bacteria (Mastuti, 2016). Tannins also have chelating properties which are thought to shrink cell walls so that their growth is hampered or even dead (Sari, 2012). Fitriah et al., 2017, revealed that tannin compounds are known to interfere with peptidoglycan synthesis which causes the formation of bacterial cell walls to be imperfect, resulting in inactivation of bacterial cells in host cells. According to Aisiah (2004), tannin is a type of compound that is included in the polyphenol group. The mechanism of action of tannins is thought to be to shrink the cell walls or cell membranes, thereby disrupting the permeability of the cells themselves. As a result, cells cannot carry out living activities so their growth is stunted and they die.

## CONCLUSION

Binahong root extract at concentrations of 5%, 10%, 15% and 20% found an inhibitory zone against the growth of *S. aureus* bacteria and the minimum inhibitory concentration (MIC) of Binahong root extract against the growth of *S. aureus* bacteria at a concentration of 5%.

## REFERENCES

- Agus, R. M., Enny, F., & Dewi, K. 2013. Isolation, Identification and Testing of the Antibacterial Activity of Triterpenoid Compounds from Binahong (*Anredera cordifolia* (Ten.) Steenis) Leaf Extract against *Staphylococcus aureus* and *Escherichia coli*. *Chem Info Journal*. Vol 1 (1). 328 – 336.
- Ani, U., Dwi, K., Diah, TM. 2012. Effect of Giving Binahong (*Anredera cordifolia* (Ten) Steenis) Leaf Extract on the Healing of *Staphylococcus aureus* Infected Wounds in Mice. *Science Health Analysis*. 01 (2) : 68-75.
- Anggun, A. W. 2012. Test of the Antibacterial Activity of Ethanol Extract of Binahong Leaves (*Anredera cordifolia* (Ten.) Steenis) Against *S. aureus* Bacteria In Vitro, Thesis. Medical School. University of Jember.
- Danan, Habibah SS, Rahmawati I. 2019. The influence of binahong leaves (solution *Anredera cordifolia* (Ten) Steenis) gingivitic) healing in SMP 13 Cempaka Banjarbaru students. *J Health Polytechnic Health Scale*. Banjarmasin;10 (2):91-98.
- Davis, W. W., & Stout T. R. 1971. Disc plate method of microbiological antibiotic assay. *American Society for Microbiology*, 4(22). p 666-670.
- Fatima Y. 2013. Antibacterial effect of peel and seed extracts of pulasan fruit (*nephelium mutabile*) against *staphylococcus aureus* and *escherichia coli* in vitro. *Animal Husbandry Journal*;10(1):31-38.
- Fitriah, Mappiratu, Prismawiryanti. 2017. Test of the antibacterial activity of Johar plant leaf extract (*Cassia siamea* Lamk) from several levels of solvent polarity. *Covalent*. 3(3): 242-251.
- Gibson, G.G., Skeet, P. 1991. *Introduction to Drug Metabolism*, translated by Iis Aisyah b., 1-30, Publisher, University of Indonesia, Jakarta.
- Ginting P, Faisal H, Hanum SF, From RW. 2020. Activity gel of binahong leaf extract (*Anredera cordifolia* (ten.) steenis) against cut wound healing infected with *staphylococcus aureus* bacteria in rabbits (*oryctolagus cuniculus*). *Pharmaceutical World Journal*; 4(3):116-125.
- Jamilah, M. 2010. Comparison of the Effectiveness of Toothpaste Containing Betel Leaf Extract and Fluorine on the Growth of *Streptococcus mutans* (In Vitro). Thesis. Faculty of Dentistry. University of North Sumatra.

- Kusuma. 2009. *Staphylococcus aureus* paper. Padjajaran University. Bandung.
- Manik, D.F., Hertiani, T., and Anshory, H. 2014. Correlation analysis between flavonoid levels and the antibacterial activity of ethanol extract and cherry leaf fractions (*Muntingia calaburam* L.) against *Staphylococcus aureus*. *Khazanah*, 6(2):1-11.
- Manoi F. 2009. Binahong (*Anredera cordifolia*) as medicine. *Industrial Plant Research and Development Newsletter*. 15 (1):p 3-5.
- Mastuti, R. 2016. *Secondary Metabolites and Plant Defense*. 3rd Ed. Malang, Brawijaya University.
- Mathers, C., T. Boerma & Fat D. M. 2008. *The Global Burden of Disease 2004 Update*. World Health Organization.
- Nasronuddin. 2007. *Infectious Diseases in Indonesia. Current and Future Solutions*. Airlangga University Press: Surabaya.
- Noorhamdani, A. S., Sudiarto, & Vita, U. 2010. Test of Binahong Leaf Extract as an Antimicrobial against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Thesis. Medical School. Brawijaya University. Poor.
- Otieno, J.N., Kennedy M.M.H., Herbert V.L., & Rogasian L.A.M. 2008. Multi Plant or Single Plant Extracts, Which Is the Most Effective for Local Healing in Tanzania?. *Afr. J. Trad. CAM*. 5(2): 165-172.
- Oxoid. 2006. *Oxoid Manual*. Edition 9. Oxoid Limited : Bandung.
- Perwita, F.A. 2011. *Purple Leaf Extract Technology in 70% Ethanol Using the Percolation Method*. Faperta. Sega Maret University, Serakarta.
- Pratama, M. R. 2005. The effect of miswak (*Salvador persica*) wood powder extract on the growth of *Streptococcus mutans* and *Staphylococcus aureus* bacteria using the Agar Plate Diffusion Method. Thesis. Faculty of Mathematics and Natural Sciences. Biology Study Program. Sepuluh November Institute of Technology, Surabaya.
- Prayudi Y. *Warta Research Institute for Spices and Medicinal Plants*. 2009.p.6.
- Rachman A, Wardatun S, Wiendarlina IY. 2018. Isolation and Identification of Saponin Compounds from Methanol Extract of Binahong Leaves (*Anredera cordifolia* (Ten.) Steenis). *Online Journal of Pharmacy Students*.; 1(1).
- Ravi, L., Manasvi, V., and Praveena, L.B. 2016. Antibacterial and antioxidant activity of saponin from *Abutilon indicum* leaves. *Asian Journal Pharm*, 9:344-347.
- Rimporok S, Kepel BJ, Siagian KV. 2015. Testing the effectiveness of binahong (*anredera cordifolia steenis*) leaf extract on the growth of *streptococcus mutans* in vitro. *Unsrat*;4(4):15-21
- Saputra S. Effect of Binahong (*Anredera Cordifolia Steenis*) Leaf Extract on the Growth of *Salmonella Typhi*. *Journal*. 2008. p.1-2.
- Sari, N.E. 2012. Test of the Inhibitory Power of Breadfruit Leaves (*Artocarpus altilis folium*) against *Candida albicans* fungi and *Bacilus subtilis* bacteria using the diffusion method. *Scientific Writing, AKAFARMA Lampung*.
- Sudarmono, P., 1993, *Textbook of medical microbiology: Genetics and Resistance, Literacy Development*, University of Indonesia, Jakarta.
- Sulistiyani S. 2013. Testing the Effect of 70% Ethanol Extract of Binahong Leaves (*Anredera cordifolia Steenis*) on Alt (Alanine Aminotransferase) Levels in Male Wistar Rats (*Rattus norvegicus*) Induced with Paracetamol. *Medical Journal*. p.3.
- Susanti, N. 2016. Antimicrobial activity of jeringau rhizome extract against the growth of *Candida albicans*. *Biodjati Journal*, 1(1):55-58.
- Sulastrianah., Imran, and Fitria, E.S. 2014. Test of the inhibitory power of soursop leaf extract (*Annona muricata* L) and betel leaf (*Piper betle* L) on the growth of *Escherichia coli* bacteria. *UHO Journal*, 1(1):76-84.
- Tille, P. M. 2017. *Bailey & Scott's Diagnostic Microbiology*. In *Basic Medical Microbiology* (fourteenth, p. 45). St. Louis Missouri: Elsevier.
- Wahyono, H. 2007. The role of clinical microbiology in treating infectious diseases. Inauguration Speech Paper for Professor in Microbiology, Faculty of Medicine, Diponegoro University

- Ying L, Hernawan I. Inhibitory Power of Binahong Leaf Extract Against Polybacteria in Recurrent Aphthous Stomatitis (SAR). *Oral Medicine Dental Journal*. 2011;3(2):18-25.
- Yuliana, S.R.I., Leman, M.A., and Anindita, P.S. 2015. Test of the inhibitory power of banana stem saponin compounds (*Musa paradisiacal*) on the growth of *Candida albicans*. *e-GiGi Journal*, 3(2):2.
- Zimbardo, M.J., D.A. Power, S.M. Miller, G.E. Wilson, and J.A. Johnson. 2009. *Difco and BBL Manual, Manual of Microbiological Culture Media*. Second Edition. Becton, Dickinson and Company. Maryland. America.