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SCREENING THE ANTIBACTERIAL POTENTIAL OF *Avicennia marina* MANGROVE LEAF EXTRACT AS A NATURAL ANTIBIOTIC INGREDIENT

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ABSTRACT

Bacterial resistance to antibiotics is a global health problem caused by two factors, namely natural resistance and acquired resistance. Several factors can cause problems. The problem in this research is whether *Avicennia marina* mangrove leaf extract found in the mangrove ecosystem in Bandar Bakau, Pangkalan Sesai Village, Dumai City, can be used as an antibiotic. This research was conducted in January-March 2024. This research aimed to determine the potential of mangrove leaf extract *A. marina* as an antibiotic against pathogenic bacteria *Vibrio* sp, *Aeromonas* sp, and *Pseudomonas* sp. The results of this research will provide benefits in the form of information regarding the potential of *A. marina* mangrove leaf extract as an antibiotic. The methods used in this research are survey methods and experimental methods. The samples were then analyzed using the paper disk diffusion method at the Marine Microbiology Laboratory, Faculty of Fisheries and Marine Science, Universitas Riau. The data obtained is then presented and explained descriptively. The research results showed that antibiotics added to *A. marina* leaf extract could inhibit the growth of *A.hydrophila* bacteria based on the inhibition zone formed of 11mm. *A.marina* contains alkaloids, terpenoids, and flavonoids and has antibacterial properties against *A.hydrophila* bacteria. Based on the data, it can be concluded that *A.marina* leaf extract can be used against pathogenic bacteria.

Keywords: Bacterial Resistance, *Avicennia marina*, *Aeromonas hydrophila*, Antibiotics

1. INTRODUCTION

Mangroves are tropical coastal vegetation communities adapted to muddy or tidal inundation. In general, mangroves are shrubs that grow below high tide levels. Mangrove trees live in a community that people often call mangrove forests. Mangroves are rainforest plants easily found in coastal areas and provide many benefits for human life, ranging from ecology and nutrition to medicine. Mangroves are widely used in the food sector, one of which is the leaves of *Avicennia marina* mangrove.

Mangrove api-api is a mangrove plant in the Avicenniaaceae and Verbenaceae families. It inhabits relatively soft or shallow muddy soils with sandy subsoil, low organic matter, and high salinity[1]. According to Aini[1], *Avicennia marina* has smooth bark with greyish-green mottled scales.

Mangrove leaves contain antibacterial substances in the form of bioactive compounds such as steroids, triterpenoids, saponins, flavonoids, alkaloids, and tannins that can inhibit the growth of microorganisms so that they cannot be used as food preservatives[2].

Avicennia marina leaf extract is an alternative phytopharmaceutical material that can have antibacterial activity. Antibacterials can inhibit or kill bacteria with the cause of infection. Infections are caused by pathogenic bacteria or microorganisms, where microbes enter the

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body tissue and multiply in the tissue. Generally, bacteria are pathogenic, but the body can accept some bacteria if they are in the proper location[4].

Vibrio alginolyticus bacteria are gram-negative bacteria found in the marine environment because they are halophilic. *V. alginolyticus* attacks grouper fish with a weak immune system, causing infection and injury to the fish body and fish death. *A.hydrophila* bacteria can be detrimental to aquaculture activities because it causes damage to skin tissue and causes death.

2. RESEARCH METHOD

Time and Place

This research was conducted from February to March 2024 at the Marine Microbiology Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Riau.

Method

The survey method is observation and data collection directly in the field. Determination of the sampling point is done purposively, namely, determining the location intentionally by paying attention and considering the conditions in the research location. Existing data is qualitatively presented descriptively, and descriptive analysis is used to view and present information and data from research in the form of images, graphs, and tables; then, the data is analyzed using the ANOVA test. The dose of mangrove leaf extract in the antibacterial test used is 12.5%, 25%, 50%, 100%.

Procedures

Sampling

Sampling is done randomly where the samples taken can represent the type of mangrove *A. marina* contained in the zoning that has been determined. *A.marina* mangrove leaves were taken as much as ± 200 g. Samples of *A. marina* were obtained and then brought to the Marine Microbiology Laboratory for cleaning. *A. marina* leaves were put into a sterile container and given ethanol solution for 2 minutes. Samples given ethanol are soaked with sodium hypochlorite for hypochlorite, and the sample is washed with distilled water and dried.

Maceration Process

Extraction of *A. marina* mangrove leaves by maceration method using methanol solvent maceration was carried out for 2 x 24 hours at room temperature with methanol solvent p.a as much as 1L for 50 g mangrove samples soaked and shaken using a shaker in Erlenmeyer flask to dissolve the sample to get filtrate After maceration 2 x 24 hours the extract solution or macerate obtained is filtered using cotton to separate the filtrate and the resulting residue The filtrate is concentrated using a rotary evaporator at a temperature of 75ºC so that a thick extract is obtained.

Preparation of *A.marina* Mangrove Leaf Extract

The maceration results obtained after a few days are put into the sample flask using a funnel. The flask is filled to the limit point of the sample flask. The next step is to attach the sample flask to the rotary, which was previously smeared with vaseline, then lower the sample flask until a quarter of the part is submerged in water, the temperature is set to 40-50ºC, turn on the vacuum pump while setting the speed level 4, the thickened extract is removed from the rotary flask and stored.

Phytochemical Test

Phytochemical tests are carried out to determine the type of secondary metabolites contained in mangrove extracts[5], namely:

Alkaloid

A total of 2 mL of extract was evaporated into a porcelain cup. The resulting residue was then dissolved with 5 mL of 2 M HCl. The solution obtained was divided into 3 test tubes. The first tube served as a blank, added with three drops of 2 M HCl. The second tube added three drops

of Dragendorff reagent, and the third added three drops of Mayer reagent[5].

Phenolic

The sample extract was taken as much as 1 mL, and phenolic testing was carried out by adding 1% $FeCl_3$ reagent to the extract; if there was a black color change, it showed phenolic compounds[5].

Flavonoids

A total of 2 mL of extract was added with enough hot water, then boiled for 5 minutes and filtered. Filtrate as much as 5 mL, add 0.05 mg and 1 mL of concentrated HCl, and shake vigorously. The red, yellow, or orange formation indicates a positive test[5].

Tannins

1 mL of extract was added with 10% iron (III) chloride solution. If a dark blue or greenish-black color occurs, it indicates the presence of tannins[5].

Steroids

A total of 2 mL of extract was added to anhydrous CH_3COOH as much as ten drops and concentrated H_2SO_4 as much as two drops. The solution was shaken gently and left for a few minutes. A blue or green indicates the presence of steroids, and color triterpenoids give a red or purple colour[5].

Saponins

A total of 2-3 mL of extract was put into a test tube, then 10 mL of hot water was added and shaken vigorously for 10 seconds, then cooled, and then one drop of HCl 2 N was added. A positive test is indicated by forming a stable froth as high as 1-10 cm for not less than 10 minutes[5].

Pathogenic Antibacterial Activity Test

Antibacterial activity tests were performed using the disc diffusion method. The disc diffusion method is the most commonly used method for determining antimicrobial antibiotic susceptibility. This method uses filter paper discs that serve as containers for antibacterial substances.

The results can be observed after an 18-24 hours incubation at 37°C[6]. Solutions of mangrove extract *A. marina* concentrations of 100%, 50%, 25%, and 12.5% were made by dissolving 4 mL of extract in 4 mL of methanol to obtain a concentration of 12.5%. Ampicillin antibiotics were then used as markers to inhibit the growth of test bacteria. Then, a methanol solution was used to show that the solvent of the extraction process could not inhibit the growth of test bacteria. Bacteria were inoculated into the media using cotton swabs with the spread plate method, and paper disks containing each concentration of extract, ampicillin, and methanol were placed on the agar media, and the bacteria were gently pressed to absorb them. Then, Incubate for 24 hours at 35°C.

Data Analysis

Descriptive analysis is used to view and present information and data from research in the form of images, graphs, and tables. Then, the data is analyzed using the ANOVA test.

3. RESULT AND DISCUSSION

Water Quality

The salinity at the three research stations was an average of 28.8%, with the highest at station II at 29.5%. The temperature at the three stations ranged from 29-30 oC, with the highest at station I, 32ºC, and an acidity (pH) of 7 ppt.

Extraction of *A.marina* Mangrove Leaves

The extraction that has been done gets the yield results as in Table 1.

Table 1. Yield results of *A. marina* mangrove leaf extracts

Sampling Location	Station	Rendemen (%)	Soaking Time (hour)
PAB Dumai	1	3,97	26
	2	5,38	30
	3	3,68	26

Phytochemical Test of *A.marina* Leaf Extracts

Phytochemical tests are carried out to detect secondary metabolite compounds of plants based on their classification as initial information in knowing the class of chemical compounds that have biological activity from a plant. This phytochemical test was conducted to determine the active compounds' content in *A. marina* mangrove leaves. Phytochemical test results on mangrove leaf extract *A. marina* can be seen in Table 2.

Table 2. Phytochemical test of *A. marina* leaf extracts

Sampling Location	Station	Alkaloid	Flavonoid	Saponin	Tanin	Terpenoid
PAB Dumai	1	+	-	+	+	+
	2	+	-	+	+	+
	3	+	-	+	+	+

Table 3 shows that the phytochemical test of *A.marina* leaf extract contains alkaloid, tannin, flavonoid, and steroid compounds. Alkaloid test results obtained positive results, as indicated by a change in the color of the extract. In the Mayer reagent, the extract turns red to brownish and forms a precipitate; in the Wagner reagent, the extract turns brownish and forms a precipitate. In the saponin test, negative results were characterized by no foam formation. In the tannin test, positive results were marked by a color change to dark green. In the flavonoid test, positive results were marked by a change in color from reddish yellow to red. In the steroid test, positive results were marked by a change in color to green.

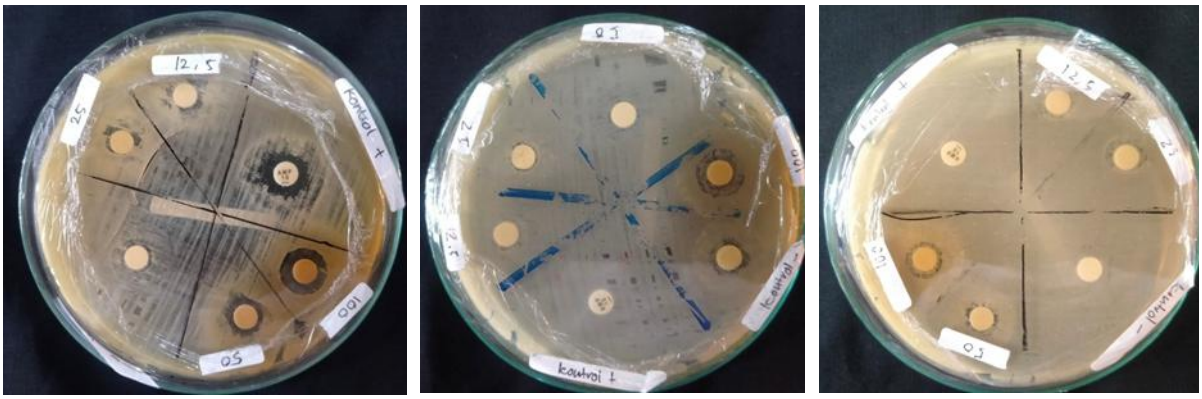
In the terpenoid test, there was no color change to purple. The results of the phytochemical test of *A. marina* mangrove leaf extract are characterized by a change in color in the extract after being dripped with reagents. It is known that the alkaloid, tannin, flavonoid, and steroid tests obtained positive results, while the saponin and terpenoid tests obtained negative results.

Phytochemical tests are carried out to determine the type of secondary metabolite group contained in the sample. The principle of this phytochemical method is the color change by a color reagent, where the resulting color change is then matched with a color standard. The presence of a color change that matches the standard color indicates a positive result for the compound group. Usman[7] suggests the presence of the group of secondary metabolite compounds contained in the extract, such as color changes, precipitation, or foam formation, by the reagent used.

The results of the phytochemical test show that the secondary metabolites generally contained in *A. marina* leaf extracts are alkaloids, flavonoids, tannins, and steroids. Syawal et al.[7] explain that the mode of action of antibacterial substances such as alkaloids and flavonoids against bacteria is thought to inhibit the work of bacterial enzymes, resulting in disruption of metabolism or death of bacterial cells and inhibition of enzyme formation in the form of extra-cellular toxins which are virulence factors in bacteria.

Antibacterial Activity of Mangrove Leaf Extract of *A. marina*

The results of the antibacterial activity test of *A. marina* mangrove leaf extract (after deducting the diameter of the disc paper by 6 mm) against *A. hydrophila* from Bandar Bakau Dumai showed an average clear zone diameter of 2.9-5.5 mm. The results of the antibacterial activity test of *A. marina* mangrove leaf extract against Vibrio alginolyticus showed an average clear zone diameter of 2.25-4.5 mm. The results of the antibacterial activity test of *A.marina* mangrove leaf extract against Pseudomonas aeruginosa showed an average clear zone diameter of 2.25-3.58 mm Figure 1.



(a) (b) (c)

Figure 1. Zone of inhibition of *A. marina* leaf extract against bacteria (a) *A. hydrophila* bacteria, (b) *P. aeruginosa* bacteria, (c) *V. alginolyticus* bacteria

In Figure 1, (a), (b), and (c), a visible inhibition zone formed around the disc paper, which indicates there are colonies of *bacteria A. hydrophilla, P. aeruginosa,* and *V. alginolyticus* against *A. marina* mangrove leaf extract. The inhibition zone at each concentration was averaged to compare extracts against pathogenic bacteria *A. hydrophila, P. aeruginosa,* and *V. alginolyticus*.

The diameter of the inhibition zone of *A. marina* leaf extract is best at a concentration of 100%, namely 11 mm in *A. hydrophyla* bacteria, and the lowest is in P. aeruginosa, with a result of 2.5 mm. At a concentration of 50%, the best result is 7.2 mm in *V. alginolyticus* bacteria, and the lowest is in *A. hydrophila* bacteria, resulting in 2 mm. Then, at a concentration of 25%, the best results were 3.5 mm in *V. alginolyticus* bacteria and the lowest in *A. hydrophila* bacteria, resulting in 2 mm. At a concentration of 12.5%, the best results in *V. alginolyticus* bacteria were 5.65 mm, and the lowest was P. aeruginosa bacteria, which resulted in 2.2 mm. In the positive control, the best results were at a concentration of 100%, with a result of 17.45 mm.

Avicennia marina extract can inhibit bacterial growth at each concentration. The best concentration to inhibit the growth of pathogenic bacteria is 100%, and the lowest result is 12.5%. This follows the hypothesis that the higher the concentration value of the extract, the greater the inhibition zone produced. It is proven that the 100% concentration has the largest average inhibition zone.

Based on the results obtained against pathogenic bacteria A. *hydrophila, A. marina* extract at concentrations of 12.5%, 25%, and 50% is categorized as weak, while at 100% concentration, it is classified as strong. According to Nopiyanti et al.[9], it is powerful if the inhibition diameter is ≥ 20 mm, substantial if 10-20 mm, moderate if the inhibition zone is 5-10 mm, and weak if the inhibition zone is ≤ 5 mm. Based on the ANOVA test at 6, we obtained a P ≥ 0.05 that the mangrove leaf extract of *A. marina* can inhibit the growth of *A. hydrophila* bacteria by determining the difference in each concentration. A Post Hoc further test was carried out with the LSD method, and the results were obtained, namely at a concentration of P ≤ 0.05 at a concentration of 12.5%, 25%, 50%, and 100%, meaning that it has a significant difference.

Based on the clear zone formed in *V. alginolyticus*, bacteria are categorized as weak (≤ 5 mm), while at 25% concentration are classified as moderate (5-10 mm). V. alginolyticus bacteria are gram-positive bacteria. Gram-positive bacteria have a thick peptidoglycan layer of 20-80 nm, while gram-negative bacteria have a thin peptidoglycan layer of 2-7 nm. The weight of peptidoglycan in Gram-negative bacteria is only 5-10% of the weight of the cell wall. This causes gram-negative bacteria to be more sensitive to antibacterial substances. The research results by Hendrawan et al.[10]

showed that mangrove *A. marina* leaf extract with a concentration of 100 mg/mL did not have an inhibition zone against *V. alginolyticus* bacteria.

Based on the ANOVA test, the value of $P \leq 0.05$ was obtained, which means that the extract of *A. marina* mangrove leaves can inhibit the growth of *V.alginolyticus* bacteria to determine the difference in each concentration. A further Post Hoc test was carried out with the LSD method. The results obtained, namely at a concentration of $P \leq 0.05$ at a concentration of 12.5%, 50%, and 100%, which means it has a significant difference, and at a concentration of 12.5% and 25% has $P \leq 0.05\%$, which means it has a significant difference.

Based on the clear zone formed in *P. aeruginosa*, bacteria are categorized as weak (≤ 5 mm), while at 100% concentration, it is classified as moderate (5-10 mm). *P. aeruginosa* bacteria are gram-negative with thin peptidoglycan[6]. The mechanism of action of antibacterial compounds includes inhibiting cell wall synthesis, inhibiting microbial cell membrane integrity, inhibiting microbial cell protein synthesis, disrupting microbial cell metabolism, and inhibiting nucleic acid and protein synthesis. Antibacterial compounds that attack will damage the cell wall or prevent its synthesis, so it will cause the formation of cells that are sensitive to osmotic pressure, known as trauma[11].

Based on the ANOVA test, the value of $P \geq 0.05$ was obtained, which means that the extract of A. marina mangrove leaves can inhibit the growth of *P. aeruginosa* bacteria by determining the difference in each concentration. A further Post Hoc test was carried out with the LSD method and the results obtained, namely at a further Post Hoc with the LSD method and the results obtained, namely at a concentration of $P \leq 0.05$ at a concentration of 12.5%, 25%, 50%, and 100%, which means that it has a significant difference. Based on the clear zone formed on *P. aeruginosa* bacteria referred to by Nopiyanti et al.[9]. The research results by Gabariel et al.[12] showed that the antibacterial activity of *A. marina* mangrove leaf extract obtained the highest results at a concentration of 100%, an average of 5.2 mm. In addition, the same concentration produces an inhibition zone against *E. coli* bacteria 5.07 mm, and *V. alginolyticus* 5.2 mm.

The inhibition zone is formed in the antibacterial activity test because *A. marina* leaf extract has antibacterial compounds that can inhibit bacterial growth. Phytochemical test results show the presence of compounds that can inhibit bacterial growth, such as alkaloids, flavonoids, tannins, and steroid compounds contained in *A. marina* extracts. Based on the discussion, it shows that the mechanism of alkaloids, flavonoids, tannins, and steroids contained in *A. marina* leaf extract has a role as an antibacterial resource against bacteria *A. hydrophila*, *V. alginolyticus* and *P. aeruginosa*. The mechanism of action of these active substances is by inhibiting cell wall synthesis, inhibiting cell membrane function, and inhibiting protein synthesis.

The positive control used was chloramphenicol ten µg. The selection of this antibiotic is based on the fact that chloramphenicol antibiotics are bacteriostatic with a broad spectrum that is active against gram-negative and gram-positive bacteria. This is based on the statement of Egra et al.[13] that chloramphenicol is a broad-spectrum antibiotic that can inhibit gram-positive and gram-negative bacteria. According to Devi & Tuty[14], using positive control aims to see the picture of the killing of test bacteria seen from the clear zone.

The negative control used in this study was distilled water. Negative control is used to see the effect of distilled water as a diluent for *A. marina* extract samples. It is known that distilled water has no activity against test bacteria, so there is no influence between the solvent and the test bacteria used. The purpose of using controls in this study is to determine the presence of factors that affect the diameter of the clear zone,

such as the quality of the media used in contamination[15].

4. CONCLUSION

A. marina mangrove leaf extract contains secondary metabolite compounds: saponins, flavonoids, steroids, and tannins. *A. marina* mangrove leaf extract can potentially inhibit the growth of pathogenic bacteria *A. hydrophila* with the diameter of the inhibition zone in the strong category (>10-20 mm), while the bacteria *P. aeruginosa* and *V. alginolyticus* are included in the medium category (5-10 mm). The highest zone of inhibition was found in *A. hydrophila* bacteria. The zone of inhibition formed in each of these bacteria rises proportional to the concentration of the extract used. Mangrove *A. marina* leaf extract can be used as a natural antibiotic to inhibit the growth of pathogenic bacteria.

Based on the research conducted, it is recommended that further research be conducted on fractions that are not continued and that the structure of the compounds obtained using NMR be identified to ensure the actual structure of the compounds.

REFERENCES

1. Afzal, M., Masood, R., Jan, G., Majid, A., Fiaz, M., Shah, A. H., Alam, J., Mehdi, F.S., Abbasi, F.M., Ahmad, H., Islam, M., Inamullah., & Amin, N.U. Efficacy of *Avicennia marina* (Forsk.) Vierh. Leaves Extracts againts Some Atmospheric Fungi. *African Journal of Biotechnology*, 2011; 10(52): 10790- 10794.
2. Aini, R. *Identifikasi Keanekaragaman Pohon Mangrove di Kawasan Wisata Hutan Mangrove Teluk Benoa Bali sebagai Dasar Pembuatan Sumber Belajar Biologi*. Universitas Muhammadiyah Malang. Malang, 2017
3. Anggraini, R.R., Hendri, M., & Rozirwan, R. Potensi Larutan Bubuk Daun Mangrove *Bruguiera gymnorrhiza* sebagai Pengawet Alami. *Maspari Journal*, 2018; 10(1): 51-62.
4. Pramanayudha, G., Khotimah, S., & Rahmayanti, S. Uji Aktivitas Senyawa Kitoson terhadap *Neisseria gonorrhoeae* yang Diisolasi dari Pasien dengan Penyakit Infeksi Seksual secara in Vitro. *Jurnal Mahasiswa PSPD FK Universitas Tanjungpura*, 2018; 4(3).
5. Dari, D.W., & Junita, D. Karakteristik Fisik & Sensori Minuman Sari Buah Pedada. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 2020; 23(3): 532541.
6. Pelezar, J.M., & Chan, E.C.S. *Dasar-Dasar Mikrobiologi Jilid I*. Universitas Indonesia. Jakarta, 2008; 954-955.
7. Usman, U. Uji Fitokimia dan Uji Antibakteri dari Akar Mangrove *Rhizopora apiculata* terhadap Bakteri *Escherichia coli* dan *Staphylococcus aureus*. *Jurnal Kimia dan Pendidikan*, 2017; 2(3): 169 – 177.
8. Syawal, H., Hakim, L., & Effendi, I. Phytochemical Analysis of *Rhizopora apiculata* Leaf Extract and Its Inhibitory Action against *Staphylococcus aureus, Aeromonas hydrophila* and *Pseudomonas aeruginosa*. *Aquaculture, Aquarium, Conservation & Legislation*, 2020; 13(4): 2242 - 2249.
9. Nopiyanti, H.T., Fitriani, A., Isnaini, I., & Melki, M. Screening of *Nypa fructions* as Antibacterial of *Bacillus subtillis, E.coli* and *S. Aureus. Jurnal Maspori*, 2016; 8(2): 83-90.
10. Hendrawan, H., Zuraida, I., & Pamungkas, B.F. Aktivitas Antibakteri Ekstrak Methanol *Xylocarpus granatum* dari Pesisir Muara Badak. *Jurnal Perikanan Tropis*, 2015; 20(2): 253- 259.
11. Panjaitan, R.S., Warganegara, W., & Madayani, F. Aktivitas Antibakteri Ekstrak Lipid Sargassaum polycistum terhadap *Bacillus cereus* dan *Staphylococcus aureus*. *Jurnal Kimia dan Pendidikan*, 2017; 3(3): 29-39.

12. Gabariel, E., Yoswaty, D., & Nursyirwani, N. Inhibition of *Xylocarpus granatum* extract against the Growth of Pathogenic Bacteria (*Pseudomonas aeruginosa, Escherichia coli*, and *Vibrio alginolyticus*). *Jurnal Perikanan dan Kelautan*, 2019; 24(2):114-118
13. Egra, S., Mardhiana, M., Roffin, M., Adiwena, M., Jannah, N., Kuspradini, H., & Mitsunaga, T. Aktivitas Antimikroba Ekstrak Bakau (*Rhizophora mucronata*) dalam Menghambat Pertumbuhan *Ralstonia solanacearum* Penyebab Penyakit Layu. *Agrovigor: Jurnal Agroekoteknologi*, 2019; 12(1): 26- 31.
14. Devi, S., & Mulyani, T. Uji Aktivitas Antibakteri Ekstrak Etanol Daun Pacar Kuku (*Lawsonia inermis* Linn) pada Bakteri *Pseudomonas aeruginosa*. *Journal of Current Pharmaceutical Sciences*, 2017;1(1): 30- 35.
15. Suciari, L.K., Mastra, N., & Widya, C.D. Perbedaan Zona Hambat Pertumbuhan *Staphylococcus aureus* Pada Berbagai Konsentrasi Rebusan Daun Salam (*Syzygium polyanthum*) secara In Vitro. *Meditory*, 2017; 5(2): 92-100