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INHIBITION OF MANGKOKAN LEAF (*Nothopanax scutellarium* Merr.) ETHANOL EXTRACT ON THE GROWTH OF *Salmonella typhimurium* BACTERIA

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ABSTRACT

Typhoid fever is an infectious disease of the digestive tract caused by the bacterium *Salmonella typhimurium*. Mangkukan leaf (*Nothopanax scutellarin* Merr.) can be an alternative treatment for that disease because it contains flavonoids, saponins, tannins, and alkaloids. This study aimed to determine the ability of Mangkukan leaf extract to inhibit the growth of *S. Typhimurium* bacteria. This study used a factorial completely randomized design consisting of two factors, namely the concentration of the extract and the age of the inoculum with 4 repetitions. The concentration of the extract used was 5%, 10%, 20%, 40%, and 80%, equipped with the positive control (chloramphenicol), and negative control (aqua dest), while the age of the inoculum was based on the growth phase of the test bacteria, namely 3 hours, 6 hours, 18 hours and 24 hours. Antibacterial activity testing was carried out using the Disc Diffusion method (Kirby-Bauer Test). The test concentrations of 5%, 10%, 20%, 40%, and 80% resulted in inhibition zone diameters of 3.41 mm, 5.4 mm, 6.04 mm, 7.43 mm, and 9.48 mm, respectively. The 80% concentration of ethanol extract from the Mangkukan leaf was effective in inhibiting the growth of *S. typhimurium* bacteria with an antibacterial effectiveness value of 60.81%.

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1. INTRODUCTION

One of the infectious diseases that is still a health problem in the world, especially in developing countries, is typhoid fever. In the world, there are 16 million to 33 million cases per year, and 200 -600 thousand deaths are caused by this disease every year. Almost 80% of typhoid fever cases occur in Asia (Zaki & Karande, 2011). In 2007, *the Communicable Disease Centre* (CDC) of Indonesia reported that the prevalence of typhoid fever was 358-810 per 100,000 population with 64% occurring in children aged 3-9 years (Moehario, 2009). According to the Ministry of Health of the Republic of Indonesia (2010), there were 41,081 cases of typhoid fever patients hospitalized and 279 of them died.

Typhoid fever is a disease caused by infection of *Salmonella typhimurium* bacteria in the small intestine. S. *typhimurium* bacteria are the most common infectious and isolated pathogenic agents in foodstuffs (Kasim, 2020). According to Poeloengan *et al.* (2006), infections caused by *S. typhimurium* are closely related to human and environmental hygiene conditions. The disease can be transmitted through foods such as meat, eggs, and water that have been contaminated with *S. typhimurium* bacteria. The incubation period for *S. typhimurium* bacteria infection ranges from 4 to 72 hours after consumption of food or water that has been contaminated *with S. typhimurium* bacteria.

So far, the administration of antibiotics has been a treatment effort to overcome infectious diseases caused by *S. typhimurium bacteria.* Excessive and uncontrolled use of antibiotics can result in bacteria becoming resistant to these antibiotics (Jawetz *et al.,* 2005). This encourages the importance of excavating sources of medicines from natural materials in the form of plants. Plants can be an alternative treatment for reducing antibiotic resistance (Pandey & Mishra, 2010). One of the plants is the mangkokan plant (*Nothopanax scutellarin* Merr.) (Dalimartha, 1999).

Mangkokan is a popular yard ornamental plant and medicinal plant in Indonesia. This plant is often grown as an ornamental plant or hedge plant, it can also be found wild in fields and riverbanks. The use of mangkokan leaves by the community is believed to be able to overcome wounds, swelling, and the smell of sweat on the body. This is in line with the theory that states that the benefits of mangkokan leaves include as an antibacterial, smoothing the digestive system, preventing hair loss, treating wounds, anti-inflammatory, improving blood circulation, preventing the appearance of anemia symptoms, and an antioxidant for the body (Hanum & Ardiansyah, 2017). According to Ahdiyani & Purwani (2015) that the metabolite compounds contained in mangkokan leaves are flavonoids, tannins, saponins, and alkaloids.

Research by Primadiamanti *et. al*. (2020), showed that the antibacterial activity of mangkokan leaf extract at a concentration of 100,000 ppm was able to inhibit the growth *of Staphylococcus aureus* with an inhibitory zone diameter of 15.69 mm. According to research by Jahari (2013), mangkokan leaf ethanol extract is able to inhibit the growth of *Staphylococcus epidermidis* bacteria with an inhibition zone diameter of 12.33 mm at a concentration of 16%.

Extracts from various plants with their antibacterial properties, have shown an important role in inhibiting pathogenic bacteria. Therefore, it is necessary to develop alternative treatments by utilizing secondary metabolite compounds contained in various plants that have the potential to reduce the use of synthetic antibiotics. Based on the description above, research is needed to determine the antibacterial effectiveness of mangkokan leaf ethanol extract (*N. scutellarium* Merr.) in inhibiting the growth of *S. typhimurium bacteria.*

2. RESEARCH METHODS

a. Media and bacteria preparation

This study is an experimental study, to determine the effectiveness of ethanol extract of mangkokan leaves (*N. scutellarium* Merr.) in inhibiting the growth of *S. typhimurium*. The research design waas Factorial Complete Random Design with treatments of test bacteria (with different inoculum ages and 5 variations) and extract concentrations. There were also positive (chloramphenicol) and negative (aquaadest) controls. Each treatment was repeated 4 times so 112 experimental units were used.

The subject of this study was mangkokan leaf extract (*Nothopanax scutellarium* Merr.) with concentrations of 5%, 10%, 20%, 40%, and 80%. The object of this study is the bacterium *Salmonella typhimurium* ATCC 14028.

Before conducting the research, equipment such as Petri dishes, test tubes, cuvettes, and pipette tips were washed under running water. Then dried by aerating. Sterilization was carried out using an autoclave at a temperature of 121^{o}C with a pressure of 1 atm for approximately 15 minutes. After the sterilization process is complete, all tools are stored in the oven with temperature 60 ^{o}C until the equipment is dry.

Twenty eight grams of *Nutrient Agar* (NA) powder was dissolved with 1 liter of aquaadest. The solution was heated using *a hot plate magnetic stirrer* until homogeneous and boiling, then poured into 5 test tubes of 5 mL as NA *slant media.* After sterilization, the media is poured into

petri dishes aseptically in the *Laminar Air Flow Cabinet* as NA plate media. 13 grams of *Nutrient Broth* (NB) powder was dissolved in 1 liter of aquaadest. The solution was heated using *a hot plate magnetic stirrer* until homogeneous and boiling then poured into a 100 mL Erlenmeyer measuring 25 mL. Then sterilized using an autoclave with a temperature of 121 ^{o}C and a pressure of 1 atm for 15 minutes.

S. Typhimurium *bacteria* were rejuvenated by taking 1 ose and inoculated on NA *slant* media by continuous streak method. The bacteria are incubated at room temperature for 24 hours (Radji, 2011).

b. Mangkokan Leaf Extract

The method of making extracts is a modification of the method carried out by Khunaifi (2010). The leaf samples used were old (dark green) and healthy (no wounds and defects were found on the leaves). 800 grams of mangkokan leaves were washed with running water, then cut into small pieces (size 2x2 cm) and dried for 5 days. Next, the leaves were dried in an oven at 50^{o}C for 3 days. The sample were then blended to a fine powder (simplicia) and sifted using a sieve.

180 grams of the leaves powder was placed into a glass vessel. Leaf powder was macerated 3 times using 96% ethanol solvent in a tightly closed glass vessel and left at room temperature for 16 hours and stirred every 8 hours. The ratio between simplicia and solvent is 1:3 (for maceration I) and 1:2 (for maceration II and III). The maceration results were filtered using aseptic sterile Whatman No.1 filter paper so that filtrates were obtained. After filtering, the filtrate obtained was collected to be evaporated using *a rotary vacuum evaporator* at a temperature of 55^{o}C and then evaporated with a *water bath* until a thick extract of Mangkokan leaves was produced. Obtained viscous extract made into a 100% stock solution by dissolving as much as 20 grams of thick extract with sterile aquatics until the volume reaches 20 mL. 100% concentration extract stock solution diluted with a sterile aqueous aqueduct to obtain solutions with concentrations of 5%, 10%, 20%, 40%, and 80% (v/v) (Harborne, 1987; Septianingsih *et. al.*, 2012).

c. Creation of Bacterial Growth Curve

The method of making the bacterial growth curve was carried out by the method carried out by Sharah *et al.* (2015). S. *typhimurium* bacterial isolate of 1 ose was inserted in 25 mL *of sterilized NB (Nutrient Broth*) media. Then it was incubated in a *shaker incubator* at room temperature and speed of 120 rpm for 24 hours so that a bacterial culture was obtained. *Sterile Nutrient Broth* media without bacterial culture was used as control. The results were then measured by *UV-Vis spectrophotometer* at a wavelength of 600 nm. After obtaining a bacterial culture with an absorbance value of 0.8-1, this culture was divided into 18 Erlenmeyer containing 20 mL of NB (*Nutrient Broth*) media of 2 mL each and then incubated in *a shaker incubator* at room temperature and speed of 120 rpm. Absorbance value was measured every 3 hours for 24 hours and a bacterial growth curve is made so that the logarithmic phase of bacteria can be known. The bacterial growth curve is obtained by plotting between times incubation with its absorption value.

d. Test Solution Preparation

The test solution used was mangkok leaf extract with a concentration of 5%, 10%, 20%, 40%, and 80%. The solvent used in the dilution of the extract is aquaades. The concentration of mangkokan leaf extract is made using the following formula:

$$V_1 \, x \, N_1 = V_2 \, x \, N_2$$

e. Bacterial Suspension Manufacturing

The rejuvenated S. typhimurium *bacterial isolate* culture was taken as many as 1 ose and then put into an Erlenmeyer containing 25 mL of NB (*Nutrient Broth*) media that had been sterilized and incubated in *a shaker incubator* at room temperature at 120 rpm for 24 hours.

f. Antibacterial Compound Activity Test

The activity test of the antibacterial compounds used was by the *Disc Difussion* method (*Kirby Bauer*) (Figure 1) referring to Das *et. al.* (2010). The media used is the NA *plate* media. Bacterial suspension of 0.1 mL with measured cell density from a spectrophotometer with *optical density* value (OD600=1) was inoculated on NA plate media with spread *plate* technique and then flattened with drigalski. Then *the blank disk* was soaked in a test solution at each concentration (5%, 10%, 20%, 40%, and 80%), positive control (chloramphenicol), and negative control (aquaades) for 20 minutes. Furthermore, *the blank disk* is placed on the NA *plate* media that has been inoculated with *Salmonella typhimurium culture*. The sample was incubated at 37ºC for 24 hours. The measurement of the inhibition zone is carried out every 3 hours for 24 hours.

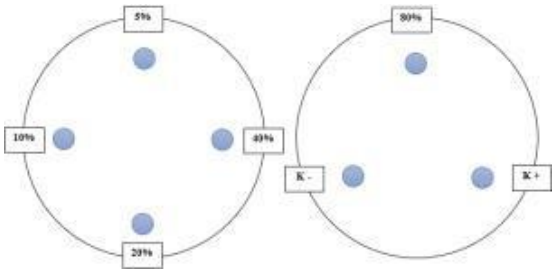

Figure 1. Antibacterial Activity Testing Research Design

g. Data Analysis Techniques

The data was carried out using the SPSS (*Statistical Product and Service Solution*) version 20.0 program with the ANOVA (*Analysis of Variant*) technique. The ANOVA test aims to determine whether there is an effect of ethanol extract of mangkokan leaves (*N. scutellarium* Merr.) in inhibiting the growth of the *S. typhimurium* bacteria tested. If it shows significant results then the Duncan post hoc analysis was used to find out which treatment has different effect.

Antibacterial effectiveness of the concentration of Mangkokan leaf extract (*Nothopanax scutellarium* Merr.) against antibiotics is calculated based on the equation according to Arora and Bhardwaj (1997):

$$E = D/Da \times 100\%$$

E: antibacterial effectiveness (%)
D: diameter of the incubation zone of antibacterial extract (mm)
Da: diameter of antibiotic inhibition zone (mm)

3. RESULTS AND DISCUSSION

a. Bacterial Growth Curve

The results of the measurement *of the optical density* of *Salmonella typhimurium suspension* bacteria in the form of a growth curve can be seen in Figure 2. Based on the growth curve of *S. typhimurium* bacteria shown in Figure 2, it is known that no lag phase (adaptation phase) was formed, the initial log phase at the 3rd hour, the final log phase at the 6th hour, the initial stationary phase at the 18th hour, and the final stationary phase at the 24th hour.

Four inoculum age points were determined on the curve that represent several phases of bacterial growth. Treatment of 3-hour-old bacterial inoculum as the initial log phase, 6-hour-old bacterial inoculum as the final log phase, 18-hour-old bacterial inoculum as the initial stationary phase, and 24 hours bacterial inoculum as the final stationary phase. Each of these age treatments was tested for its antibacterial activity against Mangkokan leaf extract.

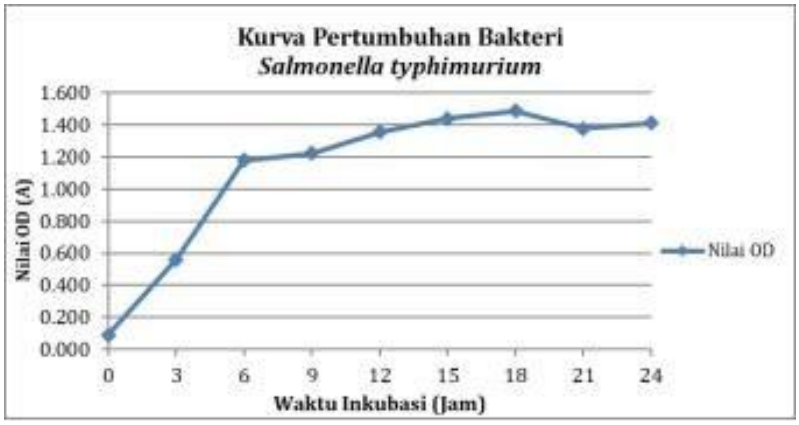

Figure 2. Bacterial Growth Curve *Salmonella typhimurium*

b. Antibacterial Activity Testing

Based on the *Kolmogorov-Smirnov normality test*, *a* significance value of 0.003 ($p<0.05$) was obtained which indicates that the data is not normally distributed. Furthermore, in the homogeneity test, a significance value of 0.000 ($p<0.05$) was obtained, which means that the data variants were not homogeneous. Therefore, *the Kruskal-Wallis non-parametric test* was used.

In each phase of bacterial growth, a significance value of 0.673 ($p>0.05$) was obtained, indicating that the age treatment of the inoculum in each phase of bacterial growth did not have a significant effect on the formation of bacterial growth inhibition zones. This shows that at the age of the inoculum, is able to produce an inhibitory effect on the growth of *S. typhimurium bacteria.* Both low and high number of bacterial cells can still be inhibited by mangkokan leaf extract. In the treatment of inoculum age, the area of the inhibition zone produced cannot be compared, because each age of the inoculum has a different number of cell optical densities resulting in different inhibition zones.

This is comparable to the theory that antibacterial activity is affected by the amount of optical density of bacterial cells present in the suspension. The more concentrated the suspension of the test bacteria used, result in the growth of bacteria on the medium to be denser so that the sensitivity to the antibacterial compounds in the extract will be weaker and the area of the resulting inhibition zone will be smaller. On the other hand, if the suspension of the test bacteria used is thinner where the number of optical densities of bacterial cells is low, the area of the inhibitory zone formed will be larger (Utami, 2017).

In the treatment of mangkokan leaf extract cocentrations, a significance value of 0.000 ($p<0.05$) was obtained, indicating that there was an influence on the formation of the growth inhibition zone of *S. typhimurium bacteria.* From *Duncan analysis,* it was found that mangkokan leaf extract different concentrations provided a significantly different antibacterial inhibitory effect on the growth *of S. typhimurium bacteria.* The difference in the diameter of the inhibition zone produced is due to the difference in the diffusion speed of antibacterial compounds in the agar medium and the concentration of different antibacterial compounds can also give results of different inhibition zone diameters, which can be seen in Table 1.

Table 1. *Post Hoc Analysis* of Mangkokan Leaf Extract Concentration on Antibacterial Activity

Treatment	Average Resistance Zone
Aquades	0a
5%	4.4b
10%	7,6c
20%	9.2d
40%	11,8e
80%	13,9f
Chloramphenicol	24.0g

Antibacterial activity of mangkokan leaf extract (*N. scutellarium* Merr.) with several extract concentrations of 5%, 10%,20%, 40%, and 80% and variations in inoculum age. The test bacteria were 3 hours, 6 hours, 18 hours, and 24 hours resulting in different average inhibition zone diameters and antibacterial power as shown in Table 2.

Table 2. Inhibition of Antibacterial Activity Mangkokan Leaf Extract

Treatment	Installment Zone Obstacles	Antibacterial Power
Aquadest	0	No inhibition zone
5%	3.41	Weak
10%	5.4	Keep
20%	6.04	Keep
40%	7.43	Keep
80%	9.48	Keep
Chloramphenicol	19.13	Strong

Based on Table 2, it is known that the average inhibition zone produced by mangkokan leaf extract (*N. scutellarium* Merr.). At a concentration of 5%, it is included in the category of inhibitors with weak inhibitory ability. Meanwhile, concentrations of 10%, 20%, 40%, and 80% are included in the category of inhibitors with moderate inhibitory capabilities. This is based on the theory according to Davis & Stout (1971) which states that the provisions of antibacterial power are as follows: the ≥20mm resistance area is included in the very strong category, the 10-20mm resistance area is in the strong category, the 5-10mm resistance area is in the medium category, and the resistance area of 5mm or less is included in the weak category (Mpila *et. al.*, 2012).

An increase in the concentration of mangkokan leaf extract given can result in a larger diameter of the inhibition zone. The higher the concentration of extracts, the more active compounds that function as antibacterial, so that the ability to inhibit the growth of *S. typhimurium* bacteria to a greater extent which is characterized by an increase in the inhibition zone produced. This is by the theory that the levels of active compounds contained in high-concentration extracts are greater than those at low concentrations. Thus, the activity of antibacterial power is affected by concentration. Antibacterial activity is influenced by several factors, including the concentration of extracts, the content of antibacterial chemically active compounds, the diffusion power of the extract, and the type of bacteria that are induced (Jawetz *et. al*., 2004).

The results of the fourth antibacterial test of bacterial age treatment, showed that the positive control treatment of chloramphenicol had antibacterial activity against *S. typhimurium* bacteria while the negative control was that the aqualasts did not have antibacterial activity. The inhibitory zone formed on chloramphenicol is larger compared to mangkokan leaf extract. The use of chloramphenicol as a positive control is due to its broad spectrum against Gram-positive bacteria and Gram-negative bacteria. Chloramphenicol can inhibit microbial protein synthesis by binding to ribosomes subunit 50s and inhibiting *peptidyl transferase enzymes* so that peptide bonds are not formed in microbial protein synthesis (Gunawan, 2011). The use of aqueous water as a negative control does not form a clear zone, so it can be ensured that the inhibition zone produced purely from mangkokan leaf extract is not affected by solvents. Aquadests are polar solvents with neutral properties (pH=7) in a pure, colorless, tasteless, and odorless state. The aqueous consists of two hydrogen atoms and one oxygen atom with the chemical formula H2O, which is polar due to the difference in charge. Aquaades are good solvents because of their polarity, so they can dissolve active compounds of tannins and flavonoids that have activities that inhibit bacterial growth (Khafidhoh, 2015).

The ability of mangkokan leaf extract to inhibit the growth of *S. typhimurium* bacteria is due to the content of secondary metabolite compounds in the form of flavonoids, alkaloids, saponins, and tannins (Khare, 2007). The mechanism of action of flavonoids as antimicrobials can be divided into 3, namely inhibiting nucleic acid synthesis, inhibiting cell membrane function, and inhibiting energy metabolism (Hendra *et. al.*, 2011). The antibacterial mechanism of flavonoids that inhibit nucleic acid synthesis is the A and B rings which play an important role in the process of intercalation or hydrogen bonding by accumulating nucleic acid bases that inhibit the formation of

DNA and RNA. The location of hydroxyl groups at 2', 4' or 2', 6' hydroxylated in the B ring and 5,7 hydroxylated in the A ring plays an important role in the antibacterial activity of flavonoids. Flavonoids cause damage to the permeability of bacterial cell walls, microsomes, and lysosomes as a result of the interaction between flavonoids with bacterial DNA (Cushnie *et. al.,* 2005).

The antibacterial effect of tannins is through reactions with cell membranes, enzyme inactivation, and functional inactivation of genetic material. The mechanism of action of tannins as antibacterial is to inhibit the enzyme reverse transcriptase and DNA topoisomerase so that bacterial cells cannot be formed (Nuria*et al.*,2009). Tannins have antibacterial activity related to the ability to inactivate microbial cell adhesins, inactivate enzymes, and interfere with protein transport in the inner layers of cells (Cowan, 1999). Tannin also has a target on the cell wall polypeptide so that the formation of the cell wall becomes less perfect. This causes bacterial cells to be lysed due to osmotic and physical pressure so that bacterial cells will die (Sari, 2011). The complexity of iron ions with tannins can explain the toxicity of tannins. Microorganisms grown under aerobic conditions require iron for a variety of functions, including the reduction of DNA ribonuclerotide precursors. Bacterial cell reverse transcriptase enzymes and DNA topoisomerase cannot be formed by the strong iron-binding capacity of tannins (Akiyama *et. al.,* 2001).

The mechanism of action of saponins as antibacterial is that they can cause leakage of proteins and enzymes from within the cell (Madduluri *et. al.,* 2013). Saponins can be antibacterial because their surface-active substances are similar to detergents, as a result of which saponins will decrease the surface tension of the bacterial cell wall and damage the permeability of the membrane. The damage to the cell membrane greatly interferes with the survival of bacteria (Harbone, 2006). Saponins diffuse through the outer membrane and vulnerable cell walls and then bind to the cytoplasmic membrane thereby disrupting and reducing the stability of the cell membrane. This causes cytoplasm to leak out of the cell resulting in cell death. Antimicrobial agents that interfere with the cytoplasmic membrane are bactericidal (Cavalieri *et.al.,* 2005).

The mechanism of action of alkaloids as an antibacterial is by interfering with the components that make up peptidoglycan in bacterial cells, so that the cell wall layer is not formed completely and causes the death of the cell (Darsana *et. al.,* 2012). Another antibacterial mechanism of alkaloids is that the alkaloid component is known to be a DNA interactor and inhibits the topoisomerase enzyme of bacterial cells (Karou, 2005).

Active compounds such as flavonoids, alkaloids, saponins and tannins contained in mangkokan leaf extract can inhibit the growth of *S. typhimurium* bacteria. These phytochemical compounds work synergistically so that they can increase their effectiveness and activity in inhibiting the growth of *S. typhimuriumw* bacteria. This shows that mangkok leaves have the potential and can be used to treat infectious diseases caused by *S. typhimurium* bacteria as evidenced by the growth inhibition process characterized by the formation of a clear zone around the *blank disk*.

Observation of the antibacterial activity of mangkokan leaf extract on the growth of *S. Typhimurium* bacteria was carried out every 3-hour interval for 24 hours, to determine the optimal time of mangkokan leaf extract in inhibiting the growth of *S. typhimurium bacteria.* The results of the statistical analysis of incubation time on antibacterial activity in each phase of bacterial growth are shown in Table 3.

Table 3. Incubation Time Analysis of Antibacterial Activity by *Mann-Whitney Test*

Incubation Time	Sig Value			
	3 Hours Lifespan	6 Hours Lifespan	Age 18 Hour	Age 24 Hour
15th hour	0,609	0,701	0,609	0,609
18th hour	0,701	0,701	0,701	0.609
21st hour	0,701	0,701	0,701	0,701

In this *Mann-Whitney test*, each inhibition zone of all concentrations at different incubation

times was paired with one another. This installation was carried out to find out if there was a significant difference in the diameter of the inhibition zone between the incubation time pairs. The results of *the Mann-Whitney test* in Table 3. show that the sig value of all treatments is >0.05, which means that there is no significant difference between the incubation times.

The antibacterial effectiveness of the concentration of mangkokan leaf extract against antibiotics was obtained by comparing the antibacterial inhibitory power with the inhibitory power of the positive control (chloramphenicol). The results of the effectiveness of the antibacterial inhibition of mangkokan leaf extract against *S. typhimurium* bacteria are shown in Table 4.

Table 4. Effectiveness of Inhibition of Mangkokan Leaf Extract on Bacterial Growth

Treatment	Drag Effectiveness (%)			
	3 Jam	6 Jam	18 Jam	24 Jam
5%	21.29	17.82	14.43	19.63
10%	33.79	29.55	29.74	32.52
20%	39.11	37.84	37.06	38.54
40%	50.81	48.07	49.84	48.37
80%	60.81	54.62	57.7	58.94

Based on Table 4, the highest percentage of inhibition effectiveness is 60.81% in 3-hour-old bacteria with a concentration of 80%. This can occur because the number of active compound levels at a concentration of 80% is higher than that of other extract concentration treatments, resulting in the highest antibacterial effectiveness against the growth of *S. typhimurium bacteria*. The effectiveness of the inhibition of mangkokan leaf extract against *S. typhimurium* bacteria is due to the content of bioactive compounds in mangkokan leaf extract that can damage the protein synthesis system, cell wall damage that causes lysis so that cell wall damage can interfere with the synthesis mechanism of bacterial cell walls.

According to Pratiwi (2008), antibacterial testing in the log phase (exponential phase) has the best inhibitory activity between the lag phase (adaptation phase) and the stationary phase. This can be due to the test bacteria in the log phase experiencing high metabolic activity and. The most sensitive condition is because bacteria will depend on the environment in which they live, so in this phase, bacteria also become more sensitive to antibiotics. This log phase is a suitable phase for antibacterial testing. When an antibacterial substance is to be tested for its antibacterial activity, the test bacteria used must be in the active phase of cell division at a constant rate. Microorganisms that grow quickly and actively will be more sensitive to antibiotic drugs Than with microorganisms that are in the resting phase (Brooks *and. ly.* 2013).

4. CONCLUSIONS

Mangkokan leaf extract has an antibacterial effect on the growth of *S. typhimurium bacteria*. The concentration of mangkokan leaf extract had a significant effect, while the age of the bacterial inoculum had no significant effect on inhibiting *S. typhimurium*. The concentration of mangkokan leaf extract was 80% effective in inhibiting the growth *of S. typhimurium* bacteria with an antibacterial effectiveness value of 60.81%.

5. REFRENCES

Ahdiyani, Ifa, & Purwani, K.I. 2015. Effect of Mangkokan Leaf Extract (*Nothopanax scutellarium*) as a Mosquito Lavicide *Culex* sp. *Journal of Science and Art.* Sepuluh November Institute of Technology. Vol.4, No.2.

Akiyama, H. K. Fujii, O. Yamasaki., T. Oono. K. Iwatsuki. 2001. Antibacterial Action on Several Tannin Against *Staphylococcus aureus. Journal of Antimicrobial Chemotherapy.* 48: 487-491.

Arora, D. S. & Bhardwaj. 1997. Antibacterial activity of some medicinal plants. *Geo. Bios,* 24:127-

131.

Brooks, G. F., Butel, J. S., and Morse, S. A. (2013). *Medical Microbiology*. Jakarta: Salemba Medika.

Cavalieri, S. J., I. D. Rankin, R. J. Harbeck., R. S. Sautter., Y. S. McCarter., S. E. Sharp., J. H. Ortez., dan C. A. Spiegel. 2005. Manual of Antimicrobial Susceptibility Testing. USA: American Society for Microbiology.

Cowan, M. M. 1999. Plant Products as Antimicrobial Agents. Clinical Microbilogy Reviews. 12: 564-582.

Cushnie, T. P. Tim, Lamb, Andrew J. 2005. Antimicrobial Activity of Flavonoids. *International Journal of Antimicobial Agentsl.* 26: 343-356.

Dalimartha. 1999. *Atlas of Indonesian Medicinal Plants*. Jakarta: Independent Publishers.

Darsana, I. Besung, I. Mahatmi, H. 2012. The Potential of Binahong Leaves (*Anredera cordifolia*) in Inhibiting the Growth of *Escherichia coli* Bacteria in Vitro. Indonesia Medicus Veterinus.

Das, K., RKS Tiwari, and DK Shivastava. 2010. Techniques for Evaluation of Medical Plant Products as Antimicrobial Agent: Current Methods and Future Trends. *Journal of Medicinal Plants Research* 4(2): 104-111.

Davis, W. W., & Stout, T. R. 1971. Disc Plate Method of Microbiological Antibiotic Assay. *Apply Microbiology*, 22(1):659- 665.

DepKes RI. 2007. *Ministerial Decree Appendix Health Number: 381/Menkes/SK/III/2007 regarding the National Traditional Medicine Policy.* Jakarta: DepKes RI.

Gunawan, Sulistia Gan. 2011. *Pharmacology and Therapeutics.* Jakarta: FKUI Publishing Agency.

Hanum, G. R. and Ardiansyah, S. 2017. Mangkokan Extract Soap (*Nothopanax scutellarium* Merr.) as an Antibacterial against *Staphylococcus aureus. Stigma Journal of science.*

Harbone, J. B. 2006. *Phytochemical Methods, 2nd Edition*. Bandung: ITB.

Hendra, R., Ahmad S., Sukari A., Sukhor M.Y., Oskoueian E. 2011. Flavonoid Analyses and Antimicrobial Activity of Various Parts of *Phaleria macrocarpa* (Scheff.). Boerl Fruit. Int J Mol Sci. 12: 3422=3431.

Jahari, Faradila. 2013. Antibacterial Activity Test of Mangkokan Leaf Ethanol Extract (*Nothopanax scutellarium* Merr.) against Bacteria Causing Body Odor by Agar Diffusion Method. *Thesis.* Makassar: Faculty of Health Sciences, UIN Alauddin Makassar.

Jawetz, E., Melnick, J. L. and Adelberg. 2004. *Medical Microbiology 23rd Edition.* Translator and editor of the medical microbiology section, Universitas Airlangga. Jakarta: Salemba Medika. 2005. *Medical Microbiology*. 23rd edition. Translation: Huriwati Hartanto *et. al*. Jakarta: ECG Medical Book Publisher.

Karou, Damintoti. Savadago. Aly. 2005. Antibacterial Activity of Alkaloids from *Sida acuta.* African Journal of Biotechnology. 4(12): 1452-1457.

Kasim, Vivien Novarina A. 2020. *Effect of Lime Peel Extract (Citrus aurantifolia) on Expression of mRNA Toll- LikeReceptors 4 (TLR4) and Soluble Interleukin 6 (IL-6) Genes in Salmonella typhi-Induced Mice*. Dissertation-S3 thesis. Makassar: Hasanudin University.

Khafidhoh, Z., Dwi S. S., Iswara A. (2015). Effectiveness of kaffir lime peel infusion (*Citrus hystrix* DC.) on the growth of *Candida albicans* causing canker sores in vitro. *University Research Coloquium*. 31-7.

Khare, C. P. 2007. Indian Medical Plants. USA: Springer Science. Role of Explant and NAA on Callus Induction of Potato (*Solanum tuberosum*). *American Journal of Life Science.* 5(5).

Khunaifi, M. 2010. Antibacterial Activity Test of Binahong Leaf Extract (*Anredera cordifolia* (ten.) Steenis) against *Staphylococcus aureus* and *Pseudomonas aeruginosa bacteria. Thesis.* Malang: UIN Malang.

Li, H. Wang, Z. Liu, Y. 2003. Review in The Studies on Tannins Activity of Cancer Prevention and Anticancer. Zhong-Yao-Cai. 26 (6): 444-448.

Madduluri, Suresh, Rao, K. Babu. Sitaram, B. 2013. In Vitro Evaluation of Antibacterial Activity of Five Indegenous Plants Extract Against Five Bacterial Pathogens of Human. *International Journal of Pharmacy and Pharmaceutical Sciences.* 5(4): 679-684.

Moehario, Lucky H. 2009. The Molecular Epidemiology of *Salmonella typhi* Across Indonesia Reveals Bacterial Migration. *Journal Infect Dev Ctries,* 3(8): 579-584.

Mpila D.A., Fatimawali & Wiyono, W.I. 2012. Antibacterial Activity Test of Mayana Leaf Ethanol Extract (*Coleus atropurpureus* L. Benth) against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* in Vitro. Manado: Faculty of Mathematics and Natural Sciences, Sam Ratulangi University.

Nuria, Maulita Cut, Faizatun, Arvin, Sumantri. 2009. Antibacterial Activity Test of Ethanol Extract of Castor Leaves (*Jatropha curcas* L.) against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Salmonella typhi* ATCC 1408. *Mediagro.* 5(2):26-37.

Pandey, R., & Mishra, A. 2010. Antibacterial Activities of Crude Extract of Aloe Barbadensis to Clinically Isolated Bacterial P. Applied Biochemistry and Biotechnology,160(5).

Poeloengan, M., Komala I., and Noor S. M. 2006. Dangers of *Salmonella* sp. to Health. *National Workshop on Zoonotic Diseases*. Bogor: IACRI.

Pratiwi, S. T. 2008. *Pharmaceutical Microbiology.* Jakarta: Erlangga

Primadiamanti, A., Winahyu, D. A., and Ramadhana, Y. T. 2020. Antibacterial Activity Test of Mangkokan Leaf Extract (*Nothopanax scutellarium*) against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Journal of Pharmaceutical Analysts.* Lampung: Malahayati University.

Radji, M. (2011). *Microbiology Textbook Pharmacy and Medicine Student Guide.* Jakarta: EGC Medical Book.

Sari, F. P. and S. M. Sari. 2011. Extraction of Antimicrobial Active Substances from Iodine Plants (*Jatropha multifida* Linn.) as Alternative Raw Materials for Natural Antibiotics. Semarang: Faculty of Engineering, Diponegoro University.

Septianingsih, U., Susanti H., and Widyaningsih W. (2012). Inhibition of Xanthine Oxidase Activity by Sambiloto Root Ethanol Extract (*Andrographis paniculata* Ness.) in Vitro. *Scientific Journal of Pharmacy*, 2 (2): 153-163.

Sharah, A., R. Kamila and Desmelati. 2015. Creation of Growth Curve of Isolated Lactic Acid Bacteria from Puffer Fish (*Rastrelliger* sp.). *www.portalgaruda.org.* Accessed on Tuesday, September 7, 2021 at 20.34 WIB.

Utami, N. A. (2017). Test of bacteriostatic inhibition of tomato extract (*Lycopersicon esculentum* Mill) against the growth of *Staphylococcus epidermidis bacteria*. *Thesis.* Faculty of Mathematics and Natural Sciences, Sanata Dharma University, Yogyakarta.

Zaki, S.A. and Karande, S. 2011. Multidrug Resistant Typhoid Fever: A Review. *Journal Infect Developing Countries*, 5 (5): 324-337.