Indonesian Journal of Bioscience (IJOBI)
Vol.1 No.2, Page 57-65, October 2023, E-ISSN: 3032-0194 | P-ISSN: 3032-0216
DOI: https://doi.org/10.21831/ijobi.v1i2.215

Antibacterial Activity of Green Meniran (*Phyllanthus niruri* Linn) Herbal Extract on The Growth of *Salmonella typhimurium*

Aulia Erta Nafisah[a], Bernadetta Octavia[a], Endang Gati Lestari[b]
[a]Biology Education Department, FMIPA, Yogyakarta State University
[b] Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, BRIN

Article Info

Article history:

Received : March, 2nd 2024
Revised : March, 19th 2024
Accepted : March, 31st 2024

Keyword:

Meniran green
Extract
Salmonella typhimurium
Antibacterial
Disc diffusion

ABSTRACT

Salmonella typhimurium is a bacteria that contaminates food that cause digestive disease, gastroenteritis, and food poisoning. The green meniran (*Phyllanthus niruri* L.) contains flavonoids, alkaloids, saponins, and tannins. This research aims to know the ability of green meniran extract as an antibacterial in inhibiting the growth of *Salmonella typhimurium*. This research design used a factorial completely randomized design consisting of two factors the concentration of extract 5%, 10%, 20%, 40%, 80%, equipped with chloramphenicol as positive control and sterile aquadest as negative control and the inoculum age of bacteria growth phase. This antibacterial test method used Kirby-Bauer disc diffusion. The results showed that the green meniran extract had antibacterial activity on the growth of *Salmonella typhimurium*. Concentrations of 5%, 10%, 20%, 40%, and 80% produced inhibition zone diameters of 6.7 mm, 8.5 mm, 9.9 mm, 12 mm, and 14.6 mm. A concentration of 80% was effective in inhibiting the growth of *Salmonella typhimurium* with an antibacterial effectiveness value of 58.95%.

Corresponding Author:

Aulia Erta Nafisaha
Departement of Biology
Universitas negeri Yogyakarta
Indonesia
Email: istuhanan.2018@student.uny.ac.id

1. INTRODUCTION

Disease caused by bacterial infection is a health problem in developing countries, including Indonesia. Bacteria that often attack humans include *Salmonella typhimurium* which is pathogenic. Contamination by Salmonella sp bacteria in food results in digestive tract infections such as gastroenteritis and food poisoning (foodborne disease) with clinical symptoms of nausea, vomiting, diarrhea, fever, and stomach cramps (Brook et al., 2013). Infectious disease caused by *Salmonella typhimurium* is closely related to human hygiene and the environment.This infectious contagious disease can be transmitted through food such as eggs, meat, and water contaminated with Salmonella sp bacteria (Poeloengan et. al., 2006).

So far, therapeutic efforts have been made to treat infections caused by *Salmonella typhimurium* bacteria, including the use of antibiotics. Antibiotics are antibacterial agents produced by bacteria or fungi that are used to treat infectious diseases (Jawetz et al., 2004). Uncontrolled use of antibiotics for a long time and inappropriate doses will reduce their activity and cause bacteria to become resistant to antibiotics and can cause residues that are harmful to human health (Brook et al.,

2013). *Salmonella typhimurium* bacteria have resistance to several antibiotics including chloramphenicol, amoxicillin, ampicillin, and co-trimoxazole (Sandika and Jhons, 2017).

Based on these problems it is necessary to find natural materials as a treatment, one of which is by exploring natural materials. The use of natural materials derived from plants as traditional medicines to overcome health problems is increasingly widespread in society. Plants can produce secondary metabolites that can be used to obtain antibacterial substances (Das et al., 2010). Green meniran (*Phyllanthus niruri* L.) is a herbaceous plant that grows wild and is available in abundant quantities but has not been widely used. Ethnobotanically, green meniran is used to treat diarrhea, fever, malaria, thrush, kidney stones, diabetes, and anti-inflammatories (Sulaksana and Jayusman, 2004).

The 96% ethanol extract of green meniran herb contains flavonoids, saponins, alkaloids, and tannins which have antibacterial activity (Mangunwardoyo et. al., 2009). Extracts from various plants have shown an important role in inhibiting pathogenic bacteria with their antibacterial abilities. Therefore, it is necessary to develop alternative treatments using secondary metabolites from natural materials that have the potential as antibacterial agents in controlling Salmonella sp. bacterial infections. It is necessary to study and analyze the antibacterial activity of green meniran herb extract on the growth of Salmonella sp. This study aims to determine the ability of green meniran extract as an antibacterial in inhibiting the growth of *Salmonella typhimurium*.

2. RESEARCH METHOD

2.1. Types of research

This type of experimental research, tested the inhibitory activity of green meniran herb extract on the growth of *Salmonella typhimurium* bacteria with a completely randomized factorial design using inoculum age treatment in each phase of bacterial growth and 5 variations of extract concentrations along with positive and negative controls.

2.2. Time and Place of Research

The research was conducted at the Microbiology Laboratory, Yogyakarta State University FMIPA Laboratory Building from March to May 2021.

2.3. Research Subjects and Objects

The research subjects were green meniran extract concentrations of 5%, 10%, 20%, 40% and 80%, and the age of the inoculum growth phase of the test bacteria. The object of this study was the zone of inhibition of *Salmonella typhimurium* growth during the 24-hour incubation time.

2.4. Research procedure

2.4.1.Making Simplicia

Green meniran herb samples are washed using running water until clean, then drained and air-dried for 5 days. Then the samples were dried in an oven at 50°C for 3 days. The dried samples were mashed with a blender and then sieved using a sieve to obtain green meniran herb simplicia.

2.4.2.Making Green Meniran Extract

Green meniran simplicia was macerated using 96% ethanol solvent in a tightly closed glass vessel for 3x24 hours. The comparison between simplicia and solvent is 1:3 (for maceration I) and 1:2 (for maceration II and III). The maceration results were filtered using Whatman No. filter paper. 1 sterile so that the filtrate is obtained. Furthermore, the filtrate was evaporated using a rotary vacuum evaporator at a temperature of 55ºC and then evaporated with a water bath to produce a thick extract of green meniran. The viscous extract obtained was made into a 100% concentrated stock solution by mixing 20 grams of the viscous extract added with sterile distilled water to a volume of 20 ml. Green meniran herbal extract with a concentration of 100% was diluted with sterile distilled water to obtain solutions with concentrations of 5%, 10%, 20%, 40% and 80% (v/v) (Harborne, 1987; Septianingsih et. al., 2012).

2.5. Media Creation

2.5.1.Media Nutrient Broth (NB)

Nutrient broth (NB) media was prepared by dissolving 8 grams of NB powder in 1000 mL of distilled water, then homogenized using a hotplate. Once homogeneous, the media was poured

into an Erlenmeyer and then sterilized using an autoclave at 121°C with a pressure of 1 atm for 15 minutes.

2.5.2.Nutrient Agar (NA) Media

Nutrient agar (NA) media was prepared by dissolving 28 grams of NA powder in 1000 ml of distilled water, then homogenized using a hotplate. Once homogeneous, the media was poured into an Erlenmeyer and then sterilized using an autoclave at 121°C with a pressure of 1 atm for 15 minutes. Next, the media was poured into a petri dish aseptically in the LAF and allowed to stand until it solidified.

2.5.3.Rejuvenation of *Salmonella typhimurium*

A total of 1 loop was inoculated on the mediaNutrients Agar slantusing the continuous streak method. Then incubated at 37ºC for 24 hours (Radji, 2011).

2.5.4.Making Bacterial Growth Curves

A total of 1 ose culture of bacterial isolates *Salmonella typhimurium* inoculated into an Erlenmeyer flask containing 25 ml of Nutrient Broth medium then incubated in an incubator shaker at 120 rpm at 37°C for 24 hours. Sterile Nutrient Broth Media without bacterial culture was used as a control. The absorbance of the bacterial culture was measured using a UV-visible spectrophotometer at a wavelength of 600 nm. At every 3-hour interval, absorbance measurements were carried out to obtain the bacterial growth curve (Rosmania and Yanti, 2020).

2.6. Antibacterial Activity Test

Antibacterial activity testing was carried out using the Kirby Bauer disc diffusion method referring to Das et. al. (2010). A total of 0.1 ml of optical density bacterial suspension (OD600 = 1) was inoculated on NA plate media using the spread plate technique. Then the sterile disc paper was immersed in the green meniran extract test solution at concentrations of 5%, 10%, 20%, 40%, 80%, positive control (3% chloramphenicol) and negative control (aquades) for 20 minutes. Then the disc paper was placed on the NA plate media which had been inoculated with *Salmonella typhimurium* bacteria. After that, incubated in an incubator at 37°C for 24 hours. The diameter of the inhibition zone was indicated by the formation of a clear zone around the disc paper.

2.7. Data Collection Instruments and Techniques

The tools used include Laminar Air Flow(LAF), oven, hotplate, magnetic stirrer, autoclave, vortex, analytical balance, rotary vacuum evaporator, water bath, shaker incubator, and spectrophotometer,

The materials used included green meniran herb samples, *Salmonella typhimurium* isolates, 70% alcohol, 96% ethanol, Nutrient Broth, Nutrient Agar, chloramphenicol, disc paper, Whatman No. filter paper. 1, ,

The data collection technique was carried out by measuring the diameter of the inhibition zone for the growth of *Salmonella typhimurium* using a vernier caliper in millimeters (mm).

2.8. Data analysis technique

Data were analyzed using SPSS version 22.0 with ANOVA test in Completely Randomized Factorial Design. If the data is not normally distributed, then use the Kruskal-Wallis nonparametric test. If the results show a significant or significant effect, then continue with Duncan's Post Hoc test to find out between treatments having the same or different inhibitory effect.

The antibacterial effectiveness of the concentration of green meniran extract against antibiotics is calculated based on the equation according to Arora and Bhardwaj (1997) in Aziz (2017), namely:

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

3. RESULTS AND ANALYSIS

3.1. Bacterial Growth Curve

Measurement results optical density *Salmonella typhimurium* bacterial suspension in the form of a growth curve can be seen in Figure 1.

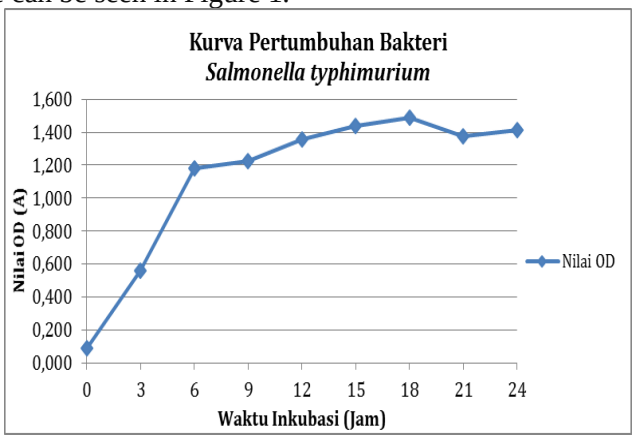

Figure 1. Bacterial Growth Curve *Salmonella typhimurium*

Based on the bacterial growth curve *Salmonella typhimurium.* As shown in Figure 6, it is known that the lag phase (adaptation phase) occurs at 0 to 3 hours, and the logarithmic phase occurs at 6 to 18 hours. Furthermore, at 18 to 24 hours, *Salmonella typhimurium* bacteria underwent a stationary phase. Age in each phase of bacterial growth used for testing antibacterial activity, namely the age of inoculum 3 hours (lag phase), 6 hours and 18 hours (log phase), and 24 hours (stationary phase). Antibacterial activity testing used three phases of bacterial growth, namely the lag phase, log phase, and stationary phase to determine the effectiveness of the green meniran herb extract tested at the age of each growth phase of the *Salmonella typhimurium* bacteria against the inhibition of antibacterial activity.

3.2. Antibacterial Activity Test

Antibacterial activity test of green meniran herb extract was analyzed using the SPSS program. Based on the Kolmogorov-Smirnov normality test, a significance value of 0.074 ($p>0.05$) was obtained which indicated that the data were normally distributed. Furthermore, in the homogeneity test, a significance value of 0.000 ($p <0.05$) was obtained, meaning that the data variant was not homogeneous. Therefore, the non-parametric Kruskal-Wallis test was used.

In the age treatment of inoculums for each phase of bacterial growth, a significance value of 0.357 ($p>0.05$) was obtained, indicating that the age of inoculums in each phase of bacterial growth did not have a significant effect on the formation of zones of inhibition of *Salmonella typhimurium* growth, both at 3 hours of inoculum age, 6 hours, 18 hours or 24 hours. This shows that the age of the inoculum in each growth phase of the given bacteria is able to produce an inhibitory effect on the growth of Salmonella sp. Both in a low number of bacterial cells and a high number of bacterial cells, green meniran herb extract can inhibit . In the inoculum age treatment it was not obtained how wide the inhibition zone was produced,

The ability of an antibacterial substance is influenced by the type, concentration (cell density), age, and condition of the bacteria. The higher the concentration of bacteria present, the longer it takes to kill the bacteria. The high concentration (cell density) of the suspension of pathogenic bacteria being resisted can affect the performance of the active antibacterial substances contained in the extract (Fardiaz, 1992).

In the concentration treatment of green meniran herb extract, a significance value of 0.000 ($p <0.05$) was obtained, indicating that there was an effect of variations in the concentration of green

meniran herb extract on the formation of inhibition zones for the growth of *Salmonella typhimurium* bacteria. After that, Duncan's Post Hoc analysis was carried out, it was found that between treatments the concentration of green meniran herb extract had a significantly different antibacterial inhibition effect on the growth of *Salmonella typhimurium* bacteria. The difference in the diameter of the resulting inhibition zone is due to the difference in the rate of diffusion of the antibacterial compound in the agar media and the concentration of the different antibacterial compounds can also produce different diameters of the inhibition zone, which can be seen in Table 1.

Table 1. Effect of Concentration of Green Meniran Herb Extract on Inhibition Power of Salmonella typhimurium

Treatment	Average Zone of Inhibition	Category Antibacterial Power*
Aquades (%)	0[a]	There is no inhibition zone
5	6,7[b]	Currently
10	8.5[c]	Currently
20	9,9[d]	Currently
40	12.0[e]	Strong
80	14.6[f]	Strong
chloramphenicol	25.5g	Very strong

Note: Notation of numbers marked with different letters indicates a significant difference based on Duncan's test (<0.05). *The category of anti-bacterial power refers to Davis and Stout (1971).

In the treatment of inoculum age did not show a significant difference, so to see the strength of antibacterial inhibition at all ages of the bacterial growth phase of each concentration was averaged so that the ability of antibacterial inhibition was known in the treatment of extract concentrations of 5%, 10% and 20% included in the category of medium inhibition, extract concentrations of 40% and 80% were included in the category of strong inhibition, while chloramphenicol as a positive control produced a very strong category of inhibition (Table 1).

The results obtained showed that increasing the concentration of the green meniran herb extract given could inhibit the development of bacteria by looking at the inhibition zone which was getting bigger. The higher the concentration of the extract used, the greater the content of active compounds that function as antibacterials, so that the ability to inhibit bacterial growth*Salmonella sp*the greater which is marked by an increase in the inhibition zone.

Green meniran herb extract at the highest concentration showed the widest inhibition zone results on bacterial growth*Salmonella sp.*According to Ajizah (2004), the concentration of the extract increases, the content of bioactive substances also increases. The higher the concentration of an extract, the higher the secondary metabolite compounds contained therein so that the antibacterial effectiveness increases (Reference). This is indicated by the larger the diameter of the clear zone formed as an inhibitory force and the stronger the category of antibacterial activity.

Several factors affect antibacterial activity, including extract concentration, content of antibacterial chemical active compounds, extract diffusing power, and type of bacteria (Jawetz*et. al*., 2004).The ability of green meniran herb extract to inhibit bacterial growth*Salmonella sp*due to the presence of secondary metabolite compounds in the form of flavonoids, alkaloids, saponins and tannins (Mangunwardoyo et. al., 2009). The content of phytochemical compounds contained in the *Phyllanthus niruri* L. plant, which has the same kinship in the same genus as Phyllanthus amarus, contains 19.01% of flavonoids, 32.37% of alkaloids, 23.70% of saponins and 19.65% of tannins. % (Sherif et. al., 2019).

The mechanism of antibacterial action for each secondary metabolite is not the same. Flavonoids as antibacterial work to damage the bacterial cell membrane by forming complex compounds with extracellular proteins that can cause damage to the bacterial cell membrane followed by uncontrolled entry of water into the bacterial cell.i which triggers swelling which causes the

bacterial cell membrane to lyse until the release of intracellular compounds from the cell (Putri, 2014).

Alkaloid compounds can form complex compounds with proteins through hydrogen bonds which can inhibit protein formation. The mechanism of action of alkaloid compounds in inhibiting bacterial growth has similarities with the mechanism of action of the antibiotic chloramphenicol, namely by inhibiting the formation of protein synthesis so that it can interfere with the metabolic activity of bacterial cells because all metabolic activity of bacterial cells is catalyzed by enzymes which are proteins (Munfaati*et. al.*,2015).

Saponins as antibacterial work by reducing the surface tension of the cell wall and damaging the permeability of the membrane. Damage to cell membranes can interfere with the survival of bacteria. 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 (Jawetz et al., 2004). This situation causes the cytoplasm to leak out of the cell and intracellular compounds to come out, resulting in an imbalance or disruption of the stability of the bacterial cell membrane so that the bacterial cell experiences lysis (Samputri, 2020).

Tannin compounds also have the ability to interfere with protein transport in the inner layer of cells, inhibiting nucleic acid synthesis,and inactivates the function of the genetic material so that bacterial cells cannot form (Nuria et. al., 2009). These phytochemical compounds work synergistically so as to increase their effectiveness and activity in inhibiting the growth of Salmonella sp. bacteria.

Chloramphenicolas a positive control is a broad-spectrum antibiotic that is active against Gram-positive and Gram-negative bacteria that are pathogenic. Chloramphenicol works by inhibiting protein synthesis needed for the formation of bacterial cells which is inhibited by the peptidyl transferase enzyme which acts as a catalyst for peptide bonds during the bacterial synthesis process, so that chloramphenicol inhibits the function of RNA from bacteria (Ganiswarna, 1995).

The use of distilled water as a negative control to demonstratedistilled water as a diluent solution of the extract had no effect on the inhibition zone of antibacterial activity. Aquades did not produce a clear zone around the disc paper, so the inhibition zone which was produced as a pure antibacterial activity came from the herbal extract of the green meniran.

Observation of antibacterial activity of green meniran herb extract against bacterial growth*Salmonella sp*performed every 3-hour interval during the 24-hour incubation period, to determine the optimal time for the green meniran herbal extract to inhibit the growth of Salmonella sp. The results of the statistical analysis of incubation duration on antibacterial activity in each phase of bacterial growth can be seen in Table 2.

Table 2. Effect of Incubation Time on Antibacterial Activity

Incubation Hour To-	**Average Inhibition Zone (hours)**			
	3	6	18	24
3	9,7[a]	8,6[a]	9,2[a]	8,3[a]
6	10.5[b]	9,5[b]	9,9[b]	9,3[b]
9	11.3[c]	10.2[c]	10.8[c]	10.4[c]
12	11.9[d]	10.6[d]	11.3[d]	11.3[d]
15	12.7[e]	10,9e	11.8[e]	12.2[e]
18	13.5[f]	11,2e	12.0[f]	12.8[f]
21	13.9g	11.4f	12.3g	13.1g
24	14.2g	11.5f	12.5g	13,3h

Note: Notation of numbers marked with different letters indicates a significant difference based on Duncan's test (<0.05).

Based on Table 2, it is known that the diameter of the antibacterial inhibition zone continues to increase as the incubation period increases. Antibacterial activity was shown starting at the 3rd hour of incubation. The larger the diameter of the clear zone formed indicates bacterial growth *Salmonella sp*increasingly hampered. The incubation period affects the size of the diameter of the

antibacterial inhibition zone of the extract of the green meniran herb. At the 3rd to 21st hour of incubation there was a significant difference in the inhibition zone of antibacterial activity, whereas at the 24th hour of incubation there was no significant increase in the diameter of the inhibition zone so that the optimal incubation time in inhibiting the growth of *Salmonella typhimurium* occurred at 21st.

The antibacterial effectiveness of green meniran extract concentration on antibiotics was obtained by comparing the antibacterial inhibition with the inhibition of the positive control, namely the antibiotic chloramphenicol. The results of the effectiveness of the antibacterial inhibition of green meniran extract against bacteria*Salmonella typhimurium*shown in Table 3.

Table 3. Effectiveness of Antibacterial Inhibitory Power of Green Meniran Herb Extract against*Salmonella typhimurium*

Treatment %	**Inhibitory Effectiveness (%)**			
	3 hours	6 Hours	18 Hours	24 hours
5	27.01	25,33	25.01	27,20
10	36,86	33,19	30.98	33,33
20	40,51	38,43	37,25	39.85
40	49,27	45.85	44,31	49,43
80	56,57	58.95	57,25	56,32

Based on Table 3 it is known that the concentration of 80% green meniran herb extract shows an effective concentration which is able to produce antibacterial effectiveness above 50%. This happened because the levels of active compounds at a concentration of 80% were higher compared to the lower treatment. The 6-hour inoculum age treatment resulted in the highest antibacterial effectiveness in inhibiting bacterial growth*Salmonella sp*that is equal to 58.95%.

According to Rahmawati (2015), antibacterial testing in the log phase (exponential phase) has the best inhibitory activity between the lag phase (adaptation phase) and the stationary phase. This could be due to the test bacteria in the log phase experiencing high metabolic activity and the most sensitive conditions because the bacteria will depend on the environment in which they live, so that in this phase the bacteria become more sensitive to antibiotics.

The log phase is the right condition 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 (Pratiwi, 2008). Microorganisms that grow rapidly and are active will be more sensitive to antibiotics compared to microorganisms that are in the resting phase (Brook et. al., 2013).

4. CONCLUSION (11 Pt)

Green meniran herb extract has an antibacterial effect on the growth of *Salmonella typhimurium* bacteria. Increasing the concentration of green meniran extract had the effect of increasing the diameter of the inhibition zone, while the age of the bacterial inoculum did not show a difference in the diameter of the inhibition zone on the growth of Salmonella sp. At a concentration of 80% it can inhibit the growth of Salmonella sp bacteria by 58.95%.

Suggestion

Suggestions for further research can be carried out by isolating and measuring the levels of active compounds contained in the green meniran herb extract in order to determine the type of active compound that is most influential in inhibiting bacterial growth.

ACKNOWLEDGEMENTS (11 Pt)

The author would like to thank (the funders) who have provided financial support for this research. (This explanation is made if this research gets funding from other parties)

REFERENCES

Ajizah, A. (2004). Sensitivity of *Salmonella typhimurium* to Psidium guajava LJ Leaf Extract Bioscientiae, 1 (1): 31-38.

Aziz. (2017). Effectiveness of Inhibitory Power of Tiwai Onion (Eleutherine americana) Extract on the Growth of Aeromonas hydrophila Bacteria in Vitro. Journal of Harpodon Borneo, 10 (1): 68-74.

Brook, GF, Butel, JS, and Morse, SA (2013). Medical Microbiology. New York: Salemba Medika.

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

Fardiaz, S. (1992). Food Microbiology. Jakarta: Gramedia Media Utama. Ganiswarna, VHS (1995). Pharmacology and Therapy. Jakarta: Department of Pharmacology, Faculty of Medicine, University of Indonesia.

Jawetz, E., Melnick, JL, and Adelberg, EA (2004). Medical Microbiology. Jakarta: EGC.

Mangunwardoyo, W., Cahyaningsih E., and Age T. (2009). Extraction and Identification of Meniran (*Phyllanthus niruri* L.) Antibacterial Compounds. Indonesian Pharmaceutical Sciences, 7 (2): 57-63.

Munfaati, PN, Evie R., and Guntur T. (2015). Activity of Antibacterial Compounds Meniran Herb Extract (*Phyllanthus niruri* L) on the Growth of Shigella dysenteriae Bacteria in Vitro. Bio Lantern Journal, 4(1): 64–71.

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

Poeloengan, M., Komala I., and Noor SM (2006). Danger of Salmonella sp. on Health. National Workshop on Zoonotic Diseases. Bogor: Balitvet.

Pratiwi, ST (2008). Pharmaceutical Microbiology. Jakarta: Airlangga.

Daughter, DN (2014). Antibacterial Activity Test of Kenikir (Cosmos caudatus Kunth.) Leaf Methanol Extract against Salmonella typhi Bacteria. Thesis. Faculty of Science and Technology, State Islamic University of Maulana Malik Ibrahim, Malang.

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

Rahmawati, M. (2015). Antimicrobial Activity Test of Ethanol Extract and Water of Pacing Rhizome (Costus spiralis) against Escherichia coli, Shigella dysenteriae, Salmonella typhimurium, Bacillus subtilis, Staphylococcus aureus and Candida albicans Fungi. Thesis. Faculty of Medicine and Health Sciences, Syarif Hidayatullah State Islamic University, Jakarta.

Rosmania and Fitri Yanti. (2020). Calculation of the number of bacteria in the Microbiology Laboratory uses the development of the Spectrophotometry method. Journal of Science Research, 22(2): 76-86.

Samputri, RD, Angeline NT and Ratna W. (2020). Antibacterial Activity Test of Kamandrah Seed Ethanol Extract (Croton tiglium L.) on the Growth of Salmonella typhi by Disc Diffusion Method (Kirby-Bauer). Journal of Herb-Medicine, 3(3):19-33.

Sandika, J. and Jhons FS (2017). Sensitivity of Salmonella typhi Causes Typhoid Fever to Several Antibiotics. Majority Journal, 6(1):41-45.

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

Sherif, UI, Yusuf AA, Alawode AR, Obiekezie CI, Okunlola BM, Abdulrazaq OM, Ariyeloye SD, and Lawal B. (2019). Phytochemical composition and biochemical effect of Phyllanthus amarus in albino rats. GSC Biological and Pharmaceutical Sciences, 8(1): 128–133.

Sulaksana J. and Jayusman DI (2004). Meniran, Cultivation and Utilization for Medicine. Jakarta: Independent Spreader.