Journal of TROPICAL BIODIVERSITY E-ISSN : 2774-4116

Antibacterial Bioactivity from Extract of Reundeu Caret (*Staurogyne longata*) and Honje (*Etlingera hemisphaerica*)

Noverita[1] and Ernawati Sinaga [1]
[1]Faculty of Biology, Universitas Nasional, Jakarta

Corresponding author: *ernawatisinaga@unas.co.id*

Abstract

Reundeu caret (*Staurogyne longata*) and Honje (*Etlingera hemisphaerica*) are two examples of plants commonly used by rural tribal communities as medicinal ingredients, one of which is by the Baduy tribal community. These two plans are usually used by local people to treat infectious diseases (wounds) and stomach aches. Therefore, it is necessary to prove the medical potential of these plants. This study was conducted with the aim of knowing the antibacterial potential of the extracts of the Reundeu caret (*Staurogyne longata*) and Honje (*Etlingera hemisphaerica*) plant extracts against the growth of *Escherichia coli, Salmonella typhi, Staphylococcus aureus*, and *Bacillus cereus*. The method used in this study is the Disc Diffusion method. The results showed that Reundeu caret and Honje plant extracts were only able to inhibit the growth of *Staphylococcus aureus* and *Bacillus cereus.* The antibacterial activity of the plant extracts of Rendeu Caret and Honje was moderate to strong. This study concluded that Honje and Reundeu caret plant extracts were more potential to be used to treat infectious diseases (wounds).

Keywords: Antibacterial, Honje (*Etlingera hemisphaerica*), Reundeu caret (*Staurogyne longata*),
zone of inhibition

INTRODUCTION

Indonesia is a country that has the largest population in the world, so many problems arise in society, one of which is health. Health is one of the problems that are closely related to medicinal ingredients. We can find various types of drugs in hospitals, pharmacies, drug stores, and even stalls. The sources of the basic ingredients for making these drugs are very diverse, some are made from natural chemicals and some are made from synthetic chemicals. The use of drugs derived from synthetic chemicals raises problems because these materials when used in excess will be harmful to the wearer. One of the many efforts currently being made is to look for natural medicinal ingredients sourced from medicinal plants.

The use of plants as medicinal ingredients is usually done by people who live in rural areas, especially by traditional people who live far from urban areas and are very tied to the culture of their community, such as the Baduy tribe who live in Kanekes village, Leuwidamar district, Lebak regency. Reundeu caret (Staurogyne longata) and

Honje (Etlingera hemisphaerica) are two examples of plants commonly used by the Baduy people for food and medicine. According to Mariani (2015), Reundeu caret is a plant that is commonly found in the mountains in the tropics. For the Sundanese people, these plants are used as fresh vegetables, besides that these plants are also used as medicinal ingredients.

Honje (*Etlingera hemisphaerica*) or better known as kecomrang is a group of spice plants from the Zingiberaceae (ginger-ginger) tribe. These plants have sour flowers and fruit with a distinctive fragrant smell, are mixed ingredients, and at the same time flavoring spices for various dishes in Indonesia. Besides, this plant is also efficacious as a medicinal ingredient, which is used as a drug for skin diseases, cancer, tumors.

The potential of these plants as medicinal ingredients is related to the content of their bioactive compounds. According to Mariani (2015), Reudeu caret contains bioactive compounds in the form of flavonoids, saponins, steroids/triterpenoids, tannins, and phenols. Meanwhile, according to Jackie et al. (2011), Honje (*Etlingera hemisphaerica*) contains bioactive compounds in the form of glycosides, polyphenols, and flavonoids. The treatment method used by the community is very simple, namely by pounding this material until smooth, then smeared on the sick part, or by brewing it with hot water and drinking it.

To prove the efficacy of plants as medicinal ingredients, especially drugs for infection by microorganisms (bacteria, fungi, and viruses), it is necessary to prove medically through antimicrobial tests, one of which is antibacterial. The test bacteria commonly used in research are bacteria that are commonly found in everyday life, and have a fairly high infection rate, and are opportunistic to other organisms. These bacteria can be from groups of Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus cereus* which produce thermostable toxins, and can also come from groups of Gram-negative bacteria such as *Escherichia coli* and *Salmonella typhi* that cause infections in the digestive tract (Adams and Moss, 2008).

Seeing the potential that exists in the two plants (*Reundeu caret* and *Honje*), and there is still little information regarding their antibacterial potential, this study was conducted to know the antibacterial power of the plant extracts of Reundeu caret (*Staurogyne longata*) and Honje (*Etlingera hemisphaerica*) against bacterial growth *Escherichia coli, Salmonella typhi*, *Bacillus cereus*, and *Staphylococcus aureus*.

The hypotheses to be tested in this study are:
1. Reundeu caret (Staurogyne longata) and Honje (Etlingera hemisphaerica) extracts have antibacterial activity against the test bacteria used.
2. The test bacteria had different responses to the Reundeu caret and Honje plant extracts.

METHOD

A. Place

A sampling of medicinal plants Reundeu caret (*Staurogyne longata*) and Honje (*Etlingera hemisphaerica*), was carried out in the village of the Baduy Dalam tribe,

Kanekes Village, Lebak Banten Regency, and the antibacterial activity testing was carried out at the Microbiology and Genetics Laboratory of the National University Integrated Lab, Bambu Kuning, Pasar Minggu South Jakarta.

B. Materials and Tools

The materials used in this study include extracts of medicinal plants Reundeu caret (*Staurogyne longata*) and Honje (*Etlingera hemisphaerica*), isolates of *Staphylococcus aureus*, *Bacillus cereus*, *Staphylococcusaureus*, and *Escherichia coli*, Mueller-Hinton Agar (Oxoid) media, Bacto Agar (Difco), Nutrient Agar (NA) media (Oxoid), Nutrien Broth (NB) media (Oxoid), 80% Methanol, 1% H2SO4 solution, 1% BaCL2 solution, Cloramphenicol antibiotic 10 g (AMP 10) (Oxoid), and aquadest.

The equipment used in this study included: blender, oven, autoclave, centrifuge, vaporizer (Evaporator Butchi 461), shaker, vortex, digital scale, petri dish, laminar air flow, incubator, Erlenmeyer flask, beaker glass, electric stove, sterile swab, tweezers, measuring flask, volumetric pipette, filter paper, bunsen burner, test tube, stirring rod, loop needle, aluminum foil, measuring cup, cotton swab, lighter, label paper, marker pen, paper disc, pH universal, bulb, and millimeter ruler.

Procedure

1. Preparation of Growth Medium and Sterilization of Equipment

a. NA (Nutrient Agar)

Nutrien Agar (NA) media was prepared by: weighing 23 grams of Nutrient Agar powder, adding 5 grams of Bacto Agar, then dissolving in 1 L of distilled water, stirring until the mixture was smooth while heated until boiling. After that, it was put into a prepared test tube of as much as 5 mL for oblique culture, and the rest was put into an Erlenmeyer. Then covered with cotton, sterilized in an autoclave at 121^{o}C, pressure 1-2 atm for 15 minutes.

b. NB (Nutrient Broth)

Nutrient Broth (NB) media was prepared by: weighing 23 grams of sodium broth powder, then dissolved in 1 L of distilled water, stirred until the mixture was smooth while heated until boiling. After that, it was put into a prepared test tube of 5 mL as many as 10 tubes and the rest was put into an Erlenmeyer. Then covered with cotton, sterilized in an autoclave at 121^{o}C, pressure 1-2 atm for 15 minutes.

c. Mueller-Hinton Agar

This medium was made by weighing 38 grams of Mueller-Hinton Agar (MHA), adding 6 grams of Bacto Agar, then dissolved in 1 L of distilled water, and heated to boiling. The media was then sterilized by autoclaving for 15 minutes at 121^{o}C (1-2 atm pressure). After sterilization, the media from the autoclave was allowed to stand until the temperature reached 45^{o}C and poured into sterile Petri dishes. This medium is used to test bacteria.

d. Tool Sterilization

Petri dishes, test tubes, Erlenmeyer, and other glassware to be used are sterilized in an oven at 180oC for 2 hours after previously being washed, dried, and wrapped in paper.

2. Sample Preparation

Medicinal plants Reundeu caret (*Staurogyne longata*) and Honje (*Etlingera hemisphaerica*), first dried in an oven at 50^{o}C, then crushed using a blender until smooth, then sieved using the smallest size sieve with a diameter of 150 mesh.

3. Extraction of medicinal plants Reundeu caret (*Staurogyne longata*) and Honje (*Etlingera hemisphaerica*)

The powdered sample was extracted by: weighing 100 grams of plant powder and then adding 500 ml of 80% methanol as a solvent, then the sample was shaken for 12 hours and macerated for 24 hours at room temperature. The macerated plant extract was filtered using filter paper to form two parts in the form of filtrate and dregs. Furthermore, the dregs were added with 250 ml of methanol and shaken, then macerated again as in the previous work, this was done three times.

The extracts were mixed and centrifuged at 3500 rpm for 15 minutes and then filtered again. The resulting supernatant was evaporated at a temperature of 40^{o}C with a vacuum rotary evaporator until it was dry which was marked by the presence of sediment. Furthermore, it is used to test the antibacterial activity.

4. Preparation of Test Bacteria

A total of one ose colony of test bacteria (*Staphylococcus aureus* and *Escherichia coli*) was dissolved in 0.85% physiological NaCl solution and the number of test bacteria used was homogenized using the McFarlan standard 0.5 (bacterial density 1.5 x 108) on a black background, and bright light. Mc.Farlan turbidity standard 0.5 was prepared by mixing 0.5 ml of 1% BaCL2 solution with 9.5 ml of 1% H2SO4.

The sterile swab was dipped into a mixture of test bacteria with 0.85% physiological NaCl, then drained by pressing the end of the swab, and rotating it against the inner wall of the tube to remove excess fluid. Then the swab is applied to the surface twice, namely horizontally and vertically so that bacterial growth can be evenly distributed.

5. Antibacterial Test

The antibacterial activity test was carried out using the Kirby-bauer (disc) diffusion method. Each blank paper disc was heated in an oven at 70^{o}C for 15 minutes. Then each disc was given a solution of plant extract with a predetermined concentration (7.5%; 10%; 12.5%) with a 0.25 L micropipette. The discs were allowed to stand for 15 minutes before being placed on the test medium. Ampicillin 10µg antibiotics and 80% methanol for each test bacteria were used as positive and negative controls.

The Petri dishes were then incubated at 37°C for 24 hours. The diameter of the inhibition zone (marked by the formation of a clear zone) formed around the disc was measured using a ruler (mm). In measuring the inhibition zone, concentrations with a diameter of 6 mm (equal to the diameter of the disc) were declared to have no inhibition zone, while concentrations with a diameter of more than 6 mm were declared to have an inhibition zone.

D. Research Design

The design used in this study was a factorial completely randomized design (CRD), as factors were the concentration of medicinal plant extracts (7.5%; 10%; 12.5%; chloramphenicol as a positive control and methanol as a negative control) and 4 types of bacteria. test (*E. coli, Salmonella typhi, Bacillus cereus,* and *S. aureus*). The antibacterial test was carried out with 3 replications. The number of treatments was 60 studies consisting of 4 types of test bacteria (E. coli, and S. aureus) x 3 concentrations of macrofungi extract (7.5%; 10%; 12.5%) and 2 controls (positive control ampicillin and methanol. negative control). The inhibition zone formed was observed as a parameter.

RESULT

A. Inhibition Zone Diameter of Reundeu Caret (*Staurogyne longata*) and Honje (*Etlingera hemisphaerica*) plant extracts against four types of bacteria tested.

1. *Escherichia coli* and *Salmonella typhi* bacteria,

The ability of Reundeu Caret and Honje plant extracts to inhibit the growth of *E. coli* and *S. Typhi* bacteria is shown in Table 1.

Table 1. Average Inhibition Zone Diameter of Plant Extracts and Honje against *Eschericia coli* and *Salmonella typhi* bacteria

Test Bacteria	Plant Extract	Extract Concentration (%)	Average Inhibition Zone Diameter (mm)
E. coli	Reundeu caret (*Staurogyne longata*)	50	6
		25	6
		12.5	6
		6.25	6
	Honje (*Etlingera hemisphaerica*)	50	6
		25	6
		12.5	6
		6.25	6
	Control +		33

	Control -		6
S. typhi	Reundeu caret (*Staurogyne longata*)	50	6
		25	6
		12.5	6
		6.25	6
	Honje (*Etlingera hemisphaerica*)	50	6
		25	6
		12.5	6
		6.25	6
	Control +		31
	Control -		6

Information: Control + = Kloramphenicoi
Control - = Metanol 70%
6 mm = No growth inhibition zone

2. *Staphylococcus aureus* and *Bacillus cereus* bacteria

The ability of Reundeu Caret and Honje plant extracts in inhibiting the growth of *S. aureus* and *B. cereus* bacteria is shown in Table 2.

Table 2. Average Inhibition Zone Diameter of Plant Extracts and Honje against *S. aureus* and *B. cereus* bacteria.

Test Bacteria	Plant Extract	Extract Concentration (%)	Average Inhibition Zone Diameter (mm)
S.aureus	Reundeu caret (*Staurogyne longata*)	50	9.5
		25	8.5
		12.5	7.25
		6.25	6.5
	Honje (*Etlingera hemisphaerica*)	50	9
		25	8
		12.5	7
		6.25	6.5
	Control +		38
	Control -		6
B. cereus	Reundeu caret (*Staurogyne longata*)	50	10
		25	8
		12.5	7
		6.25	6.5
	Honje (*Etlingera*	50	10.5
		25	9

hemisphaerica)		
	12.5	7.75
	6.25	6.75
Control +		24
Control -		6

Description: Control + = Chloramphenicoi
Control - = Metanol 70%
6 mm = No growth inhibition zone

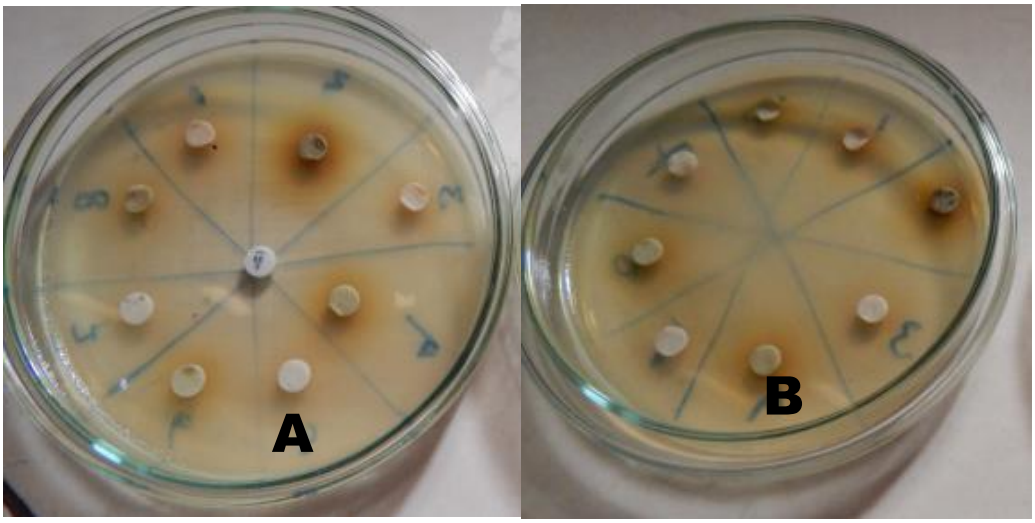

Figure 1. Antibacterial Bioactivity of Rendeu caret and Honje plants against the growth of *E.coli* and *S.typhi* bacteria
Description: A. *Escherichia coli* bacteria B. *Salmonella typhi* bacteria

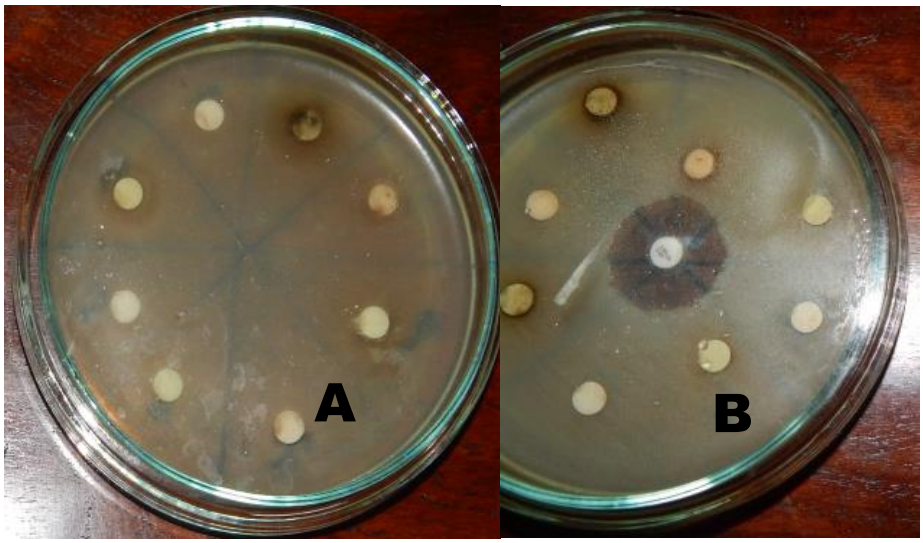

Figure 2. Antibacterial Bioactivity of Rendeu caret and Honje plants

against the growth of *S.aureus* and *B.cereus* bacteria
Description: A. *S.aureus* bacteria B. *B.cereus* bacteria

The inhibition zone of Rendeu Caret and Honje plants against *B. cereus* bacteria was greater than that of *S. aureus*, but the difference was not too significant (Figure 3).

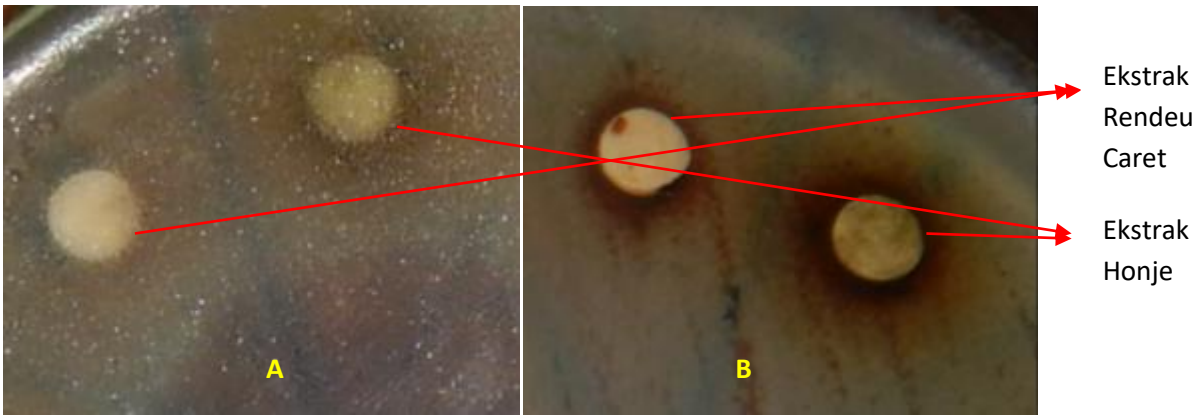

Figure 3. Comparison of Inhibitory Zones of Rendeu Caret and Honje plant extracts against
B. cereus and *S. aureus bacteria*
Description: A. Growth of S. aureus bacteria B. Growth of B. cereus bacteria

B. Effect of Concentration of Rendeu Caret Plant Extract and Honje Plant Extract on the Growth of Test Bacteria

The results of the observation of the antibacterial activity test of Reudeu Caret and Honje plant extracts with different concentrations against the test bacteria *S. aureus* and *Bacillus cereus* showed that there was an effect of concentration treatment on the growth of these bacterial colonies (Figure 4), but there was no effect on the bacteria *E.coli* and *S. typhi.* The average inhibition zone of the Rendeu Caret and Honje plant extracts with different concentrations against B. cereus and S. aureus bacteria can be seen in Figure 4.

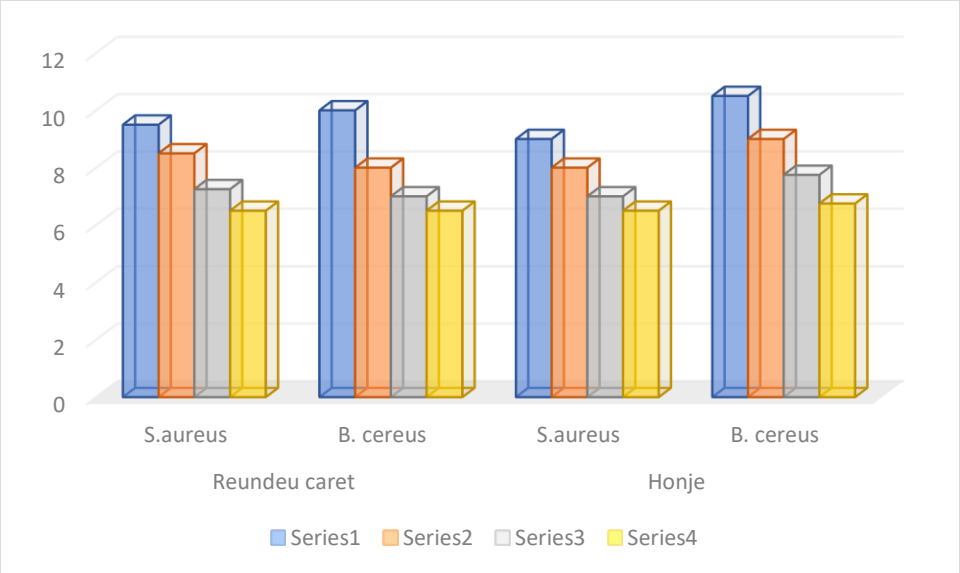

Figure 4. Bar Diagram of Average Inhibitory Zone Diameter (mm) Rendeu Caret and Honje Plant Extracts with Different Concentrations Against Staphylococcus aureus and Bacillus subtilis Bacteria

Description: Series 1= 50% Series 2 = 25% Series 3= 12,5% Series 4=6,25%

DISCUSSION

Table 1 and Table 2 above show that the extracts of the Rendeu caret plant and the Honje plant were only able to inhibit the growth of *S. aureus* and *B. cereus* bacteria. The ability of these plant extracts to inhibit the growth of S. aureus and B. cereus bacteria because the active compounds produced by these plants are able to damage the test bacterial cells, this can be seen from the inhibition zone produced which is larger than 6 mm (Figure 2), while for bacteria *E. coli* and *Salmonella typhi* did not form an inhibition zone at all (the diameter of the inhibition zone was only 6 mm) (Figure 1). According to Jackie et al. (2011) and Mariani (2015), the main bioactive compounds contained in the two plants are; saponins, flavonoids, and steroids/triterpenoids.

Saponins are bioactive compounds produced by certain plants. These compounds are antibacterial, can interfere with the permeability of microbial cell membranes, resulting in cell membrane damage so that various important components from inside microbial cells, such as proteins, nucleic acids, nucleotides, and others can come out of the cell (Astuti et al., 2011). Furthermore, Umar et al. (2012) stated that microbial cells in this case bacteria can be damaged by bioactive compounds such as flavonoids produced by plants. These flavonoids contain phenolic compounds which is acidic alcohol, known as carbolic acid. Phenol works to denature proteins and damage cell membranes, phenol binds to proteins through hydrogen bonds which causes the protein structure to be damaged.

Bacteria *S. aureus* and *B. cereus* are two examples of bacteria that belong to the group of Gram-positive bacteria, while *E. coli* and *S. typhi* are a group of Gram-

negative bacteria. These two groups of bacteria have different cell wall structures. The cell wall of Gram-positive bacteria consists of one layer, a thick layer of peptidoglycan, and a thin fat layer of 1-4%, while Gram-negative bacteria consist of 3 layers, namely lipoproteins, outer membrane phospholipids, and lipopolysaccharides, with lipid content ranging from 11%- 22%. The constituent components of the layered Gram-negative cell walls, especially the phospholipid content, make it difficult for chemical components (bioactive compounds) that are antibacterial to penetrate the cell walls of these Gram-negative bacteria (Poeloengan and Praptiwi, 2010).

The inhibition zone of Rendeu Caret and Honje plants against *B. cereus* bacteria was greater than that of *S. aureus*, but the difference was not too significant (Figure 3). According to Jawetz et al (1996), the size of the diameter of the inhibition zone is what determines the potential of an antibacterial compound.

The data shown in Figure 4 shows an increase in the inhibition zone of the Rendeu Caret and Honje plant extracts against *S. aureus* and *B. cereus* bacteria in line with the increase in the concentration of the extract. The smallest average inhibition zone (6.5 mm) resulted from treatment using an extract concentration of 6.25% against *S. aureus* and *B. cereus* bacteria, and the largest average inhibition zone (10.50 mm) was formed by treatment using an extract concentration of 50% against B. cereus bacteria.

The increase in the diameter of the inhibition zone for the growth of the test bacteria was due to the increasing number of plant extracts given. According to Jawetz et al (2001), the higher the concentration of an extract, the higher the inhibitory ability of an extract, then according to Kavitha et al. (2012) and Ramadani (2013), increasing the concentration of an antibacterial substance, will increase the content of active compounds produced by these substances so that the antibacterial activity is greater.

When observed from the average inhibition zone produced and associated with the grouping of antibacterial activity, the antibacterial activity of these two plant extracts was classified as moderate to strong, which was 6.5 - 10.5. According to Morales et al. (2003), antibacterial activity by active ingredients was grouped into 4 categories, namely weak activity (<5 mm), moderate (5-10 mm), strong (10-20 mm), and very strong (>20-30 mm).

Standard antibiotic Chloramphenicol 30µg formed an inhibition zone for the growth of the four colonies of test bacteria. The average inhibition zone formed by chloramphenicol 30µg against *E. coli* was 33 mm, against *S.typhi* by 31 mm, against *S.aureus* by 38 mm, and against *B.cereus* by 24 mm. Based on these data, it is known that the antibacterial properties contained in the plant extracts of Rendeu Caret and Honje are not as effective as the standard antibiotics used. Antibiotic Chloramphenicol 30 g affects four types of bacteria, both Gram-positive and Gram-negative, this antibiotic has a broad spectrum. Meanwhile, the plant extracts of Rendeu Caret and Honje were only able to inhibit the growth of Gram-positive bacteria, so it can be said to have a narrow spectrum.

CONCLUSION

The results of the study entitled "Antibacterial Bioactivity of Reundeu caret (*Staurogyne longata*) and Honje (*Etlingera hemisphaerica*) Plant Extracts", several conclusions can be drawn.

1. Reundeu caret (*Staurogyne longata*) and Honje (*Etlingera hemisphaerica*) plant extracts were able to inhibit the growth of *Staphylococcus aureus* and *Bacillus cereus* bacteria, and were unable to inhibit the growth of *Escherichia coli* and *Salmonella typhi* bacteria.
2. There was an increase in the inhibition zone in line with the increase in the concentration of the extract.
3. Antibacterial activity The Honje (*Etlingera hemisphaerica*) plant extract was not much different from the Reundeu caret (*Staurogyne longata*) extract in inhibiting the growth of *Staphylococcus aureus* and *Bacillus cereus* bacteria.
4. The antibacterial activity of Rendeu Caret and Honje plant extracts was moderate to strong.

ACKNOWLEDGMENT

Thank you to the Universitas Nasional and LPPM Unas who have assisted in funding this research, and also to the Microbiology and Genetics laboratory staff who have helped during research in the Laboratory.

REFERENCES

-, R., Ramadani, N. Y., -, F., & -, H. (2013). AKTIVITAS ANTIBAKTERIAL EKSTRAK ETANOL DAN REBUSAN SARANG SEMUT (Myrmecodia sp.) TERHADAP BAKTERI Escherichia coli. Jurnal Medika Veterinaria, 7(2). https://doi.org/10.21157/j.med.vet..v7i2.2938

Anisomeles malabarica (Linn.) R. Br. ex Sims. (n.d.). In SpringerReference. Springer-Verlag. https://doi.org/10.1007/springerreference_68066

Astuti, S. M., Sakinah A.M, M., Andayani B.M, R., & Risch, A. (2011). Determination of Saponin Compound from Anredera cordifolia (Ten) Steenis Plant (Binahong) to Potential Treatment for Several Diseases. Journal of Agricultural Science, 3(4). https://doi.org/10.5539/jas.v3n4p224

Fadhilla, R., Aditya Putri Iskandar, E., & Dewantari Kusumaningrum, H. (2012). AKTIVITAS ANTIBAKTERI EKSTRAK TUMBUHAN LUMUT HATI (Marchantia paleacea) TERHADAP BAKTERI PATOGEN DAN PERUSAK PANGAN. Jurnal Teknologi Dan Industri Pangan, 23(2), 126–131. https://doi.org/10.6066/jtip.2012.23.2.126

Jackie, T., Haleagrahara, N., & Chakravarthi, S. (2011). Antioxidant effects of Etlingera elatior flower extract against lead acetate - induced perturbations in free radical scavenging enzymes and lipid peroxidation in rats. BMC Research Notes, 4, 67. https://doi.org/10.1186/1756-0500-4-67

Karim, M. (2017). ANALISIS FENOLIK DAN DAYA HAMBAT DAUN BINAHONG (Anredera cordifolia (ten.) Steenis) TERHADAP BAKTERI Eschericia coli DAN Staphylococcus aureus. Indonesian Chemistry and Application Journal, 1(1), 1. https://doi.org/10.26740/icaj.v1n1.p1-9

Kurnia, K. A., Widyatamaka, S. Q., Paujiah, S., & Prayuda, E. M. (2021). Isolasi Senyawa Turunan Kuinon dari Tanaman. Syntax Idea, 3(6), 1361. https://doi.org/10.36418/syntax-idea.v3i6.1275

Melkianus, B., Fatimawali, F., & Sudewi, S. (2019). UJI AKTIVITAS ANTIBAKTERI EKSTRAK KULIT BUAH MANGGIS (Garcinia mangostana L.) TERHADAP

BAKTERI Klebsiella pneumonlae. PHARMACON, 8(1), 88. https://doi.org/10.35799/pha.8.2019.29241

MORALES, G., SIERRA, P., MANCILLA, A., PAREDES, A., LOYOLA, L. A., GALLARDO, O., & BORQUEZ, J. (2003). SECONDARY METABOLITES FROM FOUR MEDICINAL PLANTS FROM NORTHERN CHILE: ANTIMICROBIAL ACTIVITY AND BIOTOXICITY AGAINST Artemia salina. Journal of the Chilean Chemical Society, 48(2). https://doi.org/10.4067/s0717-97072003000200002

Täufel, A. (1995). M. R. Adams und M. O. Moss: Food microbiology. 398 Seiten, zahlr. Abbildungen und Tabellen. The Royal Society of Chemistry, Cambridge 1995. Peris: 22,50 £. Food / Nahrung, 39(5–6), 542. https://doi.org/10.1002/food.19950390534