Jurnal Perikanan S4

Fisheries Journal, 14(2), 972-980 (2024)
http://doi.org/10.29303/jp.v14i2.870

ANTIBACTERIAL EFFECTIVENESS OF VARIOUS TYPES OF HERBS EXTRACTS ON THE DIAMETER OF THE GROWTH INHIBITION ZONE OF *Aeromonas salmonicida*

Efektivitas Antibakteri dari Berbagai Jenis Ekstrak Herbal Terhadap Diameter Zona Hambat Pertumbuhan *Aeromonas salmonicida*

Nashir Arifin*, Maria Agustini, Sri Oetami Madyowati, Achmad Kusyairi

Aquaculture, Faculty of Agriculture, Dr. Soetomo University

Semolowaru Street No.84, Menur Pumpungan, Sukolilo District, Surabaya, East Java 60118

*Corresponding author: nasirarifin21@gmail.com

(Received May 20th 2024; Accepted June 26th 2024)

ABSTRACT

The use of chemicals such as antibiotics, immunostimulants and vaccines is one of the efforts that can be made to prevent disease attacks on fish. However, using chemicals such as antibiotics over a long period of time with inappropriate use will make disease-causing bacteria become resistant to these materials. Therefore, it is necessary to look for herbal ingredients as a substitute for antibiotics, for example the Chinese betel plant, anting anting plant and basil plant. The aim of this research was to determine the effect of different herbal plant extracts in inhibiting the growth of *Aeromonas salmonicida* bacteria. The research method used was experimental with an experimental design using a completely randomized design (CRD) consisting of 3 treatments, 9 replications. The Kirby-Bauer disc diffusion method was used in this research. Chinese betel simplicia, anting earrings and basil were soxhleted with 95% ethanol solvent until the solution was colorless and concentrated using a rotary evaporator. Next, an antibacterial test was carried out. The research results showed that the highest inhibitory power for the growth of Aeromonas salmonicida bacteria was obtained in the treatment of Chinese betel extract (*Peperomia pellucida* L) with an average of 7.75 mm, then in the treatment of anting anting extract (*Acalypha indica* L) with an average of 6.61 mmm. in the basil extract treatment (*Ocimum sanctum* L) the lowest growth inhibition zone was obtained, namely an average of 5.67 mm. Based on the Anova test with F count (0.000) > F table at a significance level of 5%, which means it shows that herbal plant extracts in each treatment have a significant effect on the growth inhibition of *Aeromonas salmonicida* bacteria.

Keywords: *Acalypha indica* L, *Aeromonas salmonicida*, Herbal Plant, *Ocimum sanctum* L, *Peperomia pellucida* L

ABSTRAK

Penggunaan bahan kimia seperti antibiotik, imonustimulan, dan vaksin merupakan salah satu upaya yang dapat dilakukan dalam mencegah terjadinya serangan penyakit terhadap ikan. Namun, penggunaan bahan kimia seperti antibiotik dalam jangka waktu lama dengan penggunaan yang kurang tepat akan membuat bakteri penyebab penyakit akan menjadi resisten terhadap bahan tersebut. Oleh karena itu, perlu dicari suatu bahan herbal sebagai penganti antibiotik, misalnya pada tanaman sirih cina, tanaman anting anting dan tanaman kemangi. Tujuan dari penelitian ini adalah untuk mengetahui pengaruh ekstrak tanaman herbal yang berbeda dalam menghambat pertumbuhan bakteri *Aeromonas salmonicida.* Metode penelitian yang digunakan adalah eksperimental dengan rancangan percobaan menggunakan rancangan acak lengkap (RAL) yang terdiri dari 3 perlakuan, 9 ulangan. Metode Disc diffusion Kirby-Bauer digunakan dalam penelitian ini. Simplisia sirih cina, anting anting dan kemangi disoxhletasi dengan pelarut etanol 95% sampai larutan tidak berwarna dan dilakukan pemekatan dengan rotary evaporator Selanjutnya dilakukan uji antibakteri. Hasil Penelitian menunjukan dimana daya hambat pertumbuhan bakteri *Aeromonas salmonicida* tertinggi diperoleh pada perlakuan ekstrak sirih cina (*Peperomia pellucida* L) dengan rata-rata sebesar 7,75 mm, kemudian perlakuan ekstrak anting anting (*Acalypha indica* L) dengan rata-rata sebesar 6,61 mm. pada perlakuan ekstrak kemangi (*Ocimum sanctum* L) merupakan zona hambat pertumbuhan terendah diperoleh yaitu rata-rata sebesar 5,67 mm. Berdasarkan uji Anova dengan F hitung (0,000) > F tabel pada taraf signifikan 5%, yang artinya menunjukan bahwa ekstrak tanaman herbal pada masing-masing perlakuan berpengaruh nyata terhadap daya hambat pertumbuhan bakteri *Aeromonas salmonicida*.

Kata Kunci: *Acalypha indica* L, *Aeromonas salmonicida*, *Ocimum sanctum* L, *Peperomia pellucida* L, Tanaman Herbal

INTRODUCTION

One of the problems that often hampers fisheries cultivation is disease attacks, which always cause losses and the most severe impact is production failure (Wirawan *et al*., 2018). The emergence of disease in fish is caused by interactions between pathogens, the environment and the host. One of the pathogens that causes disease in cultivation businesses is bacteria. Bacteria are organisms that are often found in waters, are very small, do not have a cell nuclear membrane, and are one of the pathogens that cause disease in cultivation businesses. The bacteria *A. salmonicida, E. tarda, and E. ictaluri* are some of the main causes of fish infections.

The *A. salmonicida* bacteria is a pathogen that is dangerous for the cultivation of salmonid and non-salmonid fish, as well as cyprinidae, especially goldfish (Rahmawati, 2021). A. salmonicida can cause damage and various histopathological changes to the kidneys, spleen, intestines and liver (Fajrin, 2020).

One way to prevent diseases that attack fish is to use chemicals such as antibiotics, immunostimulants and vaccines. Long-term use of antibiotics with inappropriate use will make disease-causing bacteria become resistant to these substances (Payung & Manoppo, 2015). The government currently prohibits the use of antibiotics in preventing fish diseases and recommends the use of natural ingredients because they are not carcinogenic and are more environmentally friendly (Azhar *et al*., 2020). The right action to overcome this problem is to use herbal plants, because herbal plants not only have antimicrobial effects, but can also increase the fish's immunity to environmental changes. Therefore, as a substitute for antibiotics, herbal plants such as Chinese betel, anting-anting, and basil are thought to inhibit the growth of *A. salmonicida* bacteria.

Peperomia pellucida L., or Chinese betel, is a traditional medicinal plant that has a long history of use in various cultures. Chinese betel plants are easy to find in humid environments that are not exposed to sunlight, and maintenance is very fast and inexpensive (Putrajaya *et al*., 2019). Ethanol extract of Chinese betel leaves (*Peperomia pellucida*) contains compounds that have antibacterial potential such as flavonoids, steroids, terpenoids, alkaloids, saponins and tannins (Endriyanto, 2023). The earring plant (*Acalypha indica* L.) can also be used as medicine. This plant has branching stems and long rough outlines that can reach a height of thirty to fifty centimeters. The shape of the leaves is round and oval with alternate leaves. Single sexual flowers grow in the leaf axils (Amelia, 2018). This plant contains phytochemicals that have pharmacological properties. These phytochemicals include phenolic compounds, flavonoids, alkaloids, steroids, saponins, and tannins, which have antioxidant, antibacterial, and antiparasitic properties (Saefurohman *et al*., 2023). Then basil (*Ocimum sanctum* L.) is a plant that grows widely in tropical areas such as Indonesia, and is usually consumed as a vegetable or fresh vegetables. Basil is reported to have anticancer, antioxidant, anti-inflammatory, antidiabetic, antimicrobial and antistress properties. Some of the secondary metabolites contained in basil are simple phenolics, polyphenols (flavonoids, coumarins, neolignans), essential oils, triterpenoids, sesquiterpenoids, steroids, glycosides, and cerebrosides (Singh & Chaudhuri, 2018).

The aim of the research carried out was to determine the effect of different herbal plant extracts in inhibiting the growth of *Aeromonas salmonicida* bacteria.

METHODS

Place and Time

Research activities were carried out at the Laboratory of the Marine and Fishery Products Quality Control and Supervision Agency (BP2MHKP), Puspa Agro, Sambikerep, Jemundo, Taman, Sidoarjo Regency, from 20 to 30 March 2024.

Tools and Materials

The tools used are petri dishes, spoons, micro pipettes, hot plates, funnels, cotton, tissue, 250 ml Elenmeyer flasks, measuring cups, magnetic stirrers, filter paper, tube needles, sterile cotton swabs, label paper, masks, latex gloves, aluminum foil, autoclave, incubator, analytical balance with an accuracy of 0.01g, BSC (biological safety cabinet), test tubes, tweezers, glass bottles, Soxhlet, heating mantles, and rotary evaporator. The ingredients used are Chinese betel extract, earring extract, basil extract, distilled water, 70% alcohol, 0.5 McFarland solution, disc paper, Muller Hinton Agar (MHA), Brain Heart Infusion Broth (BHIB), *Aeromonas salmonicida* bacteria, 95% ethanol, and Tryptic Soy Agar (TSA).

Research Methods

The method used is the most scientifically reliable (most valid) experimental method, because it is carried out by strictly controlling confounding variables outside those being experimented with (Arsyad & Fatmawati, 2018). This research used a Completely Randomized Design (CRD) using 3 different treatments, namely treatment SC (Chinese betel extract (*Peperomia pellucida* L) with a concentration of 100%), treatment A (anting-anting extract (*Acalypha indica* L) with a concentration of 100%), and treatment K (basil extract (*Ocimum sanctum* L) with a concentration of 100%) and 9 repetitions.

The treatment determination was based on preliminary tests on Chinese betel extract with a concentration of 100%, anting-anting extract with a concentration of 100%, and basil extract with a concentration of 100% against the bacteria *A. salmonicida, A. hydrophila*, and *E. tarda*. The results obtained in the preliminary test were that Chinese betel extract obtained an inhibition zone for the bacteria *A. salmonicida* 8.4 mm, *A. hydrophila* 3.2 mm, and *E. tarda* 0 mm; earring extract of *A. salmonicida* 8.0 mm, *A. hydrophila* 5.5 mm, and *E. tarda* 0 mm; basil extract *A. salmonicida* 6.9 mm, *A. hydrophila* 2 mm, and *E. tarda* 0 mm.

Research Preparation

1. Sample Preparation

Chinese betel plants, earrings and basil were obtained from Jl. Nangka 1 Taman Sidoarjo. Fresh samples were collected by separating them from the dirt contained in the samples, washing them using running water, then chopping them. Next, it is dried using a dehydrator at a temperature of 50°C until the leaves are completely dry and ground using a blender until simplicia powder is produced.

2. Making Extracts

30 g of Chinese betel simplicia (*Peperomia pellucida* L.), anting-anting (*Acalypha indica* L), and basil (*Ocimum sanctum* L) were weighed, then wrapped in filter paper, then placed in a Soxhlet tube. Then soxhletation was carried out using 420 mL of 95% ethanol and stopped when the liquid in the Soxhlet tube was colorless. The extraction that has been obtained is evaporated in a rotary evaporator until a thick extract is obtained (Tiah *et al*., 2018).

3. Sterilization of Tools and Materials

Sterilization in microbiology is a way to kill and eliminate all organisms found on an object (Cahyani, 2014). Sterilization of equipment is carried out using an autoclave at 121ºC at 1 atm pressure for 15 minutes and in an oven at 180ºC for 2-3 hours. Sterilization of materials was carried out using an autoclave at a temperature of 121ºC at a pressure of 1 atm for 15 minutes.

Research Implementation

1. Rejuvenation of Pure Culture Test Bacteria

A total of one pure culture colony of *A. salmonicida* bacteria obtained from the BP2MHKP Laboratory was taken using a sterile tube from the pure culture, and then inoculated in BHIB media then incubated in an incubator at a temperature of 27ºC-30ºC for 1x24 hours.

2. Aeromonas salmonicida Bacterial Culture

Pure culture of test bacteria that has been rejuvenated in BHIB media for 24 hours at a temperature of 27-30°C. then propagated in TSA medium and incubated for 24 hours at 27-30°C.

3. Resistance Testing

Take the bacterial culture of A. salmonicida which has been measured to a McFarland standard of 0.5 with a cotton swab, spread evenly on the surface of the MHA media until the surface contains the bacterial culture. Soak the paper discs in the extract according to the treatment, wait until it dries, transfer the paper discs to MHA media and incubate the media at 27-30°C for 24 hours.

Observation Stage

The inhibition zone formed after 24 hours is measured using a caliper and the strength of the inhibition zone is interpreted. The presence of antibacterial activity against the *A. salmonicida* bacteria was indicated by the formation of an inhibition zone around the paper disc containing the test solution.

Table 1. Classification of Responses to Bacterial Growth Inhibition

Inhibition Zone Diameter	Growth Barrier Response
<5 mm	Weak
5-10 mm	Medium
10-19 mm	Strong
20-30 mm	Very Strong

Source: Pramadani (2022)

Data Analysis

In this study, ANOVA data analysis was used to determine the effect of treatment. An analysis of variation (ANOVA) is carried out, then if there is an influence on the treatment, then the Duncan test is continued with a level of 5% by comparing the calculated F with the F table:

- If calculated F > F table 5%, then the treatment results in very significantly different results.
- If calculated F < F table 5%, then it is not significantly different.
- If F table 5% < F count < F table 1%, then the treatment causes significantly different results.

RESULT

Based on the results of observations, it shows that Chinese betel extract, anting-anting, and basil have the ability to inhibit the growth of Aeromonas salmonicida bacteria. These results can be seen in Table 2 and Figure 1 below:

Table 2. Range of Values, Average, Standard Deviation of *A. Salmonicida* Bacterial Growth for Each Treatment During the Study

Extract	Bacterial Growth Range (mm)	Average Bacterial Growth (mm)	Standard Deviation (SD)
Chinese betel	6 – 8.5	7.75	0.6521
Anting-anting	5.5 - 8	6.61	0.8710
Basil	4 – 7.5	5.67	0.8497

Source: Primary Data (2024)

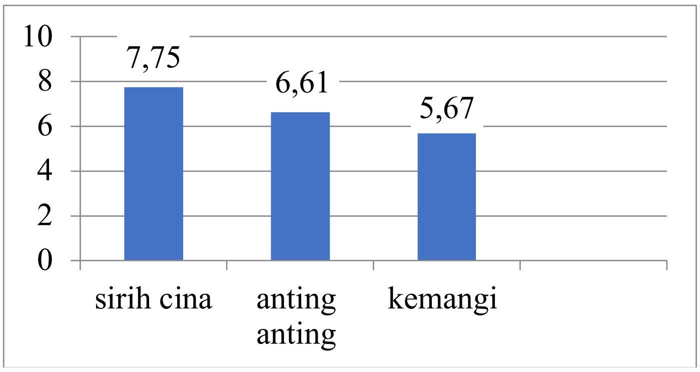


Figure 1. Diagram of the Effect of the Extract on the Growth Inhibition Zone of *A. salmonicida* Bacteria

To determine the effect of the response of Chinese betel extract (*Peperomia pellucida* L.), anting-anting (*Acalypha indica* L), and basil (*Ocimum sanctum* L.) on the inhibition zone of *Aeromonas salmonicida* bacteria, an ANOVA (Analysis of Variance) test was carried out. Results are shown in the table below:

Table 3. ANOVA Test Results for the Growth of *A. salmonicida* Bacteria

ANOVA

bakteri_as

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	19,494	2	9,747	15,343	,000
Within Groups	15,247	24	,635		
Total	34,741	26			

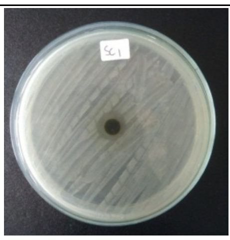
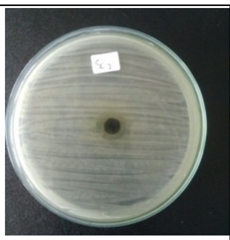
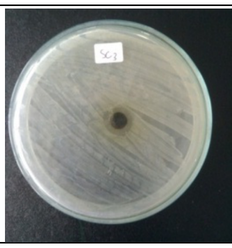
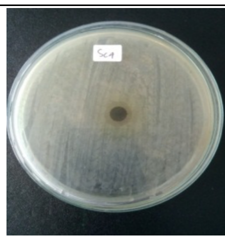
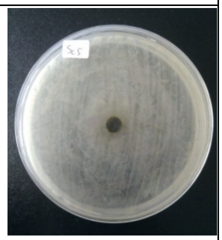
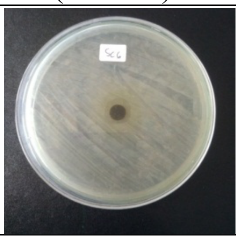
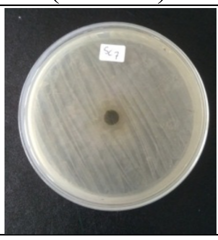
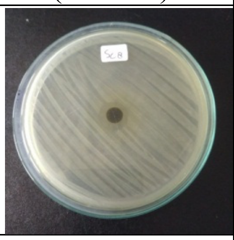
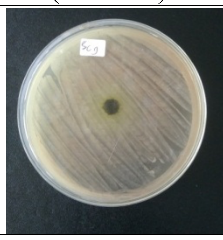
Based on the results of the ANOVA test, F count > F table means that there is a significant influence so it is necessary to carry out further tests, namely the multiple comparison test using the Duncan test. The results are presented in the table below:

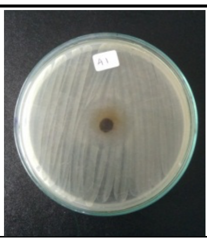
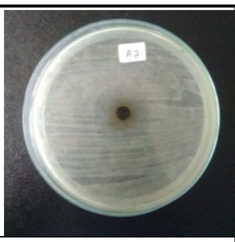
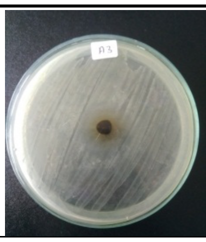
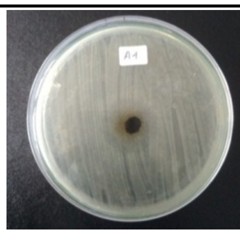
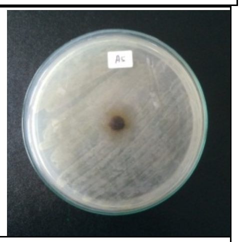
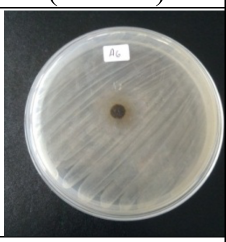
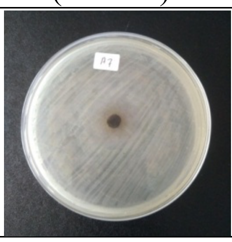
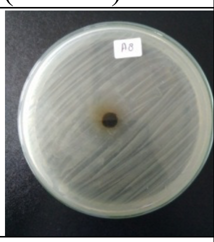
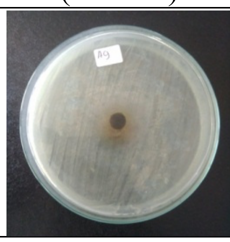
Table 4. Differences in the Notation of Duncan's Test Results on the Average Growth of *A. salmonicida* Bacteria

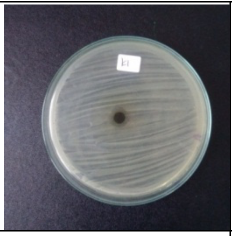
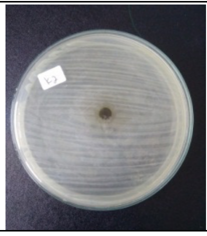
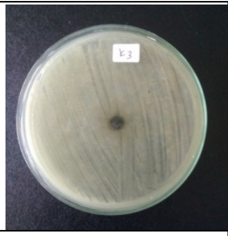
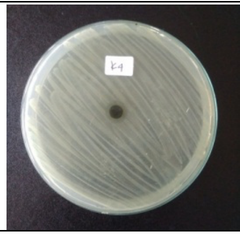
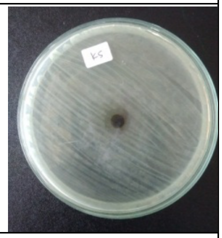
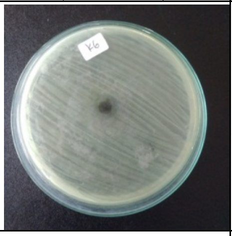
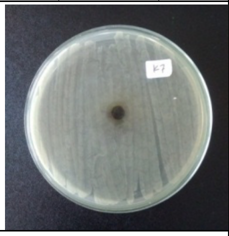
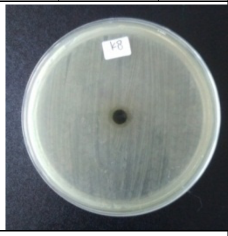
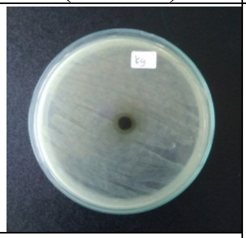
Duncan[a]

Tanaman_herbal	N	Subset for alpha = .05 1	2	3
kemangi	9	5,678		
anting_anting	9		6,611	
sirih_cina	9			7,756
Sig.		1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 9,000.

Treatment CB (Chinese Betel)				
				
Treatment CB 1 (8.0 mm)	Treatment CB 2 (8.5 mm)	Treatment CB 3 (8.0 mm)	Treatment CB 4 (7.0 mm)	Treatment CB 5 (8.0 mm)
				
Treatment CB 6 (7.0 mm)	Treatment CB 7 (8.5 mm)	Treatment CB 8 (8.0 mm)	Treatment CB 9 (6.8 mm)	

Treatment A (Anting Anting)				
				
Treatment A 1 (6.5 mm)	Treatment A 2 (6.7 mm)	Treatment A 3 (6.0 mm)	Treatment A 4 (5.5 mm)	Treatment A 5 (7.3 mm)
				
Treatment A 6 (5.5 mm)	Treatment A 7 (6.5 mm)	Treatment A 8 (7.5 mm)	Treatment A 9 (8.0 mm)	

Treatment B (Basil)				
				
Treatment B 1 (5.5 mm)	Treatment B 2 (4.8 mm)	Treatment B 3 (5.5 mm)	Treatment B 4 (4.4 mm)	Treatment B 5 (7.0 mm)
				
Treatment B 6 (6.6 mm)	Treatment B 7 (6.0 mm)	Treatment B 8 (5.1 mm)	Treatment B 9 (6.2 mm)	

DISCUSSION

Observation results show that Chinese betel extract, anting-anting, and basil can inhibit the growth of *Aeromonas salmonicida* bacteria. The clear zone around the paper disc on the media growing *A. salmonicida* bacteria indicates the presence of inhibition. The sensitivity of microbes to antibiotics or antimicrobials used as test materials is indicated by the diameter of the clear zone around the disc (Wendersteyt, 2021). In the Chinese betel extract treatment, the average zone of inhibition of bacterial growth was 7.75 mm; earring extract treatment was 6.61 mm; and basil of 5.67 mm. According to Pramadani (2022), the inhibition zone formed in the agar diffusion test measuring less than 5 mm is categorized as weak, 5-10 mm is categorized as medium, 10-19 mm is categorized as strong, and 20-30 mm is categorized as very strong.

The ANOVA test showed significant results ($P = 0.000 < \alpha\ 0.05$), meaning that different herbal plant extracts had a significant effect on the growth of *Aeromonas salmonicida* bacteria. Based on the results of the 5% ANOVA test, different herbal plant extracts had a significant effect ($p < 0.05$) on inhibiting the growth of *A. salmonicida* bacteria. The highest inhibitory power was obtained in the treatment of Chinese betel extract (*Peperomia pellucida* L.) with an average of 7.75 mm, then anting-anting extract (*Acalypha indica* L.) with an average of 6.61 mm, and basil extract treatment (*Ocimum sanctum* L.) showed the lowest growth inhibition zone with an average of 5.67 mm. The difference in the inhibition zone comes from the activity of dissolved bioactive compounds, including flavonoids. The antibacterial mechanism of flavonoids inhibits nucleic acid synthesis, cell membrane function, and bacterial metabolism in cells (Mutmainnah, 2020). Flavonoids have the ability to inhibit cell membrane function, causing damage and release of intracellular compounds through the formation of complex compounds from extracellular proteins. In addition, cell membrane permeability will be disturbed and the binding of enzymes such as phospholipase will be hampered. This compound has the ability to stop cytochrome C reductase, which stops metabolic formation and also stops energy metabolism by stopping the use of oxygen. The differences in inhibition zones are influenced by the flavonoid content in each extract. Research conducted by Sadiyah (2023) showed that the total flavonoid content in Chinese betel extract (*Peperomia pellucida* L.) was

68.42 mgQE/g. Research conducted by Wijayanti (2022) stated that the results of total flavonoid calculations in anting-anting leaf extract (*Acalypha indica* L.) were 61.8 mgQE/g, and research by Wibawa (2023) stated that the total flavonoid compounds in basil extract (*Ocimum sanctum* L.) of 35.891 mgQE/g extract.

CONCLUSION

Based on this research, the conclusion obtained is that the highest inhibition of the growth of *Aeromonas salmonicida* bacteria was obtained in the treatment of Chinese betel extract (*Peperomia pellucida* L.) with an average of 7.75 mm, then the treatment of anting-anting extract (*Acalypha indica* L.) with an average -average 6.61 mm. In the basil extract treatment (*Ocimum sanctum* L.), the lowest growth inhibition zone was obtained, namely an average of 5.67 mm.

ACKNOWLEDGEMENT

The researcher would like to thank his parents, as well as the supervisors and laboratory of the Marine and Fishery Products Quality Control and Supervision Agency who have facilitated this research activity.

REFERENCES

Amelia, A. (2018). Khasiat Tanaman Anting-Anting (*Acalypha indica* L.). *Majalah Farmasetika, 3*(1), 7-11.

Arsyad, N., & Fatmawati, F. (2018). Penerapan Media Pembelajaran Berbasis Multimedia Interaktif Terhadap Mahasiswa IKIP Budi Utomo Malang. *Jurnal Agastya, 8*(2), 189-198.

Azhar, F., Junaidi, M., Muklis, A., & Scabra, A. R. (2020). Penanggulangan Penyakit MAS (*Motile Aeromonas Septicemia*) pada Ikan Nila Menggunakan Ekstrak Temulawak (*Curcuma xanthorriza* Roxb). *Jurnal Abdi Insani Universitas Mataram, 7*(3).

Cahyani, V. (2014). *Petunjuk Praktikum Mikrobiologi Pangan*. Surakarta: Universitas Sebelas Maret.

Endriyatno, N. C., & Puspitasari, N. D. (2023). Formulasi krim ekstrak daun sirih cina (*Peperomia pellucida* L) dengan variasi konsentrasi trietanolamin dan asam stearat. *Universitas Haji Sumatra Utara, 3*(1).

Fajrin, A. R. (2020). *Histopatologi ikan Nila (Oreochromis niloticus) yang diinfeksi Aeromonas salmonicida* [Skripsi]. Fakultas Kedokteran Hewan, Universitas Gadjah Mada. Retrieved from http://etd.repository.ugm.ac.id/

Mutmainnah, B., Baktir, A., & Ni'matussahroh. (2020). Characteristics of Methicillin-Resistant Staphylococcus aureus (MRSA) and Methicillin Sensitive Staphylococcus aureus (MSSA) and their inhibitory response by ethanol extract of Abrus precatorius. *Biodiversitas, 21*(9), 4076-4085.

Payung, C. N., & Manoppo, H. (2015). Peningkatan Respon Kebal Non-spesifik dan Pertumbuhan Ikan Nila (*Oreochromis niloticus*) melalui Pemberian Jahe, *Zingiber officinale*. *Jurnal Budidaya Perairan, 3*(1), 11-18.

Pramadani, R. (2022). *Uji Aktivitas Antibakteri Ekstrak Daun Kersen (Muntingia calabura L) Terhadap Bakteri Aeromonas salmonicida, Edwardsiella ictaluri dan Edwardsiella tarda* [Skripsi]. Universitas Islam Riau.

Putrajaya, F., Hasanah, N., & Kurlia, A. (2019). Daya hambat ekstrak etanol daun suruhan (*Peperomia pellucida* L.) terhadap pertumbuhan bakteri penyebab jerawat (*Propionibacterium acnes*) dengan metode sumur agar. *Sekolah Tinggi Ilmu Kesehatan Persada, 3*(2).

Rahmawati, A. R., Ulkhaq, M. F., Susanti, D., Kenconojati, H., & Fasya, A. H. (2021). Identifikasi Bakteri *Aeromonas salmonicida* dan *Edwardsiella ictaluri* pada Ikan Hidup yang Akan Dilalulintaskan dari Daerah Istimewa Yogyakarta. *Journal of Marine and Coastal Science, 10*(2).

Sadiyah, W. V. (2023). Perbandingan kadar flavanoid total ekstrak etanol hasil metode maserasi dan sokletasi tumbuhan sirih cina (*Peperomia pellucida* L. Kunth). STIFI Bhakti Pertiwi Palembang.

Saefurohman, A., Shobri, M. M., & Wibowo, W. E. (2023). Tropical bioscience journal of biological science, 3(2).

Sartika, R., Melki, & Purwiyanto, A. I. S. (2013). Aktivitas Antibakteri Ekstrak Rumput Laut (*Eucheuma cottoni*) Terhadap Bakteri *Escherichia coli, Staphylococcus aureus, Vibrio cholera*, dan *Salmonella typhosa*. *Maspari Journal, 5*(2), 98-103.

Singh, D., & Chaudhuri, P. K. (2018). A Review Phytochemical and Pharmacological Properties of Holy (*Ocimum sanctum* L.). *Industrial Crops & Products, 118*, 367-382.

Tiah, T., Vilya, S., & Lenggo, E. (2018). Aktivitas Daya Hambat Minyak Atsiri dan Ekstrak Etanol Daun Sirih Merah (*Piper crocatum* Ruiz) Terhadap *Candida albicans*. *Sainstech Farma, 11*(2).

Wendersteyt, V. N., Wewengkang, S. D., & Abdullah, S. S. (2021). Uji aktivitas antimikroba dari ekstrak dan fraksi ascidian *Herdmania momus* dari perairan pulau Bangka Likupang terhadap pertumbuhan mikroba *Staphylococcus aureus, Salmonella typhimurium* dan *Candida albicans*. *UNSRAT Manado, 10*(1).

Wibawa, H. M. (2023). Skrining fitokimia dan penetapan kadar flavanoid total ekstrak daun kemangi (*Ocimum sanctum* L) menggunakan spektrofotometri UV-visible. Universitas Ngudi Waluyo.

Wijayanti, S., Astuti, S., & Amin, F. (2022). Uji aktivitas antibakteri salep ekstrak daun anting-anting (*Acalypha indica* L) terhadap bakteri *Escherichia coli* dan *Staphylococcus aureus*. Sekolah Kimia Analisis Kimia.

Wirawan, I. K. A., Suryani, S. A. M. P., & Arya, I. W. (2018). Diagnosa, Analisis dan Identifikasi Parasit yang Menyerang Ikan Nila (*Oreochromis niloticus*) pada Kawasan Budidaya Ikan di Subak "Baru" Tabanan. *Gema Agro, 23*(1), 63-78.