Effectiveness of methanol extract hydroid aglaophenia cupressina lamoureoux as antimicrobial in resistant Methicilline Staphylococcus Aureus (MRSA), Shigella sp., Malassezia furfur, and Candida albicans

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Abstract. Currently there are many studies that utilize potential of marine resources, because there are many potential bioactive compounds to be developed, especially to overcome multi-drug resistant (MDR) against antibiotics. Hydroid Aglaophenia cupressina Lamoureoux is a marine invertebrate that is often found around spermonde waters containing bioactive compounds that can be developed as a basic ingredient in medicine. This study aims to determine the effectiveness of methanol extract from the Aglaophenia cupressina L. hydroid in inhibiting the growth of Shigella sp. Sd 1617 bacteria, Methicillin-Resistant Staphylococcus aureus (MRSA) ATCC 25923 and Malassezia furfur fungi ATCC 14521, and Candida albicans ATCC 10231. In this study, the bioactivity test of methanol extract was carried out by swab method on NA and PDA medium. The concentration of methanol extract used is 5%, 10%, 20% and 40%. Data obtained from measurements of inhibition zone diameter during the incubation period of 24 hours to 48 hours in the Shigella sp. and Methicillin-Resistant Staphylococcus aureus (MRSA). While for the Malassezia furfur and Candida albicans fungi incubated for 48 hours to 72 hours. Data obtained in descriptive analysis. The results showed that methanol extract from Aglaophenia cupressina L. hydroid has potential as an antimicrobial which is bactericidal at all test concentrations against the bacterium Shigella sp. and Methicillin-Resistant Staphylococcus aureus (MRSA). While for the Malassezia furfur and Candida albicans fungi incubated for 48 hours to 72 hours. Data obtained in descriptive analysis. The results showed that methanol extract from Aglaophenia cupressina L. hydroid has potential as an antimicrobial which is bactericidal at all test concentrations against the bacterium Shigella sp. and Methicillin-Resistant Staphylococcus aureus (MRSA) is bacteriostatic (5%, 10%, 40%), bactericidal at a concentration of 20%. Against the fungi Malassezia furfur showed fungistatic properties in all variations in extract concentration, and against Candida albicans was fungicidal at concentrations of 5% and 10%, while concentrations of 20% and 40% were fungistatic.

1. Introduction

Multi-drug resistant (MDR) is found in several types of bacteria and fungi. Therefore, it is necessary to find potential sources of new antibiotics to overcome problems caused by bacterial and fungal infections [1]. Currently more research is directed at extracting marine resources because most of its natural resources have not been fully utilized and some marine organisms produce chemical compounds that are not present or rarely found in living organisms on land [2], [3]. This is marked by
collaboration between researchers from various institutions with pharmacologists that have resulted in a great advance in the discovery of medicines from marine biota.

According to [4] the development of new drugs originating from marine biota is due to the high marine biodiversity and the unique structure of the secondary metabolites it produces. This can be done by isolating bioactive compounds from natural marine materials, which are safe for health but also capable of activating bacteria and fungi [5]. Marine animals have a unique body defense form, one of which is in marine toxin form [6]. Marine toxin produced by marine biota comes from secondary metabolites, rich in chemical compounds, with more produced than land animals that have physical defenses [7]. Marin toxin is used in the self defense system, to maintain life and avoid interference from other organisms in the environment. These secondary metabolites have pharmacological activity so that they have the potential to be isolated and utilized in the field of medicine [8].

One of the marine biota that is rich in bioactive compounds, namely the genus Aglaophenia, is one of the marine invertebrates of the Cnidaria phylum. This phylum has the unique morphology of a plant-like body and has stinging cells or nematosyst which are used as self-defense, containing chemical compounds that when touched cause itching and even irritation to the skin. Aglaophenia lives attached to corals, sponges, sand and others [9].

Research on natural material from the Aglaophenia genus has been carried out on several species, including Aglaophenia pluma and Aglaophenia cupressina L. In A. pluma three alkaloid types were obtained with β-carboline framework. Whereas from Aglaophenia cupressina Lamoureux hydroid besides producing secondary metabolites in the form of histamine and tridentatols A, which indicates having antioxidant activity. Aglaophenia cupressina L. hydroid compounds also contain chemical compounds alkaloids, diterpenes, steroids, prostaglandins, histamine liberators and proteins in nematocytes that can inhibit the growth of pathogenic fungi [10]. The results of the study by Johannes E. [9], A. cupressina hydroid contain compounds (1) carboxylic acid compounds, namely hexadecanoic acid, (2) alkaloid groups are considered as new compounds and are temporarily named as aglao E. Unhas, and (3) class steroids, namely β-sitosterol. The three compounds have antimicrobial activity against Staphylococcus aureus, Salmonella thyphii, and E. coli.

There are still many other bioactive compounds from Aglaophenia cupressina Lamoureux hydroids that need to be investigated in biochemical and pharmacological fields [5]. Based on the description above, a study was conducted on the effectiveness of methanol extract from Aglaophenia cupressina L. hydroid as an antimicrobial against Shigella sp. bacteria, Methicillin-resistant Staphylococcus aureus (MRSA), and Malassezia furfur, and Candida albicans.

2. Materials and Methods
The materials used in this study were Aglaophenia cupressina L. hydroid, Shigella sp. SD 1617 isolate, Methicillin Resistant Staphylococcus aureus ATCC 25923 isolate, Malassezia furfur ATCC 14521 isolate, Candida albicans 10231 isolate, PDA (Potato Dextrose Agar) medium, NA medium (Agar Nutrient), amoxicilin, ketoconazole, NaCl, distilled water, methanol, alcohol 70%.

2.1. Extraction
The dry sample was cut into small sizes then weighed, macerated with methanol with a ratio of 1: 2 for 3 × 24 hours. The maceration process is repeated with the same volume ratio up to three times. The results of maceration are then removed by using a rotary evaporator.

2.2. Preparation and Rejuvenation of Bacteria and Test Fungi
The test bacteria used were Shigella sp. and Methicillin-resistant Staphylococcus aureus (MRSA) derived from pure cultures taken using a round ose, then inoculated by scraping on the slanted Nutrient Agar (NA) medium, while the test fungus used was Malassezia furfur taken from the available pure culture, carried out aseptically with a rounded ose and streaked on a sloping Potato Dextrosa Agar (PDA) medium. Each was incubated at 37°C for ± 24 hours.
2.3. Preparation of Test Bacterial and Fungi Suspensions

*Shigella sp* and Methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria that have been rejuvenated for ± 24 hours, one ose taken suspended into a sterile physiological NaCl solution of 0.9%. Then dilution of the test bacterial suspensions was carried out until 25% transmittance was obtained on the spectrophotometer, with a wavelength of 580 nm. As a blank, 0.9% sterile NaCl is used. *Malassezia furfur*, and *Candida albicans* that have been rejuvenated, are taken one ose then suspended or diluted using a 0.9% NaCl solution which is sterile and homogenized. The suspension is measured using a spectrophotometer to obtain a transmittance value of 25%.

2.4. Preparation of Test Bacterial and Fungi Control Solutions

The control solution used for bacteria is a solution of Amoxicillin and oxacillin as a positive control. While negative controls are used methanol. The control solution used was ketoconazole solution as a positive control and methanol was used as a negative control.

2.5. Preparation of Test Solution Concentration

Methanol extract from *Aglaophenia cupressina* L. hydroid was weighed as much as 4 g and dissolved in 10 ml of methanol to obtain a solution with a concentration of 40%. Then the test solution was made with a concentration of 5%, 10%, 20%, and 40% to determine the size of the ability of the extract to inhibit growth of microbes tested.

2.6. Inhibition Zone of Test Bacteria and Fungi

The test uses a agar diffusion method and a blank disk with a diameter of 5 mm. Sterile Medium Nutrient Agar (NA) is cooled at a temperature of 40°C - 45°C, then poured aseptically into a 15 ml petri dish and let it solidify. After solidifying, the suspension of *Shigella sp.* and Methicillin-resistant *Staphylococcus aureus* (MRSA) each of which had been suspended was scratched on the surface of the medium using a swab. then the blank disk is placed aseptically with sterile tweezers on the surface of the medium with a distance of one blank disk with another 2-3 cm, but previously the blank disk is immersed with methanol extract from *A. cupresina* hydroid at concentrations of 5%, 10%, 20% and 40 %, positive control with amoxicillin for *Shigella sp.* whereas oxallin for MRSA. Negative control with methanol for 15 minutes in vial bottles. Then incubated at 37°C for 24 hours and 48 hours. This test was carried out by looking at the formation of clear zones in each extract concentration.

The test uses a agar diffusion method and a blank disk with a diameter of 5 mm. Sterile Potato Dekstrose Agar (PDA) medium cooled at 40⁰C-45⁰C. Then poured aseptically into each petri dish of 15 ml and allowed to solidify. After solidifying, a suspension of *Malassezia furfur* and *Candida albicans* was applied on the surface of the medium using a swab. Then the blank disk is placed aseptically with sterile tweezers on the surface of the medium with a distance of one blank disk with another 2-3 cm, but previously the blank disk is immersed with methanol extract from hydroid *A. cupresina* L. at a concentration of 5%, 10%, 20% and 40%, positive control with ketoconazole and negative control with methanol for 15 minutes in vial bottles. Then incubated at 37°C for 48 hours and 72 hours.

2.7. Measurement of Inhibition Zone Diameter

Observations were made by measuring the diameter of the inhibition zone of growth of *Shigella sp.*, Methicillin-resistant *Staphylococcus aureus* (MRSA), and *Malassezia furfur, Candida albicans* around the blank disk using the calipers. Measurements will be made after incubating 1-2 x 24 hours on the test bacteria and 2-3 x 24 hours on the test fungi. This is done to determine the ability of methanol extract from the hydroid to inhibit or kill the growth of fungi and pathogenic bacteria tested.

2.8. Data Analysis

Measurement data were analyzed by comparing the diameter of control zone (positive control and negative control) and each type of sample concentration during 24-48 hour observation on *Shigella*
sp., Methicillin-resistant *Staphylococcus aureus* (MRSA), whereas in *Malassezia furfur,* and *Candida albicans* which is 48 - 72 hours. The inhibitory test results were analyzed descriptively and displayed in the form of drawings.

3. Results and Discussion
Hydroid *Aglaophenia cupressina* L. The results of maceration and evaporation obtained 12 grams of methanol extract.

![Whatman 42 screening](image1.png) ![Hydroid extract results](image2.png)

Methanol solvents are used because the solvent can dissolve almost all secondary metabolites both polar and non-polar. Methanol also has a relatively low boiling point so that it evaporates easily [11]. According to [12] methanol is able to attract more number of secondary metabolites compared to ethanol, so generally methanol solvents are used in maceration.

3.1. Bioactivity Test of Methanol Extract from *Aglaophenia cupressina* L. As Antibacterial

3.1.1. *Shigella sp*
Diameter of inhibition zone that formed from various concentrations of *A. cupressina* L. hydroid methanol extract against *Shigella sp.* after incubation for 24 hours and 48 hours, can be seen in Figure 3 and 4.

![Incubation for 24 hours](image3.png) ![Incubation for 48 hours](image4.png)
Table 1. Methanol extract of A. cupressina L hydroid against Shigella sp bacteria at 24 hours and 48 hours incubation

| Treatment | Inhibition Zone (nm) |
|-----------|----------------------|
|           | 24 hours | 48 hours |
| C = 5%    | 8.0      | 9.5      |
| D = 10%   | 8.5      | 10.0     |
| E = 20%   | 9.0      | 10.5     |
| F = 40%   | 10.25    | 12.0     |
| B = positive control | 13.0 | 14.0 |
| A = negative control | 0 | 0 |

Table 1 shows an increase in clear zones formed from the measurement results 24 hours to 48 hours. This indicates that methanol extract from hydroid A. cupressina L. is able to kill the growth of Shigella sp or bactericide. The results of measuring the diameter of inhibition zone are then made in the form of histograms as seen in Figure 5.

Figure 5. Histogram of inhibition zone diameter to methanol extract from A. cupressina L. hydroid to Shigella sp at 24 hours and 48 hours incubation

Figure 5 shows the measurement results on the diameter of inhibition zone in test bacteria with the bioactivity of antibacterial compounds of methanol extract at 24 hour incubation with concentrations of 5%, 10%, 20%, and 40% and positive controls obtained by resistance diameter 8.00 mm, 8.50 mm, 9.00 mm and 10.25 mm, positive control 13.00 mm. After incubation for 48 hours, the diameter of the inhibition zone increases. At concentrations of 5%, 10%, 20%, 40%, respectively 9.50 mm, 10.00 mm, 10.50 mm and 12.00 mm, positive controls 14.00 mm. While negative controls do not form a inhibition zone.

According to [13] if the diameter of the resistance zone is 20 mm or more, the inhibitory activity is categorized as very strong, 10-20 mm categorized as strong, 5-10 mm categorized as moderate, and 5 mm or smaller categorized as weak. In Figure 2 shows the size of the inhibition zone diameter at each extract concentration with incubation for 2 x 24 hours has different potential in the process of inhibiting the growth of Shigella sp. namely at a concentration of 5% and 10% which are 9.50 mm and 10.00 mm in diameter are categorized as being, concentrations of 20% and 40% each with a diameter
of 10.50 mm, and 12.00 mm is categorized as strong. So that the measurement results of the inhibitory test that has been obtained prove that methanol extract from *A. cupressina* L. hydroid has the potential as an antimicrobial. If the concentration is increased, the strong inhibition category can be very strong according to the opinion of [14] and the higher the concentration of extract given, the greater the diameter of the clear zone formed. While the positive control in the test bacteria using amoxicillin because it has been shown to have antibacterial properties, where the content of compounds contained in it affects the cell wall of bacteria that causes lysis.

3.1.2. Methicillin Resistance to *Staphylococcus aureus*

![Figure 6. Incubation for 24 hours](image)

![Figure 7. Incubation for 48 hours](image)

| Treatment          | Inhibition Zone (nm) |
|--------------------|----------------------|
|                    | 24 hours  | 48 hours |
| C = 5%             | 12.0       | 9.0      |
| D = 10%            | 10.5       | 8.25     |
| E = 20%            | 7.0        | 7.25     |
| F = 40%            | 7.5        | 6.5      |
| B = positive control | 0          | 0         |
| A = negative control | 0           | 0        |

Based on table 2, *Aglaophenia cupressina* L. methanol hydroid extract appears at a concentration of 5%, 10% and 40% there was a decrease in the diameter of the barrier zone from 24 hours to 48 hours. This indicates that the three variations of the concentration only inhibit bacterial growth or are bacteriostatic. In contrast to the concentration of 20% there is an increase in the diameter of the clear zone formed from 7.00 to 7.25 in measurements 24 hours to 48 hours. At a concentration of 20% it is lethal or bacteriocide. The results of measuring the diameter of inhibition zone are then made in the form of histograms as seen in Figure 8.
Figure 8. Histogram of inhibition zone diameter to methanol extract from A. cupressina L. hydroid to Methicillin resistant Staphylococcus aureus at 24 hours and 48 hours incubation.

Figure 8. shows that at a concentration of 20%, the inhibition zone is seen to increase from 7-7.25 mm at measurements 42 hours to 48 hours. Conversely, a concentration of 40% shows a decrease in the inhibition zone formed from 24 hours to 48 hours. According to [15], antibacterial are said to have high activity against microbes if the minimum inhibitory concentration value is low but has a large inhibitory power. A compound is said to have antibacterial activity if the diameter of inhibition zone is formed ≥ 6 mm. This is supported by the opinion [13], the 20 mm inhibition zone is classified as potentially very strong, 11-20 mm has strong potential, potentially moderate 5-10 mm diameter and 0-4 mm means weak inhibition. Then it can be said that Aglaophenia cupressina L. hydroid extract at a concentration of 20% is potentially moderate in inhibiting test bacteria (MRSA). The difference between lethal and inhibition of each concentration of extract is thought to be caused by the diffusion rate of the active substance in the extract [14]. In general, the diameter of the inhibition zone tends to increase in proportion to the increase in concentration of an extract but when viewed from the above data (Figure 4), there is a decrease in the size of the inhibition zone when concentration increases. This is because the increase in concentration causes the viscosity of extract solution to increase, thus affecting the speed of diffusion. In addition, the increase in concentration caused the solubility of antibacterial active ingredient extract to experience a decrease or saturation, thus affecting the ability of microbial growth inhibition [16].

[17] states that Aglaophenia cupressina L hydroid contains secondary compounds in the form of steroids, terpenoids, phenolics, saponins, alkaloids, flavonoids and carboxylic acids. The content of steroid/ triterpenes according [18] can damage the plasma membrane of bacterial cells, causing cytoplasm leakage and cell death. Positive control (oxacillin) does not form a clear zone. It should be noted that methicillin and oxacillin were penicillin derivatives found in 1959, as antibiotics for the treatment of infections against Staphylococcus aureus. But in 1961 Staphylococcus aureus was resistant to methicillin [19].
3.2. Bioactivity Test of Methanol Extract from Aglaophenia cupressina L. as an Antifungal

3.2.1. Malassezia furfur

The results of the inhibition zone diameter at several concentrations of methanol extract from hydroid A. cupressina L. against Malassezia furfur were characterized by the formation of zones that were not overgrown with test fungi and commonly called clear zones after incubation for 48 hours and 72 hours which can be seen in Figure 9 and 10 below.

![Figure 9. Incubation for 48 hours](image1)

![Figure 10. Incubation for 72 hours](image2)

Table 3. Methanol extract of A. cupressina L hydroid against Malassezia furfur at 48 hours and 72 hours incubation

| Treatment       | Inhibition Zone (nm) |
|-----------------|----------------------|
|                 | 48 hours | 72 hours |
| C = 5%          | 8.50      | 0        |
| D = 10%         | 9.00      | 7.50     |
| E = 20%         | 10.00     | 6.50     |
| F = 40%         | 11.00     | 9.00     |
| B = positive control | 17.00   | 14.00    |
| A = negative control | 0       | 0        |

Table 3 shows that methanol extract from A. cupressina L hydroid has antifungal activity against Malassezia furfur which is characterized by the formation of clear zones around blank discs with 48 hours and 72 hours incubation time. The results of measuring the diameter of inhibition zone are then made in the form of histograms as seen in Figure 11.
The measurement results showed that the diameter of inhibition zone methanol extract with an incubation period of 48 hours, at concentrations of 5%, 10%, 20%, and 40% having an inhibition zone diameter of 8.5 mm, 9 mm, 10 mm and 11 mm respectively and in the control positive with a diameter of 17 mm. After 72 hours incubation, the diameter of inhibition zone decreased at concentrations of 10%, 20%, and 40% and positive controls, with diameters formed 7.5 mm, 6.5 mm, 9 mm and 14 mm, while at a concentration of 5%, no clear zone is formed.

Generally the diameter of the inhibition zone tends to increase in proportion to the increase in extract concentration but in fact from the histogram data above there is a decrease in the area of the inhibition zone when concentration increases. This is presumably due to a decrease in the solubility of the active substance (saturated) so that it can affect the absorption of active substances through blank discs, and affect the ability to diffuse extracts into the agar media, further affecting the killing power and resistance of these fungi [20]. Increased concentration causes the viscosity of the extract solution to increase, thus affecting the speed of diffusion, and influencing the solubility of antifungal active substances which results in the extract experiencing a decrease or saturation which affects the ability to inhibit fungal growth [21].

Positive control using ketoconazole on test fungi because it has been proven effective in inhibiting fungal growth. The research of [22] states that ketoconazole has both systemic and non-systemic antifungal activity, which can cause irregularities in the fungal cytoplasmic membrane. According to [23] ketoconazole and fungal membrane components can form hydrophobic interactions, change membrane permeability and transport functions of essential compounds, causing metabolic imbalances that inhibit growth or cause cell death and also inhibit ergosterol biosynthesis in fungal cells.

Figure 11. Histogram of inhibition zone diameter to methanol extract from A. cupressina L. hydroid to Malassezia furfur at 48 hours and 72 hours incubation
3.2.2. *Candida albicans*

**Table 4.** Methanol extract of *A. cupressina* L. hydroid against *Candida albicans* at 48 hours and 72 hours incubation

| Treatment       | Inhibition Zone (nm) |
|-----------------|----------------------|
|                 | 48 hours | 72 hours |
| C = 5%          | 9.00     | 9.50     |
| D = 10%         | 10.00    | 10.01    |
| E = 20%         | 8.25     | 8.00     |
| F = 40%         | 10.75    | 9.75     |
| B = positive control | 14.75   | 32.50    |
| A = negative control | 0       | 0        |

Table 4 shows the results of the obstacle zone at 48 hours and 72 hours incubation, with different sizes at each concentration (5%, 10%, 20% and 40%). The results of measuring the diameter of inhibition zone are then made in the form of histograms as seen in Figure 14.

**Figure 14.** Histogram of inhibition zone diameter to methanol extract from *A. cupressina* L. hydroid to *Candida albicans* at 48 hours and 72 hours incubation
Figure 14 shows the incubation time of 48 hours to 72 hours, at concentrations of 5% and 10%, the diameter of the inhibition zone has increased (9-9.5, 10-10.01) which is fungicidal. While the concentration of 20% and 40% has decreased (fungistatic), the inhibitory zone area becomes (8.25-8.00 mm, and 10.75-9.25 mm). The results of positive control measurements using ketoconazole were (14.75 mm –32.5 mm). The increase in activity at a concentration of 5% and 10% is probably due to the amount of substances in hydroid methanol extract that is dissolved optimally so that the diffusion process to the media is also maximal. This causes the power to kill and inhibition of hydroid methanol extract to the Candida ablicans test fungus increases. The decrease in activity at concentrations of 20 & 40% is probably due to a decrease in solubility of active substances which can affect the absorption of active substances through paper disc, which in turn affects the ability to diffuse extracts into the media, marked by decreased antifungal activity [24].

4. Conclusion
Methanol extract from the hydroid A. cupressina L. is bacteriocidal towards the growth of Shigella sp. Bacteria, while the bacteria Meticillin Resistant Staphylococcus aureus continues to be bacteriostatic in concentrations of 5%, 10%, and 40%, and bactericidal at a concentration of 20%. Methanol extract from the hydroid A. cupressina L. is fungistatic against the fungus Malassezia furfur, while the fungi Candida albicans at a concentration of 5% and 10% are fungicidal, and are fungistatic at concentrations of 20% and 40%.

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