

Antibacterial Candidate Of Chaetoceros Calcitrans Against Aeromonas Salmonicida Bacteria Based In Vitro

Febby Hadi Setyawan, Sri Andayani, Maftuch

Abstract: Fish disease is an adverse thing in aquaculture's activities. One of the most harmful fish diseases is the bacterial infection of *Aeromonas salmonicida*. This bacterium can cause furunculosis. Control of bacterial diseases usually uses antibiotics, therefore we need another alternative, namely to use antibacterial from *Chaetoceros calcitrans*. The results showed that this chaetoceros has antibacterial namely terpenoids. MIC results showed that with a dose of 0.1 ppm chaetoceros extract was able to inhibit bacterial growth. From the results the disc diffusion test showed 45.3 ppm has the largest inhibitory zone against *A. salmonicida* with an average of 11.4 mm which is included in the strong category.

Index Terms: Antibacterial, *Aeromonas salmonicida*, *Chaetoceros calcitrans*, MIC, Disk diffusion

1. INTRODUCTION

Aquaculture activities are very potential activities in the community. This activity has the potential to improve the community economy, open up employment opportunities and opportunities for investment [1]. However, there are challenges in aquaculture activities, namely the decline in production due to disease. This fish disease can cause economic losses. This disease is usually caused by several types of pathogens such as viruses, parasites, fungi, and bacteria [2]. One of the fish diseases that can harm cultivation activities is a bacterial infection of the genus *Aeromonas*, with species of *Aeromonas salmonicida* [3]. *A. salmonicida* is a pathogenic bacterium in fish. These bacteria are categorized in quarantine fish pests and diseases written in the Ministry of Maritime Affairs and Culture Regulation No. 91 of 2018 [4]. *A. salmonicida* is a gram-negative and rod-shaped bacterium and is facultative anaerobic [5]. *A. salmonicida* is a bacterium that can cause furunculosis. Fish infected with this bacterium will experience skin sores, damage to the gills, hemorrhage, acute bleeding, and ulcers [6]. Treatment in bacterial infections usually uses antibiotics, but these antibiotics can cause negative impacts such as residues on the body of the fish and bacterial resistance [7]. Another alternative to reducing the use of antibiotics is to use natural antibacterial. *Chaetoceros calcitrans* is plankton that has high nutrition and antibacterial compounds such as terpenoids, flavonoids, tannins, and steroids, so it can be used as an antibacterial candidate [8,9]. Therefore, the aim of this study is to analyze the potential of *C. calcitrans* in inhibiting the growth of *A. salmonicida* bacteria.

2 MATERIAL AND METHOD

2.1 Microalgae Extraction

C. calcitrans powder were collected from Brackishwater Aquaculture Center (BBAP) Situbondo, East Java, Indonesia.

- Febby Hadi Setyawan is a student of masters degree program in Faculty of Fisheries and Marine Science, University of Brawijaya, Malang, East Java, Indonesia E-mail: febyhadi4@gmail.com
- Sri Andayani and Maftuch as lecturer in Faculty of Fisheries and Marine Science, University of Brawijaya, Malang, East Java

[10] *C. calcitrans* powder (100g) macerated with ration of 1:5 (w/v), with 500 ml Etanol (polar) for 3 days. After 3 days, the filtering was done using filter paper. Then, the filtrate was evaporated using a rotary vacuum evaporator with a temperature of 40°C and a rotation speed of 80 rpm. the extract obtained in the form of paste is stored in a small container and put in the refrigerator.

2.2 Phytochemical analysis

The phytochemical analysis was carried out in accordance with Simaremare method [11]. It was aimed to observe the active compound in crude extract. Compounds were analyzed include: flavonoids, alkaloids, phenolic, steroids, tannins and terpenoid.

2.3 Bacterial Preparation

Bacteria *A. salmonicida* obtained from the fish quarantine standard test center (BUSKIPM) Jakarta. Bacteria were grown on TSA (Trypticase Soy Agar) media and cultured on TSB (Trypticase Soy Broth) media with a density of 10⁷.

2.4 Minimum Inhibition Concentration (MIC)

This antibacterial test includes the Minimum Inhibition Concentration test and the disk diffusion test. According to [7], MIC is the lowest concentration of antibacterial ingredients that can inhibit growth. MIC testing method is done by liquid dilution method. This MIC test refers to the research of [12] with modified, MIC can be done using a sterile TSB which is inserted in a test tube as much as 5 ml. Then coarse extract of *C. calcitrans* plankton is given a test tube containing TSB with different doses in each tube. Doses used in the MIC test are 1000 ppm, 100 ppm, 10 ppm, 1 ppm, 0.1 and 0.01 ppm. Positive control was given synthetic antibacterial (Chloramphenicol) 5 ppm and negative control without administration of extract. Then each test tube was given 100 µl bacterial isolates, then incubated at 32°C for 24 hours. The media was checked for turbidity and absorbance was measured using a spectrophotometer with a wavelength of 570 nm and the MIC test was also based on the indicator of turbidity on TSB media that had been planted with bacteria and extracts with different doses and incubated for 24 hours compared to the control tube.

2.5 Disk Diffusion Test

Disk diffusion test was carried out to determine the inhibition of crude extract of *C. calcitrans* which was seen from the clear zone around the disc paper. The disk diffusion test is carried out by immersing the disc in a solvent containing an extract based on MIC test results. Then the disc is placed on the media which has been inoculated by bacteria by the scatter method. Then, incubated at 30 for 24 hours. after 24 hours a clear zone was observed and the inhibition response was classified. Classified of inhibition zone conducted by [13], <5mm (weak), 5-10mm (medium), >10-20 mm strong and >20-30mm (very strong).

3 RESULT AND DISCUSSION

3.1 Yield of Microalgae Extraction

Maceration using the ratio of simplicis and solvent (1: 5) with maceration time for 3 days obtained crude extracts after being evaporated with an evaporator speed of 80 rpm with a temperature of 40°C obtained crude extracts with 1.5 gram ethanol solvent. The yield of this crude extract is then calculated for its reduction of simplicia by calculating the final weight of the extract compared to the weight of the initial simplicia. the results obtained from the extraction process with ethanol solvent get a yield of 75% (Table 1).

TABLE 1
YIELD OF MICROALGAE EXTRACTION

Extract	Dry Weight	Extract Weight	Rendement
Etanol	200 gram	1.5 gram	0,75 %

Yield uses percent (%) units. The higher the yield value produced indicates the extract value produced more and more. The quality of the extract produced is usually inversely proportional to the amount of rendamen produced. The higher the rendered value produced, the lower the quality obtained [14].

3.2 Phytochemical Analysis

Phytochemical tests on *Chaetoceros calcitrans* were tested using powder, then reacted using reagents to form different colors according to the indicator (Table 2). Phytochemical screening results showed that the positive contained triterpenoids, marked by the formation of a brownish ring in the test solution after adding 2 ml of concentrated sulfuric acid through the tube wall. Several studies have shown that contain secondary metabolites of triterpenoids showing that these compounds have antibacterial activity, namely monoterpenoid linalool, diterpenoid, phytol, triterpenoid saponin, triterpenoid glycoside [15].

TABLE 2
PHYTOCHEMICAL TEST RESULT

No.	Identifikasi Senyawa	Parameter	Hasil
1.	Flavonoid	Brick Red, Pink, Dark Red	Negatif
2.	Tanin	Blackish Green, Blackish Blue	Negatif
3.	Terpenoid		
	• Steroid	Bluish Green	Negatif
	• Triterpenoid	Orange, Brownish Orange	Positif
4.	Fenol	Blackish Green, Blackish Blue	Negatif

3.3 Minimum Inhibiton Concentration (MIC)

MIC test using a dose of 0.01 ppm, 0.1 ppm, 1 ppm, 10 ppm, 100 ppm and 1000 ppm of the dose seen a clear tube and approaching positive control as the MIC value. The results of the mic test are presented in Table 3.

TABLE 3
MIC TEST RESULT

No.	Concentration (ppm)	Absorbance	Colour
1	0.01	0,3310	clear
2	0.1	0,3350	clear
3	1	0,5820	turbid
4	10	0,7952	turbid
5	100	0,9310	turbid
6	1000	1.0080	turbid
8	K(+)	0,2970	clear
9	K(-)	1,0690	turbid

The results above indicate that a dose of 0.1 ppm can inhibit the bacteria *A. salmonicida*, because the dose approaches the positive control value and the choice of the dose because it is an effective dose because the bacteria *A. salmonicida* is a sensitive bacterium and is included in the HPIK bacteria (Pests and Quarantine Fish Diseases). MIC test results by looking at the change in clear conditions for the first time and measuring the absorbance value using a spectrophotometer then the value used as a MIC dose is 0.1 ppm because it has a clear color and approaches positive control. The result is because *C. calcitrans* extract has antibacterial compounds, so it can inhibit the growth of *A. salmonicida* bacteria and cause the media in the tube to become clear. According to [10], *Chaetoceros* is a natural food that can inhibit the growth of some pathogenic bacteria. According to [16], *C. calcitrans* has antibacterial compounds in the form of terpenoid compounds which can inhibit bacterial growth.

3.4 Disk Diffusion Test

To find out the inhibitory ability of *C. calcitrans* extract, it is needed an inhibitory test using disc paper. According to [17], this disk test is the most widely used method for determining bacterial sensitivity to various drugs. Barriers will be seen as areas that do not show the growth of bacteria around the paper disk. The results showed that treatment D with a dose of 45.3 ppm had the largest inhibitory zone in inhibiting the growth of *A. salmonicida* bacteria and the treatment with the smallest inhibitory zone was in treatment A with a dose of 0.3 ppm (Fig. 1).

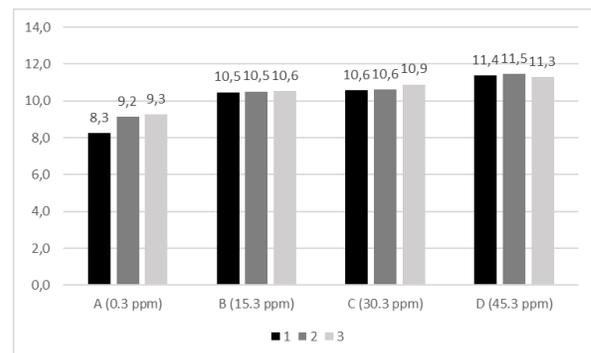


FIG 1. PRESENTATION OF TOTAL WHITE BLOOD CELLS

MIC (Minimum Inhibition Concentration) and disc tests that have been carried out show that the extract of *C. calcitrans* can inhibit the growth of *A. salmonicida* bacteria, it can be

seen from the inhibited zone formed. The bioactive compound is also very important in inhibiting the growth of *A. salmonicida* is a terpenoid compound. The antibacterial mechanism of terpenoid compounds is to damage the structure of the cell wall and interfere with the work of active transport and ionic strength in the cytoplasmic membrane of bacteria. Furthermore, these compounds will denature and activate proteins such as enzymes that affect cell metabolism [18].

3 CONCLUSION

Based on the results of research on the inhibitory test *Chaetoceros calcitrans* against *Aeromonas salmonicida* bacteria can be concluded as follows:

- *C. calcitrans* extract can inhibit the bacteria *A. salmonicida* and is bacteriostatic (can only inhibit without killing bacteria)
- The best dose is treatment D with a dose of 45.3 ppm with an average inhibition zone value of 11.4 mm and included in the strong category.

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