ANTIMICROBIAL ACTIVITY OF GALANGAL EXTRACT TO AGAINST *Aeromonas hydrophila* WHICH ATTACKED SANGKURIANG CATFISH

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ABSTRACT

Motile Aeromonas Septicemia (MAS) is a freshwater fish disease which is often the cause of aquaculture failure. Treatment of MAS is usually done by giving antibiotics, but long-term use of antibiotics can cause residues in fish. Galangal is a natural ingredient that can be utilized because it contains active ingredients such as flavonoids, essential oils, tannins and saponins which are antibacterial. The method used is experimental with Completely randomized design (CRD) used six treatments and three repetitions for the in vitro test while for the in vivo test used six treatments and two repetitions. The in vitro test treatment was carried out at a concentration of 250 mg/L (A), 500 mg/L (B), 750 mg/L (C), 1000 mg/L (D) and 1250 mg/L (E) while the in vivo test was carried out at a concentration of 0 mg/L (A), 250 mg/L (B), 500 mg/L (C), 750 mg/L (D), 1000 mg/L (E) and 1250 mg/L (F). The bacteria used was Aeromonas hydrophila with a density of 108 CFU/mL. The sangkuriang catfish used for in vivo tests was 5-7 cm in size. Parameters observed were the diameter of the inhibition zone and fish mortality (LC_{50} 24 hours). Inhibition zone data were analyzed using the F test and 24-hour LC_{50} data were analyzed descriptively. The results showed that galangal extract at a concentration of 1250 mg/L could inhibit the growth of Aeromonas hydrophila in the strong category with an average inhibition zone diameter of 11.11 mm and a 24-hour LC_{50} value obtained at a concentration of 760.29 mg/L The conclusion of this study is that galangal extract can be used to treat catfish infected with Aeromonas hydrophila with concentrations below 760.29 mg/L.

Keyword : - *Aeromonas hydrophila, Galangal Extract, In Vitro Test, In Vivo Test, Sangkuriang Catfish*

1. INTRODUCTION

Fishery is one sector that is expected to realize the welfare of the community and fisheries. The aquaculture sector is a sector that can be relied upon in building and shaping prosperity for Indonesia and the people in it. Catfish is a fishery commodity that is widely cultivated by fish farmers and the general public. The type of catfish that is widely cultivated in West Java, such as in the Depok, Bogor and Sumedang areas is the sangkuriang catfish (*Clarias gariepinus*) (Kiagus, 2018). National catfish production continues to increase from year to year, in 2010 catfish production in Indonesia amounted to 242 tons, this figure continued to increase in 2014 production reached 679 tons and until 2020 national catfish production reached 993,768.29 tons. (Statistics-KKP, 2020). One of the diseases that often attacks cultivated fish including catfish is MAS (*Motile Aeromonas Septicamea*) which is caused by infection with the *Aeromonas hydrophila* bacteria. According to research conducted by Agustini (2014), this disease attack causes the death of fish fry up to 90%. As a result of the high mortality rate of fry due to *Aeromonas hydrophila*, it is necessary to carry out countermeasures. In general, cultivators deal with sick fish due to bacterial attack by using antibiotics such as oxytetracycline and ampicillin (Arifin et al., 2013). According to Octaviana (2017), the use of antibiotics can have negative effects, including causing residues in fish and can endanger human health if humans consume them. Therefore, it is necessary to use alternative medicinal ingredients that are safer for fish and the environment. According to Sari et al., (2017), galangal extract can treat tilapia infected with *Aeromonas hydrophila*.

The content of galangal compounds consists of flavonoids, essential oils, tannins and saponins which can act as antibacterial. However, the use of galangal extract to inhibit the growth of *Aeromonas hydrophila* bacteria and its toxicity to catfish fry is unknown, so the purpose of this study was to find the concentration of galangal extract that could inhibit the growth of *Aeromonas hydrophila* and obtain a concentration that caused 50% death (LC_{50} 24 hours) in catfish.

2. RESEARCH METHOD

2.1 Material Tools

The tools used for the *in vitro test* include autoclaves, digital scales, hot plates, magnetic stirrers, petri dishes, loop needles, 15 paper discs, bunsen burners, erlenmeyer flasks, incubators, calipers, laminar air flow, precision micro pipettes 10 – 100 µL, 6 test tubes, cotton, gauze and heat-resistant plastic while the tools used for the *in vivo* test include 12 aquariums measuring 40 x 30 x 30 cm^3, fiber tubs with a volume of 300 L, aerators, plastic hoses , measuring cups, digital scales and scoops. The materials used for the *in vitro* test included pure isolates of *Aeromonas hydrophila* bacteria as much as 1 culture tube obtained from the Depok Fish Disease Control Research and Development Installation, nutrient agar (NA), 0.9% physiological NaCl and distilled water. *In vivo* included 120 sangkuriang catfish fry obtained from farmers in Cileunyi, Bandung, 20 kg of galangal dried for 14 days then macerated using 20 L of 96% ethanol for 3 days and obtained galangal extract as much as 297.02 gr and *Aeromonas hydrophila* bacteria with a density of 10^8 CFU/mL.

2.2 Method

The method used was experimental with a completely randomized design (CRD) using six treatments and three repetitions for the *in vitro* test while the *in vivo* test used six treatments and two repetitions. The treatments used for in vitro and in vivo tests were galangal extract concentrations of 0 mg/L, 250 mg/L, 500 mg/L, 750 mg/L, 1000 mg/L and 1250 mg/L.

2.3 Procedure

The zone of inhibition test was carried out to determine the ability of galangal extract as an antibacterial in inhibiting the metabolism of *Aeromonas hydrophila*. This test used the agar plate diffusion method with six concentrations of galangal extract, namely 0 mg/L, 250 mg/L, 500 mg/L, 750 mg/L, 1000 mg/L and 1250 mg/L with three replications. Treatment 0 mg/L (control) using distilled water. Materials and equipment are sterilized using an autoclave. Paper discs that had been soaked with galangal extract were put into a petri dish containing NA media and 1 mL of *Aeromonas hydrophila* inoculation (bacterial density was 10^8 CFU/mL) then incubated for 24 hours at 30°C. The diameter of the inhibition zone produced in the form of a clear zone in this test was then measured using a digital caliper. The 24-hour LC_{50} test (Lethal Concentration 50%) was carried out to determine the maximum limit for the use of galangal extract which resulted in 50% mortality in sangkuriang catfish fry after soaking for 24 hours. The treatment used in the LC_{50} test was immersing fish in a solution of galangal extract with concentrations of 0 mg/L, 250 mg/L, 500 mg/L, 750 mg/L, 1000 mg/L and 1250 mg/L for two repetitions. Prior to the LC_{50} test, catfish fry were adapted for three days in a fiber tub. Galangal extract was put into the aquarium according to the treatment. Fish were randomly added to all 10 aquariums each. LC_{50} test values were analyzed using SPSS software.

2.3 Data analysis

Inhibition zone test data were analyzed using the F test. If there was a difference between the treatments, it was continued with Duncan's multiple range test with a 95% confidence level. 24-hour LC_{50} data were analyzed descriptively.

3. RESULTS AND DISCUSSION

3.1 Inhibition Zone Test (*In Vitro* Test)

Test the antibacterial activity of galangal extract using concentrations of 250 mg/L, 500 mg/L, 750 mg/L, 1000 mg/L and 1250 mg/L. Based on observations, the greater the concentration, the greater the inhibition zone formed (Fig -1).

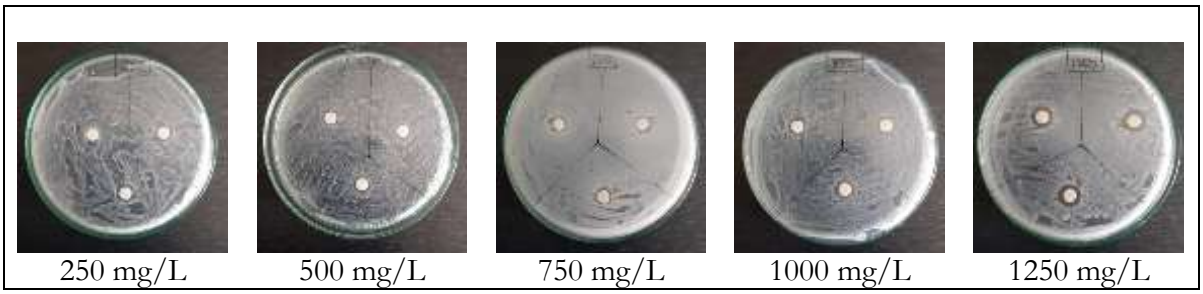

Fig -1 : Test the zone of inhibition of *Aeromonas hydrophila* bacteria

The results of the zone of inhibition test (*in vitro*) showed that galangal extract at a concentration of 1250 mg/L (treatment E) had the largest average zone of inhibition, 11.11 mm, while at a concentration of 250 mg/L (treatment A), the average result of the zone of inhibition was the smallest 6.80 mm. Treatment E was significantly different from all replicates, but treatment C was not significantly different from treatment D and treatment B (Table -1). Galangal extract with a concentration of 1250 mg/L belongs to a relatively strong category. According to Dindayani (2021), the classification of the inhibitory response is in a weak category if the clear zone diameter is ≤ 5 mm, medium category if the clear zone diameter is 5–10 mm, strong category if the clear zone diameter is 10-20 mm and very strong if the clear zone diameter is ≥ 20 mm. The ability of galangal extract to inhibit the growth of *Aeromonas hydrophila* is because it contains antibacterial compounds, one of which is flavonoids. The content of flavonoids in galangal extract can prevent the release of toxins by *Aeromonas hydrophila* bacteria. In the opinion of Rahmadona et al., (2020), the mechanism of action of flavonoids is by denaturing cell proteins in bacteria and forming complex compounds against extracellular proteins which will disrupt the permeability of bacterial cells, cell membranes will be damaged irreparably. Apart from flavonoids, saponins and tannins also contribute to inhibiting the growth of *Aeromonas hydrophila*. Saponins are active compounds that have the ability to damage membrane permeability, causing the bacterial cell wall to be destroyed (Vieira, et al., 2001). The mechanism of action of saponins is by interfering with the stability of the bacterial cell membrane, causing bacterial cell lysis (Zahro & Agustini, 2013). Tannin is a compound that has antibacterial and antioxidant properties. The mechanism of action of tannins is to precipitate bacterial proteins resulting in inactivation of bacterial enzymes and inactivation of cell wall proteins (Naim, 2004). According to Zahro & Agustini (2013), tannins have astringent properties (substances that can shrink). The mechanism of action of tannins is to damage cell membranes by binding to metal ions such as Cu and Fe. Tannins at low concentrations can inhibit bacterial growth and high concentrations can inhibit fungal growth (Apriasari et al., 2013).

Table -1: Galangal extract inhibition zone

Treatment	Inhibition zone diameter (mm) repetition-			Average
	1	2	3	
A (250 mg/L)	6.69	7.34	6.38	6.80^{d}
B (500 mg/L)	7.18	7.78	7.96	7.64^{c}
C (750 mg/L)	7.39	8.14	8.18	7.90^{bc}
D (1000 mg/L)	8.32	8.77	8.82	8.64^{b}
E (1250 mg/L)	11.59	10.99	10.74	11.11^{a}

3.2 24-hour LC_{50} test (*in vivo* test)

The results of the 24-hour LC_{50} test can be seen in Table 2. Table 2 shows that a concentration of 760.29 mg/L caused the death of more than 50% of sangkuriang catfish fry within 24 hours. Therefore, the safe concentration limit for the treatment of catfish infected with *Aeromonas hydrophila* is below the concentration of 760.29 mg/L. Data from the LC_{50} test results analyzed using SPSS software showed that a concentration of 760.29 mg/L could kill 50% of the total fish, while a concentration of 107.78 mg/L could kill 45% of the total fish (Table -2). This shows that concentrations below 760.29 mg/L are safe concentrations for treating fish. There are active compounds in galangal extract which, when administered at high concentrations, can be toxic. At high concentrations the content

of the active ingredients contained in the extract is also high. Among the active ingredients contained in galangal extract are saponins. At high concentrations saponins can be toxic to fish. According to Purbosari et al., (2022), saponins can be toxic to fish and amphibians, saponins can also kill protozoa and molluscs. This is in accordance with Pasaribu's statement (2019), saponins can be toxic or also called sapotoxins for cold-blooded animals such as fish, by destroying red blood cells through a hemolysis reaction. In the opinion of Yulistyana et al., (2020), for cold-blooded animals such as fish, saponins are toxic. Saponins will foam when shaken with water and will produce sugar and sapogenesis when hydrolyzed. Sapogenin has the property of hemolyzing blood and can irritate the digestive tract mucosa in cold-blooded animals. This is in accordance with the opinion of Sariri & Yakin (2020), saponins can hemolyze red blood cells and inhibit chymotrypsin enzymes which result in inhibition of fish productivity and growth. The same thing was stated by Yudhistira et al., (2020), fish will experience nervous disorders and lose balance because saponin metabolite compounds inhibit the binding of oxygen to the gill filaments.

Table -2 : Estimation of LC/EC values and 95% confidence limits of galangal extract based on probit analysis

Probability		95% confidence limits for concentration
		Estimate
Probit	.300	0,219
	.350	1,90
	.400	14,81
	.450	107,78
	.500	760,29
	.550	5363,04
	.600	39039,07
	.650	303778,16
	.700	2639890.54

4. CONCLUSIONS

Galangal extract at a concentration of 1250 mg/L was able to inhibit the growth of *Aeromonas hydrophila* bacteria in the strong category with an average diameter of the inhibition zone of 11.11 mm and the 24 hour LC_{50} test obtained a concentration value of 760.29 mg/L. Galangal extract can be used to treat catfish that infect *Aeromonas hydrophila* with concentrations below 760.29 mg/L.

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BIOGRAPHIES

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