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Antimicrobial activity of pandanus leaves extract to against *Aeromonas hydrophila* which attacked catfish

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Abstract
This study aims to find the concentration of pandanus (*Pandanus amaryllifolius)* leaves extract that can inhibit the growth of *Aeromonas hydrophllia* (*in vitro* test) and LC_{50}-24 hour pandan leaves extract against sangkuriang catfish fry (*in vivo test*). The research method used was experimental with a completely randomized design (CRD), using five treatments and three replications for the in vitro test, while for the in vivo test six treatments and two replications. The treatment for the in vitro test was pandanus leaves extract with a concentration of A. 600 ppm, B 800 ppm, C 1000 ppm and D 1200 ppm. While the treatment for the *in vivo test* was A. 0 ppm, B. 10 ppm, C. 100 ppm, D. 500 ppm, E.1000 ppm and F. 3000 ppm. *A. hydrophilla* bacteria used with a density of 10^8 CFU / mL Sangkuriang catfish used for the *in vivo test* measuring 5-7 cm. The 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 the 24-hour LC_{50} test dat1. a were analyzed descriptively. The results showed that pandanus leaves extract at a concentration of 1200 ppm was able to inhibit the growth of *A. hydrophila* bacteria in the strong category with an average inhibition zone diameter of 14.30 mm and an LC_{50}-24 hour value obtained at a concentration of 1737.714 ppm. The conclusion of this study is that pandanus leaves extract can be used to treat catfish that are attacked by *A. hydrophila* with a concentration below 1737,714 ppm.

Keywords: *Aeromonas hydrophilla*, pandanus leaves extract, sangkuriang catfish, *in vitro* test, *in vivo* test

1. Introduction
Pathogenic bacterial infection is the main cause of death in aquaculture activities. There are several types of pathogenic bacteria that often attack cultured fish, including *Aeromonas hydrophila* which is a gram-negative bacteria, generally attacking freshwater fish in various stages from seed to adult. The causes of this disease is called Motil Aeromonid Septicemia (MAS) or hemorrhagic septicemia. Fish attacked by MAS disease is economically very harmfull to farmers, because it can cause up to 80-100% fish mortality (Lukistyowati & Kurniasih, 2012) [1]. According to Laith and Najiah (2013) [2], symptoms appeared after infection are increased respiration followed with pale gills, lethargic, skin lesions, discoloration, hemorrhage, and bruises or ulcers on the muscles. Some fish showed fins and genital orifice base bleeding. This infection also caused kidney dropsy, enlarged liver and bile, as well as yellowish fluid accumulation inside the body cavity. Sangkuriang catfish is one type of freshwater fish that is in great demand, because it is easy to cultivate, grows fast and is easy to adapt to bad aquatic environments. As a source of animal protein, the price of sangkuriang catfish is relatively cheap, making it affordable for various groups of people. However, the obstacle faced in catfish farming is the attack of *A. hydrophila*, especially at the fry stage, this results in inhibition of consumer demand or the market for catfish fry.
Treatment of bacterial diseases in fish using antibiotics is common, but if it used for a long time with inappropriate doses, it will have a negative impact, including bacteria becoming resistant to the antibiotics given (Kapil 2005) [3]. In addition, it can cause environmental pollution and the emergence of residues in the body and humans who consumed it (Hatha *et al*. 2005) [4]. So we need alternative medicines that are safer for both fish and the environment, namely the use of antibiotics or antibacterials derived from herbal ingredients. Several types of herbs that are known to be antibacterial and their effectiveness in treating *A. hydrophila* are neem leaves (Maragathavalli *et al*. 2012) [5], noni (Pongoh and Gemaputri 2018) [6] and mango leaves (Oti and Oze 2017) [7]. These ingredients contain flavonoids, tannins, saponins, acubins,

asperuloside and alizarin as well as anthraquinone components (Kurniasih 2013) [8].

Another herbal ingredient that has the potential as an antimicrobial is the extract of pandanus leaves, because based on the results of phytochemical tests (preliminary research results), the extract of pandanus leaves contains antibacterial compounds, namely tannins, saponins, flavonoids, monoterpenoids, sesquiterpenoids, triterpenoids, quinones and alkaloids. However, the ability to inhibit the growth of *A. hydrophila* bacteria and its toxicity to catfish fry is not yet known. So the aim of this study was to find the concentration of pandanus leaves extract that can inhibit the growth of *A. hydrophila* bacteria and obtain a lethal concentration (LC_{50} - 24 hours) from pandanus leaves extract for catfish and determine the concentration of pandanus leaves extract for the treatment of catfish infected *A. hydrophila*.

2. Materials and Methods

2.1 Materials

The materials used in this research was 2000 g of wet pandanus leaves were dried for 14 days, and obtained as much as 1000 g of dry weight, and then macerated by using 96% ethanol solution as much as 20 L for three days, obtaned the pandanus leaves extract as much as 76,61g. The experimental catfish used in this study originated from Center for Research and Development of Freshwater Aquaculture (BBPBAT) in Sukabumi, West Java, sizing of 5–7 cm in total length, as much as 120 fishes. As much as 12 aquariums sizing of 40×30×30 cm^3. Aeromonas hydrophila bacteria with a density of 10^8CFU/mL.

2.2 Methods

The research method used was experimental, specific using a Completely Randomized Design (CRD) with four treatments with three replications for inhibition test (*in vitro* test) and six treatment with three replication for LC_{50}-24 hour (*in vivo* test). The treatment for the in vitro test was to use the extract of pandanus leaves with concentration A. 600 ppm, B 800 ppm, C 1000 ppm, D 1200 ppm. While the treatment for the *in vivo test* is A. 0 ppm, B. 10 ppm, C. 100 ppm, D. 500 ppm, E.1000 ppm and F. 3000 ppm.

2.3 Procedure

Zone of inhibition test (*in vitro test*)

The zone of inhibition used to test the effectiveness of pandanus leaves extract as an antibacterial to inhibit the growth of *A. hydrophila*. The zone of inhibition was used disk diffusion test with five variances of pandanus leaves extract concentration, consisted 600 mg/L, 800 mg/L, 1000 mg/L, and 1200 mg/L for 24 hours. During the test, it only used the negative control. The material and equipment were sterilized using an autoclave. The disc paper was put to a petri dish with NA medium and 1 mL of *A. hydrophila* inoculation (the bacteria density was 10^8 CFU/mL). The petri dish then was incubated for 24 hours at 30°C. The zone of inhibition was measured by using a caliper.

LC^{50} 24 hour test

The LC_{50}-24 hours test of pandanus leaves extract was done to measure the short-term poisoning potential which causes 50% of mortality. The concentration for LC_{50}-24 hours consisted six treatments (0 mg/L, 10 mg/L, 100 mg/L, 500 mg/L, 1000 mg/L, and 3000 mg/L) with three replications.The experimental fish was acclimatized in fiber container with 100 L of water for seven days. Fish with the same weight and size transferred into an aquarium with 5 L of water and stocking density of 10 fishes/aquarium. The pandanus leaves extract added into each aquarium according to the treatments. The survival rate of fish has measured with EPA probit analysis software.

2.4 Data Analysis

Zone of inhibition test (*in vitro test*) data was analyzed with an F test at 5% level, and if there was an effect on the treatment, Duncan's Multiple Range Test was performed. LC_{50} 24 hour test data was analyzed descriptively.

3. Results and Discussion

3.1 Zone of Inhibition Test (*In vitro* Test)

The antibacterial activity test of pandanus leaves extract used several concentrations, namely 600, 800, 1000 and 1200 ppm. Based on observations, the greater the concentration, the greater the inhibition zone (Figure 1).

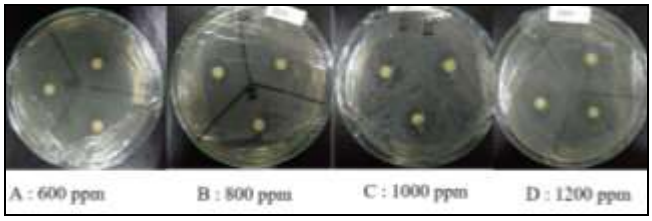

Fig 1: Inhibition test of Aeromonas hydrophila bacteria

The results of the inhibitory zone test (*in vitro*) showed that the concentration of pandanus leaves extract at 1200 ppm (treatment D) had the largest average inhibition zone of 14,3 mm, whereas at a concentration of 600 ppm (treatment A) the average smallest inhibition zone was 8.18 mm berbeda nyata dengan treatment D (Table 1). Pandanus leaves extract concentration of 1200 ppm is a relatively strong antibacterial. As opinion Susanto (2012) [9] the diameter of the inhibition zone <5 mm is categorized as low, the diameter of the inhibition zone of 5 mm – 10 mm is categorized as medium, the diameter of the inhibition zone 10 - 20 mm is categorize as strong, whereas the diameter of the inhibition zone >20 mm is categorized as very strong. The opinion of Bereksi *et al*. (2018) [10] the diameter of the inhibition zone of 20 mm is categorized as a medium inhibition zone, whereas above 20 mm is categorized as strong. According to Samsudin *et al.* (2018) [11] the diameter of inhibition zone ≤ 12 mm is categorized as low and the diameter of inhibition zone 13-14 cm is categorized as medium and the diameter of inhibition zone above 14 cm is categorized as strong in inhibiting bacterial growth.

The ability of pandanus leaves extract to inhibit the growth of *A. hydrophila* bacteria is due to the presence of secondary metabolite content including tanin, saponin, flavonoid, monoterpenoid, seskuiterpenoid, triterpenoid, kuinon dan alkaloid. Mandal (2017) [12] states the antibacterial activity of plant extracts can be attributed to the high content of phenols and flavonoids. Several studies have shown the antibacterial effect of secondary metabolites in inhibiting various types of bacteria. The mechanism of saponin in inhibiting bacterial growth by reducing surface tension, resulting in increased permeability or cell leakage and resulting in intracellular compounds to come out (Nuria *et al*. 2009) [13]. Flavonoids inhibit bacteria by forming complex compounds with extracellular and dissolved proteins so that they can damage

bacterial cell membranes and accompany the release of intracellular compounds (Cowan 1999) [14]. According to Sahu *et al.* (2013) [15] flavonoids, tannins and saponins that act as antimicrobials and have been proved to accelerate wound healing, activating macrophages, stimulating immune system as well antibacterial and antiviral effects. The content of sesquiterpenes and monoterpenes in essential oils affects the permeability and membrane activity of microbial and larvacidal proteins (Ryan and Byrne, 1988) [16]. Alkaloid compounds in inhibiting bacterial growth are by disrupting the peptidoglycan constituent components in bacterial cells so that the cell wall layer is not formed completely and causes cell death (Darsana 2012) [17]. Triterpenoids work by reacting with porin (transmembrane protein) on the outer membrane of the bacterial cell wall, forming strong polymer bonds that result in the destruction of porin (Cowan 1999) [14]. Elayaraja *et al*. (2015) [18] stated that antimicrobial activity is caused by the presence of terpenoid compounds in plant extracts that can damage the cell walls of bacteria that form complex compounds with cellular extracts, so that proteins and microbial cell walls are destroyed. Quinone as an antibacterial works by denaturing cell proteins (Chibane *et al*. 2018) [19]. The mechanism of steroid as an antibacterial is related to membrane lipids and sensitivity to steroid components that cause leakage of liposomes (Madduluri *et al*. 2013) [20].

Table 1: Inhibit Zone Pandanus leaf extract

Concentration (ppm)	Diameter zona hambat (mm) Ulangan ke			Rata-rata diameter zona hambat (mm)
	1	2	3	
600	8,88	8.94	6.73	8.18 a
800	7.28	7.81	11.54	8.87 a
1000	13.72	11.55	9.74	11.67 ab
1200	14.22	11.85	16.82	14.30 b

3.2 LC_{50} test

The result of the LC50 test showed in Table 2. It showed that as much as 1737.71 ppm of concentration caused more than 50% of mortality in Sangkuriang catfish juvenile in 24 hours, respectively. Jadi konsentrasi yang aman untuk pengobatan ikan lele yang terinfeksi *A. hydrophila* adalah di bawah 1737.71 ppm. The results of the LC50 24 h showed that the concentration of Pandanus leaf extract analyzed using EPA Probit Analysis software found a concentration 1737.71 ppm killed 50 % of total fish. While the concentration of 1121.07 ppm killed fish 15 % (Table 2). This showed that the safe concentration for disease treatment of fish was below 1737.71 ppm. Some of the active compound in Pandanus leaves extract will be toxic if the concentration is too high, especially for saponin that completely cytotoxic. In line with Septriarusli *et al.* (2012) [21], saponin is a toxin that will ruin the blood cell (hemolysis). The potential of secondary metabolites in plants as antimicrobials can be used by suppressing antimetabolic and toxic contents, one of which is to pay attention to the concentration of the plant extract (Chakraborty 2013) [22].

Table 2: Estimated LC/EC values and confidence limits base on probit analysis

Point	Exposure concentration (mm)	95% of confidence limits	
		Lower (mm)	Upper (mm)
LC/ EC 1.00	649.72	186.43	996.60
LC/ EC 5.00	866.71	341.65	1239.87
LC/ EC 10.00	1010.64	467.12	1407.91
LC/ EC 15.00	1121.07	573.06	1543.03
LC/ EC 50.00	1737.71	1205.651	2568.79
LC/ EC 85.00	2693.54	945.74	5575.00
LC/ EC 90.00	2987.84	2126.23	6862.95
LC/ EC 95.00	3484.02	2404.86	9415.84
LC/ EC 99.00	4647.58	2981.39	17315.71

4. Conclusion

1. Pandanus leaves extract at a concentration of 1200 ppm was able to inhibit the growth of *A. hydrophila* bacteria in the strong category with an average inhibition zone diameter of 14.30 mm and an LC_{50}-24 hour value obtained at a concentration of 1737.714 ppm.
2. Pandanus leaves extract can be used to treat catfish that are attacked by *A. hydrophila* with a concentration below 1737,714 ppm.

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