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Alpinia galanga Extracts for Improving Egg Hatchability and Larval Viability of Catfish

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Abstract. This research aimed to investigate the extract of the leaves and rhizomes *Alpinia galanga* as a potential compound and also to find the optimal concentration for improving egg hatchability and larval viability of catfish. Dried leaves and rhizomes of *A. galanga* were macerated and extracted with water and ethanol extracts. The extracts in concentration 600, 800 and 1,000 ppm were tested on the egg hatchability of catfish with immersion method, and challenge test with *Aeromonas hydrophyla, Pseudomonas* sp., and S*aprolegnia* spp. Next, the concentration of 800, and 1,000 ppm were tested on the larvae of catfish with immersion method and followed by challenge test with several pathogens. The result showed that water or ethanol extracts of *A. galanga* can improve egg hatchability with a value of 62.3±1.4 to 97.7±1.0 %, and larval viability of catfish of 69.4±1.0 to 90.7±2.2%. Eight hundred ppm ethanol extract of *A. galanga* rhizome has the best egg hatchability, and 1,000 ppm can improve the larval viability of catfish.

INTRODUCTION

The disease remains the major problem for fish culture, especially at the hatchery in East Kalimantan, Indonesia. Catfish are economically important aquaculture species. However, the production of catfish was threatened by diseases and especially gave severe mortalities during egg hatchability and larval stages of catfish. Generally, the pathogens that attack catfish are *Aeromonas hydrophyla*, *Pseudomonas* sp., and *Saprolegnia* spp., which usually isolated from egg, larval, and adult catfish. Among them, *A. hydrophyla* is considered as one of the most important bacterial pathogens of aquatic organism. This species causes reduce feeding, hemorrhagic, lesions, ulcer, and necrosis on the body, fin, and gills. *Pseudomonas* sp. causes ascites abdominal and cloudy of internal organs. *Saproglenia* spp. can infect the eggs of a wide variety of freshwater fish species. In fish *saprolegniasis* is associated with infections, white or grayish cotton-wool like patches found on the fin, skin, and gills of the infected host.

Some chemicals and antibiotics are often used to prevent and eradication of diseases. The formalin is widely used for treating fungal infection on fish eggs in hatchery operations to improve the hatchability and survival of larvae [1, 2]. Some of the hatcheries in East Kalimantan, Indonesia still use chemicals and antibiotics for prevention of infectious pathogens. However, these used uncontrolled and had become a new problem. The overuse of chemicals and antibiotics has become resistant and potential toxic effect on fish. The change of antibiotic-resistant pathogenic bacteria has stimulated the search for alternative antimicrobial agents from natural sources. Alternatives to the solutions to solve antibiotic resistant incident problem among pathogenic bacteria are to develop a new drug from

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natural sources such as plant [3]. For thousands of years, natural products have been used in traditional medicine all over the world and predate the introduction of antibiotic and modern drugs [4].

Alpinia galanga is used in culinary and traditional medication, especially the rhizome. It is used in dietary intake as well as in the traditional system of medicine. Earlier phytochemical analysis, the *A. galanga* contains alkaloids, saponin, glycosides, terpenoids, phenolics, flavonols, flavonoids, phytosterols, and carbohydrates [5]. The rhizome of *A. galanga* contains flavonoids, some of which have been identified as kaempferol, kaempferide, galangin, and alpinin. Galangin is a flavonoid with multiple biological activities [6]. *Alpinia galanga* is known to have antimicrobial, antioxidant, antifungal, anticancer, and gastroprotective activities [7].

Exploration of bioactive compounds from nature has received more attention from researchers as it becomes an alternative to overcome diseases [8]. This research aimed to investigate the effect of an extract of the leaves and rhizomes *A. galanga* as a potential compound and also to find the optimal concentration to improve egg hatchability and larval viability of catfish. The results of this study are expected to be used as a basis for further research, as well as an alternative to prevent and reduce the risk of microbial infection on egg and larvae of fish.

MATERIALS AND METHODS

Eggs and Larva Catfish

Brood stock of catfish from Fish Breeding Laboratory of the Fisheries and Marine Science Faculty, Mulawarman University. Each male and female broodstock of catfish was fumigated with formalin (100 ppm) for half an hour, to prevent pathogens infections. Then, the fish were acclimatized for one week. The broodstock was injected with hormone GnRH (Ovaprin) at the rate of 0.5 mL/kg of body weight. Then, female and male catfish were taken at the same pond indoor. After 12 hours they had been copulation, and then after 1-2 hours the fertilized eggs were selected and used in the test. Larval viability test used healthy and motile larva with three days old.

Alpinia galanga

A. galanga was collected from Loa Janan Kutai Kartanegara Regency, East Kalimantan Province, Indonesia. The leaves and rhizomes of *A. galanga* were washed, drained and chopped, and then dried without being exposed to direct sunlight, with a temperature of 30 ^{0}C for 18 days. Each leaf and rhizomes were macerated in two different solvents, namely water and 80 % ethanol for two days, and then the extracts were filtered three times. All concentrated were collected and then filtrated by the Rotary evaporator and evaporated with water bath [9].

Microbes

The microbes used for challenge test were *A. hydrophila*, *Pseudomonas* sp. and *Saprolegnia* spp. The microbes procured from Aquatic Microbiology Laboratory of the Fisheries and Marine Science Faculty, Mulawarman University, isolated from catfish egg. Each bacterium was cultured on Tryptone Soya Agar (TSA) at 33 °C for 24 hours and then cultured in Tryptone Soya Broth (TSB) at 33 °C for 24 hours. *Saprolegnia* spp. cultured on Potato Dextrose Agar (PDA) at pH 5.6, on 35 °C for 36 hours, then cultured on Potato Dextrose Broth (PDB) at pH 5.6, on 35 °C for 36 hours. Total bacteria or fungus concentration used for the test was 10^6 CFU/mL.

Treatment

The treatment contained four active materials with three different doses. They were ethanol extract of leaves, the ethanol extract of rhizomes, water extract of leaves, water extract of rhizome, negative control and positive control (Oxytetracycline 0.05 mg /mL). The concentration treatments of the extract were 600, 800, and 1,000 ppm, respectively. The fertilized eggs were divided into an aquarium containing 2,000 mL of water. The extracts were distributed into the aquarium, after one hour, each was challenged test with *A. hydrophyla, Pseudomonas* sp., and S*aprolegnia* spp. The treatment was repeated three times. The observation and inspection covered clinical symptom of eggs and hatchability.

Larval viability tests applied four active materials with two different doses, 800 and 1,000 ppm. The larva was taken in aquariums and treated with extracts during 30 minutes, and then after one day was subjected to challenged test with 10^6 CFU/mL dose of each disease including *A. hydrophyla, Pseudomonas* sp., and S*aprolegnia* spp. with immersion method. Larva was kept for three weeks. The observation and inspection covered clinical symptom, pathological anatomy, and viability.

RESULT AND DISCUSSIONS

The treatments of water extract and ethanol extract of *A. galanga* rhizome and leaves showed the different effect on the eggs hatchability. The egg hatchability on the ethanol extract of *A. galanga* rhizome are 76.30-97.16 %, water extract of *A. galanga* rhizome are 71.97-88.90 %, the ethanol extract of *A. galanga* leaves are 72,87-89.34 %, and water extract of *A. galanga* leaves are 62.31-78.44 %. The results of egg hatchability of catfish are presented in Table 1.

TABLE 1. The egg hatchability of catfish was treated extract of *A. galanga* and challenged by microbes

Treatments	Concentrations (ppm)	Egg Hatchability (%)		
		A. hydrophyla	***Pseudomonas* sp.**	***Saprolegnia* spp.**
Ethanol extract of rhizome	1,000	91.33±3.89	89.51±1.63	96.21±1.93
	800	89.54±3.12	82.84±2.76	97.16±0.96
	600	81.71±3.36	76.30±1.71	86.18±4.59
Ethanol extract of leaves	1,000	88.15±1.62	85.17±1.81	88.48±3.85
	800	86.49±3.94	78.82±2.62	89.34±3.01
	600	78.86±0,98	72.87±1.13	79.20±3.87
Water extract of rhizome	1,000	85.36±1.35	82.68±4.11	88.90±1.37
	800	81.47±4.30	78.55±3.06	80.73±4.66
	600	76.57±3.25	71.97±1.80	75.91±2.25
Water extract of leaves	1,000	78.44±5.24	71.53±0.56	75.84±0.77
	800	72.45±5.01	68.13±5.63	68.86±3.55
	600	67.17±0.87	62.31±1.43	64.78±2.75
Control +		85.04±6.15	84.80±4.96	82.38±6.82
Control -		58.41±1.77	60.19±8.49	52.63±2.98

The larval viability of catfish on ethanol extract of *A. galanga* rhizomes was 81.70-90.73 %, water extract of *A. galanga* rhizome was 79.41-83.42 %, the ethanol extract of *A. galanga* leaves was 70,56-78.33 %, and water extract of *A. galanga* leaves was 69.41-74.33 %. The results of the larval viability of catfish are shown in Table 2.

TABLE 2. Larval viability of catfish was treated extract of *A. galanga* and challenged by microbes

Treatments	Concentrations (ppm)	Larval Viability (%)		
		A. hydrophyla	***Pseudomonas* sp.**	***Saprolegnia* spp.**
Ethanol extract of rhizome	1,000	88.29±1.51	84.51±5.42	90.73±2.20
	800	87.33±3.51	81.70±3.02	89.79±2.32
Ethanol extract of leaves	1,000	77.33±4.04	74.79±4.51	78.33±1.53
	800	74.33±3.21	70.56±4.86	73.67±3.06
Water extract of rhizome	1,000	83.37±2.72	81.07±3.54	83.42±3.18
	800	81.77±1.08	79.41±1.03	80.45±1.72
Water extract of leaves	1,000	73.67±2.08	72.69±2.93	74.33±3.79
	800	70.33±0.58	69.41±1.01	72.00±2.65
Control +		86.00±2.73	85.29±4.50	79.67±2.52
Control -		61.15±6.36	60.67±1.15	54.59±3.68

The prevention and control of diseases are now the priority for durability of aquaculture. Therefore, it has to start from early stages of the catfish cycle including egg hatchability and larval stages. This is expected can prevent the transfer of pathogens to fry and at the end it helps reducing mortality. The observation of clinical symptom after the extract treatment, and before challenge test showed that the treatment was safe and did not cause specific clinical symptoms on eggs. After challenge test, some eggs changed in color to dense white. It indicates the eggs reaction

against pathogens so that the eggs do not hatch. Observation with a microscope, the surface of dead eggs becomes opaque, whitish, dirty and dark inside. The isolation and identification of microbes on the dead eggs were obtained microbe of challenge test. The external surface of the fish egg is easily colonized by bacteria, such as *Flavobacterium* sp., *Pseudomonas* sp., *Aeromonas* sp., and *Vibrio* sp. [10].

The fertile of eggs could hatch in 28 until 48 hours after extract treatments. In the same extract, the best egg hatchability is the eggs challenged *A. hydrophyla*, following *Saprolegnia* and *Pseudomonas*. But in the extract leaves, the best egg hatchability is the eggs challenged *Saprolegnia* Spp., following *A. hydrophyla* and *Pseudomonas*. These results show that rhizome extract of *A. galanga* is the most effective for reducing *A. hydrophyla* infections on the eggs, and the rhizome and leaf extract are effective for *Saprolegnia* Spp. *A. galanga to* have shown pronounced inhibitory activities against a wide variety of human pathogenic fungi [11]. The eggshell protects the embryo from the entry of pathogens. The hypha of *saprolegnia* can cover on the eggs surface area. The infection originates from unfertilized or dead eggs. Hyphal infection can spread quickly between eggs. Some *Saprolegnia* species can infect the eggs of a wide variety of freshwater fish species. The dead eggs can serve as a substrate for zoospore colonization, from where mycelia mats engulf and kill nearby eggs or hyphal infection [12].

In the same extract, the best larval viability is larvae of challenged *Saprolegnia* Spp., following *A. hydrophyla* and *Pseudomonas*. *A. galanga* extract more effective to prevent *Saprolegnia* than bacteria on the larvae. The larval viability of catfish was challenged *A. hydrophyla* highest than challenged *Pseudomonas*. The best larval viability of catfish is ethanol extract of *A. galanga* rhizome, following water extract of *A. galanga* rhizome, the ethanol extract of *A. galanga* leaves, and water extract of *A. galanga* leaves. The ethanol extract of *A. galanga* rhizome has inhibition zones to *A. hydrophyla* 12.33 mm*, Pseudomonas* sp. 12.00 mm, and S*aprolegnia* spp. 12.67 mm [13], and ethanol extract of *A. galanga* showed the strongest inhibitory effect against *Streptococcus aereus* [14]. The observation of clinical symptom after the conducting of extract, before challenge test, showed that the treatment was safe and did not cause death and specific clinical symptoms. The observation of pathology anatomy performed on negative control after infected by microbes revealed that the larva on seemed decline and lethargic, haemorhagi on the head, deformity of the tail. The *saprolegnia* infected can make the larva white or grayish cotton-wool like patches are found on the skin and tail.

The rhizome extract of *A. galanga* effective to protect microbial infection on the catfish larva than leave extract of *A. galanga*. Fungal infections on eggs cause disease problem which resulted in egg mortality reduces hatching of fertilized eggs and survival of larva [15]. *A. galanga* has been used as antiviral, antibacterial, anti fungal, antiamoebic, antioxidant, and gastroprotective activity [6,16]. Aqueous extract of *A. galanga* showed activity against *Klebsiella pneumonia*, *Escherichia Coli*, *Pseudomonas aeruginosin*, *S. Aureus*, and *Streptococcus pyogenes* except *Staphylococcus epidermidis* [17]. The rhizome of *A. galanga* is deposited product of metabolism of the plant. The secondary metabolites content of *A. galanga* rhizome more than the leaves. The bioactive of the plant is a product of the secondary metabolites of the plant. The bioactive of the plant have been used food and as a source of medicine. The bio-medicinal product of herbal has been used an alternative on aquaculture for growth promoting ability, tonic, and immunostimulant [18].

CONCLUSION

Both water and ethanol extracts of *A. galanga* can improve egg hatchability 62-97 %, and larval viability of catfish 69- 91%. The best extract for improving egg hatchability and larval viability were ethanol extract of *A. galanga* rhizome, followed by the water extract of *A. galanga* rhizome, the ethanol extract of *A. galanga* leaves, and water extract of *A. galanga* leaves, respectively. Eight hundred ppm ethanol extract of *A. galanga* rhizome has the best egg hatchability and 1,000 ppm can improve the larval viability of catfish. This study showed that *A. galanga* has the antimicrobial activity, and can be used as an alternative to protect the eggs and larvae of fish from pathogen infection.

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