



International Journal of Fisheries and Aquatic Studies

E-ISSN: 2347-5129

P-ISSN: 2394-0506

(ICV-Poland) Impact Value: 5.62

(GIF) Impact Factor: 0.549

IJFAS 2017; 5(6): 236-241

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www.fisheriesjournal.com

Received: 01-09-2017

Accepted: 04-10-2017

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Effectivity of sambiloto extract as medicine for catfish (*Pangasius hypophthalmus*) juveniles infected by *Aeromonas hydrophila*

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Abstract

This study aims to obtain the concentration of the most effective sambiloto extract to treat the infected catfish juveniles of *Aeromonas hydrophila*, so as to produce the highest survival rate of catfish juvenile. The research method was conducted experimentally using Completely Randomized Design (RAL) consisting of five treatments and three replications. The used treatment was soaking Siamese catfish juvenile for 24 hours in the sambiloto extract at concentrations of A (0 ppm), B (50 ppm), C (75 ppm), D (100 ppm), E (125 ppm). Test fish infected by *A. hydrophila* bacteria as much as 2 ml of NaCl / L air at concentration 10^8 CFU / mL with cohabitation method. The parameters observed were clinical symptoms, recovery process, survival rate and water quality. The results showed that the sambiloto extract was effective to treat Siamese catfish juvenile that were infected by *A. Hydrophila*, 75 ppm concentration produced the fastest recovery process that started from day 7 and the highest survival rate of 86.67%. Based on regression analysis of sambiloto extract giving effect to 91, 82% and optimum concentration to treat Siamese catfish juvenile infected by *A. hydrophila* bacteria is 84, 74 ppm.

Keywords: Sambiloto, siamese catfish juvenile, *Aeromonas hydrophila*

Introduction

Catfish is one of the important freshwater fish consumption commodities with significant economic value, the production of catfish shows a significant increase. In 2012, catfish production reaches 651,000 tons per year and in 2013 it increases to 972,778 tons per year [3]. Diseases that often attack the catfish juvenile are *A. hydrophila* bacteria that cause red spots. Treatment by way of antibiotics is often done, but this treatment can cause bacteria to become resistant and increase residuals in fish [8].

There should be a safer alternative medicine and can be used in disease control. The alternative is to use herbs of antibiotics with antibacterial properties. In sambiloto found chemicals such as *andrographolide* known as anti-bacterial [6], but there is no proper concentration in sambiloto that can treat catfish juvenile infected by *A. hydrophila*, so it is necessary to do a research on the effectiveness of sambiloto extract for treatment of catfish juvenile infected by *A. hydrophila*.

This study aims to obtain the most effective concentration of sambiloto extract in treating the infected catfish juvenile of *A. hydrophila*, so as to produce the highest survival rate of catfish juvenile.

Materials and Methods

Tool and materials used were test tube, measuring glass, ose needle, erlenmeyer, aquarium, sambiloto extract, *Aeromonas hydrophila* bacteria, 300 catfish juvenile (*Pangasius hypophthalmus*) size 5-7 cm with 20 fish/aquarium.

The research method was conducted experimentally using Completely Randomized Design (RAL) consisting of five treatments and three replications. The general model of the design used is the linear model, as follows:

$$Y_{ij} = \mu_i + \tau_i + \varepsilon_{ij}$$

The treatments in this study were:

- Treatment A: Without immersion of 0 ppm extract (control)
- Treatment B: Sambilotto extract concentration 50 ppm
- Treatment C: Sambilotto extract concentration 75 ppm
- Treatment D: Sambilotto extract concentration 100 ppm
- Treatment E: Sambilotto extract concentration 125 ppm

Research Procedure

Sambilotto extract made by macerating the dry sambilotto with 96% ethanol for 24 hours, then filtered by Whatman filter paper no. 42 . The result of maserated sambilotto was evaporated using a rotatory evaporator with temperature of 60°C to obtain a sambilotto extract.

Isolates of *A. hydrophila* bacteria originated from BBBAT Sukabumi. Formed on Na medium to multiply bacteria. Infection of *A. hydrophila* with cohabitation method and bacterial toxicity of 10⁸ CFU / ml.

Catfish juvenile that have shown clinical symptoms of *A. hydrophila* treated by immersion treatment with concentration of 50 ppm, 75 ppm, 100 ppm and 125 ppm done for 24 hours. After that observation of clinical symptoms, recovery process, survival rate and water quality for 14 days. Feeding is done 2 times a day in adlibitum.

Parameters

1. Clinical Symptoms

Clinical symptoms observed were surface damage of the body and fish behavior that included reflex test, response to feed. These observations were performed after the infections of *A. hydrophila*. The results of observation of clinical symptoms will be analyzed descriptively.

2. Recovery Process

Observations made during the recovery process were changes in fish behavior and injury to the fish body showing healing. The results of this observation will be analyzed descriptively.

3. Survival Rate

The survival percentage of catfish juvenile after *A. hydrophila* infection was obtained using the [2].

$$SR = \frac{Nt}{No} \times 100\%$$

Information

- SR : survival rate of fish (%)
- Nt : number of fish that survive at the end of research
- No : number of fish in the beginning of research

4. Water Quality

Water quality parameters studied include temperature, dissolved oxygen, and pH. These parameters were observed once a week during the study and were analyzed by comparing secondary data and discussed descriptively.

Data Analysis

The effect of immersion treatment of the sambilotto extract on the catfish juveniles on the survival rate was analyzed using ANOVA (Variant Analysis) with F test, to determine the effect of all treatments. If there is a statistical significant difference between the treatments then continued to examine using Duncan multiple-range test with 95% confidence level, to know the difference between treatments [5].

Results

Clinical Symptoms

After 3 days (96 hours) catfish juveniles showed almost the same clinical symptoms such as red spots, ulcers, Haemorage, prominent eyes (*exophthalmia*), and white eyes (*purulens*). The initial clinical symptoms seen in the 3rd day observation after infection were behavioral changes, lack of appetite, weak or abnormal swimming movement, and the appearance of red spots on the abdomen (Fig. 1).



Fig 1: Red Spots on Fish Body Part on Day 3 Post-Cohabitation

Clinical symptoms that occur on day 4 after infection were ulcers on the back surface of the body (Fig. 2a). Hemorage appears in anal and caudal fins (Fig. 2b), prominent eyes (*exophthalmia*) (Fig. 2c) and white eye (*purulens*) (Fig. 2d).



(a) Ulcer (b) Haemorage



(a) Exophthalmia (b) Purulens

Fig 2: Clinical Symptoms after Cohabitation Day 4

Figure (2) that fish infected with *Motile Aeromonas Septicemia* (MAS) disease can die instantly without any clinical symptoms such as small lesions on the surface of the body, *exophthalmia* and abscesses (Colored yellow liquid) in the abdomen [1].

Recovery process

The catfish juveniles infected with *A. hydrophila* bacteria that have been given different recovery treatments (Table 1).

Table 1: Results of Clinical Post-Treatment Observation with Sambilotto Extract

Observation days	Sambilotto Extract Concentration				
	A (0)	B (50)	C (75)	D (100)	E (125)
1	BHUEP	BHUEP	BHUEP	BHUEP	BHUEP
2	BHUEP	BHUEP	HUEP	BHEP	BUEP
3	BHUEP	BHUE	HUEP	BHEP	BUEP
4	BHEP	BHUE	HUEP	BHEP	BUEP
5	BHEP	BHUE	HUEP	BHEP	BUEP
6	BHEP	BHUE	HUEP	BHEP	BUEP
7	BHEP	BHUE	HUE	BHEP	BUEP
8	BHEP	BHUE	UE	BHEP	BUEP
9	BHE	BHU	UE	EP	UEP
10	BHE	BHU	UE	EP	UEP
11	BHE	U	U	EP	UP
12	BH	U	+	EP	UP
13	B	U	+	+	UP
14	B	+	+	+	+

Information: B = red spot
 H = haemorrhage
 U = ulcer
 E = exophthalmia
 P = Purulens
 + = healed

At treatment A (0 ppm) did not experience healing and mortality of fish juvenile increasing every day, the remaining fish from treatment A still shows red spots. Treatment B (50 ppm), D (100 ppm) and E (125 ppm) of catfish juveniles began to heal on the 9th day seen with the loss of clinical symptoms. The test fish at the C treatment (75 ppm) experienced the most rapid healing process on day 8, where

the purulens began to normal again, red spots began to disappear.

The higher concentration of sambilotto extract, the higher possibility to inhibit *A. hydrophila*. Ulcer on the surface of the body that appears begin to close. Caudal tail fins begin to improve and some fish began to heal (Fig. 3).



(a) Recovery ulcer (b) Fin damage recovery

Fig 3: Recovery process after the treatment

Healing process in fish shows that the content of antibacterial compounds in sambilotto able to inhibit the growth of *A. hydrophila* bacteria. This is in accordance with the opinion of Kardono (2003) [3], the activity of antibacterial leaf extract

of sambilotto contained in the butanol fraction consisting of four diterpen such as *andrographolide*, *neoandrographolide*, *deoxyandrographolide*, and *andrographiside*.

Table 2: Siamese catfish Juvenile Response to Feed

Observation days	Sambilotto Extract Concentration (ppm)														
	A (kontrol)			B (50)			C (75)			D (100)			E (125)		
	Replication														
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
3	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
4	-	-	-	+	+	+	+	+	++	+	+	+	+	+	+
5	-	-	-	++	+	+	+	++	++	++	+	+	+	+	+
6	-	-	-	++	++	++	++	++	++	++	+	++	+	+	+
7	-	-	+	++	++	++	++	++	++	++	++	++	+	++	++
8	-	-	+	++	++	++	++	++	++	++	++	++	++	++	++

9	-	+	+	++	++	++	++	++	++	++	++	++	++	++	++
10	+	+	+	++	++	++	++	++	++	++	++	++	++	++	++
11	+	+	+	++	++	++	++	++	++	++	++	++	++	++	++
12	+	+	+	++	++	++	++	++	++	++	++	++	++	++	++
13	+	+	+	++	++	++	++	++	++	++	++	++	++	++	++
14	+	+	+	++	++	++	++	++	++	++	++	++	++	++	++

Information: (++) Feed respon normal
 (+) Feed respon low
 (-) Feed respon none

In Table 2 it can be seen the 1st day after the treatment of sambiloto extract, catfish juveniles on all treatments did not respond to feed. This is seen with the remaining of the pellet at the bottom of the aquarium. The remaining live Siamese catfish juveniles in treatment A (control) gave a feed response less than 7 days, it is suspected that bacteria have infected the digestive part through the bloodstream, so the fish was slow

to digest the feed given.

Beginning on the 6th day of catfish juveniles in treatment B (50 ppm) showed a normal response to feed. On day 8 the D treatment test (100 ppm) and E (125 ppm) showed normal feed response. Siam catfish juveniles at C treatment (75 ppm) showed a response to normal feed starting on day 5.

Table 3: Response of Patin Fish juveniles to Surprises

Observation days	Sambiloto Extract Concentration (ppm)														
	A (0)			B (50)			C (75)			D (100)			E (125)		
	Replication														
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	+	-	+	+	+	-	+	+	-	+	-
3	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-
4	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
5	-	-	-	+	+	+	+	++	+	+	+	+	+	+	+
6	+	-	+	+	+	+	++	++	+	++	+	++	+	++	+
7	-	-	+	++	++	++	++	++	++	++	++	++	++	++	++
8	+	-	-	++	++	++	++	++	++	++	++	++	++	++	++
9	-	+	-	++	++	++	++	++	++	++	++	++	++	++	++
10	+	-	-	++	++	++	++	++	++	++	++	++	++	++	++
11	+	-	+	++	++	++	++	++	++	++	++	++	++	++	++
12	-	+	-	++	++	++	++	++	++	++	++	++	++	++	++
13	+	+	-	++	++	++	++	++	++	++	++	++	++	++	++
14	-	+	+	++	++	++	++	++	++	++	++	++	++	++	++

Information: (++) Shock respon normal
 (+) Shock respon low
 (-) Shock respon none

Table 3 shows that the juveniles of Siamese catfish after treatment with Sambiloto extract on Day 1 did not respond to shock. The catfish juvenils has changed its behavior after being infected by *A. hydrophila* bacteria, the slow fish movement and the irregular fish swimming position and always surfacing for oxygen.

In the treatment of A (control) the remaining live catfish juveniles did not show a normal response to shock, it is suspected that the treatment of A (control) was not given treatment of extracts of sambiloto, so that the catfish juveniles did not change behavior and experienced death.

On the 7th day the catfish juveniles in treatment B (50 ppm) started to gave a normal response to the shock. At treatment D (100 ppm) and E (125 ppm) began to respond normally to normal shock on day 8. It is suspected toxic substance to sambiloto extract. High content of flavonoids in the body is suspected to cause hemolytic anemia, which caused death of red blood cells due to its short life.

Of all the concentrations documented, the C treatment (75 ppm) was the fastest in responding to the normal shock on day 6. It is suspected saponin compound in sambiloto was able to inhibit the growth of *A. hydrophila* bacteria and stimulate the production of erythrocytes so that fish recovers quickly.

Survival rate of patin fish juvenile

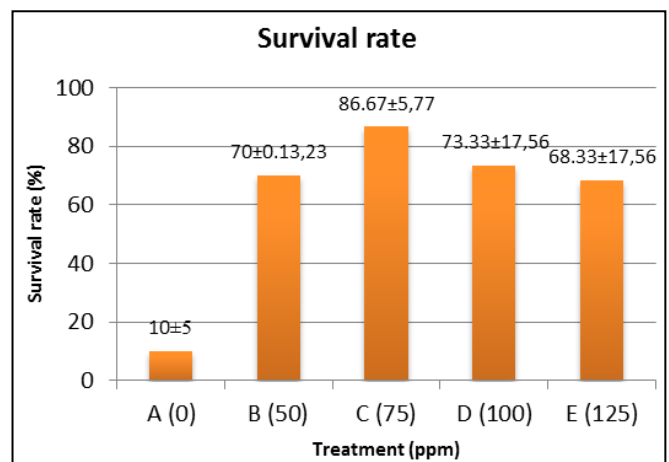


Fig 4: Graph of Survival Rate of Siamese catfish Juvenile

In Figure 4, the juveniles of Siamese catfish that were not given the treatment of the Sambiloto extract (control) resulted in the lowest survival rate of 10%. In the treatment of D (100 ppm) and E (125 ppm) tends to result in a lower survival rate of Siam catfish juvenile compared to C treatment (75 ppm).

At treatment B (50 ppm) survival rate of catfish juvenile was still low.

Treatment C (75 ppm) was the best concentration that can yield the highest survival rate of 86.67%. At this concentration the compounds contained in sambiloto flavonoids, saponins, tannins, and andrographolide work effectively in inhibiting the growth of *A. hydrophila* bacteria. Based on result of analysis variance indicate that immersion of sambiloto extract gave different effect to survival rate of catfish juveniles infected by *A. hydrophila*. Duncan test results showed that Treatment A (control) was significantly different with treatment B (50 ppm), C (75 ppm), D (100 ppm) and E (125 ppm) (Table 4).

Table 4: Average Survival (SR) of Siam Patin Fish Juveniles after Soaking Sambiloto Extract

Treatment (ppm)	Survival rate		Notation
	%	Transformation result	
A (0)	10	18.05	a
B (50)	70	57.24	b
C (75)	86.67	68.86	b
D (100)	73.33	59.81	b
E (125)	68.33	56.33	b

Information: The following value with different notation are not significant

The result of regression analysis (Fig 5) shows the effect of immersing the sambiloto extract on the survival rate of catfish juvenile infected by *A. hydrophila* bacteria.

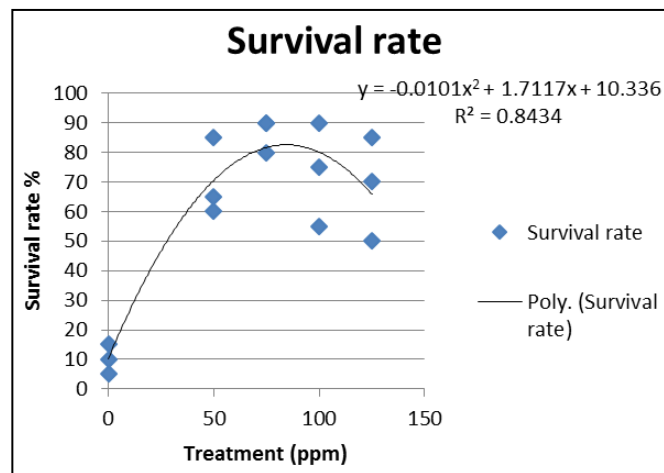


Fig 5: Graph of Relation of Sambiloto Extract Concentration on Survival Rate of Siamese catfish Juvenile

Water Quality

Table 5: Water Quality Observation Results

Treatment (ppm)	Water Quality		
	Temperature (°C)	pH	DO (mg/L)
A (0)	26,7-28	7,4-7,5	4,4-5,4
B (50)	27,3-28	7,2-7,7	4,4-4,6
C (75)	27-28	7,3-7,4	4,7-5,3
D (100)	27-27,3	7,4-7,6	4,5-4,8
E (125)	27-27,7	7,4-7,6	5,1-5,3
Optimal	25-30°C SNI	6, 5-8, 5 SNI	>4 mg/l SNI

Information: SNI (2002)

The results showed that the oxygen content at the beginning of treatment showed a range of 26.7-280C. The pH

measurements during the study showed a range of 7.2 to 7.6. The range of dissolved oxygen feeding medium of Siamese catfish during the study was in the range of 4.4-5.4 mg / L.

Discussion

Clinical symptoms

Based on observation of catfish juvenile after infected by *A. hydrophila* bacteria showed clinical symptoms of red spots, ulcers, Haemorage, prominent eyes (*exophthalmia*), and white eyes (*purulens*). The fish infected with *Motile Aeromonas Septicemia* (MAS) disease can die instantly without any clinical symptoms such as small lesions on the surface of the body, *exophthalmia* and abscesses (Colored yellow liquid) in the abdomen [1].

Recovery Process

Treatment C (75 ppm) experienced the fastest healing in which the white eye (*purulens*) began to normal again, red spots begin to disappear, *ulcer* and *exophthalmia* experienced healing. It is suspected that the compounds contained in the concentration is able to inhibit the growth of *A. hydrophila* bacteria and does not cause toxicity on the juveniles of Siamese catfish. The higher concentration of the extract of sambiloto given was able to inhibit the growth of *A. hydrophila* bacteria.

Healing in fish shows that the content of antibacterial compounds in sambiloto able to inhibit the growth of *A. hydrophila* bacteria. This is in accordance with the opinion of Kardono (2003) [3], the activity of antibacterial extract of sambiloto contained in the butanol fraction consisting of four diterpen namely *andrographolide*, *neoandrographolide*, *deoxyandrographolide* and *andrographiside*. According to Robinson 1995 in Wedayanti (2006) [10], the active substance works as an antibacterial by way of precipitating proteins, protein denaturation and fat solvents that can damage bacterial cell membranes.

In feed response and the C treatment reaction test (75 ppm) gave the fastest response improved compared to other treatments. This is suspected because the catfish juveniles have healing after the treatment of the sambiloto extract with the right concentration. The active compounds contained in sambiloto are thought to work effectively inhibiting the growth of *A. hydrophila* bacteria.

Survival Rate

Siamese catfish juveniles treated or treated with bitter extracts show higher survival rates than controls. The higher the concentration gives higher survival rate up to 75 ppm. Above 75 ppm survival rate decreased despite the healing process. This is due to the treatment of 50 ppm was not able to inhibit the growth of *A. hydrophila* bacteria resulting in low survival rates. The treatment D (100ppm) and E (125ppm) results in low survival rates, because the high concentration of sambiloto extract in treatment D and E not just inhibit bacterial growth, but also cause toxicity to catfish juveniles. High content of flavonoids in the body is suspected to cause hemolytic anemia, which caused death of red blood cells due to its short life. Lack of red blood cells cause fish to be stressed because of lack of oxygen can even cause death. Ringel *et al.*, (1994) in Samman *et al.* (1996) [9] states that toxic effects on flavonoids are acute renal failure, hemolytic anemia and thrombocytopenia.

Based on the results of analysis of survival variance at treatment B (50 ppm), C (75 ppm), D (100 ppm) and E (125

ppm) gave a real difference. This may be caused range concentrations between treatments not too far away.

Based on regression analysis quadratic relationship with equation $y = -0,0101 x^2 + 1,7117x + 10,336$ with relation of determination (R^2) = 0,8434 so that correlation relationship (R) that is 0,9182, meaning use of sambiloto extract give influence equal to 91,82% to the healing of infected catfish infected by *A. hydrophila*. The optimum value of the concentration level of the extract of sambiloto to treat the infected catfish juveniles of *A. hydrophila* was 84,74 ppm.

Water Quality

Based on the measurement of water quality, the water quality condition during the research meets the optimum standard for the siamese juvenile fishing media during maintenance. This suggests that water quality parameters are not a limiting factor during the study, so the death of Siamese juveniles is caused by the presence of *A hydrophila* bacterial infection.

Conclusion

Sambiloto extract is effective to treat Siam catfish juveniles infected by *A. hydrophila* bacteria with immersion for 24 hours, at 75 ppm concentration resulting in the fastest recovery process starting from day 7 and highest survival rate of 86.67%. Sambiloto extract gave an effect of 91.82% and optimum concentration to treat the infected Siam catfish juveniles of *A. hydrophila* of 84,74 ppm.

Acknowledgements

I would like to acknowledge the support Mrs. Elis as laboratory assistant who help this research and Mrs. Rosidah M.Si, Mr. Prof Dr. Junianto M.P., Mr Drs. Walim Lili M.Si who guide me to finish my research and this research was supported by faculty of Fisheries and Marine Sciences Padjadjaran University.

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