



**The Effect of Different Dosage of *Anredera cordifolia* (Ten.)
Steenis Leaves Extract towards the Survival Rate of
African Catfish (*Clarias sp.*) Infected by *Aeromonas
salmonicida***

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Abstract : This study aims to assess the effect of different dosage in *Anredera cordifolia* leaves extract towards the survival rate of African catfish (*Clarias sp.*) that is infected with *Aeromonas salmonicida*. The experiment of this research was carried out by using a Completely Randomized Design method with 3 replications, 1 control, and 3 treatment dosage of 0 ppm, 50 ppm, and 100 ppm. Furthermore, the fish maintenance in this study was held for 11 days; 4 days during the post-infection of *A. salmonicida* and 7 days after the soaking of *A. cordifolia* extract. The results showed that the average percentage of African catfish (*Clarias sp.*) survival rate in control indicator is 72,3% while in treatment A (0 ppm) is 43,1%. Subsequently, it is 81,1% in treatment B with the dosage of 50 ppm and increased by 90% in treatment C with 100 ppm. In other words, the highest fish survival rate is achieved in treatment C with 100 ppm formulation.

Introduction

Aeromonas salmonicida is a family of *Aeromonas sp.* bacteria with the characterization of rod-shaped, pathogenic animal, negative gram, and moves with flagella¹. Besides attacking the fish that lives in sea or salt water, this kind of bacteria also attacks the fish in which its habitat is in freshwater such as catfish².

African catfish (*Clarias sp.*) is one type of catfish which quite popular in Indonesia and has an important economic value³. This fish has a fast-growing level but vulnerable against bacterial attack⁴, one of which is the *A. salmonicida*. The disease created by *A. salmonicida* could cause a health declining even fish fatality as well as the decrease of fish farming quality and quantity².

By that, the alternative material is taken as an effort to overcome the attack of *A. salmonicida* apart from antibiotics⁵, one of the alternative materials that can be used is the *A. cordifolia* leaves. It has been noticed that there are many antibacterial compounds in *A. cordifolia* plant such as alkaloids, flavonoids, tannins, terpenoids⁶, polyphenols⁷, saponin, diterpenoid, glycosides, steroid, sesquiterpenoid⁸, total phenol, and high level of antioxidants⁹.

So, the reason why this study was established is due to the observation of survival rate that is influenced by different dosage of *A. cordifolia* leaves extract in African catfish (*Clarias sp.*) which are infected with *A. salmonicida* bacteria.

Research Methods

A. cordifolia Leaves Extraction

The dried and smoothed (crushed into a smaller form) *A. cordifolia* leaves were weighed for 125 grams and then kept in a 2000ml erlenmeyer tube. After it was settled in an erlenmeyer tube, methanol solution as much as 1000ml¹⁰ was added; the ratio of *A. cordifolia* leaves and methanol was by 1: 8¹¹. Next, it was homogenized for 60 minutes with the help of an incubator shaker in 150 rpm and set aside for 48 hours. After 48 hours, it was filtered through a filter paper and formed a filtrate and grout. The filtrate was then brought to be evaporated by using a rotary evaporator with the temperature of 50°C. As a result, an extract of *A. cordifolia* leaves was obtained.

Test Organism

The organism of this research was the African catfish (*Clarias sp.*) with the size of 12cm up to 15cm and distributed as many as 5 fish/aquariums. Before that, please noted that the fish has been adapted to the environment for 14 days. The aquarium which was used for the cultivation was 12 sterilized aquariums (30x30x30cm) with the water volume of 15 liters/aquariums.

A. salmonicida Bacteria

The *A. salmonicida* was derived from the Laboratory of Microbiology, Medical Faculty, Universitas Brawijaya. The living media of the bacteria was in the form of TSB (Tryptic Soy Broth) with a density of 10¹⁰ cells/ml.

A. salmonicida Infection

The infection of *A. salmonicida* in African catfish (*Clarias sp.*) was done by a soaking method. *A. salmonicida* was diluted to 10⁷ cells/ml by taking a 20 ml bacteria of 10¹⁰ cells/ml stock and dissolved into 20 liters of water. Then, the fish was immersed into a container with 20 liters of water that already mixed with *A. salmonicida* for 24 hours. Once the fish was kept in the water for 24 hours, the next step was to relocate the fish into aquarium for 4 days. During the 4 days' maintenance, the clinical symptom of the fish was being observed until it showed a sign of illness.

A. cordifolia Leaves Extract Immersion

The immersion of *A. cordifolia* leaves extract was implemented after the fish had shown a symptom of illness in which it occurred on the 4th day after the infection of *A. salmonicida*. The immersion process or soaking process was done by dividing the container into 3 different treatment dosage such as treatment A (0 ppm), treatment B (50 ppm), and treatment C (100 ppm). Each container was filled with 15 liters of water and diluted with *A. cordifolia* extract based on its treatment dosage. After that, every 15 fish were placed into each container for 24 hours. The dosage of leaves extract based on the results of preliminary research (LC₅₀) showed that the soaking of *A. cordifolia* extract with ≥ 200 ppm solution was toxic to African catfish (*Clarias sp.*).

Pisciculture

In this study, African catfish (*Clarias sp.*) breeding had a purpose to look into the survival rate of the fish. After the fish were kept in the *A. cordifolia* water mixture, fish in each treatment were transferred into 12 aquariums in the size of 30x30x30cm that have been labeled in accordance with its treatment and repetition. The division of aquarium was set in 9 aquariums for treatment A (0 ppm), treatment B (50 ppm), and treatment C (100 ppm), and then 3 aquariums for control indicator. The fish maintenance was carried out for 7 days. And the feeding was done *ad libitum* in every morning and evening while the cleaning was conducted in every 2 days.

Water Quality Observation

The observation of water quality was realized 2 times per day, means that it was being observed on every morning and afternoon before the feeding. The quality of the water that was examined including pH, temperature, and DO.

Data Analysis

The data analysis of survival rate was held by using an analysis of variance (ANOVA). This analysis was used to test the effect of the treatment and then continued with the use of Least Significant Difference (LSD) test at the test level of 5%.

Results and Discussion

Water Quality

The results of water quality measurements that is ranging from bacterial infections until the post soaking process can be seen in Table 1 below.

Table 1. The Results of Water Quality Measurement

No.	Water Quality Parameter	Value
1.	Temperature (°C)	25-27
2.	DO (mg/l)	4.19-7.01
3.	pH	7.6-8.6

The temperature during this research is ranging from 25°C until 27°C which means that this is still included as an optimal temperature for the maintenance of African catfish (*Clarias sp.*) that technically 25°C – 30°C⁴. The temperature below 20°C can inhibit the growth of African catfish (*Clarias sp.*)¹².

The DO measurement is resulted in 4,19 mg/l to 7,01 mg/l. This shows that the DO in treatment aquarium can be tolerated for African catfish (*Clariassp.*) fish breeding which ranging between 6,5 mg/l – 12,5 mg/l⁴. Therefore, Hermawan¹³ found that DO between 3,26 mg/l – 5,66 mg/l still considered to be relatively safe for African catfish (*Clarias sp.*) and still able to increase the growth rate of the fish.

On the other hand, the pH is found in between 7,6 – 8,6. Although the aquarium tends to be alkaline, this is preferred that it's still within the normal limit. This finding is in accordance with Augusta¹⁴, the ideal pH for freshwater fish cultivation is ranged between 7 up to 8,5 because all activities of African catfish (*Clarias sp.*) will produce the best value in that range of pH.

Survival Rate

The observation of survival rate of African catfish (*Clarias sp.*) which were infected with *A. salmonicida* and soaked with *A. cordifolia* leaves extract is presented in Table 2.

Table 2. Data Percentage of African catfish (*Clarias sp.*) Survival Rate.

Treatment Dosage (ppm)	Survival Rate (%)			Total	Average	SD
	Repetition					
	1	2	3			
KN (control)	89,96	63,41	63,41	216,78	72,3	15,32865
A (0 ppm)	39,22	39,22	50,75	129,19	43,1	6,656849
B (50 ppm)	89,96	63,41	89,96	243,33	81,1	15,32865
C (100 ppm)	89,96	89,96	89,96	269,88	90,0	0

As we can see from the results above, the highest percentage of survival rate is occurred in treatment C (100 ppm) by 90%. This number is greater than the control indicator of survival rate (fish treatment without bacterial infection and without leaves extract) which is by 72.3%. In sequence, the survival rate in treatment B (50 ppm) and treatment A (0 ppm or without the leaves extract) is 81,1% and 43.1%.

Furthermore, the calculation of variance to determine the effect of different dosage of *A. cordifolia* leaves towards the survival rate of the fish is illustrated in this following Table 3.

Table 3. Variance of African catfish (*Clarias sp.*) Survival Rate

Variance	db	JK	KT	F Count	F5%	F1%
Treatment	3	3.726,92	1242,31	9,663	4,07	7,59
Random	8	1.028,50	128,56	**		
Total	11	4.755,42				

** : significantly different

From the table, as shown above, it indicates that $F5% < F1% < F_{count}$, thus, it can be concluded that the giving of different dosage of *A. cordifolia* extract to improve fish survival rate in each treatment is significantly different. Consequently, to determine the differences of the effect in each dosage, a Least Significant Difference (LSD) at 5% and 1% was carried out. LSD can be seen in Table 4 below.

Table 4. LSD of *A. cordifolia* Extract towards African catfish (*Clarias sp.*) Survival Rate

Average Treatment	(A) 43,1	(B) 72,3	(KN) 81,1	(C) 90	Notation
(A) 43,1	0				a
(B) 72,3	29,19*				ab
(KN) 81,1	38,04**	8,85			b
(C) 90	46,89**	17,7	8,8	0	b

We can find that the different dosage of treatment A, B, and (KN, C) has a different effect towards the survival rate of African catfish (*Clarias sp.*).

The orthogonal polynomial analysis in Figure 1 is shown in order to know the response of different dosage of *A. cordifolia* extract towards the survival rate of African catfish (*Clarias sp.*).

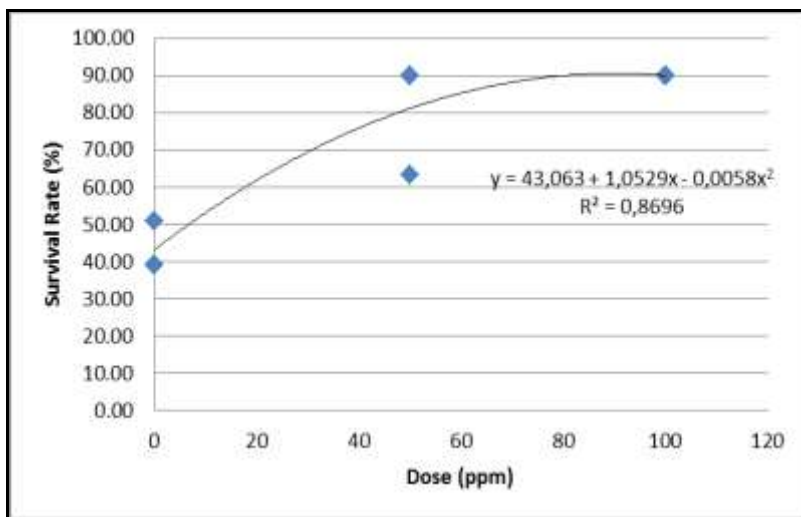


Figure 1. The Graph of African catfish (*Clarias sp.*) Survival Rate.

The equation of $y = 43,063 + 1,0529x - 0,0058x^2$ with terminated coefficient by $(R^2) = 0,8696$ is obtained from the graph that is illustrated in Figure 1 above. This means that 86% of African catfish (*Clarias sp.*) survival rate is influenced by the dosage. Therefore, it provides a fairly high correlation because the value of R^2 with a value of 0,8696 still can be said to be close to 1.

Conclusion

The results showed that the highest percentage of fish survival rate is occurred in treatment C (100 ppm) by 90%, while the lowest survival rate is happened in treatment A (0 ppm or without any leaves extract) by 43,1%, whereas, treatment B (50 ppm) have a survival rate of 81,1%. In comparison to treatment A that is without *A. cordifolia* leaves extract, fish survival rate in treatment B (50 ppm) and C (100 ppm) have a higher percentage.

This is certainly showed that the dissemination of *A. cordifolia* leaves extract in different dosage has a different effect on the survival rate of African catfish which were infected with *A. salmonicida* bacteria.

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