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Research Article

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Potential of Telang Plant (*Clitoria ternatea*) for Treatment of *Aeromonas hydrophila* Infection on Koi Fish (*Cyprinus carpio*)

Yuli Andriani[1]*, Asep Agus Handaka[1] , Rosidah[1] dan Rida Himyati H[1]

[1]*Departement of Fisheries, Universitas Padjadjaran,*
Jatinangor 45363, West Java, Indonesia

[*]*Corresponding author : yuli.andriani@unpad.ac.id*

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ABSTRACT

The aim of this study was to determine the effective concentration of telang leaf extract for the treatment of koi fish infected by the *Aeromonas hydrophila* bacteria and find out what clinical symptoms arise from *Aeromonas hydrophila* infection. The method used in this research was experimental using Completely Randomized Design (CRD) with five treatments and three replications. The treatment given was soaking koi fish infected with *Aeromonas hydrophila* in telang leaf extract for 48 hours with concentrations of 0, 150, 300, 450 and 600 ppm. Koi fish is infected with *A. hydrophila* by intramuscular injection of 0.1 ml/head with a bacterial density of 10^8 cfu/mL. The parameters observed were clinical symptoms including damage to fish body surface, fish response to feed and shock response. The data obtained were analyzed descriptively. The results showed that early clinical symptoms that arise are inflammation that is swelling and reddish color on the injection site and red spots. Other symptoms that occur are ulcers, hemorrhagic & inflammation, discoloration, exopthalmia and dropsy. The use of telang leaf extract with a concentration of 300 ppm was the best and effective treatment for treating the infected koi fish with *A. hydrophila* because within 9 days, the fish has recovered. It produced the fastest cure rate compared to other treatments.

Keywords: *Aeromonas hydrophila, koi fish, telang leaf extract, treatment, soaking.*

ABSTRAK

Tujuan dari penelitian ini adalah untuk menentukan konsentrasi efektif ekstrak daun telang untuk perawatan ikan koi yang terinfeksi oleh bakteri *Aeromonas hydrophila* dan mengetahui gejala klinis apa yang muncul dari infeksi *Aeromonas hydrophila*. Metode yang digunakan dalam penelitian ini adalah eksperimental dengan menggunakan Rancangan Acak Lengkap (RAL) dengan lima perlakuan dan tiga ulangan. Perlakuan yang diberikan adalah merendam ikan koi yang terinfeksi *Aeromonas hydrophila* dalam ekstrak daun telang selama 48 jam dengan konsentrasi 0, 150, 300, 450 dan 600 ppm. Ikan koi terinfeksi *A. hydrophila* dengan injeksi intramuskular 0,1 ml / ekor dengan kepadatan bakteri 108 cfu / mL. Parameter yang diamati adalah gejala klinis termasuk kerusakan permukaan tubuh ikan, respons ikan terhadap pakan, dan respons guncangan. Data yang diperoleh dianalisis secara deskriptif. Hasil penelitian menunjukkan bahwa gejala klinis awal yang muncul adalah peradangan yang bengkak dan warna kemerahan pada tempat suntikan dan bintik-bintik merah. Gejala lain yang terjadi adalah borok, hemoragik & inflamasi, perubahan warna, exopthalmia, dan sakit gembur-gembur. Penggunaan ekstrak daun telang dengan konsentrasi 300 ppm adalah pengobatan terbaik dan efektif untuk mengobati ikan koi yang terinfeksi dengan *A. hydrophila* karena dalam 9 hari, ikan telah pulih. Itu menghasilkan tingkat penyembuhan tercepat dibandingkan dengan perawatan lain.

Kata kunci: *Aeromonas hydrophila*, ikan koi, ekstrak daun telang, pengobatan, perendaman.

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1. Introduction

Koi fish (*Cyprinus carpio*) is a freshwater ornamental fish that is in great demand because it has a beautiful shape and color, and is also believed to bring good luck by the koi hobbies. However, there are several obstacles in the process of carrying out fish farming activities, one of the obstacles is the disease in fish caused by bacteria and can cause disability and even death. This certainly can reduce the amount of production in aquaculture activities, especially koi fish are in demand because of the beauty of their bodies, if attacked by disease will reduce the aesthetic value, which ultimately reduces consumer interest and koi fish farmers suffer losses.

One of the bacteria that attacks koi is *Aeromonas hydrophila* which causes Motile Aeromonas Septicemia (MAS) disease, *A. hydrophila* infects fish through the surface of the body or wounded gills and then enters the blood vessels and other internal organs (Ulfiana *et al*., 2012). In the healing process, farmers generally give antibiotics, but lately, treatment using antibiotics is not recommended because it is not easily biodegradable and can pollute the environment (Maulina *et al*., 2015).

There are several ways that can be done in the treatment of fish affected by *A. hydrophila*, but treatment with the soaking system is the most applicative way compared to injection and feeding because it can facilitate the treatment process, especially for treatment small sized fish on a large scale (Maisyaroh *et al*., 2018). The telang plant (*Clitoria ternatea*) is another alternative in the treatment of fish because according to Suarna (2012), telang leaves contain flavonoids and triterpenoids that function as antibacterial, then according to Al-Snafi (2013), telang plants contain saponins which are known to have an antibacterial effect, and the results of research Riswadi (2010) showed that telang leaves have antibacterial activity in steroid groups.

The purpose of this research is to determine the best concentration of telang leaf extract in the treatment of *A. hydrophila* bacterial infections that attack koi seeds and find out what clinical symptoms arise due to *A. hydrophila* infection and to provide some information about telang leaves as a treatment for *A. hydrophila* bacterial infections in aquaculture activities. Telang leaf extract concentrations used in this study were 0 ppm (control), 150 ppm, 300 ppm, 450 ppm, and 600 ppm, this was determined based on the results of the LC_{50} test and in vitro tests that had been done previously.

2. Materials and Methods

The tools and materials used in the process of making telang leaf extract are blenders to smooth the leaves of telang, scales to measure the weight of telang leaves to be used and to measure the weight of the extract produced, a 150 ml plastic measuring cup to measure the volume of methanol needed, an 80 mesh filter (0.18 mm) for sifting fine telang leaves, closed dark containers are used as a place for maceration, a spatula / stirrer for stirring ingredients, a rotatory evaporator as a filtration device and evaporation of solutions.

The extract was made using maceration method. The first step is to dry 5 kg of telang leaves that have been cut into small pieces for 7 days, the second step is to smooth the dried telang leaves using a blender, after being smooth and then macerated (put in a dark container closed and soaked with a technical methanol solution of 10 liters, stirred and allowed to stand for 2 days at room temperature), the results of maceration are filtered using Whattman paper no.42 and evaporated in rotatory evaporation at 60^{o}C at a speed of 65 rpm and produce a paste extract of ±650 grams.

The tools used in this study were 15 aquariums measuring 25 cm x 40 cm x 25.5 cm as a place for raising fish, aerators as oxygen supply, 26^{o}C aquarium heater as a regulator of water temperature in the aquarium, plastic hose with a diameter of 1.7 cm to siphon water in the aquarium, scoop to take koi fish, 1 ml syringe to infect bacteria in fish, then the material used is as much as 350 koi fish seeds koi fish size 5-7 cm in size with a weight of ± 13 g, isolates of bacteria *A. hydrophila*, distilled water used as an ingredient in the dissolution process, 0.9% physiological NaCl as a bacterial suspense solution, commercial feed PF 800 (Protein 39-41%, 5% fat, 5% fiber, 10% water content) as a source of test fish food.

The method used in this research is an experimental method using a Completely Randomized Design (CRD) with five treatments and three replications. A total of 15 aquariums, filled with 20 liters of water with a density of 1 fish/liter. All koi fish in the aquarium are infected by *A.hydrophila* bacteria through an intramuscular injection process in the fish body with a bacterial density of 10^{8} cfu/ml as much as 0.1 ml. Infected fish were observed for clinical symptoms and after

clinical symptoms appeared, the fish was soaked using telang leaf extract according to treatment. Fish are kept and observed for 14 days. During maintenance, fish are fed twice a day. The treatment given is soaking koi fish infected with *A. hydrophila* in telang leaf extract as follows :

- Treatment A : control without additional telang leaf extract
- Treatment B : 150 ppm telang leaf extract
- Treatment C : 300 ppm telang leaf extract
- Treatment D : 450 ppm telang leaf extract
- Treatment E : 600 ppm telang leaf extract

3. Results and Discussion

Observation of clinical symptoms is done by observing the wounds on the outside of the body and the behavior of koi seeds infected with *A. hydrophila* bacteria. Clinical symptoms occur after 6 hours after bacterial injection. Early clinical symptoms that arise are inflammation that is swelling and reddish color on the injection site and red patches, this symptom is on average seen in all infected koi ±12 fish in every treatment (Figure 1).

In research Haryani et al (2012) also mentioned that the first reaction of animals in cellular and vascular to bacteria that enter the body that cause damage to tissue is inflammation. This tissue damage is thought to occur due to toxins released by these bacteria and carried throughout the body through the bloodstream. This was also stated by Lallier et al. (1984), that the toxin that is spread throughout the body through the bloodstream causes hemolysis and rupture of blood vessels resulting in redness or red spots on the body of the fish.

After clinical symptoms are seen, the treatment process was carried out by soaking the koi fish using telang leaf extract according to treatment for 48 hours, then the water is replaced with new water and the fish are kept for 14 days. During the 14 days of observation after soaking, clinical symptoms that appear not only inflammation, but also other damage (Table 1).

A total of ± 300 fish (in all treatments) experienced clinical symptoms as shown in the Table 1. Viewed from Table 1, average symptoms of damage occur after 5-6 days after injection and after soaking, then improved after 7 days or 8 days later, but the results vary with each treatment. Treatment A (control), on the 3rd day the injection site became an ulcer wound (Figure 2a), this is in accordance with the research of Anyanwu et al. (2014), mentioned that the bacterium *A. hydrophila* causes injury to the body of the fish, ulcers, and bleeding. On the 8th day, treatment A (control) had exopthalmia or protruding eyes (Figure 2b), according to Asniatih et al. (2013) this is caused by the accumulation of fluid in the eye, causing the eyeballs to become concave and protrude. On the 10th day, treatment A (control) experienced clinical symptoms of dropsy or distended stomach (Figure 2c), this is caused due to inflammation and rupture of blood vessels in internal organs such as the intestine and liver caused by endotoxins (Distanaya 2016). On the 12th day, fish in treatment A (control) no longer experienced clinical symptoms of exopthalmia and dropsy because fish that experienced exopthalmia and dropsy had experienced death.

Figure 1. Inflammation at the injection site of koi (*Cyprinus carpio*)

Table 1. Observation of body damage after injection and after soaking with telang leaf extract

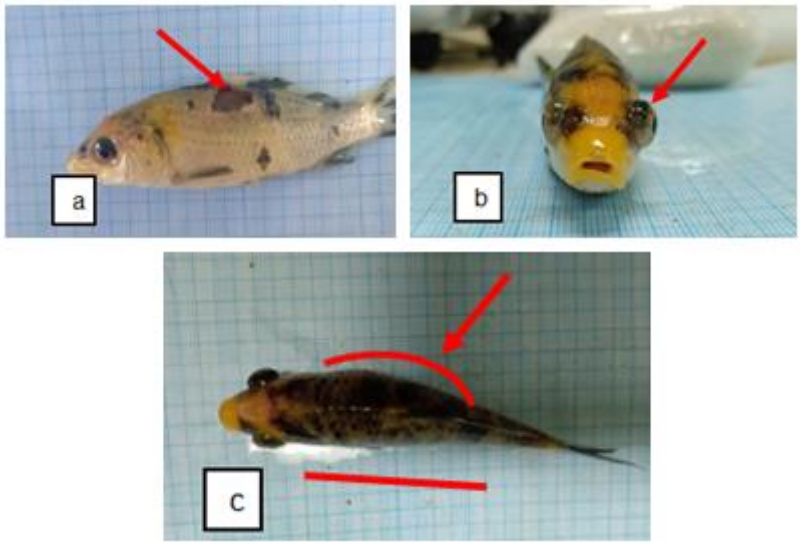
Days to-	Body Damage				
	Treatment				
	A (Control)	B (150 ppm)	C (300 ppm)	D (450 ppm)	E (600 ppm)
1	HP	HP	HP	HP	HP
2	HP	HP	HP	HP	HP
3	UHP	HP	UHP	HP	HP
4	UHP	UHP	UHP	HP	UHP
5	UHP	UHP	UHP	UHP	UHP
6	UHP	UHP	UHP	UHP	HP
7	UHP	HP	HP	HP	HP
8	UPE	P	P	P	HP
9	UPE	P	R	P	P
10	UPED	P	R	R	R
11	UPED	R	R	R	R
12	UP	R	R	R	R
13	UP	R	R	R	R
14	UP	R	R	R	R
Mortality	83.33%	56.67%	43.33%	90.00%	81,67%

Note :
U = ulcer
H = hemorrhagic & inflammation
P = pale
E = *exopthalmia*
D = dropsy
R = recover

A total of ± 300 fish (in all treatments) experienced clinical symptoms as shown in the Table 1. Viewed from Table 1, average symptoms of damage occur after 5-6 days after injection and after soaking, then improved after 7 days or 8 days later, but the results vary with each treatment. Treatment A (control), on the 3rd day the injection site became an ulcer wound (Figure 2a), this is in accordance with the research of Anyanwu et al. (2014), mentioned that the bacterium *A. hydrophila* causes injury to the body of the fish, ulcers, and bleeding. On the 8th day, treatment A (control) had exopthalmia or protruding eyes (Figure 2b), according to Asniatih et al. (2013) this is caused by the accumulation of fluid in the eye, causing the eyeballs to become concave and protrude. On the 10th day, treatment A (control) experienced clinical symptoms of dropsy or distended stomach (Figure 2c), this is caused due to inflammation and rupture of blood vessels in internal organs such as the intestine and liver caused by endotoxins (Distanaya 2016). On the 12th day, fish in treatment A (control) no longer experienced clinical symptoms of exopthalmia and dropsy because fish that experienced exopthalmia and dropsy had experienced death.

Treatment A (control) which was not soaking with telang leaf extract experienced the worst clinical symptoms and until the 14th day showed no signs of healing. Treatments B, C, D, and E treated with soaking telang leaf extract, the average surviving fish begins to heal on the 10th day and treatment C experienced the fastest recovery, which was the 9th day and treatment C had the lowest mortality value among other treatments, this shows that at that concentration, active ingredient in telang leaves, namely flavonoids, triterpenoids and saponins as antibacterials can inhibit the growth of *A. hydrophila* bacteria. In the research of Prayitno et al. (2014), the effect of ketapang leaf extract on carp infected with *A. hydrophila* bacteria experienced the fastest (ucler dried and closed) cure on day 10 with a treatment of 1500 ppm ketapang leaf extract. This difference is thought to be due difference in strength from the plant content used and differences in body strength of fish, but it can be said that telang leaves are better than ketapang leaves because the concentration of 300 ppm of telang leaf extract can produce the fastest cure, which is on the 9th day.

Not only does it function as an antibacterial, flavonoids also function to help reduce bleeding and swelling (Lesmanawati 2006), therefore, inflammation (hemorrhage) of injection marks in koi fish infected by *A. hydrophila* bacteria day by day getting better because of the healing process (Figure 3).



Note : (a) ulcer in the test fish (b) *exopthalmia* in the test fish (c) *dropsy* in the test fish

Figure 2. Body damage in treatment A (Control)

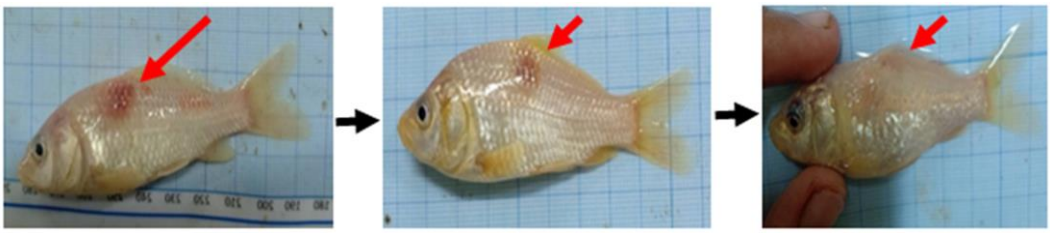


Figure 3. Reduction of inflammation after treatment with telang leaf extract

Even though fish that survive the treatments D and E experience a healing process until they finally recover, but in treatment D many fish died and several replications in treatments D and E the fish died completely, this is presumably because the concentration given is too high so that one of the antibacterial compounds in telang leaves becomes toxic to fish even though it can inhibit bacterial growth. One of the ingredients in telang leaves is saponin, evidenced by the amount of foam on the surface of the aquarium (Septiarusli 2012). Saponins are surface active compounds that are soapy, works to hemolize blood cells, and are toxic to cold-blooded animals (Faisal *et al.* 2016). Saponins are also toxic compounds that can inhibit the exchange of oxygen to breathe and eventually suffocate (Faisal *et al.* 2016).

Based on the description, telang leaf extract in fish can cure diseases caused by the *A. hydrophila* bacteria, this can be seen from the more precise dose given, the fewer clinical symptoms that arise even indicate healing in fish.

Another symptom observed is a change in behavior in fish, one of which is a decrease in appetite in fish. Decreased appetite is caused due to disturbed digestive system of fish due to inflammation that occurs in the digestive system. According to El-Ashram (2002) *A. hydrophila* bacteria cause the intestines in fish to become soft, filled with yellow mucus and feed, so the fish has a blockage in the digestive tract.

Based on observational data on the response of fish to feed (Table 2), all fish in every treatment on day 1 to day 3 have the same response to feed which tends to be low, this is in accordance with the statement of Kabata (1985) that fish will experience a decrease in response to feed given when attacked by the bacterium *A. hydrophila* and in the research of Haryani *et al.* (2012) it is also mentioned that fish experience stress after injection by *A. hydrophila* so the feed response is very little. This situation is also thought to occur because the *A. hydrophila* bacteria has produced endotoxins in sufficient quantities to make the majority of fish experience a decrease in response to feed (Distanaya, 2016).

Table 2. The results of observations on the response of feed

Days to-	Feed response														
	A (Control)			B (150 ppm)			C (300 ppm)			D (450 ppm)			E (600 ppm)		
	Replications			Replications			Replications			Replications			Replications		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1-3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4-6	+	+	+	++	+	++	++	++	++	+				+	+
7-14	++	+	++	++	++	++	++	++	++	++				++	++

Note :
+ = low response
++ = normal response
■ = no response/death

On the 4th day until the 6th day, treatments B, C, D and E show changes in feed response to normal, this shows that the active ingredient in telang leaf extract has begun to work in the healing process, but in treatment A, the 3rd test, feed response also changes to normal, this is thought to be due to the good living environment of the fish even though it was not given treatment, these fish can survive with natural body defenses and a supportive environment.

On the 7th day to the 14th day, treatment B, C, D, and E response to feed all treatments have returned to normal, it seen from the behavior of fish when fed immediately approached the feed and during the aquarium filtering process there is only a small amount of precipitated feed. This happens because the active ingredient in telang leaf extract is flavonoids, triterpenoids and saponins have worked in the healing process, also seen from clinical symptoms were on day 7 to day 14 fish treatment B, C, D, and E have decreased body damage and healing.

However, in treatment D (450 ppm), koi fish on the 2nd and 3rd tests were totally dead on the 4th day and treatment E (600 ppm) on the 1st test was totally dead on the 4th day anyway, this is caused by the content of telang leaves, namely saponins being toxic to fish in high concentrations, according to Prihatman (2001), saponin compounds are toxic to cold-blooded creatures, therefore the saponin content interferes with fish metabolism. Nevertheless, fish that still survive show a response to feed that is getting better until the last day of observation.

In addition to observing body damage and feed response, observation of the shock response was also made. Observation of the shock response is done by tapping the aquarium wall each treatment, if after the aquarium is knocked the fish reacts normally, all the fish will move away, if the fish's reaction is weak, the fish that stay away are only a little, and there is no response if the fish are silent there is no reaction at all. According to Kabata (1985), the fish's shock response is disturbed and low due to the meat muscle in the fish has been infected by the bacteria *A. hydrophila*. After soaking, the results of observations of responses to shocks differ for each treatment (Table 3)**.**

Table 3. The results of observation of shock response

Days to-	Shock Response														
	A (Control)			B (150 ppm)			C (300 ppm)			D (450 ppm)			E (600 ppm)		
	Replications			Replications			Replications			Replications			Replications		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1-3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4-6	+	+	+	++	++	++	+	+	++	+				+	+
7-14	+	+	+	++	++	++	++	++	++	++				++	++

Note :
+ = low response
++ = normal response
■ = no response/death

Viewed from Table 3, treatment A (control) showed a low shock response and nothing changes to normal until the last day of observation, this is because the fish are infected by the *A. Hydrophila* bacteria and no treatment process, while treatments B (150 ppm), C (300 ppm), D (450 ppm), and E (600 ppm) on days 1 through 3 showed a low shock response this is because fish are still stressed due to bacterial infecting activities and soaking process with telang leaf extract. On the 4th day, treatments B and C have shown normal shock responses, then on average in all treatments there was a change in the shock response to normal on the 7th day until the last day of observation, it seen from the fish's response when the aquarium wall is tapped, the fish immediately move away from the source of the knock, this is due to the soaking treatment using telang leaf extract which has inhibited the growth of *A. hydrophila* bacteria so that the condition of the fish improves and has experienced healing.

Based on the description, fish infected with the bacterium *A. hydrophila* show a shock response and a low feed response because the fish become sick, this is consistent with the statement of Rosidah *et a.l* (2018) that fish infected with the bacterium *A. hydrophila* will decrease their appetiteand the fish's response to shock is low. However, fish treated with healing showed a good response.

4. Conclusion

Telang leaf extract could be used to treat infection of *A. hydrophila* bacteria. Clinical symptoms that appear were hemorrhage, pale color, ulcer, and there are some fish that experience ulcers, exopthalamia and dropsy. The best concentration in the use of telang leaf extract to treat koi fish was 300 ppm because it reduces body damage and produces the fastest recovery, which is on the 9th day.

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