

Raw Extract Function of Yellow Root (*Arcangelisia flava* Merr) Due to the Medication of Bacterial Infection in Patin Fish (*Pangasionodon hypophthalmus*)

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ABSTRACT

Arcangelisia flava Merr is a traditional medicine and this vegetation contains bio-active compound that includes active of *saponin*, *flavonoid* and *alkaloid* as material of antimicroba. This research intended to find the accurate dosis in using yellow root vegetation and to know the function in medication of bacterial infection in patin fish (*Pangasionodon hypophthalmus*). Initial research was carried out to find out a yellow root vegetation dosage which will be used in the main research. Each of 5 patin fishes was put in the water with raw extract solution of yellow root vegetation in the initial research. Dosage of the solution was 0, 5, 10, 30, 60, and 90 ppm. The result was to place the dosage of 30 ppm, 60 ppm, and 90 ppm would be used in the main research. The main parameters which were observed were response of feeding, clinical symptom, mortality, organ change of fish which was carried out in the end of research, water quality, and body weight beginning from initial treatment when putting it in the water which contents extract of yellow root vegetation during one weeks and in the second week after bacteria infection of *Aeromonas hydrophila*. The changed of clinical symptom was observed after the infection. Results showed that during the research, the raw extract of yellow root vegetation with the dosage of 60 ppm and 90 ppm had a function in medication on bacteria infection of *Aeromonas hydrophila* in patin fish. It could be shown from positive moving reflect and response of food, the lower and decreasing of percentage level on infected fish until it was not been found. This condition was occurred on treatment B with the dosage 60 ppm and it reached 20%, the treatment C with the dosage 90 ppm and it reached 13.33%. The giving of yellow root extract with the dosis of 90 ppm was more effective in medication bacterial infection of *Aeromonas hydrophila* in patin fish (*Pangasionodon hypophthalmus*). It could be seen the number of infected fish was less than the process of fast recovery. The raw extract giving of yellow root with the dosage of 30 ppm was not effective in medication on bacterial infection of *Aeromonas hydrophila* in patin fish. It could be seen the less of positive moving reflect and the response of food. The percentage of infected fish on a treatment with the dosis of 30 ppm was 23.33% and it was found the mortality of 3.33%. Therefore yellow root vegetation is effective in medication on bacterial infection of *Aeromonashydrophila*.

Keywords: *Arcangelisia flava* Merr, bio-active, *Pangasionodon hypophthalmus*, *Aeromonas hydrophila*

INTRODUCTION

Efficiency effort of fresh water in Indonesia the longer the quicker developed such as patin fish (*Pangasionodon hypophthalmus*) which is one of the commodities that has good prospect because of the high economic value. The biggest constraint that is always occurred in industrial efficiency of fresh water is the infection. The infection is caused by virus, bacteria, fungus or parasite. It can decrease the quality on the production of fish efficiency because of the death and the failure of harvest [1].

One of the bacteria which attaches the fresh water fish is *Aeromonas hydrophila*. This kind of bacteria is motilize and causes the infection of *Motile Aeromonas Septicemia* (MAS). MAS is an infection on fish with systemic and clinical symptom like ulcer, sepsis, and widen at the skin surface [2].

Nowadays, the method that is used widely to solve the infection of efficiency fish is by using medication with chemical matter or antibiotic. This manner causes very big risk because it can resist the bacteria, needs high enough of cost, and it can pollute the environment. Antibiotic is generally given through soaking food or injection, so that the residu of antibiotic can be accumulated on the fish [3]. The other manner which is safe and kindly to environment can be carried out by using natural material. *Arcangelisia flava* Merr is one of traditional medicine vegetation for the Center of Kalimantan society especially Dayak society. This kind of traditional medicine vegetation contents bioactive compound of active *saponin*, *flavonoid* and *alkaloid* as

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antimicroba. Therefore, it is needed to observe the further usage of the vegetation as plant natural resources for controlling the pathogen of micro-organism.

MATERIALS AND METHODS

This research was conducted at the Laboratory of Fish Health in Aquaculture Department, Faculty of Agriculture, University of Palangka Raya. This research was carried out in two stages that was included the initial and main research. The initial research was intended to find the dosage of extract which would be used in the main research. The main research was carried out to know the function of extract for medication of bacterial infection in patin fish.

The equipments used in this research included aquarium with the dimension of 80 cm x 50 cm x 40 cm, double-out aerator, knife, reaction tube, bunsen, ose needle, triple balance of Ohaus, thermometer, DO meter, pH meter, and fiber tub. The materials used included yellow root vegetation, *Arcangelisia flava* Merr that was taken from the forest of Center Kalimantan, patin fish (*Pangasionodon hypophthalmus*), organ or network, Buffer Neutral Formalin (BNF) of 10 %, Formalin of 10 %, and Alcohol of 85 % and 90 %, absolute alcohol, xilol and liquid parafin, parafin, xilol, aquadest, Harris-Hematoxyline, acid alcohol, amonium, eosin, alcohol of 96%, permaunt, entelline, tissue, and label paper.

Procedure of research

Preparing of material

The sample of vegetation was dried by being windied in opened air without suffering of direct sun light. After drying, the sample was carved and then it was blended until being formed the smooth pollen, then it was sieved by using siever of 100 mesh. Result of siever was saced in the bottle and opened it closely.

Preparing of *Aeromonas hydrophila*

Stock of the bacteria was taken aseptically, then it was grown in TSA media and incubated during 24-48 hours. Bacteria sample of *Aeromonas hydrophila*.was used in this research.

Sample of fish

Patin fish (*Pangasionodon hypophthalmus*) was used as sample in this research. This sample of fish was kept with the density of 10 fishes in 45 litres of water in each aquarium.

Infectioning on sample of fish

The sample of fish was infectioned with the bacteria of *Aeromonas hydrophila* by soaking with the bacteria density of 10^6 cfu/ml. Soaking together of the bacteria of *Aeromonas hydrophila* was carried out during one week.

Initial Research

The initial research was intended to find the extract dosage which would be used in the main research. The treatment of initial research was as follow: 5 of fish samples with the bacteria of *Aeromonas hydrophila* was soaking with the bacteria density of 10^6 cfu/ml. After finding the symptom of fish which began sick, then the fish was put into stoples and to be soaked with raw extract solution of yellow root and the dosage of 0, 5, 10, 15, 30, 60, and 90 ppm. The soaking was carried out during 30 minutes. The observation of the main parameter was level of survival rate after the soaking has been carried out and clinical symptom which was appeared when the soaking. The observation was carried out during 7 days.

Main reserach

The main research was intended to know the function on row extract of yellow root in the medication of bacterial infection in the sample of fish. Dosage for the main research was determined due to the extract dosage of yellow root vegetation which gave the highest survival rate (100%) in the initial research.

The observed main parameter was the response of feeding, clinical symptom, mortality, the organ change in fish at the end of research, water quality, and the body weight from the beginning of treatment that was the first week after bacterial infection of *Aeromonas hydrophila* and the second week of soaking in the water that containing row extract solution of yellow root during 1 week. The change of clinical symptom was observed after the infection which included the inflammation, ulcer, injury, the other physical change, the reflection of running or moving, and the eating response of fish. The observation of clinical symptom and mortality was carried out every 3 hours after soaking together with the bacteria on the first day, every 6 hours on the second day, and then every 12 hours on the third day until at the end of observation during one week.

Histopatology on the sample of fish

The organ that was taken at the histopatology on the sample of fish was liver and kidney.

RESULTS AND DISCUSSION

The initial research

After being carried out the initial research, there was no died fish or to indicate the stress symptom after the soaking during 30 minutes. Therefore, it could be concluded that yellow root was safe for fish because until the dosage of 90 ppm, the fish sample was still in good condition, so the dosis of yellow root which was used in the main research was 30 ppm, 60 ppm, and 90 ppm.

The main research

Clinical symptom

The observation of clinical symptom after the soaking of yellow root vegetation due to the sample of fish during one week which was infected by the bacteria of *Aeromonas hydrophila* has given the results as follow:

Moving refection on the sample of fish

After the sample was soaked in the raw extract solution of yellow root into life media of fish on the first day, it indicated that there was different response at each treatment. At the B treatment (the dosage of 60 ppm) and the C treatment (the dosage of 90 ppm), the moving reflection of fish indicated that the response was less (K).

Feeding response on the sample of fish

Feeding response on the sample of fish from the first until the seventh day was different at each treatment especially on the fourth day. The A treatment (the dosage of 30 ppm) indicated the feeding response was less (K), but at the B treatment (the dosage of 60 ppm) and the C treatment (the dosage of 90 ppm) was still in the healthy condition.

Physical symptom on the infected bacteria of *A. hydrophila* on the fish sample

- On the first day, after in the raw extract suspension of yellow root into life media of fish, the sample of fish indicated the symptom that at the A and B treatment there was the sample of fish which was injure L) as well as inflamation (R), but at the C treatment there was the sample of fish was still injury (L).
- On the second day, the symptom of infected fish witnessed the increasing at the A treatment, the sample of fish was injury (L) as well as inflamation (R), and hemoragic (H). At the B treatment, the symptom of infected fish was less decreasing, there was the symptom of little injury (Lk), injury (L), and inflamation (R). At the C treatment, the symptom of infectioned fish was decreasing too, there was only the symptom of little injury (Lk).
- On the third and fourth day, the symptom of infectioned fish at the A treatment was increasing, there was the symptom of imflamation (R) and hemoragic (H) which the number of infectioned fish was continously increasing. At the B treatment, the symptom of infected fish was decreasing. There was the symptom of little injury (Lk) and little imflamation (Rk) with the number of fish was continuously decreasing. At the C treatment, the process of fish cure was fast which there was almost no infected fish again with the infected symptom only of little injury (Lk).
- On the fifth and sixth day, at the A treatment the infectioned fish was continuously increasing by the clinical symptom that was continuously worsening. There was the symptom of inflamation (R), hemoragic (H), dropsi (D), and mortality (M). The B and C treatment indicated the clinical symptom that was continuously improving, especially at the C treatment which almost the whole samples of fish were free from infection (cure).
- At the end of observation or on the seventh day, clinical symptom of infectioned fish at the A treatment did not indicated the improved symptom. It was different with the B and C treatment that was not found again the infectioned symptom on the sample of fish.

Based on the observation of clinical symptom during 7 days, it was concluded that the row extract dosis of yellow root at the A treatment (30 ppm) was not able to fulfill the bacterial infection of *Aeromonas hydrophila* on the sample of fish, but the raw extract dosage of yellow root at the treatment of B (60 ppm) and C (90 ppm) were effective enough to fulfill the bacterial infection with the best effectivity level at the C treatment (90 ppm). The higher cure level was at the higher dosis. It caused by the higher dosis would increase the higher content of soluted active material. Furthermore, it caused the higher ability in pressuring bacteria and at the end it would increase the cure degree of the bacterial infected fish. The result was similar with the founding of Schlegel [5]

who presented that the ability of an antimicroba to undo the ability of organism life was based on the concentration of antimicroba material.

The mechanism of pursuing on the bacterial growth by antibacterial compound could be as destroying of the cell wall. The manner was to pursue its forming or change it after finishing the form, permeability change of sitoplasm membrane that caused the material went out of cell, the change of protein molecule and nucleat acid, synthesis pursueing of nucleat acid and protein. In the pharmacy field, antibacterial material was known as the name of antibiotic such as the chemical substance which was produced by microba and it could pursue the growth of the other microba [5][6]

Percentage of cure level on the fish sample

After the soaking in the raw extract solution of yellow root on the fishsample, the percentage of fish that was varied improved based on the number and the day of indicating on the cure symptom. This condition was presented as in Figure 1.

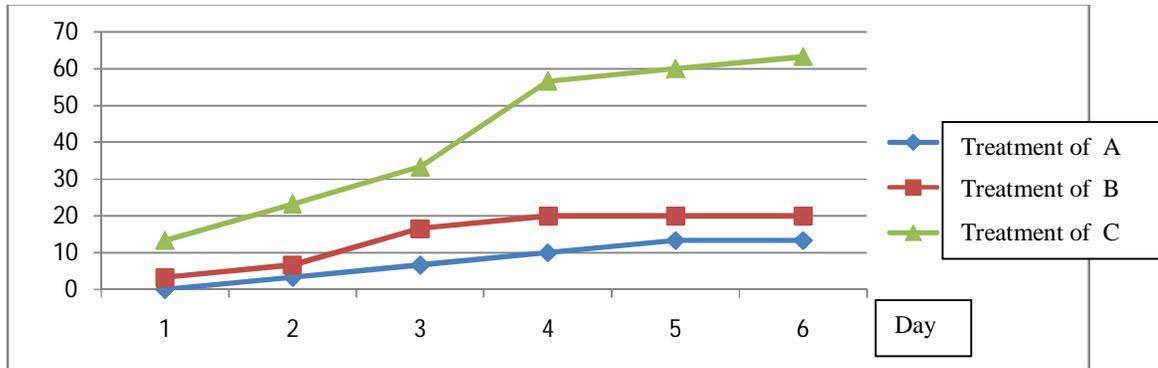


Figure 1 The percentage of cure level on the fish sample

Figure 1 presented that the precentage on the sample of fish at the treatment of A, B, and C on the first day until the seventh day that was after entering it in the raw extract material of yellow root into media of test with the different cure level at the each of treatment. The percentage of fish which was cure at the treatment of C (90 ppm) was 63.33%, the treatment of B (60 ppm) was 20.00%, and the lowest was at the treatment of A (30 ppm) that was reached 13.33%. It meant that the higher row extract dosis of yellow root was more able to medication the sick fish. The ability of a antimicroba in undoing the life ability of organism was based on the concentration of the antimicroba material [4].

Survival Rate (SR)

Survival Rate (SR) was as the level on continuity life of fish and as the number of fish which was life until at the end of observation.

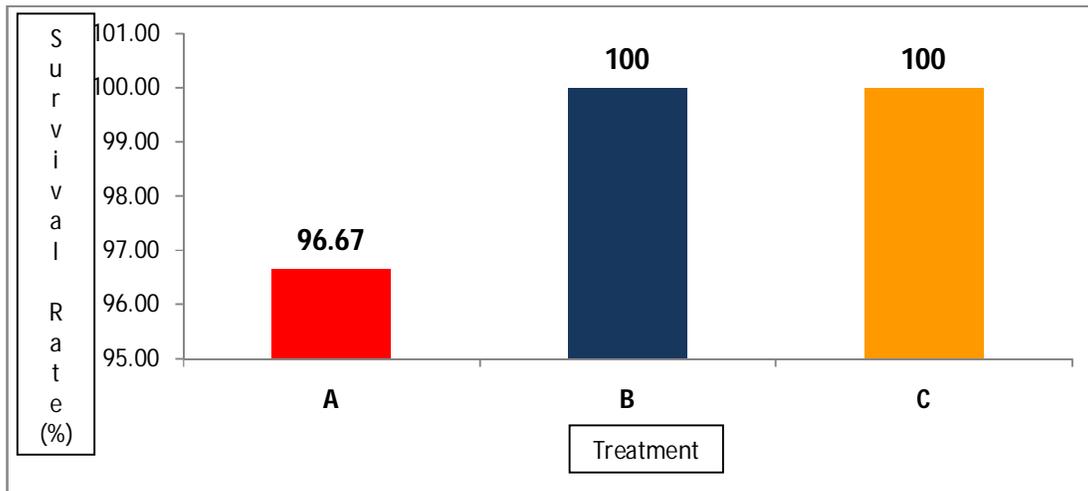


Figure 2. Graphic of survival rate on the fish sample during the observation

Figure 2 presented the survival rate on the fish sample at the each treatment from the beginning day of observation until the fourteenth day. The result of fish survival rate was 96.67% for the treatment A (30 ppm), 100% for the treatment B (60 ppm), and 100% for the treatment C (90 ppm). The result of survival rate after there was given the extract, was suitable to the observed result founded by Suryani [7]. Suryani expressed that the phytochemical test result on the extract active compound of yellow root was *saponin*, *flavonoid* dan *alkaloid*. The chemical compounds was functioned as antimicrobial. These vegetations are good for fish food because it can increase the appetite of fish and the immunity of the infection.

Weight growth on the fish sample

Data of waight growth on the sample of fish during the research was presented as in Figure 3 below.

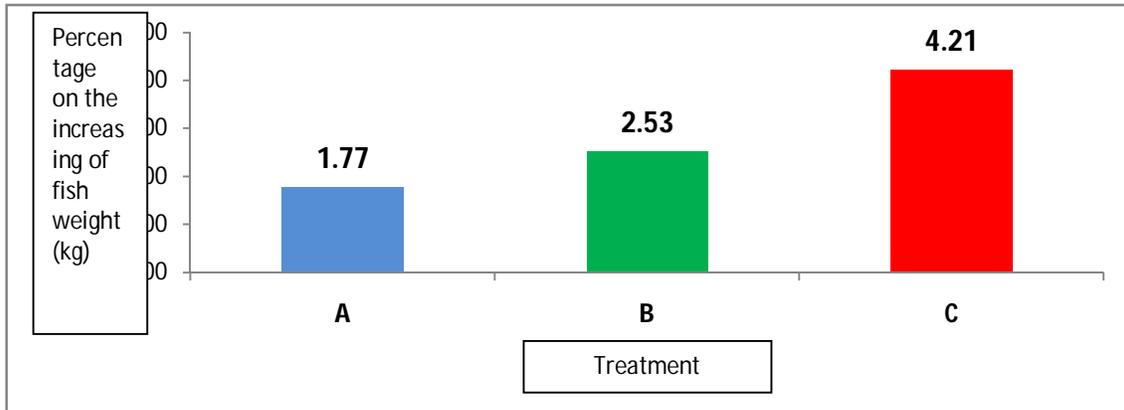


Figure 3. Percentage of weight growth on the sample of fish at the end of observation

Figure 3 presented that weight growth on the sample of fish during 14 days of observed time produced the result at the each of treatment from the beginning until the 56th was 1.77% at the treatment A (30 ppm), 2.53% at the treatment B (60 ppm), and 4.21% at the treatment of the C. If comparing among the treatment A, B, and C, the treatment of A was more low. It was caused by at the treatment A there were more fishes being infected so that the growth of fish was pursued.

Histopatology on the fish sample

Based on the result of histology on the sample of infected fish, it was obtained that at the treatment C did not indicate the changes on the inside of organ (like heart, liver, and kidney), but in the treatment A there was degeneration symptom fattyng in the organ of heart. It indicated that bacterial infection at the treatment A has caused liver damaged in the fish [8]. Result of the hystopatology observation on the sample of fish was presented as in Figure 4 and 5.

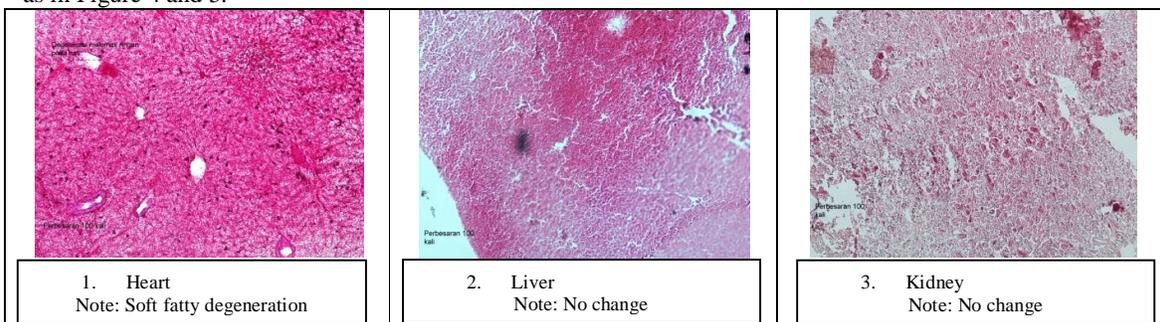


Figure 4 the result of histology observation on the sample of fish at the treatment of “A”

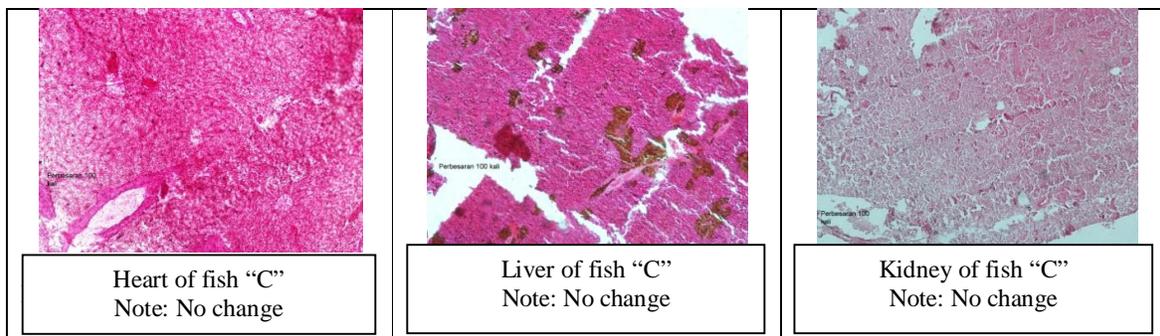


Figure 5. The result of histology observation on the sample of fish at the treatment of "C"

Water quality

The water quality which was measured during the research. Indicated that the water quality was feasible for the life of fish sample. The temperature was in the range of 26.7 °C to 27.6 °C, dissolved oxygen (DO) was in the range of 6.5 to 7.5 ppm, and pH was in the range of 7 to 8. The result was suitable to the expression of Asmawi [9] that the water quality to look after gold fish that the temperature was in the range of 25-32°C, the DO was 3 ppm, and the pH was in the range of 6.7 to 8.2.

CONCLUSION

Based on the result of the research as above, it was concluded as follows:

1. In the initial research, it was obtained the dosage range of yellow root for the main research of 30 ppm, 60 ppm, and 90 ppm. It was based on the assumption that the dosage under the 30 ppm was assumed not to be able to cure the bacterial infection of *A. hydrophila*, and if it was given the dosis that was higher than 90 ppm, the fish sample would be died.
2. In the main research, the giving on the row extract suspension of yellow root with the dosis 60 ppm and 90 ppm had the function in medication on bacterial infection of *A. hydrophila* on the sample of fish. It was seemed from the positive reflection of moving and the food responses, the decreasing on the percentage level of infected fish, and there was no death on the fish sample.
3. The giving of yellow root vegetation with the dosage of 90 ppm was more effective in the medication on the bacterial infection of *A. hydrophila* on the sample of fish. It was seemed from the number of fish which has been cured after being more infected if comparing with the dosage 30 ppm and 60 ppm and the recovery process was fast occurred.
4. The giving of yellow root vegetation with the dosis 30 ppm was less functioned in the medication on the bacterial infection of *A. hydrophila* on the sample of fish. It was seemed that there was less positive moving reflection and food response, the percentage of fish that was cure, and there was found the death at the test that was 3.33% of the distributed fish.

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