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POTENTIAL OF KALAKAI LEAF (*STENOCHLAENA PALUSTRIS*) EXTRACT AS *AEROMONAS HYDROPHILA* ANTIBACTERIA AND TOXICITY IN STRIPED CATFISH (*PANGASIUS HYPOPTHALMUS*)

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ABSTRACT
Aeromonas hydrophila is a bacteria that infects freshwater fish and causes an outbreak of motile aeromonad septicemia (MAS). One of the natural Borneo herbs that can be used in the control of the MAS, the kelakai leaf (stenochlaena palustris). The research aims to determine the inhibition of extract kelakai leaf on A. hydrophila in vitro and to analyze the toxicity a variety of doses of kelakai leaf extracted on the striped catfish (Pangasius Hypopthalmus). The method used in this study used kelakai leaf extract in vitro discus diffusion method with three solvents namely akuades, methanol and antibiotics as control, followed by toxicity test on striped catfish using random design complete with 6 treatments is K : without the extract kelakai leaf, extracts of kelakai leaf 50 mg/L (A), 100 mg/L (B), 200 mg/L (C), 400 mg/L (D), dan 800 mg/L (E). Antibacterial test extracts from kelakai akuades inhibition zone 11,02 ± 0,27 mm, extracts kelakai ethanol 8,48 ± 0,28 mm, and antibiotics (control) 11,87 ± 0,14 mm. Toxicity test for the survival of striped catfish were obtained K (control) 83,33%, A (83,33%), B (90%), C (96,67%), D (93,33%,) dan E (90 %), the result of Anova test show no difference between treatment of the meaning of all non-toxic doses of extract on striped catfish. It was determined that extracting kelakai leaf would kill a bacterium A. hydrophila and all dose of kelakai leaf akuades extract could be applied in treatment and potentially powerful to develop as a raw ingredient in controlling A. hydrophila infections in freshwater fish.

KEYWORDS: Aeromonas hydrophilla, kelakai, striped catfish, MAS.

INTRODUCTION
One of the most widely cultivated commodities in South Kalimantan is the Siamese catfish (Pangasius hypophthalamus). Obstacles faced by fish farmers in cultivation include the number of deaths in Siamese catfish fry caused by disease.

The causes of fish diseases can be divided into two groups, namely infectious diseases (viruses, bacteria, fungi and parasites), and non-infectious diseases (stress, intoxication, deficiency). One of the infectious diseases that often attack Siamese catfish farming is red spot disease or better known as MAS (Motile Aeromonad Septicemia) disease caused by A. hydrophilla bacteria (Allan & Stevenson, 1981).

Efforts to avoid the negative impact of using antibiotics can be done by using active ingredients (bioactive) from natural products where the raw materials are widely spread on the island of Kalimantan which are efficacious as traditional medicines and can be used as substitutes for synthetic drugs. one of them is kalakai plant. The plant with the Latin name Stenochlaena palustris is very well known by the people of Kalimantan as a medicinal plant.

Many studies have been carried out to prove the truth of the properties possessed by the kelakai leaves. Several studies have proven that kelakai leaves contain active substances, namely iron (Fe), calcium, vitamin C, and vitamin A. There has never been a study on the potential of kalakai for some A. hydrophila bacteria, in this study the use of kalakai leaf extract has the potential to treat bacteria. A. hydrophila causes MAS disease and its toxicity in Siamese catfish (Pangasius hypopthalmus).

RESEARCH METHODS

This research was carried out at the Fish Nutrition Laboratory and Fish Disease Pest Laboratory, Faculty of Fisheries and Marine Affairs, Lambung Mangkurat University, Banjarbaru, covering the preparation of tools and materials, extraction of kalakai leaves and bacterial rejuvenation, preparation of test fish, antibacterial test of kalakai leaf extract, and rearing of fish for testing toxicity.

The tools used are blender, scales, ruler, scissors, Erlenmeyer tube, measuring cup, hot and stirer plate, petri dish, test tube, beaker, Bunsen lamp, curved loop, measuring pipette, spatula, glass funnel, autoclave, refrigerator, vortex, 10-100 l and 100-1000 l micropipette, centrifuge, yellow tip, blue tip, gas stove, sheker, cotton, plastic, rubber band, analytical balance, incubator. 18 plastic tubs with a volume of 20 L, 1 mL disposable syringe, net scoop, hose, hematocrit capillary, microcentrifuge tube, microtube, 1 mL syringe, thermometer, and PH meter Materials used are Siamese catfish 10-12 cm in size, kalakai leaves , Aeromonas hydrophila isolates and culture media of bacterial isolates TSA, TSB GSP, fish blood samples, anticoagulant (EDTA), distilled water, methanol, and alcohol.

This research was conducted in three stages, namely preparation, in vitro test, and toxicity test. The research preparation includes the preparation of tools and materials, the preparation of the extract of the kalakai leaves, cleaned, cut into small pieces and dried using an oven at a temperature of 60 0C. The dried fenugreek leaves are mashed with a blender until they become a fine powder. Next is the rejuvenation of bacteria. Rejuvenation of A. hydrophila bacteria in stock culture was rejuvenated by culturing on GSP selective medium agar and incubating at room temperature for 18-24 hours..

In vitro test using the disc diffusion method to determine the antibacterial ability of A. hydrophila using the extract of the kalakai leaves with 2 different solvents namely aquadest, methanol, and antibiotics as a control, followed by a toxicity test on Siamese catfish using a Completely Randomized Design (CRD) with 6 treatments. and 3 replications to produce 18 experimental units, namely:
A = Soaking with kalakai leaf extract 50 mg/L
B = Soaking with kalakai leaf extract 100 mg/L
C = Soaking with Kalakai leaf extract 200 mg/L
D = Soaking with kalakai leaf extract 300 mg/L
E = Soaking with kalakai leaf extract 400 mg/L
K = Control without the use of extract

Observation Parameter:

a. Fish Survival

The survival of the fish was observed every day until the end of the treatment, the survival calculation was carried out at the end of the treatment with the following formula (Effendi, 2004).

b. Pengukuran Hematologis Ikan Patin a. Catfish Hematological Measurement

Hematology analyzer functions as a measurement and examination of blood cells in blood samples. The hematology analyzer has several advantages, namely time efficiency, sample volume, and accuracy of results. Examination using a hematology analyzer can be done quickly and only takes about 45 seconds. The blood sample used can use peripheral blood with a smaller amount of blood. The results released by this tool have gone through quality control carried out by an internal laboratory (Medonic, 2016).

c. Water quality

Pengamatan kualitas air dilakukan pada awal dan akhir perlakuan. Parameter kualitas air yang diamati adalah suhu, dan derajat kasaman (pH).

RESULTS AND DISCUSSION

3.1. Results

3.1.1 Anti-bacterial Test of Kelakai Leaf Extract In Vitro

The results of the anti-bacterial test of the in vitro disc diffusion method of macaque leaf extract are presented in table 1.

Table 1. Antibacterial Test Results of Kelakai Leaf Extract In Vitro Disc Diffusion Method.

No	Sample	Inhibition Zone (mm)
1	Kalakai leaf – aquades	11,02 ± 0,27
2	Kalakai leaf – ethanol	8,48 ± 0,28
3	Antibiotic control	11,87 ± 0,14

The results of the inhibition zone of several anti-bacterial activity test treatments using aquades and ethanol solvents with different doses gave an effect on the growth of A. Hydrophilla

3.1.2 Immersion Toxicity Test Results With Kelakai Leaf Extract

a. Fish Survival (Survival Rate)

The results of the toxicity test on the survival of catfish in the study by immersing various doses of kalakai leaf extract can be seen in Figure 1.

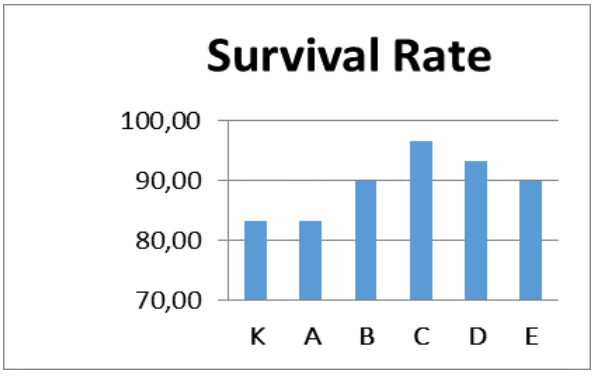

Figure 1: Catfish Survival Chart During Toxicity Test.

The results of the analysis of variance (Anova) (showing that the value of Fcount (0.81) < Ftable 5% (3.03) and Ftable 1% (4.86), it can be concluded that the treatment with kalakai leaf extract immersion has no significant effect on fish survival.

b. Fish Hematologist

• Leukocyte Value

The number of leukocytes of catfish obtained in this study ranged from 168.6–259.17x103/µL, it can be seen in the graph of the average total number of leukocytes in Figure 2.

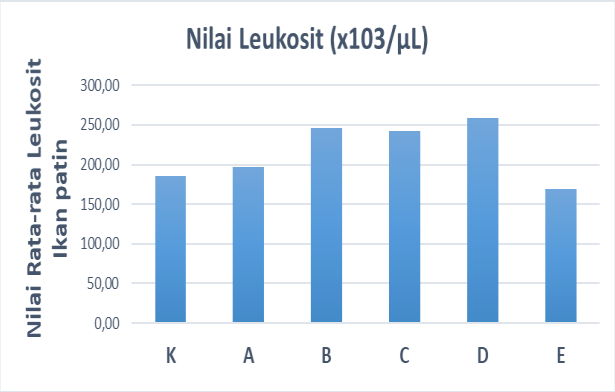

Figure 2: Graph of the average total leukocyte count.

The results of the analysis of variance (Anova) in (Appendix 3) show that the value of Fcount (0.97) < Ftable 5% (3.03) and Ftable 1% (4.86) which means H0 is accepted and H1 is rejected, it can be concluded that the treatment with leaf extract immersion Kalakai did not significantly affect the leukocyte value of fish.

• Erythrocyte Value

Erythrocyte Percentage The average percentage range of erythrocyte values after treatment can be seen in Figure 3.

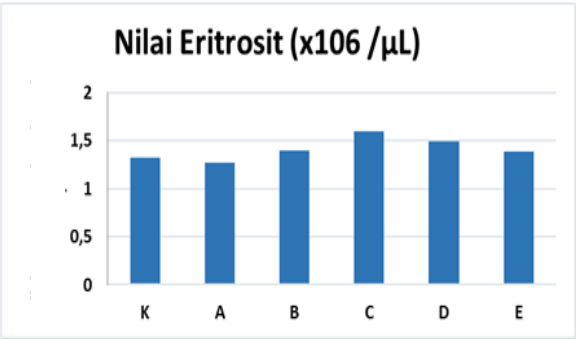

Figure 3: Graph of observation of the average total number of erythrocytes.

The results of the analysis of variance (Anova) in (Appendix 9) show that the value of Fcount (0.39) < Ftable 5% (3.03) and Ftable 1% (4.86) which means H0 is accepted and H1 is rejected, it can be concluded that the treatment by soaking the leaf extract Kalakai has no significant effect on the value of fish erythrocytes.

• Hemoglobin levels

Hemoglobin values have a range between 6.6-8.8 g/dL. the average percentage after being given treatment by soaking the extract of kalakai leaves with a maintenance time of 4 (four) days can be seen in Figure 4 below:

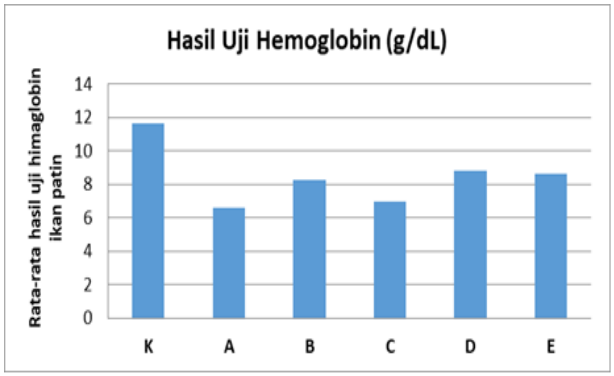

Figure 4: Graph of observation of the average level of Hemoglobin.

The results of analysis of variance (Anova) in (Appendix 12) show that the value of Fcount (0.47) < Ftable 5% (3.03) and Ftable 1% (4.86) which means H0 is accepted and H1 is rejected, it can be concluded that the treatment with leaf extract soaking Kalakai has no significant effect on hemoglobin levels in fish.

• Hematocrit Value

The percentage of hematocrit in the treatment when raising fish in healthy condition. To see a graph of the average results of the Hematocrit of fish, it can be seen in Figure 5. as follows:

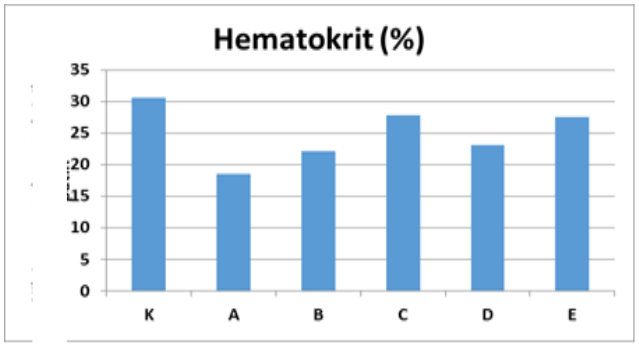

Figure 5: Graph of observation of the average value of Hematocrit.

The results of the analysis of variance (Anova) in (Appendix 15) show that the value of Fcount (0.29) < Ftable 5% (3.03) and Ftable 1% (4.86) which means H0 is accepted and H1 is rejected, it can be concluded that the treatment by soaking the leaf extract Kalakai has no significant effect on the hematocrit value in fish.

3.2. DISCUSSION

a. In Vitro Antibacterial Test of Kelakai Leaf Extract

Antibacterial test against A. hydrophilla at solvent concentrations of distilled water and ethanol, to

determine which solvent is the most active in inhibiting pathogens in freshwater organisms, namely A. hydrophila. Agar diffusion method, using disc paper (Kaur & Arora, 2009, Harlina et al., 2013). The sterile paper discs are dripped with extracts with different doses and dilutions.

After 15-30 minutes, the disc paper was attached to the semi-solid TSA media that had been inoculated with A. hydrophilla. Measurements were made after an incubation period of 24 hours at room temperature by observing the presence or absence of a clear zone formed around the chakra paper.

• Antibacterial Test of Kelakai Leaf Extract Disc Diffusion Method

The aquadest fraction gave the best effect on the growth of A. hydrophilla, with an inhibition zone of 11.02 ± 0.27 mm. The results of the inhibition test in the study with ethanol solvent 8.48 ± 0.28 showed a smaller inhibitory power than the inhibition produced by distilled water. According to Davis and Stout (1971), the criteria for antibacterial strength are as follows: a diameter of 5 mm or less is categorized as weak, an inhibitory zone of 5-10 mm is categorized as moderate, an inhibitory zone of 10-20 is categorized as strong and an inhibition zone of 20 mm or more is very strong. .

Factors that influence the inhibition zone of kalakai leaf extract on the growth of A. hydrophilla include the activity of the functional group of the substance itself, resistance of bacteria to bioactive substances, levels of bioactive substances and the amount of inoculum/density of the tested bacteria..

The inhibition zone formed due to the influence of the kalakai leaf extract was influenced by, among other things, the type of test bacteria used, which were gram-negative bacteria that had walls with a thin peptidoglycan composition. Peptidoglycan (PG) is an important web-like macromolecule that surrounds bacteria, gives them their shape, and protects them against their own osmotic pressure. Inhibition of PG synthesis causes bacterial cell lysis, making it an important target for many antibiotics (Derouaux, Sauvage, & Terrak, 2013).

This is because the ability of each method to remove antimicrobial substances contained in the leaves of the kalakai is different, according to the opinion of Takashi, et al (1998) in Fitriana, (2019) several factors affect the active power of the ingredients contained in the extract, such as the extraction method, environmental influences and so on.

Phytochemical test results on the active ingredients of the kalakai leaves contain flavonoids, alkaloids, tannins, phenols, saponins, anthraquinones, steroids and terpenoids. All types of these compounds were positively contained in the kalakai leaf extract used for this study.

The content of flavonoids has antioxidants and plays an important role in preventing cell damage. This is in accordance with the opinion of Redha (2010) which states that flavonoids are a group of phenolic compounds that have antioxidant and anti-inflammatory properties and play a role in preventing damage to cells and their cellular components by reactive free radicals. Flavonoids are antibacterial and antioxidant and are able to improve the work of the immune system because leukocytes as antigen-eaters are produced faster and the lymphoid system is activated more quickly, flavonoids are water-soluble compounds (Harborne, 1987).

The antibacterial mechanism of flavonoids is by inhibiting nucleic acid synthesis, inhibition of cytoplasmic membrane function, inhibition of energy metabolism, inhibition of attachment and biofilm formation, inhibition of porins in cell membranes, changes in membrane permeability, and attenuation of pathogenicity (Xie et al., 2014).

- **Toxicity Test Results**

Fish Survival (Survival Rate)

The results of the toxicity test on the survival of Siamese catfish in each treatment obtained K (Control) 83.33%, A (83.33%), B (90%), C (96.67%), D (93.33% ,) and E (90%). Observations were made by calculating the number of mortality for 4 days. According to Effendi (2003), the calculation of the survival rate of fish is the final number of living individuals divided by the initial number of living individuals multiplied by one hundred percent.

The survival of fish in this study, using the immersion of the Kalakai leaf extract in treatment A (50 - 800 mg/L) showed different results depending on the dose of the extracted extract, although the results of the ANOVA test showed no difference between treatments, based on the results seen in treatment C (200 mg/L) gave better results than all treatments, namely 96.67%, then there was a decrease in fish survival at a higher dose in the treatment of D (400 mg/L) and E (800 mg/L) allegedly at a dose of 200 mg/L can increase the immunity of catfish compared to higher doses.

• Fish Hematology

Hematology is closely related to pathology, especially to obtain an overview of the health condition of fish, whether they are healthy or sick. The circulatory system has many functions, in general it has a function as a transportation system including oxygen, carbon dioxide, food essences, and body metabolism. Blood carries substances from where they were formed to all parts of the body and keeps the body from functioning properly (Fujaya, 2002).

Blood parameters that can show disturbances in the fish's body are the hematocrit value, hemoglobin concentration, the number of erythrocytes (red blood

cells) and the number of leukocytes (white blood cells) (Lagler et al., 1977).

• Leukocyte Value

Observation of leukocytes aims to determine the white blood cells in the blood of fish. This leukocyte value provides clues about the health of the fish and helps to determine the occurrence of fish abnormalities. Leukocytes are responsible for the body's immune system and are responsible for destroying objects that are considered foreign and dangerous by the body such as viruses and bacteria.

Normally in healthy fish the number of white blood cells in the blood is 1% of the total blood count. The normal value of total leukocytes in freshwater fish ranges from 200-300 x 103/µL (Sri Hastuti, and Subandiyono 2015). From the normal total leukocyte values of freshwater fish, the catfish leukocyte value in this study was still in normal numbers, ranging from 1.8 to 286.8 x103/µL.

The average leukocyte value of catfish is below 200 after soaking with kalakai leaf extract so that it can reduce stress levels in fish. The leukocyte value in this study shows that the average number of leukocytes is quite low. Fish that experience stress caused by changes in environmental conditions or because of foreign objects show a response to an increase in the number of leukocytes (Hastuti and Subandiyono 2011).

Soaking kalakai leaf extract is able to be antibacterial and affect fish leukocytes. In the phytochemical tests that have been carried out, it is known that the extract of kalakai leaves contains flavonoid compounds. Flavonoids are antibacterial and antioxidant and are able to improve the work of the immune system because leukocytes as antigen-eaters are produced faster and the lymphoid system is activated more quickly, flavonoids are water-soluble compounds. According to Pradikta, Sjofian and Djunaidi (2010) in Fitriana (2019), flavonoids can act as anti-inflammatory, antibacterial, antiviral and natural antibiotics. Flavonoids can reduce the number of leukocytes due to a response to foreign objects that enter the body that are pathogenic and damaging.

• Erythrocyte Value

The value of erythrocytes after treatment and control (1.32 x 106/µL), treatment A (1.27 x 106/µL), B (1.4 x 106/µL), C (1.6 x 106/µL), D (1.49 x 106/µL) and E (1.38 x 106/µL). The value of fish erythrocytes after being given this treatment still meets the standard for the number of erythrocytes in teleost fish ranging from (1.05 - 3.0) x 106 /µL (Irianto. 2005).

According to Wedemayer and Yasutake (1977), low erythrocytes is an indicator of anemia, while a high number of erythrocytes indicates that fish are under stress. A decrease in the number of erythrocytes indicates a kidney infection, and a low erythrocyte value indicates that the fish has anemia, while a high number of erythrocytes (above normal) indicates that the fish is in a state of stress.

• Hemoglobin levels

The hemoglobin concentration of catfish in this study was still within the normal range of 6.6 – 8.8 g/dl. According to Salasia, et al (2001) The hemoglobin concentration of freshwater fish ranges from 5.05 to 8.33 g/dl. The concentration of the hemoglobin value of catfish in this study was lower than that of normal goldfish ranging from 6-10 g/dl according to Svobodova & Vyukusova, (1991) Hemoglobin is wrapped in erythrocytes, and plays a role in meeting the oxygen needs needed by the body's organs through blood circulation. The oxygen needs of cells in the body are fulfilled, causing cells to work properly because of sufficient energy. This allows individuals to work and develop perfectly (Hedayati & Tarkhani, 2014).

According to Hastuti and Subandiyono (2011), the size of the hemoglobin level in the blood indicates the ability to transport oxygen from the gills to the tissues by the blood. Most teleost fish (bone fish) have the same hemoglobin in erythrocytes as in other vertebrates. Stress conditions can affect physiological activity and hemoglobin levels in fish. The physiological state of fish blood varies greatly, depending on environmental conditions such as humidity, temperature, and pH (Adelbert, 2008).

• Hematocrit value

The hematocrit can provide clues about the health of the fish and help to determine the occurrence of abnormalities. Hematocrit is a parameter used to measure the percentage of red blood cells in the blood, by comparing the volume of red blood cells with blood plasma (Hazzulli, Setyawan, & Harpeni, 2015).

The percentage of hematocrit in treatment K (Control) was 20.57%, treatment A by soaking kalakai leaf extract (50 mg) 16.4%, treatment B by soaking kalakai leaf extract (100 mg) 22.07%, treatment C by soaking the extract Kalakai leaf extract (200 mg) was 27.77%, treatment D by soaking kalakai leaf extract (400 mg) 23.03% and treatment E by immersing kalakai leaf extract (800 mg) 20.23%.

At the time of rearing the fish in a healthy condition because the hematocrit value was above 20% except in treatment A with the immersion of kalakai leaf extract (50 mg) 16.4%, however, the fish in treatment K (Control) were still in the normal range of 20.57%. .

This is in accordance with the opinion of Fange (1992), which states that the hematocrit in a number of teslostei fish ranges from 20-40%. Increasing the hematocrit value means improving the health status of fish. This is in accordance with Hastuti & Subandiono (2012) which states that decreased hematocrit levels can be used as an

indication to determine low protein content, vitamin deficiency or the occurrence of infection in fish. During the study the value of the hematocrit level fluctuated quite a bit. Figures et al., (1990) stated that the hematocrit of fish varies depending on nutritional factors and the age of the fish.

b. Water quality

Observations of water quality were carried out as a consideration to obtain supporting data with the parameters observed were temperature and pH.

The water temperature of the maintenance medium at the end of the study by soaking the extract of kalakai leaves with different doses of 26 oC in all treatments. The optimal water temperature is in the range of 25 – 32 C. According to Irianto (2005), aquatic organisms have a degree of tolerance to temperature within a certain range which plays a very important role in growth, egg incubation, feed conversion and resistance to disease.

The degree of acidity of pH in this study had an average value taken at the end of the study when the kalakai leaf extract had different but not significant results, namely treatment K (control) 7.13, treatment A 7.27, treatment B 7.23, treatment C 7.23, treatment D 7.27, treatment E 7.00. pH is a parameter of Hydrogen ion (H+) activity in a solution expressed as an acid or a base. Aquatic biota is very sensitive to changes in pH and likes pH values ranging from 7-8.5.

The pH value greatly affects the biochemical processes of the waters, for example the nitrification process will end if the pH is low. Pure water is neutral (pH 7), in such conditions the constituent ions (H+ and OH) will dissociate in a state of equilibrium (Irianto, 2005).

CONCLUSION

Kalakai leaf extract can inhibit the growth of A. hydrophila bacteria where the aquadest kalakai leaf extract has a greater inhibitory power against A. hydrophila compared to methanol kalakai leaf extract.

Toxicity test showed that all doses of aquadest extract of the leaves given were non-toxic based on the survival and hematological parameters of catfish, all doses could be applied in medicine and had strong potential to be developed as medicinal raw materials in controlling A. hydrophila infection in freshwater fish culture

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