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EFFECTIVENESS OF BETEL LEAF EXTRACT (*Piper betle* L) AS AN ANTIBACTERIAL AGENT FOR TREATING BACTERIAL INFECTIONS (*Aeromonas hydrophila*) IN KOI FISH BREEDS

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Abstract

Betel leaf has a long history of use in traditional medicine due to its natural antibacterial properties. This study focuses on the antibacterial effects of betel leaf (Piper betle) extract, particularly against Aeromonas hydrophila infections in koi fish. The research employs an experimental approach using a Completely Randomized Design (CRD) with four treatment groups, each repeated twice. The treatments consist of: a control group (Treatment K) without betel leaf extract, Treatment A with 5 ml of betel leaf extract, Treatment B with 10 ml of betel leaf extract, and Treatment C with 15 ml of betel leaf extract. The results of the study demonstrate the significant efficacy of betel leaf extract in promoting the healing of Aeromonas hydrophila-infected fish. This is evident from observations of reduced wound diameter in fish treated with betel leaf extract. In Treatment A (5 ml dose), noticeable wound diameter reduction begins in the fourth week. Treatment B (10 ml dose) shows similar progress with visible healing in the third week. Treatment C (15 ml dose) exhibits the most promising results, with wound diameter reduction or fading evident as early as the second week. Survival rates are recorded as follows: Treatment K – 50%, Treatment A – 65%, Treatment B – 75%, and Treatment C – 90%. These findings underscore the potential of betel leaf extract as an effective antibacterial remedy in combating Aeromonas hydrophila infections in koi fish.

Keywords: *Aeromonas Hydrophilla, Betel Leaf, Koi Fish*

1. INTRODUCTION

Betel is a vine and has a greenish brown stem, heart-shaped leaves with pointed ends, has a taproot, and has a distinctive aromatic odor. Betel plants have benefits as antibacterials, namely preventing disease or bacteria in fish, this is because betel leaves (*Piper betle L*) contain chemical compounds in the form of saponins, tannins and flavonoids. These compounds in betel leaves have a function as antibacterial.

Koi fish (*Cyprinus carpio*) as an ornamental fish creature is mostly preferred by the community because it has a very beautiful color, has a diversity of types, is easy to maintain and has a high economic value. However, koi fish (*Cyprinus carpio*) are also vulnerable to bacteria and bacteria with most of the attacks, namely Aeromonas *hydrophilla* bacteria, koi fish as victims of bacterial attacks are characterized by loose scales, protruding eyes and wounds on the peeling scales. To overcome fish attacked by bacteria, efforts are needed to inhibit and kill bacteria that attack koi fish.

Efforts that can be made to treat koi fish attacked by bacteria are by giving anti-bacterial drugs. However, antibacterial drugs sold on the market are relatively expensive so it is better to use natural ingredients. Various forms of advantages obtained in the use of natural ingredients are relatively safer, easier to obtain because the plants are abundant

in the surrounding environment, do not create resistance, and are relatively harmless. As a natural ingredient that is applied as a medicine for fish infected with Aeromonas *hydrophilla* bacteria, namely betel leaves (*Piper betle L*). Betel leaves contain chemical compounds in the form of alkaloids, flavonoids, tannins, and saponins that have the function of being antibacterial. Alkaloids function as antibacterials that interfere with the peptidoglycan component in a bacterial cell, which makes the cell layer not form in an intact way or cause the intended cell to die. Flavonoids have anti-inflammatory properties, which can function to treat wounds or bleeding in the fish body. Tannins have antiseptic properties in the wounded area, and work to be bacteriostatic by being generally applied in fighting infections that occur in the wound. Saponin compounds have potential as antibacterials, the mechanism of action of saponins disrupts cytoplasmic stability which makes the cytoplasm leak and causes cells to die.

This study aims to identify the effect of betel leaf extract as an antibacterial in koi fish infected with Aeromonas hydrophilla bacteria. This investigation holds significant promise not only for the realm of aquatic health and fisheries management but also for the broader field of natural antimicrobial agents and their potential to combat bacterial infections across various species.

2. RESEARCH METHOD

This research is a qualitative type using the Experiment method with the design in the research carried out by applying a Complete Randomized Design (RAL) with 3 actions and 2 repetitions. The treatment in his research consisted of:

a. Treatment A with the administration of betel leaf extract which is 5 ml
b. Treatment B with the administration of betel leaf extract which is 10 ml
c. Treatment C with 15 ml of betel leaf extract
d. Treatment K (control) without betel leaf extract.

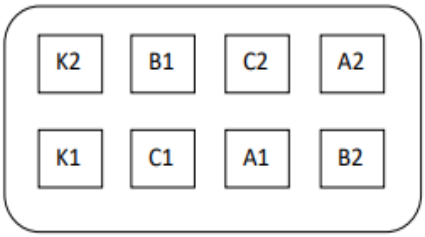

Figure 1. Placement of experimental containers

Experimental techniques are carried out by giving betel leaf extract to koi fish seeds by becoming victims of bacterial attack. While the observation technique is carried out in the field by examining the survival of koi fish attacked by bacteria after being given betel leaf extract. and for supporting parameters is a water quality test in the form of pH, temperature and oxygen that is regular.

3. RESULT AND DISCUSSION

Based on the results of research on the effect of betel leaf extract as an antibacterial on koi fish seeds infected with *Aeromonas hydropilla* bacteria carried out on their survival rate for 1 month or 4 weeks. So, the first step that needs to be done is infecting koi fish seeds with *Aeromonas hydropilla* bacteria. At first, koi fish that have been put in a container containing *Aeromonas hydrophilla* bacteria are observed by showing clinical symptoms with indicators by changing behavior and physical form. In the first week, clinical symptoms appeared with the cause due to the presence of *Aeromonas hydrophilla* bacteria, namely the behavior of koi fish after treatment that changed in the form of decreased appetite, aloof when swimming followed by less active swimming movements, fish when swimming when conditions experienced *Aeromonas hydrophilla* bacterial infection with a tilted position caused by the condition of the body loss of balance.

Based on the clinical symptoms of koi fish, all treatments are shown with symptoms of inflammation on the back, in this case because the *Aeromonas hydrophilla* bacteria use a reaction or spread in the fish body. As an indicator of inflammation of the fish body with the presence of red with visible on the body of the fish. Inflammation as the appearance of a form of symptom caused by a pathogen entering the host's body and causing infection.

The next stage is the treatment of koi fish with antibacterials made from betel extract. This is due to the content of betel leaf extract which has antibacterial and anti-inflammatory properties. The compound content with the betel leaf extract is:

a. Phenol

Phenol has the role of being a poison that can inhibit activities carried out by bacterial enzymes and can denature proteins that make metabolic activities experience bacterial cell death (Djuma et al., 2019) the content contained in betel leaves, namely phenol, has the role of being toxic to microbes that inhibit enzyme activity. The more concentration of betel leaf extract makes its ability to kill microorganisms with an increase. Not only with this, the content of betel leaves contains various phenols which become a derivative in the form of cavicol which can denature the bacterial protein (Aznury, 2021). If the increasing concentration of betel leaf extract makes the maximum phenol content, which makes the reaction stronger (Senggagau, 2019).

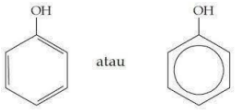

Figure 2. Structure of Phenol chemical compounds

b. Alkaloid

The immune system of fish that has increased is predicted to be caused by the presence of alkaloid compounds in the content of betel leaf extract in the bloodstream that is spread to several cells of the body, which makes an effect with several cells that are meant to be increasingly healthy, active, and structures that have improved their condition and function along with detoxification with the process of *Aeromonas hydrophilla* bacteria produced. Alkaloids as an active

ingredient with the function of being a drug and a strong activator for immune cells by being able to eliminate and kill bacteria (Candrasari et al., 2012). In a mechanism of action of an alkaloid compound as disturbing the components that make up the peptidoglycan in a bacterial cell (Dwianggraini et al., 2013)..

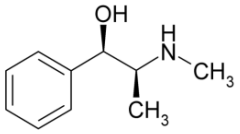

Figure 3. Structure of Alkaloid chemical compounds

c. Flavonoid

Flavonoids function as antibacterials through the mechanism of cell membrane destruction. Flavonoids have the ability as antimicrobials because the active ingredients can be used to treat infections (Safratiloa, 2021). The antibacterial mechanism in a flavonoid influences the action carried out by bacterial enzymes using a way to turn off the enzyme function against microbial cells, flavonoids have anti-inflammatory properties that make it possible to suppress the occurrence of inflammation and provide assistance in suppressing pain if there is a bleeding or swelling process that occurs in the wound in the body of koi fish.

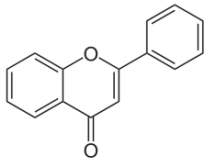

Figure 4. Structure of Flavonoid chemical compounds

d. Saponin

Saponin compounds that are lipophilic and hydrophilic which reduce cell surface tension and membrane permeability are damaged. Voltage is disturbed in the surface of the cell wall by making the cause of antibacterial contains can more easily enter a cell with subsequent cell death, another case in the permeability of the cell membrane is damaged by being able to cause interference in the survival of bacteria (Marsono et al., 2017).

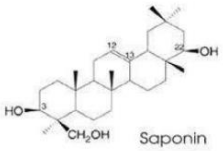

Figure 5. Structure of Saponin chemical compounds

e. Tanin

Tannin compounds as a polyphenolic compound with polar properties, tannins work as antibacterials by taking over substrates in the needs needed by bacterial growth and development. (Patil, 2015). Tannins can attack cell wall polypeptides to further cause bacterial cell walls to be damaged (Kursia et al., 2016).

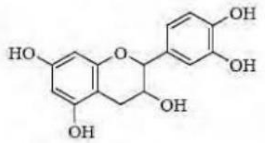

Figure 6. Structure of Tanin chemical compounds

Based on the content of compounds such as phenols, flavonoids, alkaloids, saponins and tannins in betel leaf extract make antibacterial drugs to cure diseases caused by *Aeromonas hydrophilla* bacteria that attack koi fish. In accordance with the research produced by carrying out obtained koi fish survival data based on different concentration treatments, namely the provision of betel leaf extract at a dose of 5 ml, 10 ml, 15 ml and control, namely without the provision of betel leaf extract. The data below shows the calculation of koi fish survival in the treatment with different concentrations.

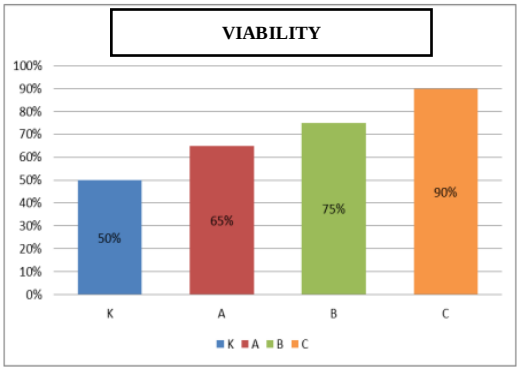

Figure 7. Viability Result

Description:
K= Control
A= Treatment A dose of 5 ml betel leaf extract
B= Treatment B dose of 10 ml betel leaf extract
C = Treatment C dose of 15 ml betel leaf extract

Survival is based on the ratio between the number of fish seeds that are alive at the end of maintenance and the number of seeds at the beginning of stocking. Seed survival was calculated with the following formula:

$$SR= Nt/No \times 100$$

Description:
SR= Survival (%)
Nt= Number of fry at the end of rearing (fry)
No= Number of seeds at the beginning of stocking (tails)

The percentage of survival is recorded in the average per repetition, of the 4 treatments are repeated 2 times. The conclusion of the survival percentage data is shown in the following table:

Table 1. Viability

Treatment	Survival Rate		Viability
	Early	End	
K	20	10	50%
A	20	13	65%
B	20	15	75%
C	20	18	90%

Fish survival is the percentage of the number of fish that live from the number of fish kept in one container at the end of maintenance. Based on the results obtained, treatment C with a dose of 15 ml betel leaf extract has a high survival rate of 90%. After that treatment B with a dose of 10 ml of betel leaf extract has a survival rate of 75%. Then treatment A with a dose of 5 ml of betel leaf extract has a survival rate of 65%, and the last with the smallest survival rate value is treatment K (control), namely without the provision of betel leaf extract with a survival rate of 50%. This is because treatment C with the highest dose has a high survival rate too, because the more the dose of betel leaf extract (Piper betle L), the faster the compounds contained in it work.

The curing time of Koi fish attacked by Aeromonas hydrophilla bacteria can be seen in the table below:

Table 2. Koi Fish Curing Time

Treatment	1st Week	2nd Week	3rd Week	4th Week
K	Invisible	Invisible	Invisible	Invisible
A	Invisible	Invisible	Invisible	Visible
B	Invisible	Invisible	Visible	Visible
C	Invisible	Visible	Visible	Visible

Based on the table above, the healing time of koi fish attacked by Aeromonas hydrophilla bacteria in treatment A with a dose of 5 ml betel leaf extract, the healing process was only seen in the third week which was marked by the shrinking size of the wound on the koi fish, then treatment B with a dose of 10 ml betel leaf extract, the healing process was seen in the second week which was marked by the shrinking size of the wound on the koi fish, and for treatment C with a dose of 15 ml betel leaf extract, the healing process was seen in the first week which was marked by the shrinking size of the wound on the koi fish.

The supporting parameter of the research is water quality. Water is a very important factor for the life of fish seeds, so water quality needs to be considered. Good water quality can improve the survival of koi fish seeds. Water quality parameters measured in this study include temperature, pH and dissolved oxygen (DO).

Table 3. Quality of Water at the Beginning of Research

Treatment	Observation					
	Early			End		
	Temperature (°C)	pH	DO (ppm)	Temperature (°C)	pH	DO (ppm)
K1	27,4	6,8	7,3	28,2	7,91	6,4
K2	27,4	6,8	7,3	28,4	7,93	6,5
A1	27,4	6,8	7,3	28,6	7,98	6,3
A2	27,4	6,8	7,3	28,8	7,96	6,4
B1	27,4	6,8	7,3	28,9	8,08	6,5
B2	27,4	6,8	7,3	28,9	8,13	6,3
C1	27,4	6,8	7,3	29,0	8,14	6,8
C2	2,7,4	6,8	7,3	29,0	8,14	6,8

A good temperature for koi fish life ranges from 25-35℃. Based on the measurement results, the temperature at the beginning of the study was controlled to remain at 27.4℃. While at the end of the study the temperature increased, which ranged from 28.6-29.0 ℃. The temperature at the end of the study rose due to organic matter in the water mixed with betel leaf extract.

Based on the results of pH measurements at the beginning of the study, it was controlled to remain at 6.8. While at the end of the study the pH increased ranging from 7.91 to 8.14. pH increases due to the content of organic matter in the water mixed with betel leaf extract. The pH range is very good for koi fish survival. According to Cholik (2005) said that if the pH of the water in the pond is around 6.5-9.0 is a good condition for the maintenance of koi fish. At the end of the study the pH increased due to the content of organic matter in the water that reacted with betel leaf extract, and the increase in pH was also caused by small alkalinity.

Based on the results of the measurement of dissolved oxygen (DO) at the beginning of the study was controlled to remain at 7.3 mg/l. While at the end of the study oxygen levels decreased ranging from 6.3-6.8 mg/l. At a dissolved oxygen content of more than 5 mg/l fish growth runs normally (Kahfi, 2016). The availability of oxygen is very influential on metabolism in the body of fish and for fish survival.

4. CONCLUSSION

The provision of betel leaf extract has a significant effect on the cure of koi fish seeds infected with *Aeromonas hydrophilla* bacteria which is characterized by a decrease in the size of the wound on koi fish seeds, so that betel leaf extract (*Piper betle* L) is effective to be used as a medicine for fish infected with *Aeromonas hydrophilla* bacteria. And the best dose of betel leaf extract as an antibacterial is in treatment C with a dose of 15 ml with an average survival rate of 90%, which is characterized by the fastest the curing time of Koi fish infected with *Aeromonas hydrophilla* bacteria, for treatment A with a dose of 5 ml betel leaf extract koi fish infected with *Aeromonas hydrophilla* bacteria began to show curing in week 3, then for treatment B with a dose of 10 ml betel leaf extract koi fish began to show curing in week 2, and for treatment C with a dose of

15 ml betel leaf extract koi fish infected with *Aeromonas hydrophilla* bacteria have begun to show curing in the first week.

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