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# Effective Use of *Mimosa pudica* Leaf Extract on *Cyprinus carpio* Infected with *Aeromonas hydrophila*

# Efektivitas Ekstrak Daun *Mimosa pudica* Pada *Cyprinus carpio* Yang Terinfeksi *Aeromonas hydrophila*

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**Putri Amelia Lestari[a] , Ismarica Ismarica[a]*, Irma dewiyanti[b] , Siska Mellisa[a] , Sari Afriani[a] , Cut Dara Dewi[a]**

[a] *Department of Aquaculture, Faculty of Marine and Fisheries, Syiah Kuala University, Jln. Meurebo Campus Usk Darussalam Banda Aceh*
[b] *Marine Science Study Program, Faculty of Marine and Fisheries, Syiah Kuala University, Jln. Meurebo Campus Usk Darussalam Banda Aceh*

**Abstract**

This study aims to determine the effect of giving Mimosa pudica leaf-soaking extract to Cyprinus carpio fish infected with Aeromonas hydrophila. This research was conducted in the Laboratory of Fish Hatchery and Breeding, Faculty of Marine and Fisheries, Syiah Kuala University and the method used a complete randomized design (CRD) with 4 treatments (A: Mimosa pudica extract 0%, B: Mimosa pudica extract 3%, C: Mimosa pudica extract 5%, D: Mimosa pudica extract 7%). The results of this study indicate that Mimosa pudica extract affects the clinical symptoms of Cyprinus carpio. Data from ANOVA analysis showed that the administration of Mimosa pudica extract had a significant effect (P<0.05) on survival with each treatment of 36.67±11.54% in treatment A, in treatment B of 56.67±5.77%, in treatment C of 66.67±5.77% and in treatment D of 83.33±5.77%. Leukocyte results in all treatments showed differences in values where each was in treatment A at 29.5 $10^4$ cells/$mm^3$ , in treatment B at 19.5 $10^4$ cells/mm3, in treatment C at 15.4 $10^4$ cells/mm3, and in treatment D at 7.21 $10^4$ cells/mm3. The haemoglobin results of all treatments also had different values, namely in treatment A of 3.4 g/dl, treatment B of 4.2 g/dl, treatment C of 5.2 g/dl, and treatment D of 6 g/dl. The conclusion from the results of this study is that 7% Mimosa pudica leaf extract shows the best results on clinical symptoms, survival, leukocyte count and haemoglobin levels.

*Keywords: Mimosa pudica, Cyprinus carpio, Aeromonas hydrophila, Leukocytes, Haemoglobin*

**Abstrak**

Penelitian ini bertujuan untuk mengetahui pengaruh pemberian ekstrak perendaman daun *Mimosa pudica* terhadap ikan *Cyprinus carpio* yang terinfeksi *Aeromonas hydrophila*. Penelitian ini dilaksanakan di Laboratorium Pembenihan dan Pembiakan Ikan, Fakultas Kelautan dan Perikanan Universitas Syiah Kuala dan metodenya menggunakan rancangan acak lengkap (RAL) dengan 4 perlakuan (A: Ekstrak *Mimosa pudica* 0%, B: Ekstrak *Mimosa pudica* 3%, C: Ekstrak *Mimosa pudica* 5%, D: Ekstrak *Mimosa pudica* 7%). Hasil penelitian ini menunjukkan ekstrak *Mimosa pudica* berpengaruh terhadap gejala klinis *Cyprinus carpio*. Data hasil analisis ANOVA menunjukkan bahwa pemberian ekstrak *Mimosa pudica* berpengaruh nyata (P<0.05) terhadap kelangsungan hidup dengan masing-masing perlakuan yaitu 36.67±11.54 % pada perlakuan A, pada perlakuan B sebesar 56.67±5.77%, pada perlakuan C sebesar 66.67±5.77% dan pada perlakuan D sebesar 83.33± 5.77%. Hasil leukosit pada semua perlakuan memperlihatkan perbedaan nilai dimana masing-masing yaitu pada perlakuan A sebesar 29.5 $10^4$ sel/mm3, pada perlakuan B sebesar 19.5 $10^4$ sel/mm3, pada perlakuan C sebesar 15.4 $10^4$ sel/mm3, dan pada perlakuan D sebesar 7.21 $10^4$ sel/$mm^3$. Hasil hemoglobin semua perlakuan juga terdapat nilai yang berbeda yaitu pada perlakuan A sebesar 3.4 g/dl, pada perlakuan B sebesar 4.2 g/dl, pada perlakuan C 5.2 g/dl, dan pada perlakuan D sebesar 6 g/dl.Penelitian ini menyimpulkan bahwa ekstrak daun *Mimosa pudica* 7% menunjukan hasil terbaik pada gejala klinis, kelangsungan hidup, jumlah leukosit dan kadar hemoglobin.

Kata Kunci : *Mimosa pudica, Cyprinus carpio, Aeromonas hydrophila, Leukosit, Hemoglobin*

## 1. Introduction

*Cyprinus carpio* (goldfish) is one type of freshwater fish that is of high economic value and has a high tolerance to different environmental conditions (Winker *et al.,* 2011). The carp farming business has several important factors, one of which is the availability of high-quality seeds (healthy, non-

* *Correspondence: [a] Department of Aquaculture, Faculty of Marine and Fisheries, Syiah Kuala University, Jln. Meurebo Kampus Usk Darussalam Banda Aceh*
*e-mail: ismarica@usk.ac.id*

defective and non-diseased seeds) which will spur the development of aquaculture quickly (Ishaqi and Sari, 2019). Poor quality seeds are caused by several factors, one of which is fish disease. One of the carp diseases that is often found is Motile Aeromonas Septicemia. Manik (2014) states that the cause of Motile Aeromonas Septicemia is the bacterium *Aeromonas hydrophila*. The disease attacks very quickly and kills cultured fish so that production levels will decrease (Noor and Jati, 2023). Economic losses are estimated at US$ 9 billion per year in aquaculture due to disease outbreaks (Subasinghe *et al*., 2001). One of the bacteria that may attack fish is *Aeromonas hydrophila* (Murwantoko *et al.,* 2013).

*Aeromonas hydrophila* is a pathogenic bacterial agent in waters that can cause great losses in aquaculture (Gilani *et al.,* 2021). carp is one of the freshwater fish attacked by *Aeromonas hydrophila* bacteria. *Aeromonas* sp. is the most widely isolated bacteria from carp farming systems (Sanyal *et al*., 2018). Jun *et al.* (2013) stated *Aeromonas* spp. is the most frequent bacteria in freshwater habitats and is always associated with severe infections in cultured fish species.

Fish that are susceptible to disease are often treated with the use of antibiotics, namely using Chloramphenicol and oxytetracycline (Igbinosa *et al*., 2012). In addition, diseased fish are also treated using other chemical drugs. Mali *et al.* (2023) stated that chemical drugs for fish treatment have been proven to prevent and inhibit the development of bacteria. However, giving chemical drugs with inappropriate doses and carried out continuously can cause microbial resistance, and pollute the environment. So, natural ingredients can be used as antibiotics that are safe for the environment.

One of the natural materials that can be used as antibiotics that are safe for the environment is *Mimosa pudica* leaves. *Mimosa pudica* leaves are often known as putri malu leaves. Fadlian *et al.* (2016) stated that *Mimosa pudica* leaf extract can inhibit the activity of pathogens and fungi. According to Styani *et al*. (2021), putri malu leaves have anti-inflammatory activity or stabilize red blood cell membranes. The results of the EPM phytochemical test, namely *Mimosa pudica* leaf extract, contain flavonoids, tannins, and gallic acid (Jannah *et al.,* 2018). Anggita *et al.* (2018) stated that *Mimosa pudica* leaves contain secondary metabolite compounds, namely flavonoids and tannins. In addition, flavonoid, steroid, and tannin compounds in *Mimosa pudica* leaf extract also function as free radical inhibitors and stabilize red blood cell membranes.

Research related to the utilization of *Mimosa pudica* leaf extract has been carried out including *Mimosa pudica* leaf extract can inhibit growth and reduce the number of microorganisms in *Euthynnus affinis* (Parnanto *et al*., 2013), the effect of giving crude extract of *Mimosa pudica* leaves on the hematology of koi fish infected with *Pseudomonas fluorescens* bacteria (Himmaty, 2017), the application of *Mimosa pudica* extract as an inhibitor of melanosis in shrimp (Jannah *et al.*, 2018), Anggita *et al. (2018*) concluded that shy daughter leaf extract has a weak inhibition against *Pseudomonas aeruginosa* bacteria, Antibacterial is a biological or chemical compound that is natural or synthetic*,* 2018), Anggita *et al*. (2018) concluded that the extract of putri malu leaves has a weak inhibition against *Pseudomonas aeruginosa* bacteria, Antibacterials are biological or chemical compounds that are natural or synthetic to inhibit bacterial growth and activity. Based on the research, research related to the effectiveness of *Mimosa pudica* leaf extract as a natural antibiotic in goldfish (*Cyprinus carpio*) seeds infected with *Aeromonas hydrophila* bacteria has never been done so it is necessary to conduct this research to add information about drugs that inhibit the growth of *Aeromonas hydrophila* bacteria in goldfish.

## 2. Materials and Methods

### *2.1. Time and Place*

The research was conducted from December 2022 to January 2023 at the Fish Hatchery and Breeding Laboratory, Faculty of Marine and Fisheries, Syiah Kuala University, and the Agriculture Laboratory, Syiah Kuala University.

### *2.2. Preparation of Mimosa pudica leaf extract*

Mimosa pudica leaves that have been picked are then washed thoroughly first, then dried for 5 days, after drying the Mimosa pudica leaves are mashed into flour using a blender. The flour was macerated for 3 days with 70% ethanol solvent. During maceration, the sample was stirred every first 6 hours and allowed to stand for the next 18 hours (Pal *et al*., 2015). The filtrate was filtered using Whatmann filter paper. Then the filtering results were evaporated using a rotary evaporator to become a thick extract. The extract was stored at refrigerator temperature for further use (Lakshmibai *et al*., 2016).

### *2.3. Preparation of maintenance media and test animals*

The maintenance medium for the test animals is a 25 L jar of 12 pieces. Jars that will be used are first washed and dried for 1 day. After that, the jar is filled with 10 L of fresh water and equipped with an aerator. After that, the water was settled for 5 days.

The test animals in this study were goldfish fry from Lambaro village, Aceh Besar Regency as many as 120 fish (1 fish/L) with a length of 8-10 cm. the fish were first acclimatized for 3 days. Acclimatization aims to adjust the fish to the new environment. Fish were fed with pellets twice a day (morning and evening).

### *2.4. Bacterial dilution*

*Aeromonas hydrophila* bacteria obtained from BPAP Ujung Batee with a density of 6 x $10^8$ cells/ml. Bacterial dilution is carried out to obtain bacteria with a bacterial density of 6 x $10^7$ cells/ml. The first stage of bacterial dilution is Nutrient agar (NA) weighed as much as 2 g and then mixed with distilled water as much as 100 ml in an erlenmeyer erlenmeyer, the solution was stirred until homogeneous and sterilized in an autoclave autoclave with a temperature of $121^0$ C for 30 minutes. The sterilized NA media was allowed to stand for a few minutes then each NA media was poured 10 ml into a petri dish and the pouring was done in an isolation room (*Laminar Air Flow*) and allowed to harden and the NA media was ready for use. Furthermore, each 1 petri dish of bacteria was filled into a test tube and then added 9 ml of distilled water, each suspension was taken as much as 1 ml filled into a test tube containing 9 ml of distilled water and serial dilution was carried out until dilution $10^7$. After dilution, bacteria were isolated using the pouring cup method by dripping 1 ml of liquid suspension at dilution $10^7$ into a petri dish, then poured NA media and incubated for 24 hours (Dermawan *et al.* 2016).

### *2.5. Bacterial infection*

Infection of *Aeromonas hydrophila* bacteria by immersion. The bacteria soaking medium is a jar with a size of 25 liters. Bacteria were immersed as much as 1 ml/L. Fish were immersed until clinical symptoms appeared (pale color, peeling scales, and often swimming to the surface.

### *2.6. Mimosa pudica leaf extract dilution*

The extract was diluted according to the concentration. Extract dilution was carried out according to the research of Hamidah *et al.* 2019. The concentration of *Mimosa pudica* leaf

extract is 3%, 5%, and 7%. The dilution carried out for a concentration of 3% is 15 ml of extract added to 485 ml of distilled water. The dilution carried out for a concentration of 5% is 25 ml of extract added to 475 ml of distilled water. The dilution carried out for a concentration of 7% is 35 ml of extract added to 465 ml of distilled water.

2.7. *Carp treatment and maintenance*

Treatment of goldfish with *Mimosa pudica* leaf extract using the immersion method. Goldfish that have been infected with bacteria are put into a container that has been installed with an aerator and *Mimosa pudica* leaf extract is added at a dose of 3, 5, 7%. Immersion of the extract was carried out for 24 hours (Hanapin, 2016). Carp blood samples were taken before and after treatment to calculate the total number of leukocytes and hemoglobin.

After the treatment process, the water in the jar was replaced with normal water and maintenance was carried out for 14 days. During maintenance, pellets were fed ad satiation with feeding frequency in the morning and evening every day. Water siphoning was done after 2 hours of feeding and water change was done once a week with 20% of the total water volume.

2.8. *Research parameters*

The parameters of this study were clinical symptoms of fish (fish body color, bleeding on the fish body, ulcers, swimming ability, damaged and whitish fins, damaged and protruding eyes, etc.), survival rate (SR), water quality (pH, temperature, and DO), and blood picture (leukocytes and hemoglobin).

Survival (SR)

$$SR = \frac{Nt}{No} x100\%$$

Description:
SR = Survival rate;
Nt = Number of live test fish at the end of the study;
No. = Number of live test fish at the beginning of the study;

Blood picture

$$Leukosit\ (10^3\ sel\ mm^{-3}) = \frac{W1 + W2 + W3 + W4}{N\ x\ P}$$

Description:
W = Number of cells;
N = Number of counting rooms (4),
P = Diluent (20 x 10).

*2.9. Data Analysis*

Survival rate data were analyzed using ANOVA (Analysis of Variances) test at 95% confidence interval. Based on the results of ANOVA, the data obtained had a significant effect ($P < 0.05$), so the data were further tested using the Duncan test. The parameters of clinical symptoms, blood picture, and water quality were analyzed descriptively.

## 3. Results and Discussion

Clinical symptoms were observed visually by observing fish behavior and damage to fish body parts for 14 days. Before being infected, the results of observations of clinical symptoms of goldfish in all treatments were still in normal condition. After being infected, the goldfish showed clinical symptoms presented in Figure 1.

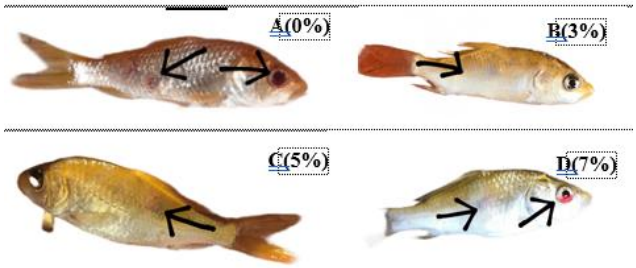


**Figure 1.** Clinical Symptoms in treatment A (0%) were red spots on the surface of the body and eyes and peeling scales; in treatment B (3%) were body discoloration, peeling scales, and excessive mucus; Treatment C (5%) were discoloration and swelling of the body; treatment D (7%) were peeling scales, body discoloration, excessive mucus, bleeding in the eyes.

After treatment, the carp showed the clinical symptoms presented in Figure 2.

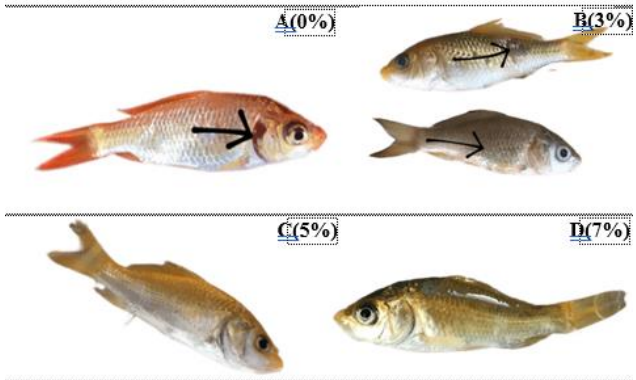


**Figure 2.** Clinical symptoms in treatment A (0%) are red spots on the body surface and peeling scales; in treatment B (3%) are peeling scales, and excessive mucus; Treatment C (5%) is mucus begins to decrease; treatment D (7%) is the condition of the fish back to normal.

Goldfish after being infected with *Aeromonas hydrophila* bacteria in all treatments (Figure 1) showed clinical symptoms similar to the research of Buda *et al.* (2023), namely clinical symptoms in goldfish (*Cyprinus carpio*) infected with *Aeromonas hydrophila* bacteria characterized by changes in shape, physique, behavior and response to feed. Clinical symptoms that appear in goldfish infected with *Aeromonas hydrophila* are red spots at the base of the tail, inflammation at the injection site, distended abdomen, and protruding eyes (Pratama *et al*., 2017). Naptipulu *et al*. (2016) stated that clinical symptoms in fish include wounds on the body surface and detached scales.

From the observation of clinical symptoms for 14 days, the results obtained showed that the fish immune system decreased which resulted in fish stress until death occurred. The cause that occurs is because the dose of *Mimosa pudica* leaf extract in treatment A (0%), treatment B (3%), and treatment C (5%) is not able to overcome the bacterial infection of *Aeromonas hydrophila* because the dose is not effective to overcome the bacterial infection, while in treatment D (7%) is seen as quite effective in overcoming bacterial infection with the highest level of effectiveness, due to the increasing dose given the increasing levels of active ingredients dissolved in it, thus increasing its ability to suppress bacteria and ultimately increasing the degree of recovery of goldfish infected with bacteria. As stated by Schlegel (1994), that the ability of an antimicrobial to negate the ability of living organisms depends on the concentration of the antimicrobial material itself. In addition to clinical symptoms, the dosage of *Mimosa pudica* leaf extract also affects the survival of *Cyprinus carpio* fish.

Survival data analyzed by ANOVA test showed significant effect ($P<0.05$). The results of Duncan's further test in treatment

A (0%) were significantly different from treatment B (3%), C (5%), D (7%) and in treatment B (3%) were not significantly different from treatment C (5%). The results of the analysis can be seen in Table 1.

**Table 1.**
Anova survival test data

| Treatment | SR (%) |
|---|---|
| A (0%) | 36.67± 11.54[a] |
| B (3%) | 56.67± 5.77 [b] |
| C (5%) | 66.67± 5.77 [b] |
| D (7%) | 83.33± 5.77[c] |

**Notes:** SR=Survival rate, same superscript indicates not significantly different while different superscript indicates significantly different.

*Mimosa pudica* leaf extract has a significant effect on survival for 14 days. The highest survival is in treatment D (7%) which is 83.33 ± 5.77 %, while the lowest value in treatment A (0%) is 36.67 ± 11.54 %. Widiastuti (2009) said the average value of goldfish survival was 97%, while according to Buda *et al.* (2023) the highest value was 80%. Anugrah (2018) states that a survival rate of >50% is classified as good, 30-50% is moderate and <30% is not good. The lowest survival rate in this study was because the fish were not given *Mimosa pudica* leaf extract treatment, while the higher fish survival rate in this study was due to fish treatment using *Mimosa pudica* leaf extract. This is because the active compounds contained in *Mimosa pudica* leaf extract affect the growth of *Aeromonas hydrophila* bacteria. These active compounds can withstand the growth of *Aeromonas hydrophila* bacteria. The results of research by Utami *et al*. (2021) stated that the use of shy daughter leaf extract for the treatment of goldfish seeds infected with *Aeromonas hydrophila* bacteria with 24-hour soaking is effective for treatment, because shy daughter leaves contain anti-bacterial compounds. The statement of Masitoh (2020) is that *Aeromonas hydrophila* bacteria can produce toxin products that can cause death even though the clinical symptoms seen from the outside are due to inflammation. In addition, fish survival is not directly influenced by feed, but due to internal factors, namely lack of adaptation and stress during maintenance (Buda *et al*., 2023). Effendi (2002) states that survival is also influenced by internal factors such as disease resistance and age.

In addition to survival, *Mimosa pudica* leaf extract also affects the blood picture of fish such as leukocytes and hemoglobin. The results of the blood picture of leukocytes and hemoglobin levels can be seen in Table 2.

**Table 2.**
Leukocyte count and hemoglobin level data

| No. | Treatment | Leukocyte count ( $10^4$ cells/mm $)^3$ | | Hemoglobin levels (g/dl) | |
|---|---|---|---|---|---|
| | | H-1 | H-3 | H-1 | H-3 |
| 1. | A (0%) | 28.4 | 29.5 | 3.4 | 3.4 |
| 2. | B (3%) | 26.1 | 19.4 | 4.2 | 4.2 |
| 3. | C (5%) | 25.8 | 15.4 | 5 | 5.2 |
| 4. | D (7%) | 22.9 | 7.2 | 5.2 | 6 |

**Notes**: H-1 = day after bacterial infection, H-3 = day after treatment.

Treatment D (7%) showed the best leukocyte results of $7.21x10^4$ cells/$mm^3$, followed by treatment C (5%) $15.4x10^4$ cells/$mm^3$, treatment B (3%) 19.4 $x10^4$ cells/$mm^3$, and in treatment A (0%) $29.5x10^4$ cells/$mm^3$. While the best Hemoglobin value results in treatment D (7%) which is 6 g/dl, then in treatment C (5%) which is 5.2 g/dl, treatment B (3%) 4.2 g/dl and in treatment A (0%) which is 3.4 g/dl.

The results that have been obtained in Treatment D (7%) is the best treatment based on the results of leukocytes obtained which is $7.21x10^4$ cells/$mm^3$. The number of leukocytes is still within the threshold. statement Lagler *et al*., (1977) that the average number of normal fish leukocytes ranges from 20,000 - 150,000 cells / $mm^3$. The number of leukocytes below the normal limit indicates that the fish has symptoms of anemia, while the number of leukocytes above the normal limit, the fish will show resistance to foreign antigens (Dayanti *et al* 2013). *Mimosa pudica* leaf extract influences the number of leukocytes. After immersion, there is a decrease in the number of leukocytes, which indicates that the fish can adapt to new environmental conditions. According to Mali *et al.* (2023), the low number of leukocyte cells indicates that the jackfruit leaf extract treatment given can be accepted by fish and can adapt to the treatment given well. According to Arindita and Prayitno (2014), the decrease in the number of leukocytes in fish is due to leukocyte cells in the blood vessels decreasing because many leukocytes move towards tissues infected by *Aeromonas hydrophila* bacteria.

Leukocytes are a blood picture that can be used as an indicator of infection in the body. The body will increase leukocyte production when a foreign body enters the body (Dianti *et al.,* 2013). In addition, leukocytes are also blood components that function as non-specific defenses that will localize and eliminate pathogens through the process of phagocytosis (Riauwaty and Syawal, 2016). Anderson and Siwicki (1993) stated that an increased leukocyte count can be a sign of infection, stress, or leukemia. Infection will show inflammation. The response will also appear due to the presence of toxic chemicals, bacteria, parasites, and viruses. In addition, leukocytes are blood components that function as a non-specific defense that will localize and eliminate pathogens through the process of phagocytosis. According to Riauwaty and Syawal (2016), the difference in total leukocytes is caused by the relatively high temperature in the fish rearing pond which eventually causes disease seeds to develop more easily and causes fish to experience stress. Giving *Mimosa pudica* leaf extract can increase the non-specific immune system characterized by a decrease in total leukocytes due to the active substance content of flavonoids tannins and saponins in the leaves of putri malu which are anti-bacterial (Ardiansyah, 2007).

In addition to leukocytes, hemoglobin levels are also observed blood picture parameters. The highest hemoglobin level was in treatment D (7%) with a value of 6 g/dl. This statement is the same as the statement of Kusrini *et al.* (2019), the range of carp hemoglobin levels is still in the normal range ranging from 6-10 (g/dl blood). It is suspected that the production of hemoglobin in the fish body is due to the active ingredients contained in *Mimosa pudica* leaf extract but also caused by physiological and environmental factors. Ardika *et al.* (2016) stated that the factor that affects the increase in hemoglobin is stress in fish. Mali *et al.* (2023) stated that hemoglobin levels were influenced by flavonoid compounds in jackfruit leaf extract. flavonoid compounds work as antibacterials that cause hemoglobin levels in the fish body to return to normal after infection with *Aeromonas hydrophila* bacteria. Flavonoid compounds play a role in the process of stabilizing red blood cell membranes where flavonoids will protect erythrocyte membranes from damage due to hypotonic solution induction. In addition, flavonoid compounds work by interacting with hypotonic solutions and will inhibit their membrane-damaging activity (Saputra, 2015).

In contrast to the above statement, *Mimosa pudica* leaf extract has no effect on water quality. Kordi (2010) states that the normal temperature in aquaculture is 27-29 ° C with DO

measurements during the maintenance of test fish obtained a value of 3.44 - 6.95 mg / l, and pH 6.5-7.5 is still in the normal category and can be tolerated by carp. The water quality data is presented in Table 3.

**Table 3.**
Water quality observation data

| Treatment | Temperature (ºC) | DO (mg/l) | pH |
|---|---|---|---|
| A (0%) | 25-28 | 3.12-6 | 7.5- 8.5 |
| B (3%) | 25-28 | 3.12-6 | 7.5- 8.5 |
| C (5%) | 25-28 | 3.12-6 | 7.5- 8.5 |
| D (7%) | 25-28 | 3.12-6 | 7.5- 8.5 |

## 4. Conclusion

The conclusion of the results of this study is that the extract of shy daughter leaves (Mimosa pudica) has a real effect on survival, hemoglobin and leukocytes with each value of 85%, 6 g/dl, and 7.2 $10^4$ cells/$mm^3$. The best dose of *Mimosa pudica* leaf extract in this study is 7% in treatment D. *Mimosa pudica* leaf extract at a dose of 7% can be used as an anti-bacterial in goldfish infected with *Aeromonas hydrophila* bacteria.

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