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EFFECTIVENESS OF NARCISSUS LEAF EXTRACT (*Crinum asiaticum* L.) FOR THE TREATMENT OF CARP (*Cyprinus carpio* L.) JUVENILE INFECTED WITH *Aeromonas hydrophila*

EFEKTIVITAS EKSTRAK DAUN BAKUNG (*Crinum asiaticum* L.) UNTUK PENGOBATAN BENIH IKAN MAS (*Cyprinus carpio* L.) YANG DIINFEKSI BAKTERI *Aeromonas hydrophila*

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Abstract

In fish farming activities, problems often arise that must be faced by farmers, the main factor causing the failure of fish farming efforts is the emergence of disease attacks. The type of disease that often attacks carp (Cyprinus carpio) is the bacteria Aeromonas hydrophila. Efforts made by farmers using artificial drugs or antibiotics. The use of antibiotics does not always have a positive effect; there are also negative effects. Therefore, natural, environmentally friendly handling is needed, and water lily leaf extract plants (Crinum asiaticum L.) are able to treat A. hyrophila infections in farmed fish. This study aims to determine the soaking of water lily leaf extract on the survival and optimal concentration that can reduce infection in carp juvenile (Cyprinus carpio). The research method used is an experiment with a Completely Randomized Design (CRD) consisting of 3 treatments and 3 replications. Treatment P1 (20 ml of water lily leaf extract), treatment P2 (40 ml of water lily leaf extract), and P3 (60 ml of water lily leaf extract). Data were analyzed using Analysis of Variance (ANOVA). If the results were significantly different, they were followed up with the Least Significant Difference (LSD) test. The results showed that the provision of water lily leaf extract (Crinum asiaticum L.) soaking had a very significant effect on the survival of carp (Cyprinus carpio) juvenile. The optimal concentration that can reduce A. hyrophila bacterial infection is in treatment P1 (20 ml) with the highest survival rate of 90%. Water quality during the study obtained a temperature value between 23-27.3 oC, pH between 6.9-8.45, and DO levels between 4.1-8.3 mg/l.

Keywords: *Aeromonas hydrophila*, water lily leaves, carp, survival

Abstrak

Dalam kegiatan budidaya perikanan kerap muncul permasalahan yang harus dihadapi para pembudidaya, faktor utama penyebab gagalnya usaha budidaya ikan yaitu munculnya serangan penyakit. Jenis penyakit yang sering menyerang ikan mas (*Cyprinus carpio*) adalah bakteri *Aeromonas hydrophila.* Upaya yang dilakukan pembudidaya dengan menggunakan obat buatan atau antibiotik. Penggunaan antibiotik tidak selamanya memberikan efek positif namun juga terdapat efek negatif oleh sebab itu, diperlukan penanganan secara alami, ramah lingkungan, dan mampu mengobati infeksi *A. hyrophila* pada ikan budidaya dengan tanaman ekstrak daun bakung (*Crinum asiaticum* L.). Penelitian ini bertujuan untuk mengetahui perendaman ekstrak daun bakung terhadap kelangsungan hidup dan konsentrasi optimal yang dapat menurunkan infeksi pada benih ikan mas (*Cyprinus carpio*). Metode penelitian yang digunakan adalah eksperimen dengan Rancangan Acak Lengkap (RAL) yang terdiri dari 3 perlakuan dan 3 ulangan. Perlakuan P1 (20 ml ekstrak daun bakung), perlakuan P2 (40 ml ekstrak daun bakung), dan P3 (60 ml ekstrak daun bakung). Data dianalisis menggunakan *Analisis of Variance* (ANOVA) apabila hasilnya berbeda nyata maka dilanjutkan dengan uji beda nyata terkecil (BNT). Hasil penelitian menunjukkan bahwa pemberian perendaman ekstrak daun bakung (*Crinum asiaticum* L.) berpengaruh sangat nyata terhadap kelangsungan hidup benih ikan mas (*Cyprinus carpio*). Konsentrasi optimal yang dapat menurunkan infeksi bakteri *A. hyrophila* yaitu pada perlakuan P1 (20 ml) dengan tingkat kelangsungan hidup paling tinggi sebesar 90%. Kualitas air selama penelitian didapatkan nilai suhu antara 23-27,3 ^{o}C, pH antara 6,9-8,45, dan kadar DO antara 4,1-8,3 mg/l.

Kata kunci: *Aeromonas hydrophila*, daun bakung, ikan mas, kelangsungan hidup

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1. Introduction

As part of efforts to meet the increasing demand for fishery products, the implementation of freshwater aquaculture systems, which have now reached the intensification stage, is not

without biological risks, namely the emergence of disease- s (Khairuman et al., 2008). Fish farming activities, including carp (*Cyprinus carpio)* farming, present many problems and challenges that must be faced by farmers. One of the most common problems and a major factor causing the failure of fish farming businesses is disease (Afrianto et al., 2015). Fish diseases are often caused by fungi, parasites, viruses, and bacteria (Suminto et al., 2013).

Fish disease is caused by microorganisms that live and grow in the fish's body, disrupting its health. The onset of disease in fish can be caused by three factors, namely poor physical condition of the fish, poor living environment, and pathogens or causes of disease (viruses, bacteria, fungi, and parasites). These three factors are closely related, because if one factor occurs, disease will definitely occur (Partosuwiryo and Yus, 2011). There are two causes of disease in fish, namely living and non-living organisms. Living organisms that cause disease in fish are parasites. Examples of parasites that attack carp are viruses, fungi, bacteria, protozoa, worms, and microscopic shrimp. Non-living causes of disease include the physical properties of water, the chemical properties of water, and feed that is unsuitable for carp. Types of diseases that often attack carp are *Icthyophthirius multifilis, Lernea, Dactylogyrus,* and *Aeromonas hydrophila (*Khairuman et al., 2008).

Aeromonas hydrophila is a gram-negative bacterium that is opportunistic and can cause mass mortality in fish in a very short time (Lukistyowati and Kurniasih, 2012). *A. hydrophila* is one of the parasites that often attacks ornamental fish or fish for consumption, with a mortality rate of up to 100%. Fish infected with this disease are characterized by clinical symptoms such as red lesions on their bodies. This bacterium infects and causes swelling and bleeding of organs and is commonly found in parts of the fish's body such as the gills, liver, skin, kidneys, and digestive tract (Kabata, 1985). A*. hydrophila* affects almost all freshwater commodities at all ages and can even become a deadly epidemic, causing enormous losses (Kamiso and Triyanto, 1992).

One medicinal plant that has potential in healing microbial infections, especially *A. hydrophila* in fish, is the calla lily (*Crinum asiaticum* L.). According to Widayani et al. (2019), calla lily leaves contain foam that is useful for maintaining wound moisture, thereby accelerating wound healing. Crinum leaves are known to contain alkaloids, triterpenoids, saponins, tannins, and flavonoids (Mentari and Hidayah, 2020). These compounds make crinum leaves effective as antioxidants and antibacterials (Riris et al., 2018). Based on this antibacterial ability, this study utilized bakung leaves to treat A*. hydrophila* infections in carp juvenile. The treatment was carried out through a system of immersion in bakung leaf extract, which is an effective method because water-soluble antibacterial compounds can be absorbed by the skin, gills, liver, and kidneys of carp juvenile (Sukamto, 2007). Therefore, it is necessary to test the effectiveness of bakung leaf extract as a treatment for A*. hydrophila* bacterial infections in carp juvenile.

2. Materials and Methods

2.1. Time and Place

The study was conducted from April to July 2024. It was carried out at the Freshwater Fish Cultivation Center (BBIAT) in Muntilan, Magelang Regency, Central Java, while the extraction of bakung leaves and the cultivation of pure *Aeromonas hydrophila* bacteria as test bacteria were conducted at the Integrated Laboratory of the Faculty of Agriculture, Tidar University, Magelang, Central Java.

2.2. Equipment and Materials

The equipment used in this study included aquariums, aerators, hoses, aeration stones, blenders, sieves, Bunsen burners, analytical scales, thermometers, pH meters, DO meters, *rotary evaporators*, and *syringes*. The materials used in this study included carp juvenile, *Aeromonas hydrophila*, bakung leaves, 95% ethanol, and distilled water.

2.3. Research Procedure

The procedures carried out during the research included:

a. Provision of media and test fish. The media used in this study was an aquarium measuring 60x20x30 cm with a water volume of 36 L. Before use, the aquarium was first washed with running water until clean, then left to stand or dried in the sun until completely dry. This study used 3 treatments with 3 replicates, so a total of 9 aquariums were used for the treatment media. Each test container was filled with 24 L of water that had been left to stand for 3 days and equipped with strong aeration to supply oxygen (Makhfirah et al., 2018). A total of 90 carp fry measuring 7-9 cm in length were tested, with each aquarium containing 10 test fish. The test fish were acclimatized beforehand so that they could adapt to the test aquarium for 5 days. During the acclimatization and adaptation process carried out in the hapa, the fish were fed floating pellets with a protein content of ± 30%. The amount of feed given is 5% of the fish's body weight per day, with a feeding frequency of twice a day at 9:00 AM and 3:00 PM. This adaptation process is carried out so that the fish do not experience excessive stress in the new environment (Fandhi, 2014).

b. The production of daylily leaf extract using the maceration method involved washing fresh daylily leaves thoroughly, then air-drying them for one day until dry. The leaves were then ground or blended until smooth. Next, 1800 grams of daylily leaf powder was weighed and extracted using the maceration method with 18 liters of 95% ethanol solvent. According to Mirani and Mangunsong (2018), the maseration process of bakung leaves was carried out in a tightly closed glass container at room temperature and avoided direct sunlight for 3 times 24 hours while stirring occasionally for 15 minutes. Stirring was carried out at least once a day. The crude extract that had been settled for 3 times 24 hours was then filtered with filter paper to obtain the filtrate and residue. The resulting maserated daffodil leaves were then evaporated using a *rotary vacuum evaporator* at a temperature of 70°C with a rotation speed of 120 rpm to obtain a thick extract of daffodil leaves by separating the extract and ethanol, thus obtaining a thick and concentrated extract from daffodil leaves. Extraction using the maceration technique has several advantages, namely its simplicity, which minimizes damage to natural materials or the content of bakung leaves (Dinda and Ridwanto, 2022).

c. The cultivation of *Aeromonas hydrophila* bacteria and the preparation of TSA (*Tryptic Soy Agar)* media for bacterial culture were carried out by weighing 20 grams of TSA media and dissolved in 500 ml of sterile distilled water in an Erlenmeyer flask, then homogenized and boiled on a hot plate while stirring continuously until completely dissolved for approximately 10 minutes. After boiling, the solution is covered with cotton and aluminum foil, then the medium is sterilized in *an autoclave* at 120°C for 15 minutes. Next, the solution is poured into sterile Petri dishes to a height of 3 mm, poured near a Bunsen burner. After pouring the

solution, the edges of the Petri dishes are heated with a Bunsen burner to prevent contamination by other organisms. The medium is left to harden and stored in an incubator at 30°C for 24 hours, after which it can be used. Media that is not used immediately can be stored in a refrigerator/cooler, wrapped individually in newspaper and placed with the Petri dish lid facing down to avoid condensation dripping onto the medium. If stored media is to be used, it must first be placed in an incubator so that the media temperature is the same as the ambient temperature. Weigh 1.3 grams of NB (*Nutrient Broth)* and dissolve it in 100 ml of sterile distilled water in an Erlenmeyer flask, then homogenize until completely dissolved. The Erlenmeyer flask is covered with cotton and aluminum foil, then the medium is sterilized in *an autoclave* at 120°C for 15 minutes. After that, the medium is cooled to 30°C. Bacterial inoculation is carried out on cold media, because bacteria will die if exposed to hot temperatures. To make it last longer, media that is not used immediately can be stored in a refrigerator. To cultivate *Aeromonas hydrophila* bacteria on TSA media, bacteria from a pure culture are taken with an ose needle that has been previously heated with a Bunsen burner. Next, they are inoculated on the media using a zig-zag streaking method. The media that has been inoculated with bacteria is incubated in an incubator at a temperature of 37°C for 24 hours. For NB medium, 4 mm of medium is poured into a test tube. Then, 5 inocula of bacteria from a pure culture are added and incubated in an incubator at 37°C for 24 hours (Irianto, 2006).

d. Injection of *Aeromonas hydrophila* bacteria into test fish. The *Aeromonas hydrophila* bacteria used in this study were obtained from the Integrated Laboratory of the Faculty of Agriculture, Tidar University, Magelang. Before injecting *A. hydrophila* bacteria into carp fry, they were diluted first. The bacteria that had been cultured in NB medium were then grown on TSA solid medium. One ose of bacteria was mixed into 5 ml of distilled water. The liquid bacterial isolate was standardized using the McFarland method, which equalizes turbidity with the McFarland standard solution and is equivalent to a bacterial density of $1.5x10^{(2)}$CFU/ml. Next, A*. hydrophila* bacteria were activated to make them more aggressive. One ose of bacterial isolate was dissolved in 3 ml of NB liquid medium, then incubated for 24 hours. The incubated bacteria were cultured on TSA slant medium and incubated for 24 hours. The grown bacteria were homogenized into 5 ml of distilled water. This bacterial isolate was then compared with the McFarland standard suspension, which equalized the turbidity with the McFarland standard solution equivalent to a density of $1.5x10^{(2)}$CFU/ml. The A*. hydrophila* bacteria obtained were then injected into the fish. The injection method used in this study was intramuscular, whereby the bacteria were injected into the back of each test fish that was to be placed in the treatment medium. The A*. hydrophila* bacteria injected into the fish was 0.1 ml/fish with a dilution dose of $10^{(2)}$cfu/ml and 0.9 ml/fish of NaCl was added, then homogenized, referring to the research by Lukistyowati and Kurniasih (2011). After infection, the fish were left undisturbed or maintained for 24 hours to observe the clinical symptoms caused by the bacterial infection. However, if some fish died before 24 hours, all fish were harvested and transferred to an aquarium containing bakung leaf extract at each concentration according to the treatment to prevent mass mortality (Rosidah et al., 2018).

e. Treatment with daffodil leaf extract was carried out after the fish were left for 24 hours after infection with *Aeromonas hydrophila* bacteria. The infected fish were then placed in aquariums containing daffodil leaf extract at concentrations corresponding to each treatment, namely A (20 ml), B (40 ml), and C (60 ml). The test fish were immersed in the bakung leaf extract at the respective concentrations for 45 minutes, according to Gasperz (1991). After the immersion process was complete, the aquarium water was replaced with normal water, and maintenance and observation were carried out for 14 days to see if there was any recovery in the fish after treatment.

2.4. Water Quality Measurement

Water quality measurements in this study included temperature, pH, and DO, which were taken in the morning between 8:00 and 9:00 a.m. and in the afternoon between 4:00 and 5:00 p.m. Temperature was measured using a thermometer, pH using a pH meter, and DO using a DO meter.

2.5. Research Parameters

In this study, the research method used a completely randomized design (CRD) with 3 treatments and 3 replicates. The treatments in this study were the use of 20 ml of daffodil leaf extract (P1), the use of 40 ml of daffodil leaf extract (P2), and the use of 60 ml of daffodil leaf extract (P3).

a. Clinical Symptoms

The clinical symptoms observed were damage to the surface of the fish's body and changes in the fish's behavior by observing the fish's response to feed and its response to shock (reflex test) by tapping the aquarium glass (Cahyono 2001). In general, the clinical symptoms of carp infected with *A. hydrophila* are that the color of the fish's body becomes slightly dark, the skin becomes rough and bleeding occurs, which then turns into ulcers. The fish become passive or often remain still and frequently open their mouths at the water surface to take in oxygen because their gills are damaged, making it difficult for them to breathe. Their stomachs appear slightly bloated, all their fins are damaged and turn white, and their eyes are damaged and slightly protruding. Observations were conducted daily during the treatment period of 45 minutes and the maintenance period of 14 days (Rosidah et al., 2018).

b. Survival Rate

The survival rate is the percentage of fish alive at the end of the treatment period compared to the initial number of fish at the start of the treatment period (Widyatmoko *et al*., 2019). The survival rate of carp was observed by counting the number of fish alive each day during the treatment period. The survival rate of carp in percentage terms is determined by dividing the number of test fish alive at the end of the study by the number of test fish alive at the beginning of the study (Effendie, 1997). According to Emu (2010), survival rate is the ratio between the number of fish alive at the beginning and the number of fish alive at the end of the cultivation period, with the following calculation formula:

$$SR = \frac{NT}{N0} \times 100\%$$

Explanation:
SR = Survival rate of test fish (%)
NT = Number of fish alive after 3 days of maintenance following anesthesia (tails)
N0 = Number of live fish before anesthesia (tails)

2.6 Data Analysis

The effect of immersing carp fry in daylily leaf extract on survival was analyzed using *Analysis of Variance* (ANOVA). If the

results were significantly different, the least significant difference (LSD) test was used to compare the values between treatments with the best response at the 0.05 level using SPSS software. Data on water quality measurements and clinical symptoms of fish were analyzed descriptively (Fandhi, 2014). According to Akib (2014), the decision criteria are as follows:

I. If Fcount ≤ Ftable with a 95% confidence interval (CI) α (0.05), then accept H_o or reject H_I, meaning that there is no effect of giving buttercup leaf extract on the survival rate of carp juvenile.
II. If Fcount > Ftable with a confidence interval (CI) of 95% α (0.05), then fail to reject H_o or accept H_I, meaning that there is an effect of giving milkweed leaf extract on the survival rate of carp fry.
III. If Fcount > Ftable, then a further test is carried out to determine the significance level of the effect of giving milkweed leaf extract on the survival rate of carp fry using the BNT (Smallest Significant Difference) test calculation formula as follows:

$$BNT\alpha = (t\alpha; dbg)\sqrt{2KTG/r}$$

Explanation:
α = 5% significance level
dbg = degrees of freedom for error
KTG = mean square error
r = number of replications
t = treatment

3. Results and Discussion

a. Water Quality Parameters

Water quality is the physical, chemical, and biological condition and properties of a body of water compared to the standards of suitability for specific requirements. The level of productivity of a body of water is determined by environmental factors, especially the suitability of water quality. Various components, including feed residues, urine, and other organic materials, affect water quality in controlled aquaculture systems (Handajani, 2011). In this study, water quality measurements were taken daily for 14 days during the maintenance period in a carp (*Cyprinus carpio)* fry aquarium. The water quality parameters observed were temperature, pH, and DO.

1. Temperature

An important water quality parameter for supporting fish survival is temperature. According to research, the optimal water temperature for each fish species varies. The optimal water temperature for the survival of carp (*Cyprinus carpio)* is 22-28˚C. (FAO, 2013). Figure 1 shows the average temperature during the 14-day maintenance period.

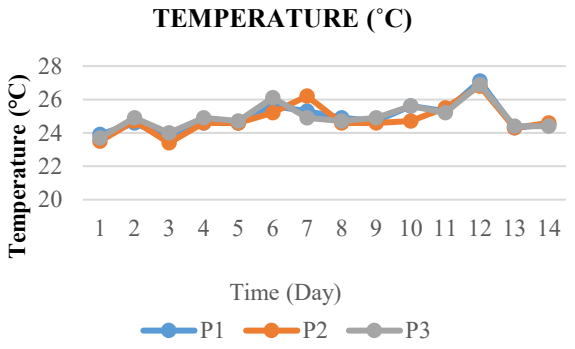

Figure 1. Average Temperature Results Over 14 Days

In this study, the temperature measured in the first week ranged from 23.6 to 26.2°C, while in the second week, the temperature measured ranged from 24.3 to 27.1°C. The difference in temperature range between the first and second weeks of the maintenance period was highly dependent on the environment and weather. This was because the weather was sunny in the first week, followed by cloudy conditions in the afternoon, so the research site was not directly exposed to sunlight. Sunlight only shone through the windows in the morning until noon. In the second week, the temperature range was one degree higher than in the first week. This was because the weather in the second week was more often sunny and cloudy, causing the temperature to be higher than in the first week. All treatments had almost the same average temperature during the maintenance period. This was because the maintenance process was carried out in the same environment, so there were no significant differences between treatments. This supports the research by Faizati et al. (2021), which states that the temperature of fish kept in the same place and in the same water will be comparable. This temperature range is still considered normal and suitable for carp (*Cyprinus carpio)* cultivation, as supported by Makaminan (2011), who stated that the optimum temperature range for carp (*Cyprinus carpio)* is 22-30 °C.

One environmental factor that has a significant impact on aquatic animals is temperature. The most important factor that greatly affects the survival and growth of fish is temperature (Widaryati, 2016). This is in line with the opinion of Ridwantara et al. (2019), namely that water temperatures that are too low or too high can affect fish growth and health. Water temperatures that are too low can inhibit fish metabolism and growth rates. In addition, low water temperatures can trigger fish diseases caused by bacteria, while water temperatures that are too high can reduce the dissolved oxygen content in the water, which can cause stress in fish and reduce their resistance to disease. Then Taufik et al. (2009) stated that carp fry can adapt and live in cold, room, and warm temperatures, but temperature fluctuations can also cause an increase in ammonia levels in the water.

2. pH

Another important water quality parameter that needs to be considered is the pH condition of the water in the cultivation environment. According to Susanto (2014), the measurement of acidity (pH) is expressed in numbers from 1 to 14. The lower the pH level in a solution, the more acidic it is ( ), and conversely, the higher the pH level, the more alkaline the solution is. The ideal pH level is 7, which is neutral. Wihardi (2014) states that the optimal pH range for carp cultivation is between 6.5 and 8.5. Figure 2 shows the average pH during the 14-day cultivation period.

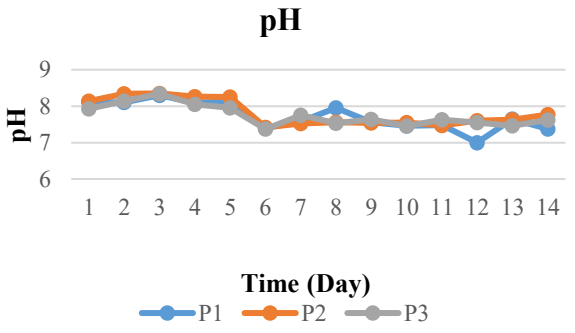

Figure 2. Average pH Results Over 14 Days

The pH measurement results in the first week of this study showed a range of 7.3-8.3, while in the second week the measured pH was in the range of 7-7.9. Based on the average pH measurement results during the maintenance period, the range was normal. This range is still within the pH range allowed for

carp cultivation. These values indicate that the pH of the maintenance tank is normal. This is in accordance with Susanto (2014), who states that the appropriate pH for carp is between 6 and 9. The pH measurement results indicate that the pH in the aquarium is still relatively safe for the life of the carp tested, due to the aquarium conditions being constantly maintained. In addition to temperature, measuring the pH of the water in this study is very important in fisheries to ensure that the water quality is good and optimal for fish growth and health. In all treatments, both in the first and second weeks, there were no significant differences in average pH during the maintenance period. This is because, according to Cahyono (2004), pH changes can be caused by low alkalinity, so drastic pH changes do not occur and water pH quality remains stable.

Water conditions that are too acidic or alkaline can cause stress in fish and affect their health (Chotiba, 2013). Fluctuations in the pH of a body of water will affect the physiological processes of organisms. According to Wihardi (2014), water pH that is too low can trigger bacterial growth. The more acidic the pH of the fish's living environment, the faster the reproduction of bacteria that cause fish diseases. The pH that most triggers bacterial growth in carp is an acidic pH of 5 and 5.5. Meanwhile, excessively high water pH can cause fish mortality if the pH exceeds 11. Fluctuating water pH levels can also negatively impact fish survival rates.

3. DO

Dissolved oxygen (DO) levels are an important limiting factor for the survival of aquatic organisms, including fish, because fish breathe through their gills by taking in dissolved oxygen in the water. According to Tatangindatu (2013), the ideal DO level for farmed fish is 4-5 mg/l. Oxygen deficiency causes stress and death in fish because their body tissues cannot bind dissolved oxygen in the blood. According to Koniyo and Panigoro (2018), the reduction in dissolved oxygen levels in water is due to the presence of organic waste materials that consume oxygen during decomposition. The solubility of oxygen in water can be influenced by temperature. In line with Boyd's (1998) statement *in* Yanuar (2017), as the water temperature decreases, the dissolved oxygen level increases, while a temperature increase of 4 °C can cause oxygen consumption to increase. Figure 3 shows the average DO during the 14-day maintenance period.

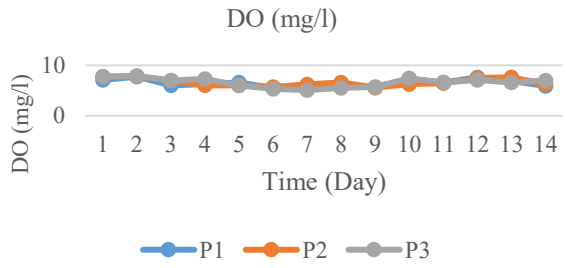

Figure 3. Average DO Results Over 14 Days

In this study, dissolved oxygen (DO) measurements in the first week showed a range of 5.1-7.8 mg/l, while in the second week, DO measurements were in the range of 5.5-7.6 mg/l. Dissolved oxygen (DO) values in this range are still within the normal DO range for carp cultivation. This is in accordance with Wihardi (2014), who states that the dissolved oxygen level in water or ponds that is good for carp growth is >4 mg/l. The dissolved oxygen measurement results during the maintenance period were relatively stable and did not experience fluctuating values. This was due to strong aeration supply activities so that the dissolved oxygen requirement was maintained. Suboptimal DO values can cause stress in fish and have a negative impact on fish health, and can cause death due to oxygen deficiency (*anoxia)* caused by the body's tissues being unable to bind dissolved oxygen in the blood (Dahril et al., 2017). Fish need oxygen for metabolic processes, so sufficient dissolved oxygen concentration in the water is very important to support the health of carp. Oxygen is one of the important limiting factors in fish farming (Muslim *et al.*, 2021).

b. *Surv*ival *Rate*

Fish survival *rate*, also known as *survival rate* (SR), is the ratio between the number of individuals alive at the end of the study and the number of individuals alive at the beginning of the study. Based on the results of a study on the treatment of carp (*Cyprinus carpio)* fry infected with *Aeromonas hydrophila* bacteria by immersion using different doses of *Crinum asiaticum* L. leaf extract, different survival rates were observed, as shown in Table 1 below.

Table 1.
Survival Rate of Carp at the End of Cultivation

Repetition	Treatment		
	P1	P2	P3
1	10	0	0
2	10	0	3
3	7	3	0
Total	27	3	3
Average	90%	10	10

Source: Personal Data (2024)

The results of the study after 14 days of carp seed maintenance showed a carp seed survival rate of 10% and 90%. The highest survival rate was observed at dose P1 (20 ml) at 90%, while the lowest survival rates were observed at dose P2 (40 ml) at 10% and at dose P3 (60 ml) at 10%. The highest survival rate of carp fry was observed in treatment P1, which is thought to be because the dose administered was still tolerable for the fish, and the quality of the water in which the fish lived supported the highest survival rate in this treatment. Meanwhile, the lowest survival rate was observed in treatments P2 and P3, due to the high mortality rate of fish during the maintenance period caused by the difference in the doses administered. The mortality of carp fry in treatments P2 and P3 occurred mostly on the first day of maintenance, because the fish could not tolerate the high concentration of daisy leaf extract administered. Mixing bakung leaf extract with different doses can treat A. *hydrophila* bacteria in carp fry and also has an impact on the survival rate of test fish. This is in line with the opinion of Dewi et al. (2019), who stated that the higher the dose of bakung leaf extract given, the higher the mortality rate in test fish. The high mortality rate in carp fry was caused by the inability of the carp fry to adapt to the bakung leaf extract administered during the treatment period.

The results of phytochemical qualitative tests conducted in Audryan's (2023) study stated that the test results of daylily leaf extract showed alkaloid levels of 33.35 mg/mL, a flavonoid content of 21.16 mg/mL, a saponin content of 25.41 mg/mL, a tannin content of 18.95 mg/mL, a glycoside content of 0.29 mg/mL, and a steroid/triterpenoid content of 0.84 mg/mL. The most abundant compound in bakung leaf extract is alkaloid. Alkaloids are known to have a wide range of pharmacological properties, including anti-cancer, anti-bacterial, anti-viral, and anti-inflammatory properties (Adamski *et al.,* 2020). The second most abundant compound is saponin. When saponins interact with bacterial cells, the bacteria will break down or lyse (Peoleongan and Pratiwi, 2010). The third most abundant compound is flavonoids. Flavonoids function as antibacterial agents that effectively inhibit the growth of viruses, bacteria, and fungi (Krisnata et al., 2014). Tannin compounds, as one of the

chemical groups with antibacterial properties, interact with cell membranes, destroy or inactivate genetic substances, and inactivate enzymes (Saad *et al.*, 2012).

The active compounds found in daylily leaf extract have been proven to be another alternative in treating fish diseases. However, when treating carp fry infected with *A. hydrophila* bacteria by soaking them in daylily leaf extract solution, the dosage of the solution mixture must be carefully considered. However, another factor that causes low survival rates in carp fry is the high alkaloid content in bakung leaves. According to Palic *et al*. (2002), these alkaloids are toxic or poisonous when used in large quantities and over a long period of time, causing death. In addition to alkaloids, bakung leaves also contain saponins. These saponins are very dangerous to fish in aquatic environments. Saponins are a type of glycoside commonly found in plants, and have foaming characteristics, so that when reacted with water and shaken, they form a long-lasting foam. Saponins are easily soluble in water and insoluble in ether. They have a bitter, pungent taste and cause sneezing and irritation of the mucous membranes. Saponins are toxins that can destroy blood cells and often cause hemolysis of red blood cells. In very dilute solutions, saponins are highly toxic to cold-blooded animals such as fish (Robinson, 1991 *in* Lukistyowati and Kurniasih 2011). This statement is supported by Lukistyowati (2012), who states that the high saponin content in daffodil leaf extract can cause foam in the water, making it difficult for fish to obtain oxygen. Saponin will enter the bloodstream through the gills, and when fish take oxygen from the water, saponin will enter the body and bind to hemoglobin, which can cause fish to become anemic and eventually die. Therefore, treatment with too high a dose results in fish mortality. The high concentration of bakung leaf extract used causes low survival rates in test fish, as the compounds in the extract become toxic, leading to fish death. The test fish that survive do so due to differences in individual fish resistance and are influenced by the quality of the water as the medium in which the fish live. The survival data of carp fry obtained from each treatment was then analyzed using *Analysis of Variance* (ANOVA). The results of the ANOVA test are shown in Table 2 below.

Table 2.
Results of ANOVA Analysis of Goldfish Survival

SK	DB	JK	KT	F-Calculated	F-Table	
					0.05	0.01
Treatment	2	12800	6400	21.33	5.14	10.92
Error	6	1800	300			
Total	8	14,600				

Source: Personal Data (2024)

Based on the results of the ANOVA test analysis, the Fcount value is > Ftable (21.33 > 5.14) with a 95% confidence interval and a significance level of α = 0.05, we accept H1 or reject H0, which means that soaking milkweed leaf extract in carp juvenile infected with A*. hydrophila* bacteria has a very significant effect on the survival of carp juvenile. Based on the ANOVA test results showing a very significant effect, a Least Significant Difference (LSD) test was conducted to determine the level of significance of the effect of bakung leaf extract on the survival rate of carp juvenile. The LSD test results are shown in Table 3 below.

Table 3.
Results of the BNT Follow-up Test on Carp Survival Rate

Treatment	Mean	Mean + LSD	Symbol
P2	10	44.59	a
P3	10	44.59	a
P1	90	124.59	b

c. Clinical Symptoms

Initial research before carp (*Cyprinus carpio*) juvenile were infected with *A. hydrophila* bacteria showed that the fish in were healthy, as indicated by their ability to swim freely, normal appetite, fresh red gills, good eye reflexes, and normal fins. Therefore, it can be said that both the fish and their aquatic environment were free from *A. hydrophila* bacteria*.* A*. hydrophila* bacteria are pathogenic bacteria, as seen during the infection process, where the carp fry appeared stressed, characterized by frequent swimming to the surface due to difficulty in obtaining oxygen and abnormal swimming patterns. In this study*,* after the fish were left for 24 hours after infection with A*. hydrophila* bacteria*,* treatment was carried out by placing the infected test fish in an aquarium containing dandelion leaf extract at each concentration according to treatment P1 (20 ml), P2 (40 ml), and P3 (60 ml). The fish were immersed in the bakung leaf extract at the respective concentrations for 45 minutes.

Within 72 hours after infection with *A. hydrophila* bacteria or on the first day of carp fry rearing*,* more than 50% of the test fish showed clinical symptoms of infection with the bacteria. Clinical symptoms observed after immersing the fish in daylily leaf extract included damage to the body surface, fish response to feed, and fish response to shock. This observation was carried out during the 14-day maintenance period of the carp fry to see whether there was any recovery from the clinical symptoms that appeared after treatment. The observations of damage to the body surface included *dropsy (*swollen belly), *exophthalmos (*damaged or protruding eyes), and *hemorrhages*, which were red spots on the surface of the fish's body. As stated by Lubis *et al.* (2014), fish samples infected with A*. hydrophila* bacteria exhibited clinical symptoms such as dark body color, bleeding on the skin leading to wounds and ulcers, bloated abdomen (*dropsy)*, damaged or protruding eyes, ragged fins, and slow movement. The clinical symptoms of damage on the surface of carp fry observed can be seen in Table 4 as follows.

Table 4.
Recovery of Damage to Carp Fry

Day	Concentration of Bakung Leaf Extract (ml)		
	P1 (20 ml)	P2 (40 ml)	P3 (60 ml)
1	-	EDH	EDH
2	-	EDH	EDH
3	-	EH	EH
4	-	EH	H
5	-	E	H
6	-	E	H
7	-	E	H
8	-	E	H
9	-	E	H
10	-	E	H
11	-	-	H
12	-	-	H
13	S	S	S
14	S	S	S

Description: (E): *Exophthalmos*; (H): *Hemorrhagic;* (D): *Dropsy;* (S): Recovered

Table 4 shows that on days 1 to 2 after treatment with bakung leaf extract at treatment concentrations P2 (40 ml) and P3 (60 ml), clinical symptoms such as *exophthalmos*, *dropsy*, and *hemorrhage were* observed in the test fish. This is in accordance with the statement by Lukistyowati and Kurniasih (2012), who reported that fish infected with A*. hydrophila* bacteria had clinical symptoms of *hyperemia* (redness) and fluid in the abdominal cavity. The appearance of wounds and bleeding on the test fish's body was caused by A*. hydrophila* bacterial toxins, one of which is hemolysin toxin. Huys *et al.* (2002) stated that hemosilin toxin plays a role in breaking down red blood cells, causing cells to escape from blood vessels and causing redness

on the skin surface. Bleeding occurred on the first day when the carp were exposed to A. *hydrophila* bacteria. This attack can cause bleeding on the surface of the fish's skin (Indriani et al., 2014). In addition, the fish appear to be oxygen-deficient, move very slowly, and always gather around the aeration stones. Clinical symptoms in carp after infection with A. *hydrophila* bacteria are characterized by behavioral changes, namely abnormal swimming, staying at the bottom of the aquarium, swimming close to the aeration, and decreased appetite. This is in accordance with the statement by Hardi *et al.* (2014), that fish infected with A. *hydrophila* bacteria cause the appearance of clinical symptoms of abnormality in swimming patterns and decreased appetite. On days 1 to 12 at treatment concentration P1 (20 ml), there were no clinical symptoms of damage to the surface of the test fish. On days 1 to 2 at treatment concentrations P2 (40 ml) and P3 (60 ml), there were clinical symptoms *of exophthalmia, dropsy,* and *hemorrhage,* respectively. On days 3 to 4, clinical symptoms of *exophthalmos* and *hemorrhage* were still present in test fish at treatment concentrations P2 (40 ml) and P3 (60 ml). On day 4, treatment P3 (60 ml) only showed clinical symptoms *of hemorrhage.* On days 5 to 10, clinical symptoms of *exophthalmos* and *hemorrhage* were observed in test fish at treatment concentrations P2 (40 ml) and P3 (60 ml). On the 11th day of maintenance, the test fish in each treatment showed signs of recovery from clinical symptoms, marked by active swimming and the disappearance of spots on the fish's body, indicating that the fish had undergone a recovery process. However, on day 12, two test fish in the P1 treatment concentration (20 ml) died due to oxygen deficiency when they were transferred to a bucket without aeration during the siphoning of the maintenance aquarium container. There were also clinical symptoms *of hemorrhage* in one test fish that died in the P3 treatment concentration (60 ml). On the 13th to 14th days of the test fish maintenance period, the fish showed signs of being healthy, such as the disappearance of damage to the surface of the fish's body, the skin and scales of the fish appearing normal again, the fish swimming actively, and taking oxygen normally. The following are images of healthy carp fry before infection with A. *hydrophila* bacteria and carp fry showing clinical symptoms of infection with A. *hydrophila* bacteria, presented in Figures 4, 5, and 6 as follows.

Figure 4. Condition of test fish before infection with *A. hydrophila* bacteria

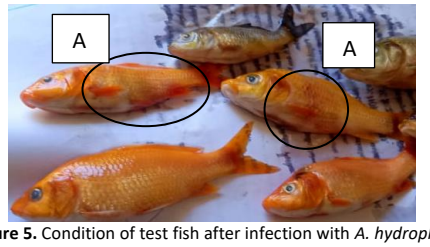

Figure 5. Condition of test fish after infection with *A. hydrophila* bacteria (A) red bruises resembling wounds *(hemorrhagic)*

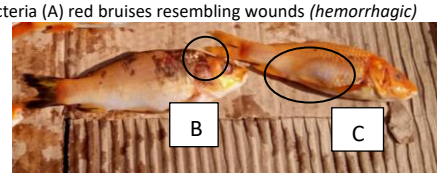

Figure 6. Condition of test fish after infection with *A. hydrophila* bacteria (B) damaged or protruding eyes (*exophthalmos*) (C) bloated abdomen (*dropsy*)

Observation of the test fish's response to feed was the same as observation of the recovery of damage to the test fish's body, which was carried out during the 14-day maintenance period. Based on the results of observing the fish's response to feed after treatment with daylily leaf extract for each different treatment, the results can be seen in Table 5 as follows.

Table 5.
Response of Carp Fry to Feed

Day	Treatment								
	P1 (20 ml)			P2 (40 ml)			P3 (60 ml)		
	1	2	3	1	2	3	1	2	3
1	-	-	-	-	-	-	-	-	-
2	+	+	+	-	-	-	-	-	-
3	+	+	+	-	-	+	-	-	-
4	++	++	++	-	-	+	-	+	-
5	++	++	++	-	-	+	-	+	-
6	++	++	++	-	-	+	-	+	-
7	++	++	++	-	-	+	-	+	-
8	++	++	++	-	-	+	-	+	-
9	++	++	++	-	-	+	-	+	-
10	++	++	++	-	-	+	-	+	-
11	++	++	++	-	-	++	-	+	-
12	++	++	++	-	-	++	-	+	-
13	++	++	++	-	-	++	-	++	-
14	++	++	++	-	-	++	-	++	-

Description: (-)No response to feed; (+)Low response to feed; (++)High response to feed (no feed leftovers)

Table 5 shows that the fish's response to each feed differs. In treatment P1 (20 ml), a low response to feed was observed from day 2 to day 3. Then, from day 4 until the end of the maintenance period, the fish consistently had a high response to feed every day until it appeared normal at the end of the observation (day 14). The test fish in treatment P2 (40 ml) showed a response to feed only on day 3, but the response to feed was still low until day 10. Then, from day 11 until the end of the rearing period, the fish's response to feed returned to normal, marked by a high response to feed. In treatment P3 (60 ml), there was no response to feed until day 3 of the rearing period, but from day 4 to day 12 of the rearing period, a low response to feed began to appear. At the end of the maintenance period on the 13th to 14th day, the fish's response to feed returned to normal, marked by a high response to feed. This low response to feed is in accordance with the statement by Affandi and Tang (2002), that fish infected with A. *hydrophila* bacteria show symptoms of reduced appetite. Stress causes fish to become weak, refuse to eat, and one of the consequences is infection, which plays a very dominant role. This is followed by the opinion of Aniputri *et al.* (2014), namely that the better the fish's feeding response, the faster the healing process occurs.

Observation of the test fish's response to shock was the same as observation of body damage recovery and response to feed conducted during the 14-day maintenance period. Based on the results of observing the fish's response to shock after treatment with daylily leaf extract for each treatment, the results are shown in Table 6 as follows.

Table 6.
Response of Carp Fry to Shock

Day	Treatment								
	P1 (20 ml)			P2 (40 ml)			P3 (60 ml)		
	1	2	3	1	2	3	1	2	3
1	-	-	-	-	-	-	-	-	-
2	+	+	+	-	-	-	-	-	-
3	+	+	+	-	-	+	-	+	-
4	++	++	++	-	-	+	-	+	-
5	++	++	++	-	-	+	-	+	-
6	++	++	++	-	-	+	-	+	-
7	++	++	++	-	-	+	-	+	-
8	++	++	++	-	-	+	-	+	-
9	++	++	++	-	-	+	-	+	-
10	++	++	++	-	-	+	-	+	-
11	++	++	++	-	-	++	-	++	-
12	++	++	++	-	-	++	-	++	-
13	++	++	++	-	-	++	-	++	-
14	++	++	++	-	-	++	-	++	-

Description: (-) No response to shock; (+) Low response to shock; (++) High response to shock (agile movement)

Based on Table 6, it can be seen that the test fish given the treatment of immersion in daylily leaf extract showed different responses to shock for each treatment. The best response or reflex to tapping the glass wall of the aquarium was observed in test fish treated with P1 (20 ml) because from day 4 to day 14 at the end of the maintenance period, the fish's movements had returned to normal, as indicated by a high response to shock, compared to treatments P2 (40 ml) and P3 (60 ml).

4. Conclusion

Based on the results of the research that has been conducted, it can be concluded that:

1. The effect of immersion in *Crinum asiaticum* L. leaf extract has a very significant effect on the survival rate of carp (*Cyprinus carpio*) juvenile infected with *Aeromonas hydrophila* bacteria.
2. The optimal concentration of *Crinum asiaticum* L. leaf extract that can reduce *Aeromonas hydrophila* bacterial infection is at a dose of P1 (20 ml) with a survival rate of 90%.

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