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EFFECTIVENESS OF *Gynura procumbens* AGAINST *Pangasius* sp INFECTED WITH *Edwardsiella tarda* BACTERIA

(Efektifitas Ekstrak Daun Sambung Nyawa Terhadap Pangasius sp Terinfeksi Bakteri Edwardsiella tarda)

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ABSTRACT

This study aimed to analyze the effect of soaking the extracts of *Gynura procumbens* leaves with different concentrations on wound healing of catfish infected with *Edwardsiella tarda* bacteria. This research was conducted at the Wet Laboratory of the Fish Quarantine and Quality Control Agency of Padang, West Sumatra. This study used an experimental method with a completely randomized design (CRD) consisting of 3 treatments and 4 replications. The treatment of this research was concentration of the *Gynura procumbens* leaves consisted of treatment P1 = 2000 ppm; treatment P2 = 3000 ppm and treatment P3 = 4000 ppm. Based on the the analysis of variance, the concentration of the leaf extract had a significant effect on wound shrinkage. Duncan's follow-up test (DMRT) showed that the treatment was P1; P2; and P3 there are significant differences in each treatment. it was shown that *Gynura procumbens* leaf extract with different concentrations had no significant effect ($p > 0.05$) on the survival of catfish. The lowest survival rate was in treatment P1 66.67% treatment P2 and P3 were 100%. The best results were found in P2 treatment of 80.20% on wound shrinkage and 100% on survival rate.

Keywords: concentration, *Edwardsiella tarda*, *Gynura procumbens*, *Pangasius* sp

ABSTRAK

Penelitian ini bertujuan untuk menganalisis pengaruh perendaman ekstrak daun sambung nyawa dengan konsentrasi berbeda terhadap penyembuhan luka ikan Patin yang terinfeksi bakteri *Edwardsiella tarda*. Penelitian ini dilakukan di Laboratorium Basah Badan Karantina dan Pengendalian Mutu Ikan kota Padang, Sumatera Barat. Penelitian ini menggunakan metode eksperimen dengan Rancangan Acak Lengkap (RAL) yang terdiri dari 3 perlakuan dan 4 ulangan. Perlakuan dalam penelitian ini adalah konsentrasi ekstrak daun sambung nyawa yang terdiri dari perlakuan P1 = 2000 ppm; perlakuan P2 = 3000 ppm dan perlakuan P3 = 4000 ppm. Berdasarkan analisis varian, konsentrasi ekstrak daun sambung nyawa berpengaruh nyata terhadap penyusutan luka. Uji lanjut Duncan (DMRT) menunjukkan bahwa perlakuannya adalah P1; P2; dan P3 terdapat perbedaan nyata pada masing-masing perlakuan. Ekstrak daun sambung nyawa dengan konsentrasi berbeda tidak berpengaruh nyata ($p>0,05$) terhadap kelangsungan hidup ikan lele. Tingkat kelangsungan hidup terendah pada perlakuan P1 66,67% perlakuan P2 dan P3 100%. Hasil terbaik didapatkan pada perlakuan P2 sebesar 80,20% pada penyusutan luka dan 100% pada tingkat kelangsungan hidup.

Kata Kunci: daun Sambung Nyawa, *Edwardsiella tarda*, konsentrasi, *Pangasius* sp

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INTRODUCTION

Catfish (*Pangasius* sp) is a freshwater commodity that has high economic value in the market and a high demand in Indonesia, especially Sumatra and Kalimantan. People like the delicious taste of fish because the high level of freshness, white meat color and low fat content (Aristasari et al., 2020). Catfish is one of the leading fish in freshwater widely cultivated. The intensification of cultivation is one of the efforts to increase catfish production (Sihombing et al., 2021). Intensive cultivation systems can support high production yields and have been widely used. Improper management in intensive cultivation systems causes disease (Fattah et al., 2021; Rachmawati and Nurhayati, 2022). Disease infection in fish can be one of the problems in aquaculture activities. Diseases can have a negative impact on the development of fish farming (Sumatorno et al., 2017).

One of the diseases that often attack catfish is MES (Motile Edwardsiella Septicemia) disease or often known as Edwardsielliosis. This disease is caused by infection with the bacterium *Edwardsiella tarda* (Kartikaningsih et al., 2018). That bacteria is a pathogenic bacterium that has a negative impact on catfish culture. Infections caused by E. tarda bacteria can reduce productivity in aquaculture. The decrease in productivity occurs due to horizontal transmission through contact between one fish and another fish through the water of cultivation media (Amanu et al., 2016; Park et al., 2012). The material that is often used to treat fish diseases caused by bacteria is the use of antibiotics. However, the use of antibiotics in a certain period of time further expands the negative impact on fish farming (Monteiro et al.,, 2018). In addition to being expensive and polluting the environment, the use of antibiotics can also cause resistance to bacteria, thus endangering humans who eat the fish (Rasul and Majumdar, 2017). So we need an alternative method using local herbal ingredients that are safer.

The use of herbal ingredients to treat fish diseases has been widely carried out because they have antibacterial properties (Karina et al., 2015). One of them is *Gynura procumbens* leaf plant (*Gynura procumbens*). *Gynura procumbens* plants contain chemical alkaloids, saponins, flavonoids and tannins. The material has a role as an antibacterial to suppress harmful bacteria and kill disease (Andleeb et al., 2014; Madhuri et al., 2012). However, the treatment of disease in catfish has not been carried out. Therefore, it is necessary to conduct a study to determine the effect of soaking the leaf extract on the wound healing of catfish infected with Edwardsiella tarda bacteria.

MATERIAL AND METHODS

This research was conducted at the Wet Laboratory of the Fish Quarantine and Quality Control Agency of Padang, West Sumatra. This study used an experimental method with a completely randomized design (CRD) consisting of 3 treatments and 4 replications. The treatment of this research was immersion of the extract of life-long leaves using different concentrations for the healing of wounds of catfish infected with *Edwardsiella tarda* bacteria. The immersion concentration of the *Gynura procumbens* leaves consisted of treatment P1 = 2000 ppm; treatment P2 = 3000 ppm and treatment P3 = 4000 ppm.

Research procedure

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The catfish used were 27 fish, which were 18-22 cm in size. Connecting Life Leaves obtained from nature. Pure isolates of *Edwardsiella tarda* bacteria were cultured in the Laboratory of the Fish Quarantine and Quality Control Agency. The bacteria were then diluted as much as 10^1. *Edwardsiella tarda* bacteria were injected at a dose of 0.1 ml/head with a concentration of 10^1 CFU/ml by inframuscular (injection into the muscle) dorsally. After the injection, clinical symptoms were observed, including wounds, behavior and fish appetite for 7 days. Then carry out the recovery process using the immersion of the leaf extract of life grafts by observing every day for 30 days. During the study, observations of water quality were carried out on the parameters of temperature, DO, pH and ammonia (NH3).

Observed variable

Wound closure was measured using the formula according to Suratman (1996) as follows:

$$Dx = \frac{(dx(1)+dx(2)+dx(3)+dx(4)}{4}$$

Dx = wound diameter x day (mm)

dx(1-4) = wound diameter measured from various directions

The percentage of wound healing was measured using the formula Nicodemu, et al., (2014) as follows:

$$P\% = \frac{(d0-dx)}{[\![d0]\!]} \text{ x } 100\%$$

P = Percentage of wound closure (%); d0 = first day wound diameter; dx = Diameter of wound on day x

The survival rate of the test fish carried out during the study was calculated by the Effendi (1992) formula:

$$SR\% = \frac{Nt}{No} \text{ x } 100\%$$

SR = Survival Rate (%) Nt = Number of fish that live at the end of the study (tails) N0 = Number of fish that live at the beginning of the study (tails).

Data analysis

The survival data obtained were analyzed by analysis of variance. To analyze the difference between treatments, it was continued with Duncan's Test. water quality data were analyzed descriptively based on the measurement results.

RESULTS AND DISCUSSION

Observation of clinical symptoms started from the first day after the fish was injected or injected with *Edwardsiella tarda* bacteria until the seventh day. On the first day the injection site only slightly swelled and there were no chronic symptoms. In the afternoon, swelling began to appear in the injection site and two days later, small ulcers began to appear on the site of the *Edwardsiella tarda* bacteria injection. On the 3rd day after the injection, the fish began to swim abnormally and swim to the surface, then on the 4th day the ulcers had started to widen and deepen. On the 5th and 6th days, the test fish had started to lose color Pigment on their body, the fish had not responded to the food given and then the fins had experienced necrosis or threshing on the fins.

The recovery process begins with the reduction of clinical symptoms caused by the *Edwardsiella tarda* bacterial infection. After the fish were soaked using the leaf extract of *Gynura procumbens* with concentrations of

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2000 ppm, 3000 ppm and 4000 ppm, they gradually began to show progress in each treatment P1, P2 and P3. It had begun to show recovery from the *Edwardsiella tarda* bacterial infection. The results of this study showed that the leaf extract of *Gynura procumbens* was able to cure catfish infected with *Edwardsiella tarda* bacteria, reducing the size of the wound on the body of the test fish and the color pigment on the body of the fish had also returned.

Based on the results of the analysis of variance on table 1, the concentration of the leaf extract had a significant effect on wound shrinkage. Duncan's follow-up test (DMRT) showed that the treatment was P1 (2000 ppm); P2 (3000 ppm); and P3 (4000 ppm) there are significant differences in each treatment. The best results were found in P2 treatment of 80.20% followed by P3 treatment of 66.03% and the last treatment P1 was 39.43%.

Table 1. data on wound shrinkage and catfish survival

Treatment	Sound shrinkage (%)	Survival rate (%)
P1	39.43 ± 17.82^{a}	66.67
P2	80.20 ± 9.33^{b}	100
P3	66.03 ± 12.58^{c}	100

Notes: P1 (2000 ppm *Gynura procumbens* leaves extract); P2 (3000 ppm *Gynura procumbens* leaves extract); P3(4000 ppm *Gynura procumbens* leaves extract)

Table 1 showed that *Gynura procumbens* leaf extract with different concentrations had no significant effect ($p > 0.05$) on the survival of catfish. The lowest survival rate was in treatment P1, namely the immersion of *Gynura procumbens* leaf extract with a concentration of 2000 ppm of 66.6%, after that treatment P2 and P3 were 100% survival rate because during the study there were no dead fish. Water quality parameters of catfish rearing in the aquarium had a temperature range of 26.8 ^{o}C to 27.68 ^{o}C. The pH of the water was 7 from the beginning of the study to the end of the study. DO water during the study ranged from 3.48-4.40 ppm. The water quality in these parameters is still in a good range for fish to live during the study.

Clinical symptoms experienced by catfish infected with *Edwardsiella tarda* bacteria such as the body of the fish experiencing ulcers, necrosis of the fins or fin loss, pale fish color, decreased appetite and unstable swimming. According to Park et al., (2012), the characteristics of the *Edwardsiella tarda* bacteria attack on lightly infected fish show small wounds, then necrosis of the skin occurs, spreads to the flesh and internal organs and loses color pigment. *Edwardsiella tarda* that infects spreads to wounds of internal organs to the flesh, skin necrosis occurs, then the wound develops in the flesh and dermis causing the skin to blister so that it loses color pigment. When the wound gets worse, it will cause a foul odor and spread throughout the body (Kartikaningsih et al., 2018; Amanu et al., 2016).

Recovery process is a process of healing or restoring the systems in the body of the test fish that are problematic after the catfish is injected with the *Edwardsiella tarda bacteria* (Riyadi et al., 2021). Observations of these clinical symptoms started from the first day after the fish were soaked in the extract of the Connect Nyawa leaf for 30 minutes (day 8) until the 21st day. In the recovery process, clinical symptoms

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are observed including wound healing, movement responses, food responses and other clinical symptoms.

According to Fauziyyah et al., (2021), improved wound conditions and clinical symptoms in catfish due to the content of alkaloid compounds, saponins, flavonoids and tannins contained in the leaves of *Gynura procumbens*. *Gynura procumbens* leaves have the potential to be antimicrobial. P2 treatment (3000 ppm) obtained the highest results because the concentration of 3000 ppm was the optimal concentration for wound healing in catfish infected with *Edwardsiella tarda* bacteria.

This means that the higher the concentration does not mean it has a good effect on healing the wounds of catfish infected with *Edwardsiella tarda* bacteria. *Gynura procumbens* leaves have many antibacterial compounds where the incorporation of excessive activity of several active compounds can reduce the content or effect of these compounds. Antagonistic interactions reduce the final activity of combining several active compounds (Kurniawan, 2021; Zapién-Campos et al., 2015).

The survival of catfish during the study in treatment P2 and P3 was the same 100% and the lowest was treatment P1 at 66.6%, this was due to the low concentration of life-suspension leaf extract. The concentration of 2000 ppm in aquarium P1 was very low when compared to other treatments, because treatment A after soaking using 2000 ppm of *Gynura procumbens* leaf extract did not produce good wound cover and low survival (Karina et al., 2015; Untari, et al., 2022).

Table 2. Water Quality of Rearing *Pangasius* sp

Treatment	P1	P2	P3
Temperature (^{o}C)	26.89-27.47	26,75-27.68	26.8-27.46
pH	6.95-7.15	7.05-7.25	7.00-7.31
DO (ppm)	3.64-3.74	3.48-3.63	3.63-4.40

Notes: P1 (2000 ppm *Gynura procumbens* leaves extract); P2 (3000 ppm *Gynura procumbens* leaves extract); P3(4000 ppm *Gynura procumbens* leaves extract)

The water quality parameters for catfish rearing in the aquarium temperature obtained were still good and suitable for fish life (Table 2). according to Sitanggang (1994), a good temperature for fish life ranges from 24 ^{o}C to 28 ^{o}C. According to Ferguson (1994), the water pH range of 6-9 is considered safe for fish. The pH content during the study was still in a good range for fish to live. A pH that is too high or too low can kill fish (Setyaningrum et al., 2020).

Water physico-chemical parameters such as dissolved oxygen (DO) concentration are the parameters that get the most attention because they reflect the water quality and health of an aquatic ecosystem. The minimum concentration limit and the role of DO for aquatic ecosystems reflect the ability of water bodies to adapt to the presence of pollutant loads. DO is very influential on fish life, especially for growth, tissue repair and reproduction (Mukti et al., 2019).

CONCLUSION

The results of this study concluded that the concentration of the leaf extract of 3000 ppm was the best concentration for wound healing in catfish infected with E. tarda bacteria with an average percentage of Sound shrinkage is 80.20% and 100% survival rate of fish.

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