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Effect of a Methanol Extract of *Avicennia marina* Mangrove Leaf to Treat the Infected North African Catfish (*Clarias gariepinus* "Burchell 1822") by *Aeromonas hydrophila*: A Field Study in Banjarnegara, Indonesia

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ABSTRACT

Banjarnegara is one of the North African catfish (*Clarias gariepinus* "Burchell 1822") cultivation centers in Central Java Province, Indonesia. However, problems are often found in the field, one of which is aeromoniasis caused by *Aeromonas hydrophila*. Extract of *Avicennia marina* can be used as an alternative natural treatment. The study aimed to conduct a field-testing using a methanol extract of *A. marina* to treat the infected North African catfish by *A. hydrophila* in the Banjarnegara region, Indonesia. The research method was experimental using a completely randomized design with 4 treatments and 4 replications, namely P0: not treated with the extract (control), P1: 0.2 g L^{-1}, P2: 0.3 g L^{-1}, and P3: 0.4 g L^{-1}. This study showed that the clinical signs in the North African catfish after infection with *A. hydrophila* were ulcers, lesions, depigmentation, abdominal dropsy, hyperemia, hemorrhagic, and exophthalmia. The recovery process appeared in P1, P2, and P3 on days 4-5, except for the control group. In addition, P1, P2, and P3 treatments could increase the survival rate ($P < 0.05$) to 74.95-84.95%, compared to the control group (P0), which was 6.6%. Meanwhile, the increase in the weight and length of the fish in all treatments were not significantly different ($P > 0.05$). In conclusion, the leaf methanol extract of *A. marina* is effective and efficient in treating *A. hydrophila* infection for the North African catfish at 0.2g L^{-1}.

INTRODUCTION

The North African catfish farming in Banjarnegara, Central Java, Indonesia, has great potential and prospective as a livelihood for the community. However, fish farming in the field often encounters obstacles, including disease problems. Aeromoniasis or motile Aeromonas septicemia (MAS) is a bacterial disease caused by *Aeromonas hydrophila* **(Rasmussen-Ivey *et al.*, 2016; Pessoa *et al.*, 2019)**. *A. hydrophila* are opportunistic and pathogenic which cause clinical signs in the infected fish and result in fatal fish mortality **(Austin & Austin 2016; Hamid *et al.*, 2017; Raji *et al.*, 2019)**. The fish infected with *A. hydrophila* often show clinical signs including hemorrhagic on the body, hyperemia, ulcer, abdominal cavity, abdominal dropsy, depigmentation and erosion of the skin, erythema and erosion of the fins, hemorrhagic of the gills, exophthalmia, congestion in the liver, kidneys, and spleen, and decreased appetite **(Dias *et al.*, 2016; Emeish *et al.*, 2018)**. MAS can cause mass death if not treated immediately and appropriately.

Synthetic antibiotics are a common treatment for fish infected by *A. hydrophila*. However, the excessive use of antibiotics brings the impact of emerging resistant bacteria in addition to environmental pollution **(Serwecińska 2020)**. Such a condition triggers the evolution of antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes (ARG) which have the potential to increase the risk of transmitting environmental resistance to humans **(Manaia, 2017)**. Previous research revealed that *A. hydrophila* is resistant to methicillin and rifampicin **(Vivekanandhan *et al.*, 2002)**, and resistant to ampicillin and erythromycin **(Mulia *et al.*, 2021)**. Therefore, it is necessary to look for bioactive compounds from natural ingredients. Plants are a source of secondary metabolite compounds that have the potential as a source of natural antibiotics **(Gorlenko *et al.*, 2020; Purbomartono *et al.*, 2023)**.

Previous studies have reported that natural ingredients from a tropical coastal plant (*Diospyros maritimea*) are proven to be antibacterial **(Isnansetyo *et al.*, 2022)**. Other studies reported that mangrove plants contain antibacterial secondary metabolites **(Arulkumar *et al.*, 2020; Syawal *et al.*, 2020; Vittaya *et al.*, 2022)**. Mangrove plants *Excoecaria agallocha* contain natural ingredients with the potential to be antibacterial to *A. hydrophila* **(Bandaranayake 2002; Dwisari *et al.*, 2016; Mulia *et al.*, 2023)**. Another potential mangrove plant is *Avicennia marina* **(Ananthavalli & Karpagam 2017; Das *et al.*, 2018)**. *A. marina* can inhibit the growth of *Staphylococcus aureus* and *Vibrio alginolyticus* **(Danata & Yamindago 2014)**. The leaves of *A. marina* contain active compounds such as flavonoids, alkaloids, terpenoids, and tannins that are antibacterial to *A. hydrophila* **(Mulia *et al.*, 2018)**. Laboratory-scale analysis on the utilization of *A. marina* leaves has indicated successful results in handling the North African catfish against *A. hydrophila* infection **(Mulia *et al.*, 2022)**.

The effectiveness of *A. marina* leaves as an antibacterial for *A. hydrophila* has not been tested in the field, even though infection has repeatedly occurred. Therefore, it is necessary to carry out field tests using *A. marina* leaves. The study aimed to conduct a field-testing using methanol extract of mangrove leaves *A. marina* to treat the North African catfish infected by *A. hydrophila* in Banjarnegara, Indonesia.

MATERIALS AND METHODS

Samples

The fish used were the North African catfish, which measured 9- 12cm long and weighed 14-23g. The field test was carried out in Kertayasa Village, Mandiraja District, Banjarnegara Regency.

Design research

The research was designed using an experimental method with a completely randomized design (CRD), consisting of four treatments and four replications. The treatments involved different doses of *A. marina* extract for the North African catfish. The treatments were as follows: P0 – no treatment with *A. marina* extract (control), P1 – a dose of 0.2g/ L of *A. marina* extract, P2 – a dose of 0.3g/ L of *A. marina* extract, and P3 – a dose of 0.4g/ L of *A. marina* extract. Each sample unit contained ten North African catfish.

Preparation of methanol extract of mangrove leaves *A. marina*

The *A. marina* leaves were taken from the mangrove forest of the Brackish Tourism area in Tritih Kulon Village, Cilacap Regency. The leaves used are leaves located in the 3-5th segment of the shoot, not too old nor too young, and free from pests. The samples that have been taken were placed in a plastic bag and were then weighed until they weighed 4kg. Methanol extract from *A. marina* leaves was made using the maceration method **(Mulia *et al.*, 2018)**.

Growth of *Aeromonas hydrophila* and restore its pathogenicity

The isolate of *A. hydrophila* strain GPl-04 was grown on glutamate starch phenol (GSP) medium (Merck, Darmstadt, Germany) and was incubated at 30°C for 24h. A single colony growing in the culture with a yellow color on the GSP medium indicated that the isolate was *A. hydrophila*. A single colony of *A. hydrophila* from GSP medium was cultured onto trypic soy broth (TSB) medium (Merck, Darmstadt, Germany) and incubated at 30°C for 24h. As much as 0.1mL of bacterial suspension from the TSB medium was injected intramuscularly into the dorsal of the healthy fish. Then, the fish was put back into the rearing container. If any fish were found dead or showed signs of MAS disease infection, re-isolation was performed. Bacterial isolation was conducted by collecting samples from the kidney using an inoculation needle, which were then streaked onto GSP medium and incubated at 30°C for 24 hours. If a colony with characteristics of *A. hydrophila* appeared on the GSP medium, it indicated that the isolate had the capability to cause MAS disease in the fish. The isolates could then be re-cultured on TSB medium and re-infected into healthy fish for further re-isolation. This process of bacterial re-infection and re-isolation could be repeated up to three times **(Mulia, 2012)**.

Aeromonas hydrophila **infection in the North African catfish**

The suspension of *A. hydrophila* at a dose based on the lethal dose$_{50}$ (LD_{50}) test (× 10^8 CFU mL^{-1}) was intramuscularly injected on the dorsal of the North African catfish by 0.1mL/ fish. Before the injection, the dorsal of the fish was smeared with 70% alcohol. Then, after the injection, the wound was smeared with betadine to prevent infection. Next, the North African catfish were put into the rearing containers. The development of clinical signs of MAS disease was observed every day, before treatment with the *A. marina* methanol extract.

Treatment of the North African catfish using the methanol extract of mangrove leaves *A. marina*

After the clinical signs of MAS disease appeared in the North African catfish, treatment with methanol extract of *A. marina* mangrove leaves was carried out at concentrations based on the assigned treatments. The methanol extract was administered by soaking the North African catfish in a container for 60 minutes, after which they were returned to the original rearing pond.

Research parameters

The parameters investigated in this research included the development of disease signs, the recovery process, fish survival rate, and the increase in length and weight of the fish. Water quality parameters, such as water temperature, pH, and dissolved oxygen (DO), were also assessed.

Fish survival rate

Determining the fish survival rates was carried out by counting the number of living fish at the end of this study based on **Effendi (2002)**:

$$SR = (N_t / N_0) \times 100$$

SR = survival rate (%)
Nt = number of animals at the end of the study
N_0 = number of animals at the beginning of the study

Development signs of fish disease

Observations on the development signs of disease were carried out after fish were infected by *A. hydrophila* showing visible clinical signs on the body such as hemorrhagic, erosion on one part of the body and fins, appearing ulcers on fish body, peeled-off scales, erythema fins, abdominal cavity, abdominal dropsy, as well as exophthalmia **(Mulia, 2012)**.

Recovery process of fish

The recovery process was done after the fish being soaked in *A. marina* extract at different concentrations based on each treatment. Daily observations were made within 30 days. The recovery process included stages of healing of the infection such as shrinking injection wounds, smaller ulcers, wound closure, and other recovery signs.

Increase in weight of the North African catfish

The increasing weight was measured to determine the growth level of the tested North African catfish. The fish are weighed once a week. The fish weight calculation was determined as outlined in the study of **Effendi (2002)**:

$$W = Wt - Wo$$

W= increasing weight (g)
Wt= final weight (g)
Wo= initial weight (g)

Increase in length of the North African catfish

The length of all the tested fish was measured at two stages: at the beginning and at the end of the study. Fish length was calculated based on the method described by **Effendi (2002)**:

$$L = L_t - L_o$$

Information:
L = increasing length (cm)
L_t = final fish length (cm)
L_o = initial fish length (cm)

Data analysis

Research data including survival rate, increasing weight, and length of fish were analyzed using the analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) at a 5% test level. The water quality variables including water temperature, pH, and DO were analyzed descriptively and qualitatively.

RESULTS

Development of clinical signs of MAS disease in the North African catfish

The development of clinical signs of MAS disease was observed after the North African catfish were infected by *A. hydrophila* up to before given treatments. Appearing clinical signs included external signs on the fish body due to *A. hydrophila* infection such as appearing ulcers and lesions on the injection wound, peeled-off scales, hemorrhagic on the abdomen and gills, abdominal dropsy and hyperemia, depigmentation of the dorsal fin, and exophthalmia. Clinical signs of MAS disease were visible 24 to 48h after the infection (Table 1).

Table 1. Development of clinical signs of motile Aeromonas septicemia (MAS) disease in the North African catfish after being infected by *Aeromonas hydrophila*

Treatment	Clinical signs of MAS disease in North African catfish
P0 (Controls)	Ulcers and lesions were found in the injection wound on the dorsal, depigmentation of the dorsal fin, peeled-off scales in the injection wound, and white membranes around the eyes, tendency to stay at the bottom of water, looking weak and decreasing appetite.
P1 (0.2 g L^{-1})	Ulcers and lesions found in injection wound on the dorsal, peeled-off scales on injection wound, abdominal dropsy, exophthalmia, unstable swimming, frequent movement to the surface, decreasing appetite.
P2 (0.3 g L^{-1})	Ulcers and lesions found in injection wounds on the dorsal, peeled-off scales on injection wounds, abdominal dropsy, and hyperemia, depigmentation of the dorsal fin, white membranes around the eyes, the tendency to stay at the bottom of water, decreasing appetite.
P3 (0.4 g L^{-1})	Ulcers were found on the injection wound on the dorsal, peeled-off scales on the injection wound, abdominal dropsy and hyperemia, hemorrhagic gills, exophthalmia, unstable swimming, frequent movement to the surface, and decreasing appetite.

Recovery process

The fish recovery process was observed after treatment with the methanol extract of *A. marina* mangrove leaves until maintenance for 30 days. In control (P0), the recovery process occurred slowly and naturally. In general, from day 1 to day 30, most of the fish were still showing clinical signs of disease. One fish of each population died each day. The healing process of the wound was only visible in some fish, most of the ulcers in the injection wound were still visible in addition to peeled-off skin, fin erosion, tendency of unstable swimming as well and decreasing appetite. Some of the fish started to show healing progress although very slowly. In the fish treated with methanol extract of mangrove leaves *A. marina,* such as P1, P2, and P3 treatments, the recovery process began to be visible from days 4 and 5, as indicated by the healing process on the wound edges, increasing appetite, and the beginning to show agile movements.

Survival rate

The survival rates of the treatments showed how effective the methanol extract of mangrove leaves *A. marina* was in treating North African catfish infected by *A. hydrophila*. The results showed that the methanol extract of *A. marina* leaves in treatments P1, P2, and P3 (P<0.05) increased the survival rate which was higher than the control treatment (P0) (Fig. 1). Meanwhile, the treatments P1, P2, and P3 were not significantly different (P> 0.05), indicating that the three treatments, namely concentrations of 0.2 g L^{-1}, 0.3 g L^{-1}, and 0.4 g L^{-1}, had relatively the same effect in treating the North African catfish infected with *A. hydrophila*.

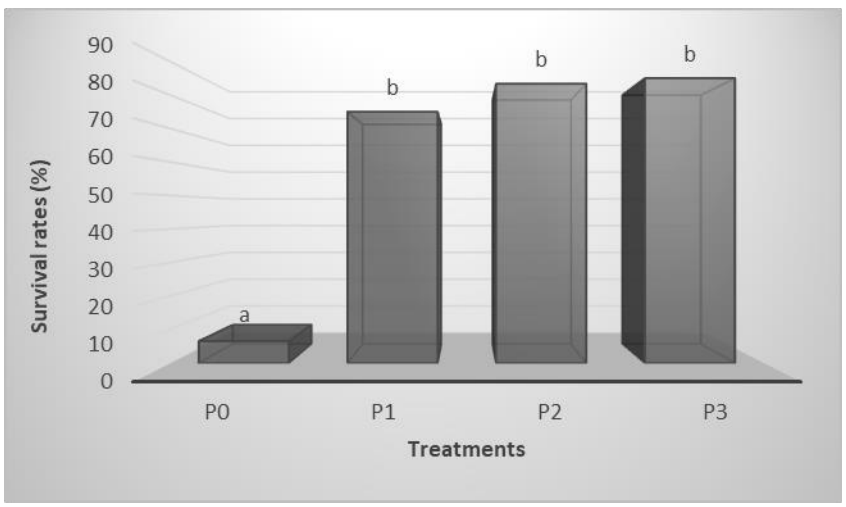

Fig. 1. The survival rate of the North African catfish after exposure to the methanol extract of *A. marina* leaves. P0: not treated with the extract (control), P1: 0.2g L^{-1}, P2: 0.3 g L^{-1}, and P3: 0.4 g L^{-1}. The same letters for each treatment are not significantly different in the DMRT test results at the 5% test level

Increase in the length and weight of the North African catfish after given the methanol extract of *A. marina* leaves treatments

The results showed that the increasing length of the North African catfish under all treatments was not significantly different (P>0.05, Table 2). Likewise, the increasing weight at the end of this study did not show a significant difference between treatments (P> 0.05, Table (3)). Thirty days of observations did not show difference in growth between the fish under the control (P0) and the methanol extract of mangrove leaves *A. marina* treatments (P1, P2, and P3).

Table 2. Increase in the length of the North African catfish after exposure to the methanol extract of *Avicennia marina* leaves

Treatment	Increasing length (cm)				Average increasing length ± Standard deviation (cm)
	1	2	3	4	
P0	0	0	1.60	2.74	1.08±1.33[a]
P1	2.67	2.23	1.90	2.52	2.33±0.33[a]
P2	1.90	1.84	1.49	2.61	1.96±0.47[a]
P3	2.29	1.11	1.46	1.83	1.67±0.51[a]

Information: Numbers followed by the same superscript are not significantly different in the DMRT test results at the 5% test level and the number following ± indicates the value of standard deviation.

Table 3. Increase in the weight of the North African catfish after exposure to the methanol extract of *Avicennia marina* leaves

Treatment	Increasing Weight (g)				Average increasing Weight ± Standard deviation (g)
	1	2	3	4	
P0	0	0	10.74	0.07	2.70±5.35[a]
P1	2.10	6.68	6.80	6.67	5.56±2.30[a]
P2	3.11	4.55	2.33	4.70	3.67±1.14[a]
P3	0.39	0.10	2.62	3.66	1.69±1.72[a]

Information: Numbers followed by the same superscript are not significantly different in the DMRT test results at the 5% test level and the number following ± indicates the value of standard deviation.

Water quality parameters

The water quality parameters observed during this study consisted of water temperature, pH, and dissolved oxygen levels that were within the normal ranges of 22–29°C, 6–7, and 5.8–8.5mg L^{-1}, respectively (Table 4).

Table 4. The parameter values of water quality

Parameter of water quality	Treatment				**NSA (2000)**	**FADC (2016)**
	P0	P1	P2	P3		
Temperature (^{0}C)	23-29	23-29	23-29	23-29	25-30	20-30
Dissolved Oxygen (mg/L)	5.8-7.3	6.2 – 8.0	5.9 – 7.5	5.9 – 8.5	>4	3-6
Acidity (pH)	6-7	6-7	6-7	6-7	6.5-8.5	6.5-8.5

DISCUSSION

Efforts for MAS disease treatment in the North African catfish were carried out in aquaculture ponds in Banjarnegara by using methanol extract from the mangrove leaves *A. marina*. Before the treatment, the North African catfish infected by *A. hydrophila* showed clinical signs of aeromoniasis that were almost like all infected fish. They included ulcers on injection wounds on the dorsal, dorsal fin depigmentation, peeled-off scales, abdominal dropsy and hyperemia, gill hemorrhage, and exophthalmia. The fish also showed less stable swimming movement, the tendency to stay on the bottom, looking weak, frequent movement to the surface, and decreasing appetite. A previous study also reported that fish infected by *A. hydrophila* showed clinical signs of hyperemia, hemorrhagic, ulcers, erythema and erosion, necrotic, abdominal dropsy, skin depigmentation, tail and fins lesions, excessive mucus production, pale gills, losing balance when swimming, and decreasing appetite **(Mulia 2007; Austin & Austin, 2016; Rozi *et al.*, 2018; El-Son *et al.*, 2019)**.

Laboratory and field-testing on the North African catfish infected by *A. hydrophila* in Banyumas obtained clinical signs of body depigmentation, erosions, ulcers, hemorrhagic, fins erythema, white soft flesh, abdominal dropsy, hemorrhagic on the gills, fins and tail lesions, less active swimming, and decreasing appetite **(Mulia & Vauziyyah, 2021; Mulia *et al.*, 2022)**. These clinical signs in the fish infected by *A. hydrophila* were assumed to be due to the bacteria producing several toxins, i.e., hemolysin, protease, elastase, lipase, cytotoxin, enterotoxin, gelatinase, caseinase, lecithinase, and leucocidin **(Silva *et al.*, 2012; Doan *et al.*, 2013)**. Hemolysin is an enzyme that plays an important role in *A. hydrophila* pathogenicity and is cytotoxic for the fish **(Rasmussen-Ivey *et al.*, 2016)**. It causes red blood cells to lyse **(Soto-Rodriguez *et al.*, 2018)**. Moreover, it damages the tissue that affects epithelial and hemorrhagic cells on the surface of the fish skin **(Li *et al.*, 2013; Sarkar *et al.*, 2020)**. Proteases are also important in bacterial pathogenicity and cause infectious processes **(Pessoa *et al.*, 2019; Fernández-Bravo & Figueras, 2020)**. It can degrade the fish epithelial mucosa, causing discoloration of the scales, as well as lesions on the skin and internal organs **(Beaz-Hidalgo *et al.*, 2013)**.

The recovery process of the North African catfish was observed due to the use of methanol extract from the mangrove leaves *A. marina* treatments and after 30 days of post-treatment. In general, the fish control experienced severe clinical signs and continued mortality up to the end of this study. Clinical signs that continued to develop included the ulcer on the injection wound that became deeper every day, peeled-off skin and flesh, erythema fins, fin erosion, unstable swimming movement, and decreased appetite. Development of *A. hydrophila* on the fish body causes clinical signs up to the end of this study with 93.4% mortality. This took place since the fish under the control treatment were not given the methanol extract of the mangrove leaves *A. marina*, and the recovery process occurred naturally. Until the end of this study, clinical signs were still visible such as smaller ulcers, pale body color, and no increase in appetite.

The different condition was shown by the fish under the methanol extract of mangrove leaves *A. marina* treatment. Observation on days 1–3 showed that the North African catfish under P1, P2, and P3 treatment exhibited an decreasing appetite compared to the case before bacterial infection. This indicated that the fish experienced stress after *A. hydrophila* infection. Behavioral changes, such as a tendency to stay at the bottom and a decrease in appetite, were observed 24 hours after *A. hydrophila* infection via intramuscular injection **(Hardi *et al.*, 2014)**. Some of the infected fish in the P1, P2, and P3 treatment groups also died. The deaths were believed to be caused by more severe signs of MAS disease compared to the other fish, including swelling around the injection site, abdominal dropsy, blue bruises inside the stomach, and hemorrhoids on the gills.

The recovery process under the P1, P2, and P3 treatments began to appear from days 4 and 5 as marked by increasing appetite. The North African catfish given with P1 treatment experienced a faster recovery process than other treatments. The recovery signs started to appear as indicated by increasing appetite, drying wound edges, and agile fish movements. The recovery process seemed to improve from day 7 to 11. Ulcer length which initially reached 0.5–1.5cm began to shrink to 0.2cm. The lesion on the injection mark began to cover as indicated by the brown color of the wound. The ulcer closed completely on day 15, followed by an increasing appetite, recovered erythema fins and fin erosion in addition to a stable swimming.

Under P2 and P3 treatments, the North African catfish recovery process began to occur on day 5 as marked by the increasing appetite and edges of the wound that started to dry. The recovery process began to improve on day 7 as marked by a drying wound, wrinkles on the wound edges, and a stop of bleeding. Changes in behavior also occurred under the treatments as marked by more agile and active fish movements, increasing appetite, and stable swimming. On day 16, catfish infected by *A. hydrophila* was completely recovered. This was marked by a closed injection wound, increasing appetite, recovered fins erythema and erosion, as well as stable swimming movement.

The North African catfish recovery process was assumed to be due to the treatment effects of methanol extract mangrove leaves of *A. marina*. Previous researchers have reported that *A. marina* contains active compounds such as flavonoids, alkaloids, terpenoids, and tannins **(Mulia *et al.*, 2018)**. Other studies also reported that *A. marina* contains active antibacterial compounds **(Das *et al.*, 2018; Okla *et al.*, 2021)**. As antibacterials, flavonoids work by damaging the cytoplasmic membrane, inhibiting porins in the cell membranes, inhibiting cell wall synthesis and nucleic acid synthesis, and influencing bacterial pathogenicity **(Wu *et al.*, 2008; Xie *et al.*, 2015; Farhadi *et al.*, 2019)**. Alkaloids can cause death to bacterial cell due to their ability to interfere with the peptidoglycan arrangement of the bacterial cell wall which prevent it from being completely formed **(Cushnie *et al.*, 2014)**.

The tannins work by inhibiting the reverse transcriptase and DNA topoisomerase enzymes that enable them to inhibit the formation of bacterial cells. They can also deactivate bacterial cell adhesion and enzymes, and interfere with the transport of protein layers in cells **(Pandey & Negi, 2018; Belhaoues *et al.*, 2020)**. Tannins can also damage cell membranes **(Fraga-Corral *et al.*, 2020)**.

The giving of *A. marina* mangrove leaf extract in an immersion was proven effective in treating the North African catfish infected by *A. hydrophila* in both laboratory-scale and field-testing in the Banyumas area. The fish recovery process could be significant in comparison to those under the control **(Mulia & Vauziyyah, 2021; Mulia *et al.*, 2022)**. Other research using the *A. marina* mangrove leaf extract addition into the feed indicated a reduction of fish susceptibility against pathogenic bacterial infections, such as *Vibrio parahaemolyticus*, *V.anguillarum*, *Pseudomonas flourescens*, and *P. aeruginosa* in marine ornamental fish **(Dhayanithi *et al.*, 2013)**.

The treatment using the methanol extract of mangrove leaves of *A. marina* for the North African catfish infected by *A. hydrophila* could increase the score of survival rate compared to the control treatment. The survival rates under P1, P2, and P3 treatments were 74.95, 83.30, and 84.95%, respectively, while the survival rate control (P0) was 6.6%. The results showed that there was no significant difference between P1, P2, and P3 treatments (P>0.05), instead, it showed a significant difference (P<0.05) with the control (P0). Such a low survival rate of fish under the control (P0) was because no treatment was given to the North African catfish infected by *A. hydrophila*. Thus, *A. hydrophila* spread to the fish's internal organs and caused periodical death. North African catfish under P1, P2, and P3 treatments showed higher survival rates because the antibacterial activity of *A. marina* mangrove leaf extract was able to inhibit the growth of *A. hydrophila*, block bacterial respiration, inhibit the action of enzymes in the cell, change the permeability of the cytoplasmic membrane, denature the bacterial cell wall, damage the bacterial cell wall, and cause nutrient leakage from within the cell, cause bacterial cell death and ultimately reduce the number of *A. hydrophila* **(Guimarães *et al.*, 2019; Yang *et al.*, 2020; Okla *et al.*, 2021)**.

The results showed that the effective and efficient treatment for *A. hydrophila* infection in the North African catfish was P1 at a concentration of 0.2g L^{-1} because at this concentration, although it was the lowest, it showed equal ability of P2 and P3 treatment (concentration 0.3g L^{-1} and 0.4g L^{-1}). Similarly, a field-test on the North African catfish treated using the methanol extract of mangrove leaves *A. marina* in the Banyumas area, effectively used a concentration of 0.2g L^{-1} **(Mulia & Vauziyyah, 2021)**.

The North African catfish showed relatively similar increasing weight and length under all treatments. This study revealed that the results of all treatments were not significantly different (P>0.05). Treatment using the methanol extract of mangrove leaves *A. marina* leaves showed no significant effect on the increase in weight and length of the fish. A similar result was also obtained on field-testing of the North African catfish given the extract of *A. marina* mangrove leaves in the Banyumas area **(Mulia & Vauziyyah, 2021)**.

Good water conditions are one of the keys to success in cultivating the North African catfish because water is the most vital medium for the fish life **(Afrianto & Liviawaty, 2016)**. The water temperature during this study ranged from 22-29°C, which was measured in the morning, mid-day, and evening. In general, the normal range for the North African catfish according to the **NSA (2000)** was 25–30°C, whereas according to **FADC (2016)** it was 20-30°C. On the 2nd day after the North African catfish recovery process, the temperature reached its lowest value, namely 22°C. However, decreasing temperature did not have a real effect on the North African catfish given the *A. marina* mangrove leaf extract. There were indications that the number of fish deaths on day 2 was only around 1-2 fish per treatment.

Dissolved oxygen levels during this study ranged from 5.8 to 8.5mg L^{-1}. The lowest oxygen level measured was 5.8mg L^{-1}, which was presumably because the measurements were taken in the morning or night. The highest dissolved oxygen level recorded was 8.5 mg/L, which is likely due to the oxygen measurement being taken during daylight hours. During the day, plants around the study area performed photosynthesis, which contributed to the increase in oxygen levels in the pond water. Additionally, aquatic plants in the rearing pond, such as moss, could produce oxygen through photosynthesis. Dissolved oxygen levels are an important factor for the survival of North African catfish. Based on the data obtained, the dissolved oxygen value remained within suitable levels. According to **FADC (2016)**, optimal dissolved oxygen levels for North African catfish growth range from 3–6 mg/L, while **NSA (2000)** suggests values above 4mg/ L.

In this study, the optimal pH value was maintained by siphoning and changing the water, keeping the pH between 6 and 7. This range was considered suitable, as the North African catfish requires a pH range between 6.5 and 8.5 **(NSA, 2000; FADC, 2016)**.

CONCLUSION

The results of field tests in the Banjarnegara area showed that a methanol extract of *A. marina* leaves was effective and efficient in controlling *A. hydrophila* infections in gourami at a dose of 0.2g L^{-1}. The mangrove leaves of *A. marina* contain antibacterial compounds that can be widely used as a natural anti-*A. hydrophila*.

ETHICAL APPROVAL

The management, conditions, and procedures of the experiment in this study were approved by the Ethical Clearance Commission of Gadjah Mada University (approval # certificate: 00137/04/LPPTI/I/201).

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