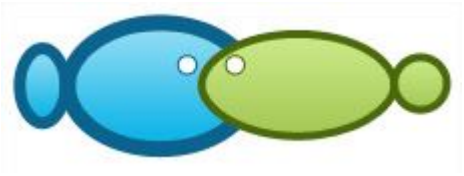

Antibacterial activity of mangrove plant (*Avicennia marina*) to control *Aeromonas hydrophila* infection in African catfish (*Clarias gariepinus*)

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Abstract. African catfish (*Clarias gariepinus*) is intensively cultured in Indonesia, and has become the most important freshwater fish species in the country's aquaculture. However, this species is highly susceptible to *Aeromonas hydrophila* infection. To control such infection, one of the counter measure methods is by using bioactive natural compounds from mangrove plants. This study evaluated the antibacterial activity in a mangrove plant (*Avicennia marina*) and determined the effectiveness of the extract to control *A. hydrophila* infection in African catfish. This study aimed to determine the development signs of fish disease, survival rates, and recovery process of treated fish after challenged with *A. hydrophila* infection. The experiment method applied was the completely randomized design (CRD) with 4 treatments of extract concentration and 4 replications, i.e., T0: control (without extract treatment), T1: 0.2 g L^{-1}, T2: 0.3 g L^{-1}, and T3: 0.4 g L^{-1}. Mangrove leaves was extracted by maceration process using methanol. The fish was treated with the extract by applying the immersion method. The recovery process was observed for 14 days after treatment. The development of disease signs, recovery process, and survival rates were observed. The data were analyzed by using analysis of variance (ANOVA) and continued with Duncan multiple range test (DMRT) at 5% test level. The development of disease signs and recovery process was analyzed descriptively. The results showed that the healing process in treated samples began to occur on day 3-4; while in control samples, it began to appear on day 7-8. Samples treated with the extract were completely recovered after 7-8 days given with treatment. While in control samples, recovery was slower and reached day 13-14. The results of statistical analysis showed that mangrove leaves extract significantly improved the catfish survival rate ($p < 0.05$). In conclusion, the extract of *A. marina* mangrove leaves at 0.2 g L^{-1} effectively and efficiently controlled *A. hydrophila* infection in African catfish. The results suggest that *A. marina* mangrove leaves extract is prospective as natural resources to develop anti *A. hydrophila* compounds.
Key Words: *Aeromonas hydrophila*, African catfish, *Avicennia marina*, mangrove, natural bactericidal.

Introduction. African catfish (*Clarias gariepinus*) is one of the potential freshwater aquaculture fish in Indonesia. The production continues to increase between the years (BPS 2021). One of the constraints in the cultivation of African catfish is bacterial disease caused by *Aeromonas hydrophila* (Anyanwu et al 2015; Alimuddin et al 2018; Mulia et al 2022). It also attacks other fish and aquatic biota, such as channel catfish (*Ictalurus punctatus*), gouramy (*Osphronemus gouramy*), Nile tilapia (*Oreochromis niloticus*), eels (*Anguilla japonica)*, common carp (*Cyprinus carpio*), Chinese giant salamander (*Andrias davidianus*), shrimp (*Litopenaeus vannamei* and *Penaeus monodon*), and Chinese stripe-necked turtles (*Ocadia sinensis*) (Wang et al 2012; Stratev et al 2015; Yano et al 2015; Guo et al 2016; Abd El Tawab et al 2017; Wimalasena et al 2017; Peatman et al 2018; Wamala et al 2018). *A. hydrophila* has rod-shape, Gram negative, facultative anaerobic, oxidase positive, catalase positive, fermentative, ferments glucose (produces acid) and does not form spores (Mulia et al 2011; Percival & Williams 2014; El-Sharaby et al 2021).

Other studies report that *A. hydrophila* comes with higher virulence potential than *A. sobria*. They also reveal the possibility of *A. caviae* in raw and ready-to-eat (RTE) to become the major threat to public health (Abd-El-Malek 2017). Virulence of *A. hydrophila* is due to the role of virulent genes in the bacteria such as aerolysin (*aer*), haemolysin (*haem*), cytotonic heat-labile enterotoxin (*alt*), cytotonic heat-stable enterotoxin (*ast*), cytotoxic heat-labile enterotoxin (*act*), polar flagellum (*fla*), and lateral flagellum (*laf*) (Yi et al 2013; Aravena-Román et al 2014; Abreu et al 2018). This condition triggers *A. hydrophila* to be able to produce exotoxin that influences its pathogenicity (Masuyer 2020; Raju et al 2020; Sarkar et al 2020). Fish infected by *Aeromonas* show clinical symptoms of septicemia, lesion, skin ulcer, fin rot, erosion, hemorrhagic, hyperemia, congestion, abscess, abdominal edema, abdominal dropsy, abdominal acites, necrosis, and exophthalmia (Kozinska & Pekala 2012; Hamid et al 2017; Wamala et al 2018; Elgohary et al 2020; Mulia et al 2020).

Infected fish due to *A. hydrophila* are mostly treated with synthetic antibiotics. However, excessive use can cause bacterial resistance, death of non-target biota, antibiotic residues in fish meat, and environmental pollution (Parker & Shaw 2011; Serwecińska 2020; Bombaywala et al 2021). Thus, considering their minor risk, the use of antibacterial compounds from natural ingredients is recommended. Secondary metabolite compound from a tropical coastal plant (*Diospyros maritima*) is proven to be antibacterial (Isnansetyo et al 2022). Other useful natural substance is the antibacterial compounds found in mangrove plants *Avicennia marina*. There are evidences of *A. marina* containing compounds as flavonoids, alkaloids, terpenoids, and tannins that are antibacterial for *A. hydrophila* (Mulia et al 2018). The extract of *A. marina* has more effective use as antibacterial than anti-fungal (Amirkaveei & Behbahani 2011). The leaves extract of *A. marina* is able to inhibit the growth of *Staphylococcus aureus* and *Vibrio alginolyticus* bacteria (Danata & Yamindago 2014). This study aimed to evaluate the antibacterial activity of a mangrove plant (*A. marina*), and determined the effectiveness of its extract to control *A. hydrophila* infection in African catfish.

Material and Method

Time and place of experimental study. This study was conducted at Laboratory of Microbiology and Laboratory of Fish and Environmental Health, Universitas Muhammadiyah Purwokerto, Indonesia in April-September 2021.

Purification of Aeromonas hydrophila. The bacterial isolate used was *A. hydrophila* GPI-04. Its purification was carried out by growing the isolate on glutamate starch phenyl (GSP) medium (Merck) at 30°C for 24 h. Single colony growing on the medium were re-cultured on tilted TSA agar medium and incubated at the same temperature and time, before stored as stock culture (Mulia 2012).

Restoring the virulence of Aeromonas hydrophila. Efforts to restore the virulence of *A. hydrophila* GPI-04 bacteria included the reinfection and re-isolation of its isolate against African catfish. The first reinfection was aimed to determine whether the pure isolates were pathogenic bacteria that caused aeromoniation disease to the catfish. Furthermore, the second and third reinfection and re-isolation were carried out to identify more complete and specific symptoms of the disease as well as to increase the virulence (Mulia 2012).

Pathogenicity test to determine the lethal dose (LD_{50}). Lethal $dose_{50}$ (LD_{50}) is a dose of bacterial suspension capable of killing 50% of the population of the sample. It was used to test its ability in killing bacteria and identify the proper concentration for testing antibacterial effectiveness of *A. marina* leaf extracts which was obtained by using methanol solvent. Single colony of GSP medium was transferred to 10 mL TSB liquid medium and incubated at 30°C for 24 h. Next, the bacteria culture in TSB medium was diluted at 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} concentrations before injected into healthy catfish samples in 0.1 mL $fish^{-1}$. Each treatment used 8 fishes and repeated twice.

Observations on the clinical symptoms of motile *Aeromonas* septicemia (MAS) disease on the samples were done every day for 7 days.

Making the leaf extract of Avicennia marina. Weighed leaves of *A. marina* were cut into small pieces and put into 60^{o}C oven for 4 x 24 hours or until completely dry. The dry pieces were weighed to obtain the dry weight and put into electric grinder to become simplicia. The extract was made through maceration method by using methanol solvent (Mulia et al 2018). The simplicia was macerated by using 80% methanol solvent for 2 x 24 hours. Methanol ratio was 1 L for 200 g simplicia. This was modified from Abeysinghe et al (2006). The solvent was then filtered through filter paper to obtain filtrate. Next, it was evaporated in rotary evaporator at 50^{o}C up until macerate was obtained. Then, the macerate was dried by using the waterbath until thick extract was obtained.

Infecting the Aeromonas hydrophila into African catfish. African catfish was injected intramuscularly as much as 0.1 mL fish^{-1} based on the result of LD_{50} dose, which was 9.52 x 10^{7} CFU mL^{-1}. Betadine was given to the injection spot on the back of the samples to prevent infection before they were returned to the rearing containers. Observation on clinical symptoms of MAS disease was done in two days before *A. marina* extract was given.

Treating the African catfish using the A. marina extract solvent. African catfish already infected with *A. hydrophila* was treated with *A. marina* extract by one hour of immersion at T0: control concentration (without extract treatment), T1: 0.2 g L^{-1}, T2: 0.3 g L^{-1}, and T3: 0.4 g L^{-1}, and four repetitions on each treatment. Observation on the fish recovery process was carried out in 14 days.

Research parameters. Research parameters consisted of the main parameter, such as signs of MAS disease, recovery process, and fish survival. Supporting parameters included water quality, temperature, pH, and level of dissolved oxygen.

Data analysis. The main parameter data were analyzed using analysis of variance (Anova) and Duncan multiple range test (DMRT) at 5% test level. The supporting parameter data were descriptively quantitative analyzed.

Results

Clinical signs development of MAS in African catfish. African catfish infected with *A. hydrophila* showed clinical symptoms of MAS disease (Table 1). Clinical signs were observed for two days before *A. marina* leaf extract treatment was given.

Table 1

The clinical signs development of MAS in African catfish infected by *A. hydrophila*

Day	*Clinical signs of MAS in African catfish*
1	Ulcers on the body, haemoragic septicemia, and peeling up of skin up to 2.5 cm, erythrema fins, white soft flesh, decreasing appetite, tendency to stay in the bottom of water.
2	Wider ulcers, haemoragic septicemia, dorsal fins erosion, loosing of appetite, lethargic and always staying in the bottom of water, in some cases floating vertically with head above the surface.

African catfish recovery process. Recovery process included the African catfish that was infected with *A. hydrophila* going through the healing. Control samples (T0) indicated recovery process occurred naturally without *A. marina* leaf extract treatment, although in slower process. The recovery process began to appear on day 7-8 as indicated by new skin formation covering wound around the abdomen. On day 9-10, dorsal fin around the

injection spot began to form. On day 11-12, the flesh around the ulcer was reddish, fish tended to actively swimming and appetite slightly increased. Until the last day of observation (day 14), the recovery process was still ongoing with fish actively moving in addition to increasing appetite.

In contrast, samples given with *A. marina* leaf extract treatments showed faster recovery process. In T1, it started on day 3-4 as indicated by the formation of new skin and flesh on the ulcer wound around the abdomen. Also, wounds on the fins hat were narrowing. On day 5-6, samples with less than 2 cm wounds were healed with ulcers that had been tightly covered by new skin, in addition to samples indicated increasing appetite and active movements. On day 7-8, the majority of samples showed full recovery, increasing appetite, active movements and zero deaths of samples. On the last day of observation (day 14), all samples were recovered and indicated with ulcers on each fish that were completely covered with skin, increasing appetite, active movements and zero deaths.

In T2, the recovery process also started on day 3-4 as indicated by disappearing wounds and ulcers on the body with only 0.7-1.2 cm remaining wounds. Samples with initial ulcers of less than 2 cm had completely recovered with fully formation of new skin. Some of the samples with initial ulcers that were wider than 2 cm experienced new skin formation around the ulcers on the stomach in addition to the flesh on the ulcer wounds beginning to reddish and newly formation of flesh beginning to cover the ribs. All samples showed active movements and increasing appetite. On day 5-6, the ulcers were narrowing (between 0.4 and 0.7 cm). Because more samples had fully recovered, all fish showed active movements and increasing appetite. On day 7-8, all of alive samples were fully recovered and indicated back to normal appetite and active swimmings.

In T3, formation of new skin around the ulcer began on day 3-4, with soft-textured white flesh and dorsal fin falling off around the infection. On day 5-6, the ulcers got narrower with new skin formation around the ulcers and reddish flesh color in addition to the fish samples moving actively and increasing appetite. On day 7-8, some fish experienced complete recovery and increasing appetite. All samples were active and none of the fish died. On day 9-10, the most of the samples had fully recovered with appetite returning to normal, moving actively and zero deaths. On the last day of observation (day 14), all of alive samples had fully recovered with appetite returning to normal, moving actively and zero death.

Survival rate. The survival rate of African catfish under *A. marina* leaf extract treatments (T1-T3) subsequent to the recovery process was 50-80%. Meanwhile, control (T0) samples exhibited survival rate of only 30% (Figure 1). The results showed highest survival rates in T1 and T2 by 70 and 80%, respectively, which were significantly different ($p = 0.000$; $p < 0.05$) from T0 (30%) and T3 (50%).

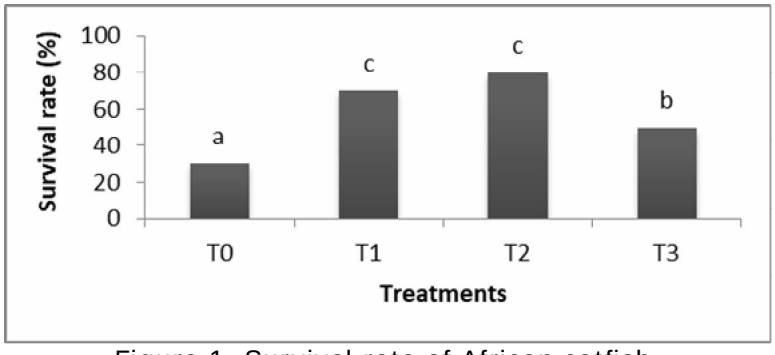

Figure 1. Survival rate of African catfish.

Water quality. Water quality parameters were closely controlled to optimize environmental rearing conditions. The measurement results showed that water temperature, pH and dissolved oxygen within the study varied between 26 and 28°C, 7-7.5, and 8.1-8.6 ppm, respectively (Table 2).

Table 2

Parameters of water quality

Treatment	*Temperature (^oC)*	*Acidity (pH)*	*Dissolved oxygen (ppm)*
T0	26-28	7.0-7.3	8.3-8.5
T1	27-28	7.0-7.4	8.2-8.6
T2	26-28	7.1-7.3	8.1-8.3
T3	26-28	7.0-7.5	8.1-8.3

Discussion. African catfish infected with *A. hydrophila* showed some clinical signs on day 1 and 2 which included sores and peeling of skin on the injection spot, flesh going white and soft, haemoragic, erythrema fins, dorsal fin erosion, decreasing appetite, less-active swimming and tendency to stay on the bottom, or in some cases, floating vertically with head above the surface.

A. hydrophila infection causes damage to skin tissue, blood vessels, and flesh. Skin wounds due to injection process were the main and fast entrance point of *A. hydrophila* infection. Initially, skin would peel off and bleed due to damage of blood vessels around the skin. The flesh uncovered with skin would turn pale-white and the texture would fall out. Results of previous studies also reported clinical signs of MAS in fish infected with *A. hydrophila*, such as haemoragic septicemia, ulcers, hyperemia, necrotic, erythrema fins, fin erosion, abdominal dropsy and decreasing appetite (Doan et al 2013; Austin & Austin 2016; Dias et al 2016; Zhang et al 2016).

Clinical signs of MAS in infected fish were assumed to be caused by the ability of *A. hydrophila* to produce a number of toxins (Silva et al 2012; Al-Fatlawy & Al-Ammar 2013). It can produce exotoxins which include hemolysin, protease, elastase, lipase, cytotoxin, enterotoxin, gelatinase, caseinase, lechitinase, and leucocidin. Hemolysin is an enzyme that can lyse red blood cells and free hemoglobin that enables much blood coming out of wounds on infected body surfaces (Huys et al 2002). Hemolysin toxin activity causes hemorrhagic on fish skin surface (Li et al 2013).

Protease is a proteolytic enzyme functioning to fight the host's body defenses from developing the disease and take over host's supply of nutrients from reproducing. Lechitinase is an enzyme with a role of destroying various tissue cells and is especially active in lysing red blood cells, while leucocidin is an enzyme that can kill white blood cells (Pelczar & Chan 2015).

The *A. marina* leaf extract treatment triggered better and faster fish recovery process than the control treatment. The recovery process under *A. marina* leaf extract treatments (T1, T2, and T3) started on day 3-4; while in the control (T0), it started on day 7-8. Samples given with extract treatment were completely recovered on day 7-8. While under the control treatment, samples recovery up to day 13-14 were slower. Indications of recovery process in African catfish included narrowing ulcer, formation of new skin and flesh on the ulcer, narrowing wound on the fin, fish swimming more actively and improving appetite. Fully recovery was indicated by healed ulcers (no visible ulcers on the fish body) and wounds on the fins, as well as no fin flakes, normal appetite, and all samples were active. The recovery process was presumed to be due to role of active compounds contained in the extract of *A. marina* which were antibacterial. Previous researches reported various secondary metabolites found in *A. marina* leaf extract, such as alkaloids, flavonoids, terpenoids, and tannins (Mulia et al 2018). Other studies also reported antibacterial active compounds in *A. marina* (Ananthavalli & Karpagam 2017; Das et al 2018; Okla et al 2021). The *A. marina* leaf extract treatment given in the feed was indicated to reduce fish susceptibility against various pathogenic bacteria infections such as *Pseudomonas flourescens*, *P. aeruginosa*, *Vibrio parahaemolyticus*, and *V. anguillarum* in marine ornamental fish (Dhayanithi et al 2013).

There are various inhibition mechanisms of microorganisms growth by antimicrobial compound. Some common mechanisms included the destruction of cell walls by inhibiting their formation or changing the structure of the cell walls after their formation, the changes in the cytoplasmic membrane permeability that cause the release

of food materials from the cell, the changes in protein and nucleic acid molecules, inhibition of enzymes and nucleic acid and protein synthesis (Tjay & Rahardja 2015).

Alkaloids contain nitrogen-alkali groups that are reactive to amino acids of those composing the bacterial cell walls and DNA. This reaction will cause structural changes of amino acids and lead to genetic changes and lysis. The antibacterial mechanism of alkaloids can also interfere with the peptidoglycan (component of cell walls) preparation in bacteria, in which the formation of cell wall layer is not full and causes bacterial death (Cushnie et al 2014). Flavonoids have excellent antibacterial activity (Farhadi et al 2018). This compound can inhibit nucleic acid synthesis and alteration in cytoplasmic membrane, as well as inhibit the porins in cells membranes, change in membrane permeability and attenuation of the pathogenicity (Cushnie & Lamb 2011; Xie et al 2015). Flavonoids can also damage the cytoplasmic membrane and suppression of cell wall synthesis caused by D-alanine-D-alanine ligase inhibition (Tamba et al 2007; Wu et al 2008).

Terpenoids are compounds with antibacterial potentials. They can damage the bacterial cell wall, cause lysis, changes of cytoplasmic membrane permeability, leakage of nutrients within the cell and denaturation of cell proteins, as well as inhibit enzymes working in the cell (Yang et al 2020). Tannins can damage cell membranes and induce the formation of complex compounds that attack bacterial enzymes thereby increasing the toxicity of tannins to bacteria. Tannins are presumably having the ability to contract cell membranes or cell walls which interfere with the permeability of bacterial cells so that they can inhibit the growth of bacterial cells (Farha et al 2020).

The survival rate of control fish (T0) was only 30%. This was significantly different from *A. marina* leaf extract (T1, T2, and T3) treatments which reached 50-80%. The fish under control treatment exhibited highest mortality of 70%. This was due to the fish immune system unable to inhibit the growth of bacteria when infected with *A. hydrophila*. Thus, the fish body continued to suffer from damage and fish died. Survived fish was 30% with on-going recovery process until the end of observation.

The survival rates between samples under *A. marina* leaf extract treatments T1, T2, and T3 were higher than the control. This was assumed to be influenced by the antibacterial compounds contained in the extract. Secondary metabolite compounds in *A. marina* leaf extract, such as polyphenols, flavonoids, and tannins have antibacterial potentials (Ravikumar et al 2009; Gnanadesigan et al 2011) are able to inhibit growth of *A. hydrophila* (Mulyani et al 2013). Antibacterial activity of *A. marina* leaf extract can either denature bacterial cell walls, block bacterial respiration or destabilize outer membranes and depletion of intracellular ATP (Vivekanandhan et al 2009).

The results showed that 0.2 g L^{-1} (T1) was the most effective and efficient concentration for treating African catfish infected with *A. hydrophila*. It was the optimum concentration to kill *A. hydrophila*. At 0.3 g L^{-1} (T2) the survival rate was not significantly different from T1. Meanwhile, at higher concentration [0.4 g L^{-1} (T3)], it was suspected that apart from killing *A. hydrophila* bacteria, it also killed its host (fish samples). The results showed that leaves of *A. marina* mangrove were prospective natural resource for the development of anti-*A. hydrophila* compounds. The use of medicine made of natural ingredients can be a solution to cope with the problem of bacterial disease in fish (Dhayanithi et al 2013).

The measurement results of water quality parameters, such as water temperature, dissolved oxygen and pH, indicated normal range of 26-28^{o}C, 8.1-8.6 ppm, and 7.0-7.5, respectively. The optimal water temperature, dissolved oxygen and pH for African catfish cultivation were 25-30^{o}C, 3-20 ppm, and 6.5-8, respectively (Nugrahajati et al 2013; Saparinto & Susiana 2013).

Conclusions. This study has successfully documented the effectiveness of *A. marina* leaf extract as an antibacterial. The leaves extract of *A. marina* mangrove plants at 0.2 g L^{-1} effectively and efficiently controlled *A. hydrophila* infection in African catfish. The results suggest that *A. marina* leaves are prospective natural resources to be developed as anti *A. hydrophila* compounds.

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Conflict of interest. The authors declare that there is no conflict of interest.

References

Abd-El-Malek A. M., 2017 Incidence and virulence characteristics of *Aeromonas* spp. in fish. Veterinary World 10(1):34-37.

Abd El Tawab A., Maarouf A., El Hofy F., El Mougy E., 2017 Detection of some virulence genes in *A. hydrophila* and *A. caviae* isolated from fresh water fishes at Qalubia Governorate. Benha Veterinary Medical Journal 33(2):489-503.

Abeysinghe P. D., Wanigatunge R. P., Pathirana R. N., 2006 Evaluation of antibacterial activity of different mangrove plant extracts. Ruhuna Journal of Sience 1:104-112.

Abreu R. E. F., Magalhães T. C., Souza R. C., Oliveira S. T. L., Ibelli A. M. G., Demarqui F. N., Gouveia J. J. S., Costa M. M., Gouveia G. V., 2018 Environmental factors on virulence of *Aeromonas hydrophila*. Aquaculture International 26(2):495-507.

Al-Fatlawy H. N. K., Al-Ammar M. H., 2013 Molecular study of *Aeromonas hydrophila* isolated from stool samples in Najaf (Iraq). International Journal of Microbiology Research 5(1):362-365.

Alimuddin, Putri F. M., Wahjuningrum D., Hardiantho D., Sunarma A., Nuryati S., 2018 Resistance against *Aeromonas hydrophila* infection and growth of second generation (F2) African catfish (*Clarias gariepinus*) using selected molecular markers. Biotropia 25(2):95-102.

Amirkaveei S., Behbahani B. A., 2011 Antimicrobial effect of mangrove extract on *Escherichia coli* and *Penicillium digitatum*. IPCBEE 9:185-188.

Ananthavalli M., Karpagam S., 2017 Antibacterial activity and phytochemical content of *Avicennia marina* collected from polluted and unpolluted site. Journal of Medicinal Plants Studies 5(3):47-49.

Anyanwu M. U., Chah K. F., Shoyinka V. S., 2015 Evaluation of pathogenicity of motile *Aeromonas* species in African catfish. International Journal of Fisheries and Aquatic Studies 2(3):93-98.

Aravena-Román M., Inglis T. J. J., Riley T. V., Chang B. J., 2014 Distribution of 13 virulence genes among clinical and environmental *Aeromonas* spp. in Western Australia. European Journal of Clinical Microbiology and Infectious Diseases 33(11): 1889-1895.

Austin B., Austin D. A., 2016 Bacterial fish pathogens. Disease of farmed and wild fish. 6th edition. Springer International Publishing, Switzerland, 732 pp.

Badan Pusat Statistik, 2021 [Indonesia Statistics 2022]. BPS-Statistics Indonesia, Jakarta. [in Indonesian]

Bombaywala S., Mandpe A., Paliya S., Kumar S., 2021 Antibiotic resistance in the environment: a critical insight on its occurrence, fate, and eco-toxicity. Environmental Science and Pollution Research 28:24889-24916.

Cushnie T. T., Lamb A. J., 2011 Recent advances in understanding the antibacterial properties of flavonoids. International Journal of Antimicrobial Agents 38(2):99-107.

Cushnie T. P. T., Cushnie B., Lamb A. J., 2014 Alkaloids: an overview of their antibacterial, antibiotic-enhancing and antivirulence activities. International Journal of Antimicrobial Agents 44:377-386.

Danata R. H., Yamindago A., 2014 Analisis aktivitas antibakteri ekstrak daun mangrove *Avicenia marina* dari Kabupaten Trenggalek dan Kabupaten Pasuruan terhadap pertumbuhan *Staphylococcus aureus* dan *Vibrio alginolyticus*. Jurnal Kelautan 7(1):13-20. [in Indonesian]

Das S. K., Samantaray D., Mahapatra A., Pal N., Munda R., Thatoi H., 2018 Pharmacological activities of leaf and bark extracts of a medicinal mangrove plant *Avicennia officinalis* L. Clinical Phytoscience 4(13):1-10.
Dhayanithi N. B., Kumar T. T. A., Balasubramanian T., Tissera K., 2013 A study on the effect of using mangrove leaf extracts as a feed additive in the progress of bacterial infections in marine ornamental fish. Journal of Coastal Life Medicine 1(3):226-233.
Dias M. K. R., Sampaio L. S., Proietti-Junior A. A., Yoshioka E. T. O., Rodrigues D. P., Rodriguez A. F. R., Ribeiro R. A., Faria F. S. E. D. V., Ozorio R. O. A., Tavares-Dias M., 2016 Lethal dose and clinical signs of *Aeromonas hydrophila* in *Arapaima gigas* (Arapaimidae), the giant fish from Amazon. Veterinary Microbiology 188:12-15.
Doan H. V., Doolgindachbaporn S., Suksri A., 2013 The LD_{50} of asian catfish (*Pangasius bocourti*, Sauvage 1870) challenge to pathogen *Aeromonas hydrophila* FW52 strain. Pensee Journal 75(10):287-293.
Elgohary I., Elatief J. I. A., Fadel N. G., Eissa A. E., Mahmoud M. A., 2020 Pathological, bacteriological and seasonal prevalence of *Aeromonas hydrophila*, *Vibrio vulnificus*, *Proteus vulgaris* and *Pseudomonas aeruginosa*; infecting *Oreochromis niloticus* in some Egyptian fish farms. Egyptian Journal of Aquatic Biology and Fisheries 24(5): 467-482.
El-Sharaby S., El-Ballal S., Ali M., El-Hady M., 2021 Molecular studies on *Aeromonas* species isolated from freshwater fishes collected from Delta region, Egypt. Journal of Current Veterinary Research 3(2):8-15.
Farha A. K., Yang Q. Q., Kim G., Li H. B., Zhu F., Liu H. Y., Gan R. Y., Corke H., 2020 Tannins as an alternative to antibiotics. Food Bioscience 38:1-10.
Farhadi F., Khameneh B., Iranshahi M., Iranshahy M., 2019 Antibacterial activity of flavonoids and their structure–activity relationship: an update review. Phytotherapy Research 33:13-40.
Gnanadesigan M., Ravikumar S., Inbanesan J. S., 2011 Hepatoprotective and antioxidant properties of marine halophyte *Lumnitzera racemosa* bark extract in CCL4 induced hepatotoxicity. Asian Pacific Journal of Tropical Medicine 4(6):462-465.
Guo S. L., Yang Q. H., Feng J. J., Duan L. H., Zhao J. P., 2016 Phylogenetic analysis of the pathogenic genus *Aeromonas* spp. isolated from diseased eels in China. Microbial Pathogenesis 101:12-23.
Hamid N. H., Hassan M. D., Sabri M. Y. M., Hasliza A. H., Hamdan R. H., Afifah M. N. F., Raina M. S., Nadia A. B. S., Fuad M. M., 2017 Studies on pathogenicity effect of *Aeromonas hydrophila* infection in juvenile red hybrid tilapia *Oreochromis* sp. Proceedings of International Seminar on Livestock Production and Veterinary Technology, pp. 532-539.
Huys G., Kampfer P., Albert M. J., Khun I., Denys R. , Swings J., 2002 *Aeromonas hydrophila* subsp. *dhakensis* subsp. nov., isolated from children with diarrhoea in Bangladesh, and extended description of *Aeromonas hydrophila* subsp. *hydrophila* (Chester, 1901) Stanier, 1943 (approved lists 1980). International Journal of Systematics and Evolutionary Microbiology 52:705-712.
Isnansetyo A., Handayani D. P., Istiqomah I., Arif A., Kaneko T., 2022 An antibacterial compound purified from a tropical coastal plant, *Diospyros maritima*. Biodiversitas 23(1):135-142.
Kozińska A., Pękala A., 2012 Characteristics of disease spectrum in relation to species, serogroups, and adhesion ability of motile aeromonads in fish. The Scientific World Journal 949358:1-9.
Li C., Wang R., Su B., Luo Y., Terhune J., Beck B., Peatman E., 2013 Evasion of mucosal defenses during *Aeromonas hydrophila* infection of channel catfish (*Ictalurus punctatus*) skin. Developmental and Comparative Immunology 39(4):447-455.
Masuyer G., 2020 Crystal structure of exotoxin a from *Aeromonas* pathogenic species. Toxins 12(397):1-14.
Mulia D. S., 2012 [African catfish vaccination]. Pustaka Pelajar, Yogyakarta. 122 pp. [in Indonesian]
Mulia D. S., Maryanto H., Purbomartono C., 2011 [Isolation, characterization, and identification of bacteria on infected African catfish in Banyumas Regency]. Jurnal Sainteks 7(1):1-15. [in Indonesian]

Mulia D. S., Choeriyah D., Maryanto H., Purbomartono C., 2018 Bactericidal prosperity from tropical mangrove, *Avicennia marina*, to control fish bacterial pathogen, *Aeromonas hydrophila* GK-01 and GPI-04 strains. Advanced Science Letters 3398-3402.
Mulia D. S., Isnansetyo A., Pratiwi R., Asmara W., 2020 Molecular characterizations of *Aeromonas caviae* isolated from catfish (*Clarias* sp.). AACL Bioflux 13(5):2717-2732.
Mulia D. S., Utomo T., Isnansetyo A., 2022 The eficacy of *Aeromonas hydrophila* GPI-04 feed-based vaccine on African catfish (*Clarias gariepinus*). Biodiversitas 23(3): 1505-1510.
Mulyani Y., Bachtiar E., Kurnia M. U. A., 2013 [The role of secondary metabolites of mangrove plants against *Aeromonas hydrophila* bacterial infection in carp (*Cyprinus carpio* L.)]. Jurnal Akuatika 4(1):1-9. [in Indonesian]
Nugrahajati P., Wargiyanto, Krisnawati M., 2013 [Secrets of business success and excellent catfish cultivation]. Lily Publisher, Yogyakarta, 122 pp. [in Indonesian]
Okla M. K., Alatar A. A., Al-amri S. S., Soufan W.H., Ahmad A., Abdel-Maksoud M. A., 2021 Antibacterial and antifungal activity of the extracts of different parts of *Avicennia marina* (Forssk.) Vierh. Plants 10(252):1-14.
Parker J. L., Shaw J. G., 2011 *Aeromonas* spp. clinical microbiology and disease. Journal of Infection 62(2):109-118.
Peatman E., Mohammed H., Kirby A., Shoemaker C. A., Yildirim-Aksoy M., Beck B. H., 2018 Mechanisms of pathogen virulence and host susceptibility in virulent *Aeromonas hydrophila* infections of channel catfish (*Ictalurus punctatus*). Aquaculture 482:1-8.
Pelczar M. J., Chan E. C. S., 2015 [Microbiology fundamentals]. UI Press, Jakarta. [in Indonesian]
Percival S. L., Williams D. W., 2014 *Aeromonas*. In: Microbiology of waterborne diseases: microbiological aspects and risks. 2nd edition. Percival S. L., Williams D. W., Gray F. N., Yates M. V., Chalmers R. M. (eds), Elsevier, London, pp. 49-64.
Raju V. S., Sarkar P., Pachaiappan R., Paray B. A., Al-Sadoon M. K., Arockiaraj J., 2020 Defense involvement of piscidin from striped murrel *Channa striatus* and its peptides CsRG12 and CsLC11 involvement in an antimicrobial and antibiofilm activity. Fish and Shellfish Immunology 99:368-378.
Ravikumar S., Ramanathan G., Subakaran M., Inbaneson J. S., 2009 Antimicrobial compounds from marine halophytes for silkworm disease treatment. International Journal of Medicine and Medical Sciences 1(5):184-191.
Saparinto C., Susiana R., 2013 [Successful hatchery of 6 types of economical freshwater fish]. Lily Publisher, Yogyakarta, 278 pp. [in Indonesian]
Sarkar P., Issac P. K., Raju S. V., Elumalai P., Arshad A., Arockiara J., 2020 Pathogenic bacterial toxins and virulence influences in cultivable fish. Aquaculture Research 52:2361-2376.
Serwecińska L., 2020 Antimicrobials and antibiotic-resistant bacteria: a risk to the environment and to public health. Water 13(3313):1-17.
Silva B. C. D., Mourino J. L. P. , Vieira F. N., Jatobá A., Seiffert W. Q., Martins M. L., 2012 Haemorrhagic septicaemia in the hybrid surubim (*Pseudoplatystoma corruscans* × *Pseudoplatystoma fasciatum*) caused by *Aeromonas hydrophila*. Aquaculture Research 43:908–916.
Stratev D., Daskalov H., Vashin I., 2015 Characterisation and determination of antimicrobial resistance of b-haemolytic *Aeromonas* spp. isolated from common carp (*Cyprinus carpio* L.). Reveu Medicine Veterinary 166(1-2):54-61.
Tamba Y., Ohba S., Kubota M., Yoshioka H., Yoshioka H., Yamazaki M., 2007 Single GUV method reveals interaction of tea catechin (-)-epigallocatechin gallate with lipid membranes. Biophysical Journal 92(9):3178-3194.
Tjay T. H., Rahardja K., 2015 [Important drugs: efficacy, use and side effects]. Edisi Ke-7. PT. Elex Media Komputindo, Jakarta, 1037 pp. [in Indonesian]
Vivekanandhan S., Misra M., Mohanty A. K., 2009 Biological synthesis of silver nanoparticles using Glycine max (soybean) leaf extract: an investigation on different soybean varieties. Journal of Nanoscience and Nanotechnology 9(12):6828-6833.

Wamala S. P., Mugimba K. K., Mutoloki S., Evensen O., Mdegela R., Byarugaba D. K., Sørum H., 2018 Occurrence and antibiotic susceptibility of fish bacteria isolated from *Oreochromis niloticus* (Nile tilapia) and *Clarias gariepinus* (African catfish) in Uganda. Fisheries and Aquatic Sciences 21(6):1-10.
Wang L., Wei Y., Yuan G., Dai M., Chen, X., 2012 Molecular characterization and virulence genes of *Aeromonas hydrophila* isolated from the Chinese giant salamander (*Andrias davidianus*). Asian Herpetological Research 3(4):303-309.
Wimalasena S. H. M. P., de Silva B. C. J., Hossain S., Pathirana H. N. K. S., Heo G. J., 2017 Prevalence and characterisation of quinolone resistance genes in *Aeromonas* spp. isolated from pet turtles in South Korea. Journal of Global Antimicrobial Resistance 11:34-38.
Wu D., Kong Y., Han C., Chen J., Hu L., Jiang H., Shen X., 2008 D-Alanine:D-alanine ligase as a new target for the flavonoids quercetin and apigenin. International Journal of Antimicrobial Agents 32(5):421-426.
Xie Y., Yang W., Tang F., Chen X., Ren L., 2015 Antibacterial activities of flavonoids: structure-activity relationship and mechanism. Current Medicinal Chemistry 22(1): 132-149
Yang W., Chen X., Li Y., Guo S., Wang Z., Yu X., 2020 Advances in pharmacological activities of terpenoids. Natural Product Communications 15(3):1-13.
Yano Y., Hamano K., Tsutsui I., Aue-Umneoy D., Ban M., Satomi M., 2015 Occurrence, molecular characterization, and antimicrobial susceptibility of *Aeromonas* spp. in marine species of shrimps cultured at inland low salinity ponds. Food Microbiology 47:21-27.
Yi S. W., You M. J., Cho H. S., Lee C. S., Kwon J. K., Shin G. W., 2013 Molecular characterization of *Aeromonas* species isolated from farmed eels (*Anguilla japonica*). Veterinary Microbiology 164(1-2):195-200.
Zhang D., Moreira G. S. A., Shoemaker C., Newton J. C., Xu D. H., 2016 Detection and quantification of virulent *Aeromonas hydrophila* in channel catfish tissues following waterborne challenge. FEMS Microbiology Letters 363:1-5.

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