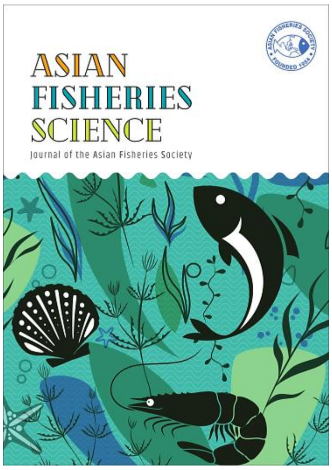

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Antibiotic Susceptibility Profiles of *Aeromonas hydrophila* Isolates From Aquaculture Farms and Response to Potential Antibacterial Plant Extracts

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Abstract

Aeromonas spp., which inhabits freshwater and marine water bodies, can be responsible for the diseases and mortalities of many different cultured fishes. In this study, the antibiotic susceptibility profiles of 20 *Aeromonas hydrophila* strains isolated from diseased freshwater fish cultured in the Red River Delta, Vietnam were categorised as non-wild-type (non-WT) strains or were resistant to at least one antibiotic. Also, *in-vitro* antibacterial activities of extracts from two local plants against several antibiotic-resistant *A. hydrophila* strains were done to screen for potential bio-antibiotic materials. The antibiotic susceptibility results showed that 25 % of bacterial strains were resistant to 3–9 antibiotics, 35 % to 2 antibiotics, and 10 % to one antibiotic. Both plants, rose myrtle seed, *Rhodomyrtus tomentosa* Hassk, 1842, extract and fermented garlic, *Allium sativum* Linnaeus, 1753, supernatant, showed inhibitory activities against antibiotic-resistant *A. hydrophila* strains. Furthermore, the *R. tomentosa* extract and the fermented *A. sativum* supernatant exhibited significant antibacterial effects to several *A. hydrophila* strains, namely *A. hydrophila* CEDMA17.021, CEDMA17.002, CEDMA17.008, and CEDMA17.009 resistant to two or more antibiotics. This study demonstrated multiple resistant profiles of *A. hydrophila* strains to different antibiotics and the inhibitory activities of *R. tomentosa* extract and the fermented *A. sativum* supernatant against antibiotic-resistant *A. hydrophila* strains. Hence, this study indicates the potential use of bio-antibiotics derived from plants to manage *Aeromonas*-related infections.

Keywords: *Allium sativum*, antibiotic resistance, plant extract, *Rhodomyrtus tomentosa*

Introduction

Disease outbreaks, especially infectious diseases, have been considered a major constraint affecting sustainable aquaculture development globally because they have annually caused the loss of at least 10 % of aquaculture's production (Adams, 2019). Amongst aquaculture bacterial pathogens, *Aeromonas* spp, including *Aeromonas hydrophila*, can be pathogenic to many freshwater fish species such as common carp (*Cyprinus carpio* Linnaeus, 1758), grass carp (*Ctenopharyngodon idella* Cuvier and Valenciennes, 1884), Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758), rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) (Pridgeon and Klesius, 2012) and different catfish species (*Pangasianodon hypophthalmus* Sauvage, 1878; *Ictalurus punctatus* Rafinesque, 1818)(Pridgeon and Klesius, 2012; Hossain et al., 2014).

The infection of farmed fish by *A. hydrophila* can be characterised as haemorrhagic septicaemia with signs of ulceration, haemorrhaging, and fin erosion (Dias et al., 2012). Antibiotics have been commonly used to combat disease outbreaks, including bacterial diseases caused by *A. hydrophila* (Haniffa and Kavitha, 2012; Hammed et al., 2015; Pham et al., 2015). Importantly, the overuse and misuse of antibiotics have been reported by farmers in freshwater aquaculture systems (Rico et al., 2013; Pham et al., 2015). In addition, antibiotics used in aquaculture are partially absorbed by fish, and the rest can remain in aquaculture systems and be present in aquaculture products, increasing antibiotic residues and antibiotic resistant bacteria (Vivekanandhan et al., 2002; Miranda et al., 2018). Multiple antibiotic resistance among *A. hydrophila* isolates are reported in many previous studies worldwide (Guz and Kozinska, 2004; Deng et al., 2016).

Many strategies have been studied and applied in aquaculture to reduce the use of antibiotics. These are applying biosecurity, vaccination and good aquaculture practices (GAPs) certification, improving farming practices and diagnostic services, developing and implementing regulations, limiting antibiotic access, developing alternative compounds for antibiotics, and others (Herriksson et al., 2018). Recently, plant extracts have been increasingly applied in aquaculture in different parts of the world to prevent and treat certain viral, parasitic, fungal and bacterial diseases, which are considered potential alternative approaches (Haniffa and Kavitha, 2012; Olusola et al, 2013; Gabriel, 2019). There is some evidence to suggest that plants can be rich resources of bioactive compounds such as tannins, alkaloids, flavonoids, phenolics, polysaccharides, and essential oils, which act against different diseases (Olusola et al., 2013; Nguyen et al., 2018; Dang et al., 2019). Plant extracts are readily available, inexpensive, and more biodegradable than antibiotics (Olusola et al., 2013; Reverter et al., 2014; Gabriel, 2019).

In a previous study, 20 *A. hydrophila* strains isolated from diseased freshwater fish cultured in the Red River Delta in Vietnam were categorised as wild type (fully susceptible, WT) or non-wild-type (non-WT) (Dang et al., 2020). The authors also reported the diameters of inhibitory zones used for categorisation of WT strains of *A. hydrophila*. The present study further analyses the percentages of these *A. hydrophila* strains categorised as non-WT to at least one antibiotic and antibiotics resistant to the bacterial strains. In addition, the study identified and screened antibacterial activities of two Vietnamese plant extracts (rose myrtle seed *Rhodomyrtus tomentosa* (Aiton) Hassk, 1842, extract and fermented garlic, *Allium sativum* Linnaeus, 1753, supernatant) against antibiotic-resistant *A. hydrophila* isolates. These plant extracts contain bioactive compounds that exhibit antibacterial effects (Lai et al., 2013; Nguyen et al., 2018; Dang et al., 2019). Such studies can better understand antibiotic resistance in Vietnamese freshwater aquaculture and help identify possible bio-antibiotic alternatives of natural plants that are safe and effective for use in aquaculture.

Materials and Methods

Bacterial isolates

A total of 20 *A. hydrophila* strains used in Dang et al. (2020) were also used in the present study for antibiotic susceptibility testing. While a total of 14 *A. hydrophila* strains (Table 1) were selected for antibacterial susceptibility testing of plant extracts. Twelve of the bacterial strains were selected from the list of 20 *A. hydrophila* strains tested for antibiotic resistance. The selected strains were isolated over 3 years (2015–2017), representing different provinces and resistance to at least two antibiotics. Two other *A. hydrophila* strains selected were CEDMA18.046 isolated from Nile tilapia (*Oreocromis niloticus* Linnaeus, 1758) in Hung Yen province in 2018 and the strain *A. hydrophila* CEDMA19.021 isolated from grass carp (*Ctenopharyngodon idella* Cuvier and Valenciennes, 1884) in Bac Ninh in 2019 (Table 1).

All 22 *A. hydrophila* strains used were isolated from freshwater fish farms with disease outbreaks in different provinces of the Red River Delta, Vietnam from 2015 to 2019, identified using API 20E and stored in glycerol at –80 °C at the Aquatic Animal Disease Laboratories of the Research Institute for Aquaculture No. 1, Vietnam.

Antibiotics

Ten antibiotics known to be widely used either legally or illegally in aquaculture in Vietnam were selected for antibiotic resistance testing. The antibiotics tested were ciprofloxacin (CIP_{5}, 5 µg), chloramphenicol (CHL_{30}, 30 µg), doxycycline (DOX_{30}, 30 µg), erythromycin (ERY_{30}, 30 µg), florfenicol (FLO_{30}, 30 µg), neomycin (NEO_{30}, 30 µg), rifampicin (RIF_{30}, 30 µg), tetracycline (TCY_{30}, 30 µg), streptomycin (STM_{10}, 10 µg) and trimethoprim/sulfamethoxazole (SXT_{25}, 1.25/23.75 µg). Ciprofloxacin and chloramphenicol are currently banned nationally for use in aquaculture (MARD, 2016).

Preparation of rose myrtle, Rhodomyrtus tomentosa *seed extract*

The extract of rose myrtle, *R. tomentosa* seed was prepared as described in Nguyen et al. (2018), and Dang et al. (2019). Briefly, the rose myrtle seeds were scrubbed and separated from the mature fruits of wild *R. tomentosa* plants harvested from the mountainsides of Phu Binh district, Thai Nguyen province, Northern Vietnam and identified to species level as described in Dang et al. (2019). The seeds were shade-drained for 60 min at room temperature, dried in an oven at 50 °C for 15 h, then ground up into a powder using a fine screen with a mesh size less than 1 mm. The *R. tomentosa* seed powder was extracted following the method optimised by Lai et al. (2014) as follows: extracting the powder using 79 % ethanol with a solid: liquid ratio of 1.20^{-1} (weight.volume^{-1}) at 85 °C for 79 min; centrifuging the extracted solution at 6,000 rpm for 10 min at 4 °C; concentrating the supernatants under reduced pressure using a rotatory evaporator at 40 °C to make the dried extract.

Preparation of fermented garlic (Allium sativum) *supernatant*

Whole dried garlic was purchased in Hai Duong province and prepared for fermentation by first

Table 1. List of *Aeromonas hydrophila* strains used for antibacterial testing of plant extracts.

No.	Bacterial strain	Host source	Year of isolation	Location (Province)
1*	*A. hydrophila* HDPT15.6	Nile tilapia (*Oreochromis niloticus* (Linnaeus, 1758))	2015	Phu Tho
2*	*A. hydrophila* CEDMA16.19	Nile tilapia (*O. niloticus*)	2016	Vinh Phuc
3*	*A. hydrophila* CEDMA16.34	Nile tilapia (*O. niloticus*)	2016	Bac Ninh
4*	*A. hydrophila* CEDMA17.002	Jewel cichlid ((*Hemichromis guttatus* Gunther, 1862)	2017	Ha Nam
5*	*A. hydrophila* CEDMA17.008	Nile tilapia (*O. niloticus*)	2017	Hoa Binh
6*	*A. hydrophila* CEDMA17.009	Nile tilapia (*O. niloticus*)	2017	Hoa Binh
7*	*A. hydrophila* CEDMA17.021	Nile tilapia (*O. niloticus*)	2017	Hai Duong
8*	*A. hydrophila* CEDMA17.044	Common carp (*Cyprinus carpio* Linnaeus, 1758)	2017	Hai Duong
9*	*A. hydrophila* CEDMA17.046	Grass carp (*Ctenopharyngodon idella* Cuvier and Valenciennes, 1884)	2017	Hai Duong
10*	*A. hydrophila* CEDMA17.047	Nile tilapia (*O. niloticus*)	2017	Hai Duong
11*	*A. hydrophila* CEDMA17.048	Grass carp (*C. idella*)	2017	Hai Duong
12*	*A. hydrophila* CEDMA17.049	Common carp (*C. carpio*)	2017	Hai Duong
13	*A. hydrophila* CEDMA18.046	Nile tilapia (*O. niloticus*)	2018	Hung Yen
14	*A. hydrophila* CEDMA19.021	Grass carp (*C. idella*)	2019	Bac Ninh

*Strains used for antibiotic susceptibility testing.

removing the skin. The cloves were then thinly sliced, mixed with rice vodka and cultured honey in the following proportions: Garlic (20 kg) + rice vodka (18 L) + cultured honey (2 L). The garlic mixture was then incubated at room temperature (23 °C–28 °C) for about 25–30 days to allow the fermentation process. Finally, after removing the garlic clove residues, the supernatant of fermented garlic was tested as a bio-antibiotic product against *A. hydrophila*.

Antibiotic susceptibility of Aeromonas hydrophila *isolates*

The disk diffusion method (Bauer et al., 1966) was performed for antibiotic susceptibility testing of 20 *A. hydrophila* strains. Briefly, suspensions of each strain of *A. hydrophila* (concentration of 10^8 cfu.mL^{-1}) were distributed over the surface of the Mueller-Hinton agar (MHA, Sigma-Aldrich, USA) plate. Antibiotic discs (Oxoid, UK) were placed in the agar plates where bacteria had been placed, and the plates were left at room temperature for 5–10 min. The plates were then inverted and incubated at 29 °C for 24 h. *Escherichia coli* ATCC 25922 strain was used as the control. Each test was repeated twice, and antibiotic susceptibility was expressed if present as the mean of inhibition diameters (mm) produced by each antibiotic.

Antibacterial activity of plant extracts

The antibacterial effects of plant extracts against 14 *A. hydrophila* strains (Table 1) were performed following the method described by Dang et al. (2019).

Preparation of *R. tomentosa* extract discs: the stock concentrations of 80, 100, 120, 140 and 160 µg.µL^{-1} of *R. tomentosa* extract were made by dissolving the dried extract in dimethyl sulfoxide (DMSO). Each diluted extract (25 µL) was then applied to a sterile paper disc (diameter = 8 mm; Advantech, Tokyo) to make extract discs with final concentrations of 2,500 µg, 3,000 µg and 3,500 µg.disc^{-1}, respectively.

Preparation of fermented garlic supernatant discs: To make discs of the fermented garlic supernatant for antibacterial activity testing, 25 µL, 30 µL and 35 µL of the supernatants were applied to a sterile paper disc (diameter = 8 mm; Advantech, Tokyo), respectively.

All extract discs, including those for *R. tomentosa* extract and the fermented garlic supernatant, were then placed onto MHA plates inoculated with *A. hydrophila* strains (10^8 cfu.mL^{-1}), followed by incubation at 29 °C for 24 h. For the control, the positive control disc contained doxycycline (30 µg), the negative control disc 25 µL DMSO and *E. coli* ATCC 25922 strain was used for quality control purposes. Each test was repeated in triplicate, and the antibacterial activity was expressed as the mean of inhibition diameters (mm) (if present) produced by each plant product.

Statistical analysis

The antibiotic susceptibility patterns of *A. hydrophila* strains were categorised as fully susceptible (wild type, WT), or manifesting reduced susceptibility (non-wild type, non-WT) using normalised resistance interpretation (NRI)-determined cut-off values (CO_{WT}) (Kronvall, 2010; Dang et al., 2020). The WT of *A. hydrophila* strains were identified following the disc zone cut-off values CO_{WT} of all the 10 tested antibiotics presented in Dang et al. (2020), which were as follows: ≥11 mm for florfenicol, ≥12 mm for erythromycin and neomycin, ≥13 mm for streptomycin, ≥14 mm for rifampicin, ≥18 mm for doxycycline, ≥19 mm for tetracycline and trimethoprim/sulfamethoxazole, ≥25 mm for chloramphenicol, and ≥34 mm for ciprofloxacin.

The susceptibilities of plant extracts to *A. hydrophila* strains were based on the measurement of diameters of the inhibitory zones induced by the diluted extracts, and the inhibitory zones were interpreted following Dang et al. (2019), with resistant or no antibacterial activity classed at (≤12 mm), intermediate antibacterial activity (13–15 mm), and susceptible or strong antibacterial activity (≥16 mm) respectively. The data were presented as mean ± SE using SPSS for Windows version 25. One-way ANOVA tested the significant differences among the means of variables at $P < 0.05$ to compare the inhibitory zones induced by different extract concentrations and by different extracts at the same concentrations.

Results

Antibiotic susceptibility patterns of Aeromonas hydrophila *strains*

By applying the disc zone cut-off values CO_{WT} of all 10 tested antibiotics to the antibiotic susceptibility data of each *A. hydrophila* strain, the antibiotic susceptibility patterns showed that 5 (25 %) strains were categorised as non-WT for 3 to 9 tested antibiotics, 35 % non-WT for 2 antibiotics, 10 % non-WT for only an antibiotic and 30 % of *A. hydrophila* strains were categorised as WT for all tested antibiotics (Table 2). The non-WT *A. hydrophila* strains that were susceptible to 2–9 antibiotics and the names of antibiotics resistant by these non-WT strains are presented in Table 3.

Table 2. Categorisation of *Aeromonas hydrophila* strains as fully susceptible (wild type, WT) or manifesting reduced susceptibility (non-wild type, non-WT) strains against tested antibiotic.

Categorisation of bacterial strain	Number of bacterial strains (n = 20)	% of bacterial strains
Non-WT for 3 to 9 tested antibiotics	5	25
Non-WT for 2 tested antibiotics	7	35
Non-WT for a tested antibiotic	2	10
WT for all tested antibiotics	6	30

Antibacterial susceptibility of Rhodomyrtus tomentosa *extract against antibiotic-resistant* Aeromonas hydrophila *isolates*

The results showed that the inhibition zones induced

Table 3. List of *Aeromonas hydrophila* strains categorised as non-wild type (non-WT) strains that were manifesting reduced susceptibility for two or more antibiotics.

Strain	Categorisation	Name of antibiotics*
A. hydrophila CED17.047	Non-WT for 9 antibiotics	CIP_5, CHL_{30}, DOX_{30}, ERY_{15}, FLO_{30}, RIF_{30}, TCY_{30}, STM_{10}, SXT_{25}
A. hydrophila CED17.048	Non-WT for 6 antibiotics	CIP_5, CHL_{30}, DOX_{30}, FLO_{30}, TCY_{30}, SXT_{25}
A. hydrophila CED17.044	Non-WT for 5 antibiotics	CIP_5, CHL_{30}, FLO_{30}, TCY_{30}, SXT_{25}
A. hydrophila CED17.020	Non-WT for 4 antibiotics	CHL_{30}, DOX_{30}, TCY_{30}, SXT_{25}
A. hydrophila CED17.021	Non-WT for 3 antibiotics	CIP_5, RIF_{30}, SXT_{25}
A. hydrophila CED16.34	Non-WT for 2 antibiotics	SXT_{25}, CIP_5
A. hydrophila CED17.046	Non-WT for 2 antibiotics	SXT_{25}, ERY_{15}
A. hydrophila CED17.049	Non-WT for 2 antibiotics	CIP_5, STM_{10}
A. hydrophila HBTT16.01	Non-WT for 2 antibiotics	CIP_5, ERY_{15}
A. hydrophila CED17.002	Non-WT for 2 antibiotics	CIP_5, STM_{10}
A. hydrophila CED17.008	Non-WT for 2 antibiotics	ERY_{15}, STM_{10}
A. hydrophila CED17.009	Non-WT for 2 antibiotics	SXT_{25}, CIP_5

*CIP-ciprofloxacin, CHL-chloramphenicol, DOX-doxycycline, ERY-erythromycin, FLO-florfenicol, NEO-neomycin, RIF-rifampicin, TCY-tetracycline, STM-streptomycin and SXT-trimethoprim/sulfamethoxazole.

by *R. tomentosa* extract against the 14 *A. hydrophila* strains ranged from 6.3 to 22.7 mm (Fig. 1). The inhibition by *R. tomentosa* extract was dose-dependent, and the inhibitory zones significantly increased with an increased concentration of extract. Among 14 *A. hydrophila* strains at the concentration of 3,500 $\mu g.disc^{-1}$, the extract showed strong antibacterial activities to 5 isolates (the inhibitory zones ≥17.3 mm); immediate antibacterial activities to 6 isolates (the inhibitory zones from 12.3 to 14.7 mm) and had no antibacterial activities to 3 isolates (the inhibitory zones ≤12 mm). Specifically, 4 out of 5 *A. hydrophila* isolates susceptible to the *R. tomentosa* extract were resistant to at least 2 antibiotics, including *A. hydrophila* CEDMA17.002; CEDMA17.008, CEDMA17.009 and CEDMA17.049 (Fig. 1; Table 3).

Antibacterial susceptibility of fermented Allium sativum *supernatant against antibiotic-resistant* Aeromonas hydrophila *isolates*

The results showed that the inhibition zones induced

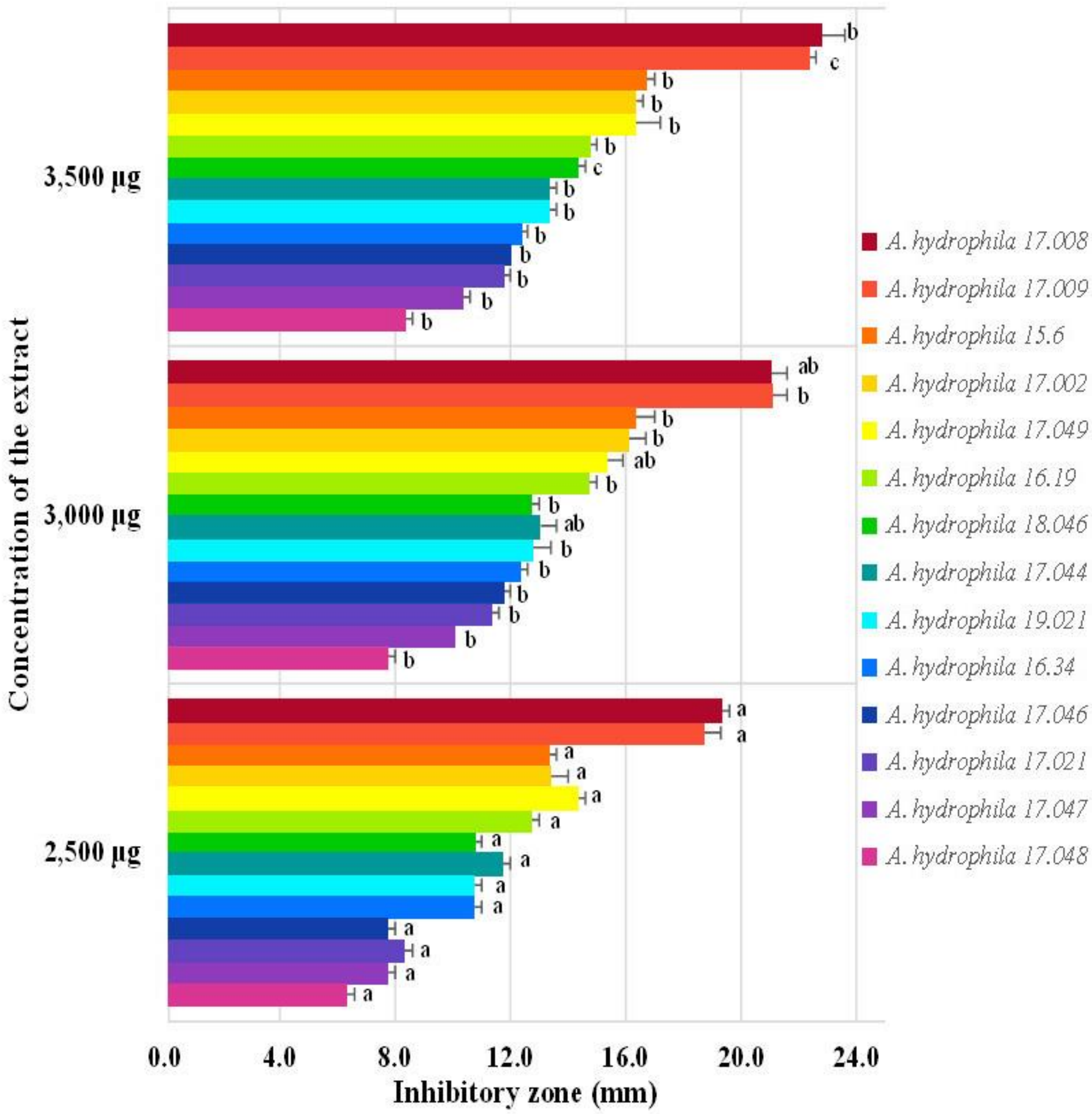

Fig. 1. Means of inhibitory zones of *Rhodomyrtus tomentosa* extract against different *Aeromonas hydrophila* strains. Each column was the average mean of three inhibitory zone diameters (corresponding to triple repeat) of the extract to each A. *hydrophila* strain. Columns with the same colour indicated the same *A. hydrophila* strain at different extract concentrations. Values with different superscripts in the same colour columns at different concentrations are significantly different ($P < 0.05$).

by the fermented garlic supernatant against *A. hydrophila* strains ranged from 8.3 to 25.0 mm (Fig. 2), indicating that the *A. hydrophila* strains were susceptible to fermented garlic supernatant.

As indicated in Figure 2, inhibition by the fermented garlic supernatant was dose-dependent, in which the induced inhibitory zones significantly increased with an increase in the applied concentration. Among 14 *A. hydrophila* strains, the supernatant had strong antibacterial activities to 3 isolates (inhibitory zones ≥21.3 mm at concentration of 35 $\mu L.disc^{-1}$); immediate antibacterial activities to 7 isolates (inhibitory zones from 12.3 to 14.7 mm at concentration of 35 $\mu L.disc^{-1}$) and had no antibacterial activities to 4 isolates (inhibitory zones ≤12 mm at concentration of 35 $\mu L.disc^{-1}$) (Fig. 2). Specifically, all 3 *A. hydrophila* isolates susceptible to the fermented garlic supernatant were also resistant to 2 and 3 antibiotics, including *A. hydrophila* CEDMA17.021; CEDMA17.008, and CEDMA17.009 (Fig. 2; Table 3).

Discussion

In many Asian countries, it has been reported that in many cases, farmers use antibiotics to treat diseased fish without any diagnostic results and also administer antibiotics as prophylaxis (Pham et al., 2015; Li et al., 2016). In addition, farmers can easily obtain antibiotics in any veterinary or even medical drugstores without a veterinary prescription in many Asian countries, including Vietnam (Pham et al., 2015). Availability and easy access to antibiotics can be the main reasons for the overuse of antibiotics in aquaculture systems. As a result, previous studies have reported antibiotic resistance to bacterial pathogens from aquaculture farming (Vivekanandhan et al, 2002; Kaskhedikar and Chhabra, 2010; Dang et

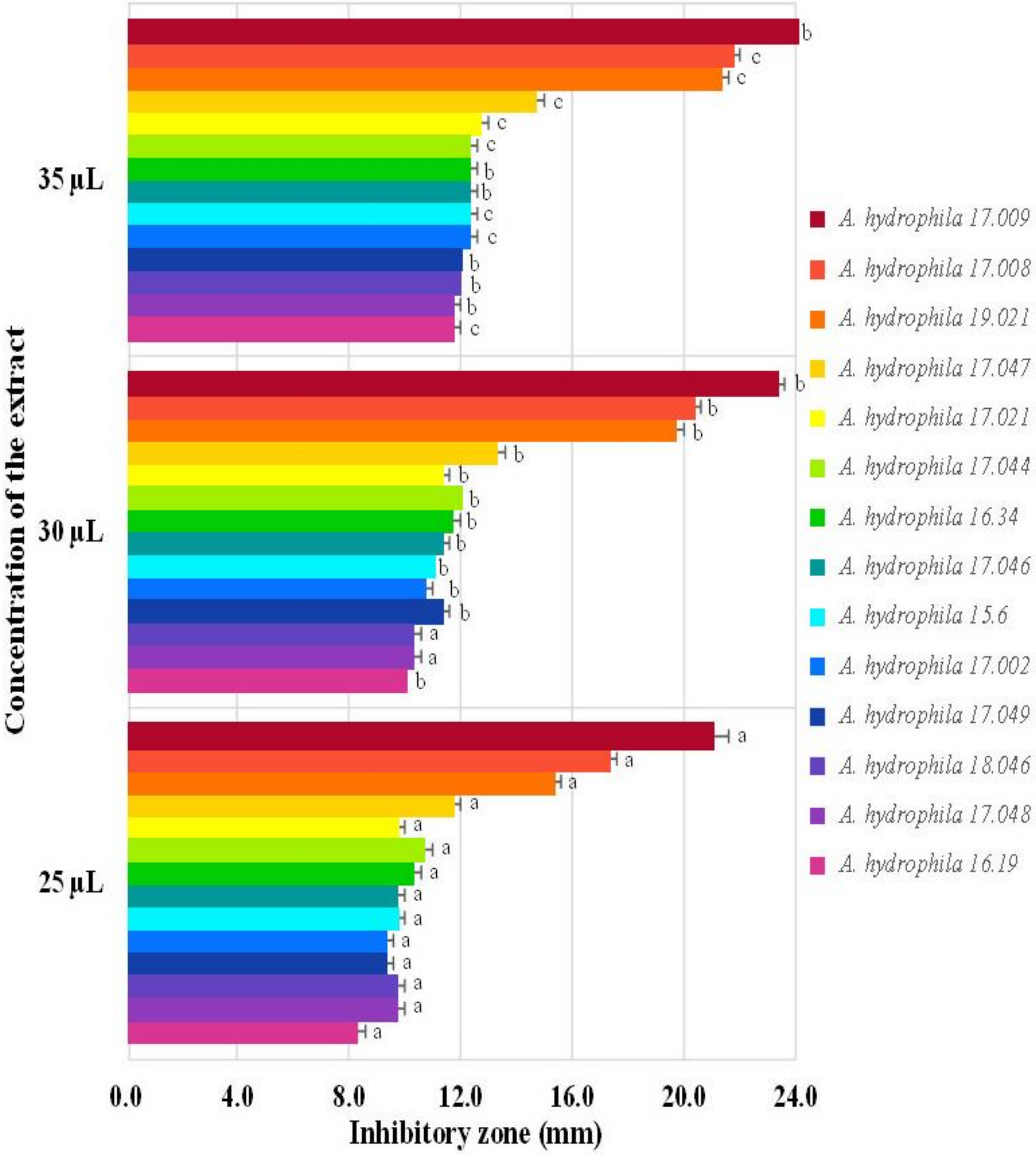

Fig. 2. Means of inhibitory zones of fermented garlic supernatant against different *Aeromonas hydrophila* strains. Each column was the average mean of three inhibitory zone diameters (corresponding for triple repeat) of the supernatant to each A. *hydrophila* strain. Columns with the same colour indicated the same *A. hydrophila* strain at different supernatant concentrations. Values with different superscripts in the same colour columns at different concentrations are significantly different ($P < 0.05$).

al., 2020) and as seen in the present study. Among 20 *A. hydrophila* strains tested in this study, 5 (25 %) *A. hydrophila* strains were categorised as non-WT for 3 to 9 tested antibiotics and 35 % non-WT for 2 antibiotics (Table 2). Dang et al. (2020) reported the highest antibiotic resistance of *A. hydrophila* to ciprofloxacin (45 %), followed by trimethoprim/sulfamethoxazole (35 %), streptomycin (25 %), tetracycline, florfenicol and chloramphenicol (20 % for each), erythromycin and doxycycline (15 % for each), and rifampicin (5 %). These findings indicated antibiotic resistance in *A. hydrophila* strains in freshwater aquaculture farming in Vietnam. Similarly, antibiotic resistance was reported for *A. hydrophila* strains isolated from different aquatic species in different aqua systems worldwide, such as cultured carp in the Czech Republic (Čížek et al., 2010), Bulgaria (Stratev et al., 2015), and China (Yang et al., 2018); cultured tilapia in Brazil (Belem-Costa and Cyrino, 2006), India (Preena et al., 2020), and Malaysia (Pauzi et al., 2020); cultured catfish in Malaysia and Nigeria (Ashiru et al., 2011; Laith and Najiah, 2014); from goldfish (*Carassius auratus* Linnaeus, 1758) (Jeeva et al., 2013), and rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) (Saavedra et al., 2004).

Multiple antibiotic resistance of *A. hydrophila* strains were found in this study, in which the highest resistance was found in the strain *A. hydrophila* CEDMA17.047 to 9 tested antibiotics, and then followed by the strain *A. hydrophila* CED17.048 (resistant to 6 antibiotics), *A. hydrophila* CED17.044 (resistant to 5 antibiotics), *A. hydrophila* CED17.020 (resistant to 4 antibiotics) and *A. hydrophila* CED17.021 (resistant to 3 antibiotics) (Table 3). These multiple antibiotic resistant *A. hydrophila* strains were isolated from diseased fish sampled in Hai Duong province in 2017 (Table 1; Dang et al., 2020). The development of multiple antibiotic resistance by *Aeromonas* spp. isolated from aquaculture systems became a significant problem many years ago (Hatha et al., 2005). Previous studies reported multi-drug resistance mediated by class I integrons to *Aeromonas* spp. isolates from freshwater fish (Deng et al., 2016). Similarly, multiple antibiotic resistance was found in *Aeromonas salmonicida* susp. *salmonicida* isolated from Atlantic Canadian aquaculture (McIntosh et al., 2008), and in many bacterial isolates such as *Escherichia coli*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Pseudomonas fluorescens*, *Pseudomonas cepacia* and *Proteus vulgaris*, in the study of Ramesh et al. (2010). The rapid increase in the number of resistant and multi-resistant *Aeromonas* spp. is due to the ability of these bacteria to transfer antibiotic resistance by mobile genetic agents (plasmids, transposons, gene cassettes, class I integrons) among bacterial populations (Patil et al., 2016; Piotrowska et al., 2017).

As illustrated in the present study, the finding of multiple antibiotic resistance amongst Vietnamese *A. hydrophila* strains poses a significant concern to the aquatic environment and aquaculture products. This calls for concerted efforts to look for alternative treatments and prevention of infectious diseases by replacing antibiotics. Plant products could replace antibiotics as they are cheaper, easily prepared and more biodegradable than synthetic antibiotics (Olusola et al., 2013; Syahidah et al., 2015; Gabriel, 2019). They can stimulate the immune system of aquatic animals and act as antibacterial, antiviral and antiparasitic agents due to their active biochemical components like alkaloids, flavonoids, pigments, phenolics, terpenoids, steroids and essential oils (Citarasu, 2010). The present study has revealed the *in vitro* antibacterial activities of the *R. tomentosa* extract and the fermented garlic supernatant tested against *A. hydrophila* strains (Figs. 1, 2). More importantly, the *R. tomentosa* extract and the fermented garlic supernatant showed strong antibacterial effects against *A. hydrophila* strains (CEDMA17.002, CEDMA17.008, CEDMA17.009, CEDMA17.049 for the *R. tomentosa* extract, and CEDMA17.021, CEDMA17.008, CEDMA17.009 for the fermented garlic supernatant, which showed antibiotic resistance to at least 2 antibiotics (Figs. 1, 2; Table 3).

Because plants are rich in a wide variety of bioactive compounds which act against different diseases through different metabolites such as immune-modulating, growth-promoting, digestive enhancing and appetite-stimulating effects; therefore, plant extracts have been recently considered as potential alternatives in place of synthetic chemicals such as antibiotics and other chemotherapeutic drugs in aquaculture (Mahdavi et al., 2013; Olusola et al., 2013; Gabriel, 2019). Antibacterial activity against antibiotic-resistant *A. hydrophila* that causes severe fish disease was also reported in other plant extracts from olive (*Oleo europaea* Linnaeus, 1753), myrtle (*Myrtis communis* Linnaeus, 1753), thyme (*Thymus vulgaris* Linnaeus, 1753), rosemary (*Rosmarinus officinalis* Linnaeus, 1753) and yarrow (*Achillea falcata* Linnaeus, 1753) (Al Laham and Al Fadel, 2014). Many plant extracts exhibited protective effects against *Aeromonas* spp., including *A. hydrophila*, infected aquatic animals in aquaculture systems (Rajendiran et al., 2008; Hammed et al., 2015; Baba et al., 2016; Nugroho et al., 2017; Palanikani et al., 2018; Nguyen et al., 2018; Dang et al., 2019).

The *R. tomentosa* extract contains bioactive compounds with antibacterial properties such as hydrolysable tannins, resveratrol, piceatannol (Lai et al., 2013; Nguyen et al., 2018). And these extracts have antibacterial effects against *Vibrio harveyi* and *V. parahaemolyticus*, which cause acute hepatopancreatic necrosis disease (AHPND) in shrimp (Nguyen et al., 2018; Dang et al., 2019). However, the present study reports for the first time *R. tomentosa* extract showing antibacterial activity against *A. hydrophila*. Similarly, garlic that contains bioactive compounds such as alliin, diallyl sulphides and allicin (Amagase and Milner, 1993) and the garlic essential oil showed a significant antibacterial effect against *A. hydrophila* in previous studies (Saleh et al., 2017). Garlic mixed in fish and shrimp pellets fed daily can protect against bacterial infection and increase feed intake (Lee and Gao, 2012; Olusola et al., 2013; Syahidah et al., 2015). In Vietnam, garlic has been used for several decades to prevent and treat bacterial diseases in freshwater farmed fish (Ha Ky et al., 1996; Do et al., 2004). In addition, it has also been reported that garlic extract could effectively control *Ichthyophthirius multifiliis* infection, the most dangerous parasite affecting freshwater fish (Bartolome et al., 2010).

Regarding the safety of these plant extracts to aquatic animals, the *R. tomentosa* extract was concluded to be safe for shrimp, as reported by Nguyen et al. (2018), and Dang et al. (2019). Garlic can safely be used by humans both internally and externally, indicating that they are likely safe for use in aquaculture. These data support that the *R. tomentosa* extract and the fermented garlic supernatant can be used as bio-antibiotic materials to prevent and treat diseases caused by *A. hydrophila* in aquaculture. Such potential and promising materials could be efficient alternatives to achieving sustainable, safer and eco-friendly fish production.

Recently, the fermented garlic supernatant used in the present study was applied in farms in Nghe An (Le et al., 2018) and Nam Dinh provinces, Vietnam, to successfully control AHPND diseases caused by *Vibrio parahaemolyticus* (Vp_{AHPND}) in shrimp. The same was also applied in Tra Vinh province, Vietnam, to successfully control ulcerative haemorrhagic disease caused by *A. hydrophila* and/or *Pseudomonas fluorescens* in snakehead (*Channa argus* Cantor, 1842) (Yen T. Pham, personal communication, 2018). These farmers use the following dosages; 7–10 mL of the supernatant.kg^{-1} of feed for 5 to 7 days and repeated every 10 days throughout the culture period. Despite these positive reports, further studies on *in-vivo* experiments of these two plant extracts against *A. hydrophila* infection must be done in fish before recommending for use in aquaculture farms.

Many applications, such as vaccination, probiotics/prebiotics, biosecurity and GAPs certification and other methods have been used in aquaculture to improve water quality and control disease outbreaks, resulting in a reduction in antibiotic use. The present study's findings of *R. tomentosa* extract effectiveness and the positive results of fermented garlic supernatant against *A. hydrophila* strains could further justify the development of natural plant-based products that can be safely used as bio-antibiotic for the control of bacterial pathogens in aquaculture.

Conclusion

Seventy per cent of *Aeromonas hydrophila* strains isolated from diseased freshwater fish cultured in the Red River Delta, Vietnam, were categorised as non-wild type (non-WT) for at least one to nine of the antibiotics. The highest resistance was to 9 antibiotics, whereas other strains showed resistance to lower numbers of antibiotics ranging from 6 to 3.

The *Rhodomyrtus tomentosa* extract and the fermented *Allium sativum* supernatant exhibited significant antibacterial effects against several *A. hydrophila* strains (CEDMA17.021, CEDMA17.002, CEDMA17.008, and CEDMA17.009) that were resistant to two or more antibiotics. This study demonstrated multiple resistant profiles of *A. hydrophila* strains to different antibiotics. In addition, the study also presented the inhibitory activities of the *R. tomentosa* extract and the fermented *A. sativum* supernatant against antibiotic-resistant *A. hydrophila* strains. Hence there is a potential for using bio-antibiotics derived from natural plant materials in the management of *Aeromonas*-related infections.

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Author contributions: Lua T. Dang: Designed experiments and prepared the draft of manuscript. Hanh T. Nguyen, Yen T. Pham, Hanh M.T. Truong: Conducted the experiments of antibiotics resistance profiles of *A. hydrophila* strains and antibacterial potentials of Vietnamese plant-extracts against antibiotic-resistant *A. hydrophila* strains. All authors contributed to the final version of manuscript.

References

Adams, A. 2019. Progress, challenges and opportunities in fish vaccine development. Fish & Shellfish Immunology 90:210–214. https://doi.org/10.1016/j.fsi.2019.04.066

Al Laham, S.A., Al Fadel, F.M. 2014. Antibacterial activity of various plants extracts against antibiotic-resistant *Aeromonas hydrophila*. Jundishapur Journal of Microbiology 7:e11370. https://dx.doi.org/10.5812/jjm.11370

Amagase, H., Milner, J.A. 1993. Impact of various sources of garlic and their constituents on 7, 12-dimethylbenz [α] anthracene binding to mammary cell DNA. Carcinogenesis 14:1627–1631. https://doi.org/10.1093/carcin/14.8.1627

Ashiru, A., Uaboi-Egbeni, P., Oguntowo, J., Idika, C. 2011. Isolation and antibiotic profile of *Aeromonas* species from tilapia fish (*Tilapia nilotica*) and catfish (*Clarias betrachus*). Pakistan Journal of Nutrition 10:982–986. https://dx.doi.org/10.3923/pjn.2011.982.986

Baba, E., Acar, U., Ontas, C., Kesbic, O.S., Yilmaz, S. 2016. The use of *Avena sativa* extract against *Aeromonas hydrophila* and its effect on growth performance, hematological and immunological parameters in common carp (*Cyprinus carpio*). Italian Journal of Animal Science 15:325–333. https://doi.org/10.1080/1828051X.2016.1185977

Bartolome, R.T., Ella, R.L.A., Garcia, A.A., Magboo, M.L.E., Papa, R.D.S. 2010. Addition of crude methanolic *Allium sativum* (garlic) extracts to commercial fish feed can potentially prevent or delay *Ichthyophthiriasis* in the black molly *Poecilia sphenops*. Acta Manilana 55:37–42.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Truck M. 1966. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology 45:493–496. https://doi.org/10.1093/ajcp/45.4_ts.493

Belem-Costa, A., Cyrino, J.E.P. 2006. Antibiotic resistance of *Aeromonas hydrophila* isolated from *Piaractus mesopotamicus* (Holmberg, 1887) and *Oreochromis niloticus* (Linnaeus, 1758). Scientia Agricola (Piracicaba, Braz.) 63:281–284. https://doi.org/10.1590/S0103-90162006000300011

Citarasu, T. 2010. Herbal biomedicines: a new opportunity for aquaculture industry. Aquaculture International 18:403–414. http://dx.doi.org/10.1007/s10499-009-9253-7

Čížek, A., Dolejská, M., Sochorová, R., Strachotová, K., Piačková, V., Veselý, T. 2010. Antimicrobial resistance and its genetic determinants in aeromonads isolated in ornamental (koi) carp (*Cyprinus carpio koi*) and common carp (*Cyprinus carpio*). Veterinary Microbiology 142:435–439. https://doi.org/10.1016/j.vetmic.2009.10.001

Dang, L.T., Nguyen, H.T., Hoang, H.H., Lai, H.N.T., Nguyen, H.T. 2019. Efficacy of rose myrtle *Rhodomyrtus tomentosa* seed extract against acute hepatopancreatic necrosis disease in Pacific whiteleg shrimp *Penaeus vannamei*. Journal of Aquatic Animal Health 31:311–319. https://doi.org/10.1002/aah.10080

Dang, L.T., Nguyen, L.H.T., Vo, C.D., Bui, V.H.T., Nguyen, L.V., and Phan, V.T. 2020. Status of Viet Nam's national action plan on antimicrobial resistance in aquaculture. Asian Fisheries Science 33.S1:112–118. https://doi.org/10.33997/j.afs.2020.33.S1.016

Deng, Y., Wu, Y., Jiang, L., Tan, A., Zhang, R., Luo, L. 2016. Multi-drug resistance mediated by class 1 integrons in *Aeromonas* isolated from farmed freshwater animals. Frontiers in Microbiology 7:935. https://dx.doi.org/10.3389%2Ffmicb.2016.00935

Dias, C., Mota, V., Martinez-Murcia, A., Saavedra, M.J. 2012. Antimicrobial resistance patterns of *Aeromonas* spp. isolated from ornamental fish. Journal of Aquaculture Research & Development 3:131. http://dx.doi.org/10.4172/2155-9546.1000131

Do, T.H., Bui, Q.T., Nguyen, H.D., Nguyen, T.M. 2004. Aquatic pathology. Agricultural Publishing House, Ho Chi Minh City. 204 pp. (in Vietnamese).

Gabriel, N.N. 2019. Review on the progress in the role of herbal extracts in tilapia culture. Cogen Food & Agriculture 5:1619651. https://doi.org/10.1080/23311932.2019.1619651

Guz, L., Kozinska, A. 2004. Antibiotic susceptibility of *Aeromonas hydrophila* and *A. sobria* isolated from farmed carp (*Cyprinus carpio* L.). Bulletin-Veterinary Institute in Pulawy 48:391–395.

Ha Ky, Bui, Q.T., Pham, T.Y., Vu, T.T., Nguyen, V.H., Doan, V.T., Le, T.H., Nguyen, H.T., Vu, V.D., Nguyen, V.T., Nguyen, T.D., Ha, T.D., Ngo, D.B., Nguyen, N.N., Do, T.H. 1996. Studies on disease preventive and treatment measures for fish and shrimp. Project report (Project No: KN-04-12, 1991-1995). Ministry of Fisheries. 150 pp. (in Vietnamese).

Hammed, A.M., Amosu, A.O., Awe, A.F., Gbadamosi, F.F. 2015. Effects of *Moringa oleifera* leaf extracts on bacteria (*Aeromonas hydrophila*) infected adults African mud catfish *Clarias gariepinus* (Burchell, 1822). International Journal of Current Research 7:22117–22122

Haniffa, M.A., Kavitha, K. 2012. Antibacterial activity of medicinal herbs against the fish pathogen *Aeromonas hydrophila*. Journal of Agricultural Technology 8:205–211.

Hatha, M., Vivekanandhan, A.A., Joice, G.J., Christol. 2005. Antibiotic resistance pattern of motile aeromonads from farm raised fresh water fish. International Journal of Food Microbiology 98:131–134. https://doi.org/10.1016/j.ijfoodmicro.2004.05.017

Henriksson, P.J.G., Rico, A., Troell, M., Klinger, D.H., Buschmann, A.H., Saksida, S., Chadag, M.V., Zhang, W.B. 2018. Unpacking factors influencing antimicrobial use in global aquaculture and their implication for management: a review from a systems perspective. Sustainability Science 13:1105–1120. https://doi.org/10.1007/s11625-017-0511-8

Hossain, M.J., Sun, D., McGarey, D.J., Wrenn, S., Alexander, L.M., Martino, M.E., Xing, Y., Terhune, J.S., Liles, M.R. 2014. An Asian origin of virulent *Aeromonas hydrophila* responsible for disease epidemics in United States-farmed catfish. mBio 5:e00848-14. https://doi.org/10.1128/mBio.00848-14

Jeeva, S., Packia Lekshmi, N.C.J., Raja Brindha, J., Vasudevan, A. 2013. Studies on antibiotic susceptibility of *Aeromonas hydrophila* isolated from gold fish (*Carassius auratus*). International Journal of Current Microbiology and Applied Sciences 2:7–13.

Kaskhedikar, M., Chhabra, D. 2010. Multiple drug resistance in *Aeromonas hydrophila* isolates of fish. Veterinary World 3:76–77.

Kronvall, G. 2010. Normalized resistance interpretation as a tool for establishing 556 epidemiological MIC susceptibility breakpoints. Journal of Clinical Microbiology 48:4445–4452. https://doi.org/10.1128/jcm.01101-10

Lai, T.N.H., Andre, C.M., Chirinos, R., Nguyen, T.B.T., Larondelle, Y., Rogez, H. 2014. Optimisation of extraction of piceatannol from *Rhodomyrtus tomentosa* seeds using response surface methodology. Separation and Purification Technology 134:139–146. https://doi.org/10.1016/j.seppur.2014.07.032

Lai, T.N.H., Herent, M.F., Quetin-Leclercq, J., Nguyen, T.B.T., Rogez, H., Larondelle, Y. 2013. Piceatannol, a potent bioactive stilbene, as major phenolic component in *Rhodomyrtus tomentosa*. Food Chemistry 138:1421–1430. https://doi.org/10.1016/j.foodchem.2012.10.125

Laith, A., Najiah, M. 2014. *Aeromonas hydrophila*: antimicrobial susceptibility and histopathology of isolates from diseased catfish, *Clarias gariepinus* (Burchell). Journal of Aquaculture Research and Development 5:215. https://doi.org/10.4172/2155-9546.1000215

Le, V.H., Nguyen, T.H., Le, H.T., Nguyen, C.T., Nguyen, C.H., Nguyen, T.H., Tran, N.A., Nguyen, T.H., Le, T.H., Pham, T.Y. 2018. Studies on solutions to control acute hepatopancreatic necrosis disease in shrimp in Nghe An province. Project report. 91 pp. (in Vietnamese).

Lee, J.Y., Gao, Y. 2012. Review of the application of garlic, *Allium sativum*, in aquaculture. Journal of the World Aquaculture Society 43:447–458. https://doi.org/10.1111/j.1749-7345.2012.00581.x

Li, K., Liu, L., Clausen, J.H., Lu, M., Dalsgaard, A. 2016. Management measures to control diseases reported by tilapia (*Oreochromis* spp.) and whiteleg shrimp (*Litopenaeus vannamei*) farmers in Guangdong, China. Aquaculture 457:91–99. https://doi.org/10.1016/j.aquaculture.2016.02.008

Mahdavi, M., Hajimoradloo, A., Ghorbani, R. 2013. Effect of *Aloe vera* extract on growth parameters of common carp (*Cyprinus carpio*). World Journal of Medical Sciences 9:55–60. http://dx.doi.org/10.5829/idosi.wjms.2013.9.1.75128

MARD (Ministry of Agriculture and Rural Development). 2016. Circular 10/2016/TT-BNNPTNT on the list of veterinary drugs permitted for circulation, banned from use in Vietnam, published codes for imported veterinary drugs allowed to circulate in Vietnam. Issued June 1, 2016. 1035 pp. (in Vietnamese).

McIntosh, D., Cunningham, M.B., Fekete, F.A., Parr, E.M., Clark, S.E., Zainger, Z.B., Danner, G.R., Johnson, K.A., Beattie, M., Ritchie, R. 2008. Transferable, multiple antibiotic and mercury resistance in Atlantic Canadian isolates of *Aeromonas salmonicida susp. salmonicida* is associated with carriage of an IncA/C plasmid similar to the *Salmonella enterica* plasmid pSN254. Journal of Antimicrobial Chemotherapy 61:1221–1228. https://doi.org/10.1093/jac/dkn123

Miranda, C.D., Godoy, F.A., Lee, M.R. 2018. Current status of the use of antibiotics and the antimicrobial resistance in the Chilean salmon farms. Frontiers in Microbiology 9:1284. https://doi.org/10.3389/fmicb.2018.01284

Nguyen, H.T., Dang, L.T., Nguyen, H.T., Hoang, H.H., Lai, N.H.T., Nguyen, T.H.T. 2018. Screening antibacterial effects of Vietnamese plant extracts against pathogens caused acute hepatopancreatic necrosis disease in shrimps. Asian Journal of Pharmaceutical and Clinical Research 11:77–83. https://doi.org/10.22159/ajpcr.2018.v11i5.23618

Nugroho, R.A., Manurung, H., Nur, F.M., Prahastika, W. 2017. *Terminalia catappa* L. extract improves survival, hematological profile and resistance to *Aeromonas hydrophila* in *Betta* sp. Archives of Polish Fisheries 25:103–115. https://doi.org/10.1515/aopf-2017-0010

Olusola, S.E., Emikpe, B.O., Olaifa, F.E. 2013. The potentials of medical plant extracts as bio-antimicrobials in aquaculture. International Journal of Medicinal and Aromatic Plants 3:404–412.

Palanikani, R., Soranam, R., Chanthini, K.M.P. 2018. Pathogenicity and control of *Aeromonas hydrophila* and *A. veronii* in Indian major carps (*Catla-catla*) by the effect of herbal supplement of *Andrographis paniculata* (*Lamiales: Acanthaceae*). International Journal of Fisheries and Aquatic Studies 6:361–370.

Patil, H.J., Benet-Perelberg, A., Naor, A., Smirnov, M., Ofek, T., Nasser, A., Minz, D., Cytryn, E. 2016. Evidence of increased antibiotic resistance in phylogenetically-diverse *Aeromonas* isolates from semi-intensive fish ponds treated with antibiotics. Frontiers in Microbiology 7:1875. https://doi.org/10.3389/fmicb.2016.01875

Pauzi, N.A., Mohamad, N., Azzam-Sayuti, M., Yasin, I.S.M., Saad, M.Z., Nasruddin, N.S., Azmai, M.N.A. 2020. Antibiotic susceptibility and pathogenicity of *Aeromonas hydrophila* isolated from red hybrid tilapia (*Oreochromis niloticus* × *Oreochromis mossambicus*) in Malaysia. Veterinary World 13:2166–2171. https://doi.org/10.14202/vetworld.2020.2166-2171

Pham, D.K., Chu, J., Do, N.T., Brose, F., Degand, G., Delahaut, P., De Pauw, E., Douny, C., Nguyen, K.V., Vu, T.D., Scippo, M.L., Wertheim, H.F.L. 2015. Monitoring antibiotic use and residue in freshwater aquaculture for domestic use in Vietnam. EcoHealth 12:480–489. http://dx.doi.org/10.1007/s10393-014-1006-z

Piotrowska, M., Przygodzińska, D., Matyjewicz, K., Popowska, M. 2017. Occurrence and variety of beta-lactamase genes among *Aeromonas* spp. isolated from urban wastewater treatment plant. Frontiers in Microbiology 8:863. https://doi.org/10.3389/fmicb.2017.00863

Preena, P., Dharmaratnam, A., Swaminathan, T.R. 2020. Antimicrobial resistance analysis of pathogenic bacteria isolated from freshwater Nile tilapia (*Oreochromis niloticus*) cultured in Kerala, India. Current Microbiology 77:3278–3287. https://doi.org/10.1007/s00284-020-02158-1

Pridgeon, J.W., Klesius, P.H. 2012. Major bacterial diseases in aquaculture and their vaccine development. CAB Reviews Perspectives in Agriculture Veterinary Science Nutrition and Natural Resources 7:1–16. http://dx.doi.org/10.1079/PAVSNNR20127048

Rajendiran, A., Natarajan, E., Subramanian, P. 2008. Control of *Aeromonas hydrophila* infection in spotted snakehead, *Channa punctatus*, by *Solanum nigrum* L., a medicinal plant. Journal of the World Aquaculture Society 39:375–383. https://doi.org/10.1111/j.1749-7345.2008.00163.x

Ramesh, S., Manivasagan, S., Ashokkumar, S., Rajaram, G., Mayavu, P. 2010. Plasmid profiling and multiple antibiotic resistance of heterotrophic bacteria isolated from Muthupettai mangrove environment, southeast coast of India. Current Research in Bacteriology 3:227–237. https://dx.doi.org/10.3923/crb.2010.227.237

Reverter, M., Bontemps, N., Lecchini, D., Banaigs, B., Sasal, P. 2014. Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives. Aquaculture 433:50–61. https://doi.org/10.1016/j.aquaculture.2014.05.048

Rico, A., Phu, T.M., Satapornvanit, K., Min, J., Shahabuddin, A.M., Henriksson, P.J.G., Murray, F.J., Little, D.C., Dalsgaard, A., van den Brink, P.J. 2013. Use of veterinary medicines, feed additives and probiotics in four major internationally traded aquaculture species farmed in Asia. Aquaculture 412–413:231–243. https://doi.org/10.1016/j.aquaculture.2013.07.028

Saavedra, M.J., Guedes-Novais, S., Alves, A., Rema, P., Tacão, M., Correia, A., Martínez-Murcia, A. 2004. Resistance to beta-lactam antibiotics in *Aeromonas hydrophila* isolated from rainbow trout (*Oncorhynchus mykiss*). International Microbiology 7:207–211. https://doi.org/10.2436/IM.V7I3.9472

Saleh, E.A., Morshdy, A.E.M.A., Mohamed, A.M., El-Sobary, B. 2017. Prevalence of *Aeromonas* species and their herbal control in fish. Global Veterinaria 18:286-293. https://doi.org/10.5829/idosi.gv.2017.286.293

Stratev, D., Daskalov, H., Vashin, I. 2015. Characterisation and determination of antimicrobial resistance of β-haemolytic *Aeromonas* spp. isolated from common carp (*Cyprinus carpio* L.). Revue Médecine Véterinaire 166:54–61.

Syahidah, A., Saad, C.R., Dauh, H.M., Abdelhadi, Y.M. 2015. Status and potential of herbal applications in aquaculture: A review. Iranian Journal of Fisheries Sciences 14:27–44. https://doi.org/10.22092/IJFS.2018.114421

Vivekanandhan, G., Savithamani, K., Hatha, A.A.M., Lakmanaperumalsamy, P. 2002. Antibiotic resistance of *Aeromonas hydrophila* isolated from marketed fish and prawn of South India. International Journal of Food Microbiology 76:165–168. https://doi.org/10.1016/s0168-1605(02)00009-0

Yang, Y., Miao, P., Li, H., Tan, S., Yu, H., Yu, H. 2018. Antibiotic susceptibility and molecular characterization of *Aeromonas hydrophila* from grass carp. Journal of Food Safety 38:e12393. https://doi.org/10.1111/jfs.12393