



# In Vitro Antibacterial Activity of Tropical Plant Extracts Against Fish Pathogens in Vietnam

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## Abstract

Crude methanol extracts of 43 Vietnamese plants were screened in vitro for their antibacterial activity against three common freshwater fish pathogens, including *Aeromonas hydrophila*, *Edwardsiella ictaluri* and *Streptococcus agalactiae*. The agar disc diffusion method was used to evaluate the antibacterial activity, followed by minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined using the broth dilution method. Among the 43 plants screened, eight plant extracts (*Bouea oppositifolia*, *Wedelia chinensis*, *Terminalia catappa*, *Punica granatum*, *Sonneratia caseolaris*, *Sonneratia ovata*, *Ludwigia hyssopifolia* and *Phyllanthus urinaria*) exhibited wide-spectrum antibacterial activity to all three common freshwater fish pathogens. Six plant extracts (*Ageratum conyzoides*, *Alpinia galanga*, *Borassus flabellifer*, *Abutilon indicum*, *Eupatorium odotatum*, and *Scoparia dulcis*) might be good candidates for the prevention of co-infection of *E. ictaluri* and *S. agalactiae* in tilapia whereas *Muntingia calabura* and *Camellia sinensis* could be applied to striped catfish to combat *E. ictaluri* and *A. hydrophila*. Through MIC and MBC determination, *L. hyssopifolia*, *A. galanga*, *Ageratum conyzoides* extracts showed a bactericidal activity to *A. hydrophila*, *E. ictaluri* and *S. agalactiae*, respectively, while the other extracts could prevent the growth of tested bacteria. The screening results suggested the potential application of plant extracts as alternative therapeutic agents against bacterial infections in aquaculture.

**Keywords:** *Aeromonas hydrophila*, antibacterial activity, *Edwardsiella ictaluri*, plant extracts, *Streptococcus agalactiae*

## Introduction

Bacterial diseases continue to severely constrain the sustainable aquaculture industry due to its high mortality level and heavy economic losses (Toranzo et al., 2005; Pridgeon and Klesius, 2012). In Vietnam, reports of losses resulting from bacteria in striped catfish (*Pangasianodon hypophthalmus* (Sauvage, 1878)), mainly by *Edwardsiella ictaluri* and *Aeromonas hydrophila*, ranged from 30 % to 60 % (Crumlish et al., 2002, 2010; Phan et al., 2009). Besides, outbreaks of *Streptococcus agalactiae* infection in tilapia (*Oreochromis* spp.) have been reported in many Asian countries leading to a 90 % mortality rate (Mian et al., 2009; Abuseliana et al., 2010; Ha et al., 2011; Li et al., 2014). Control and treatment of bacterial infections commonly rely on the use of chemical agents,

particularly antibiotics in aquaculture ponds (Rico et al., 2013; Pham et al., 2015). However, the improper use of antibiotics is the main reason for the emergence and selection of antibiotic resistant-bacteria (Tu et al., 2008; Quach et al., 2014; Sun et al., 2020). The resistant bacteria or their genes can be easily transferred to humans via food consumption, direct contact, or the environment (Evans et al., 2009; Sreedharan et al., 2012). Thus, developing reliable alternative therapies against bacterial pathogens is crucial for improving quality and quantity in aquaculture production.

Currently, attention has been drawn to plant extracts as a promising alternative to antibiotics due to their easy availability, relatively low cost and antipathogenic properties (Reverter et al., 2014;

Stratev et al., 2018). Researchers from various fields have investigated local plants with a new view of their antibacterial usefulness in reducing and replacing antibiotic and chemical usage in aquaculture (Razak et al., 2019). With the tropical climate zone, the Mekong Delta in Vietnam has a rich diversity of medicinal plants used as food, feed and traditional medicine (Britta et al., 2016). Furthermore, the Mekong Delta is the main production site of freshwater fish such as striped catfish, tilapia or red tilapia (*Oreochromis* spp.). Therefore, the present study aims to investigate the *in vitro* antibacterial activity of Vietnamese plant extracts, against three common pathogenic bacteria, including *Aeromonas hydrophila*, *Edwardsiella ictaluri*, and *Streptococcus agalactiae* that pose the most significant threat to cultured freshwater fish in Vietnam.

## Materials and Methods

### Preparation of plant extracts

Fresh plants were identified (Supplementary Table 1) and collected from regions including Hau Giang, An Giang, Vinh Long, Dong Thap, Ben Tra, Tra Vinh, Long An provinces and Can Tho city in Mekong Delta, Vietnam. The voucher specimens were deposited in the College of Natural Science, Can Tho University, Vietnam. All collected plants were washed with tap water and oven-dried at 45 °C for 7 days. During the oven-drying process, plants were mixed twice a day. After drying, they were blended into powder form using an electric blender. Then 100 g of plant powder were macerated in 1 L methanol (Chemsol, Vietnam) 5 times, each 24 h at room temperature (28 °C). The extracts were filtered through Whatman filter paper with 11 µm pore size (Advantec, USA) and evaporated in a rotary evaporator with rotation speed of 50 rpm. The crude extracts were dissolved in 100 % DMSO (VWR Prolabo, France) to obtain a final concentration of 400 mg.mL<sup>-1</sup>.

### Maintenance and preparation of bacterial inocula

Three isolates of fish pathogenic bacteria, including *A. hydrophila*, *E. ictaluri* and *S. agalactiae* were isolated from diseased farm cultured striped catfish (*A. hydrophila* and *E. ictaluri*) and red tilapia (*S. agalactiae*), identified by PCR technique (Panangala et al., 2007; Rodkhum et al., 2012) then stored in glycerol stocks at -80 °C until use. These isolates were recovered on tryptone soya agar (Himedia, India) plates and incubated at 28 °C for 16–48 h. A colony was picked for re-confirmation by Gram staining. Then several colonies were suspended and well-mixed in sterilised sodium chloride solution (0.85 % NaCl). Afterwards, each bacterial inoculum was adjusted to a concentration of 10<sup>8</sup> CFU.mL<sup>-1</sup> (Abs<sub>610</sub> = 0.08) using a spectrophotometer (S-220, Boeco, Germany). The bacterial inoculum was directly used for the antibacterial activity test, except for *A. hydrophila* inoculum, which was diluted to 10<sup>6</sup> CFU.mL<sup>-1</sup>.

### Preparation of paper discs

Paper discs (8 mm, Advantec, Japan) were placed in Petri dishes and impregnated with crude extract (50 µL). Then the paper discs were air-dried in a sterilised flow cabinet for 30 min. Based on the antibiogram of tested bacteria (Supplementary Table 2), the selected positive controls were doxycycline 30 µg (Abtek, UK), florfenicol 30 µg (Abtek, UK) and ampicillin 10 µg (BD BBL, USA) for *A. hydrophila*, *E. ictaluri* and *S. agalactiae*, respectively. DMSO was used as a negative control.

### Antibacterial activity test

The agar disc diffusion method was used to screen the antibacterial activity of the plant extracts (Oonmetta-aree et al., 2006). The bacterial inoculum was spread on Mueller Hinton agar (MHA; Himedia, India) and kept for about 15 min in a flow cabinet to allow the surface of the agar plate to dry. The prepared paper discs were placed onto MHA plates inoculated with the respective bacterium, followed by incubation at 28 °C for 16–48 h. The antibacterial activity of each plant extract was determined by measuring the diameter of the inhibitory zone forming around the paper disc. All of the experiments were performed in triplicate, and the inhibitory zone of each plant extract was calculated as mean ± standard deviation (SD).

### Determination of minimum inhibitory concentration (MIC)

The plant extracts which showed strong inhibition were selected to determine the MIC using the broth dilution method (Oonmetta-aree et al., 2006). Briefly, a series of concentrations of plant extract was prepared by two-fold serial dilution, ranging from 50 mg.mL<sup>-1</sup> to 0.024 mg.mL<sup>-1</sup>. In this method, paper discs were impregnated with 63 µL of plant extracts and air-dried in a sterile flow cabinet. The impregnated paper discs were then immersed into 1 mL nutrient broth (Difco, USA) which contained 10<sup>6</sup> CFU.mL<sup>-1</sup> of *A. hydrophila* or 10<sup>7</sup> CFU.mL<sup>-1</sup> of *E. ictaluri*. In the case of *S. agalactiae*, the impregnated paper disc was immersed into 1 mL Luria broth containing 10<sup>7</sup> CFU.mL<sup>-1</sup> of bacteria. Tubes were inoculated with broth medium in the absence of bacteria (blank control sample), bacteria with medium (positive control sample), and bacteria with respective antibiotics (negative control samples). The test tubes were incubated at 28 °C for 16–48 h with gentle shaking. The MIC of the plant extracts was considered the lowest plant extract concentration that inhibited the visible growth of bacteria. The MIC experiment was performed in triplicate.

### Determination of minimum bactericidal concentration (MBC)

The tube which showed no turbidity of the bacteria

and the last tube that showed the turbidity from the MIC test was used for further MBC testing. The MBC was the lowest plant extract concentration that killed 99.9% of tested bacteria. The determination of MBC was performed using the method of Oonmetta-aree et al. (2006). One-hundred microliter of culture medium used in the MIC test were spotted on TSA plate (HiMedia, India), incubated at 28 °C for 16–48 h and counted in the grown colonies. The concentration of plant extracts that produced less than 20 colonies was considered as MBC value.

## Results

### Antibacterial activity of plant extracts against *A. hydrophila*, *E. ictaluri* and *S. agalactiae*

In this study, we screened the antibacterial activities of 43 crude methanol extracts from 27 different botanical families (Supplementary Table 1) against 3 aquatic pathogens, including two Gram-negative (*A. hydrophila* and *E. ictaluri*) and one Gram-positive bacteria (*S. agalactiae*). The screening results using the disc diffusion method are shown in Table 1. There was no inhibition zone of negative controls (DMSO), while the positive controls showed strong antibacterial activities, including doxycycline against *A. hydrophila* ( $20.0 \pm 0.58$  mm), florfenicol against *E. ictaluri* ( $48.7 \pm 2.08$  mm), and ampicillin against *S. agalactiae* ( $31.7 \pm 0.83$  mm).

According to Abd-El-Aziz and Sallam (2021), the antibacterial activity can be classified as strong (>20 mm), moderate (16–19 mm), mild (12–15 mm) and less than 12 mm is considered inactive. Most of the plant extracts showed inhibitory activity against at least one tested bacterium, with the diameter of inhibitory zones ranging from 8.4 to 47 mm. The strongest antibacterial activity against *A. hydrophila*, *E. ictaluri*, and *S. agalactiae* was demonstrated by *B. oppositifolia*, *S. caseolaris*, and *Alpinia galanga* with the mean of an inhibitory zone as  $31.0 \pm 1.00$  mm,  $46.0 \pm 4.10$  mm and  $47.0 \pm 1.00$  mm, respectively.

Among tested extracts, *Bouea oppositifolia*, *Wedelia chinensis*, *Terminalia catappa*, *Punica granatum*, *Sonneratia caseolaris*, *Sonneratia ovata*, *Ludwigia hyssopifolia* and *Phyllanthus urinaria*, were found to be of broadest antibacterial spectrum, which showed moderate to strong activity against all of the tested bacteria with the zone of inhibition from 17 to 46 mm. Although, *Alpinia galanga*, *Borassus flabellifer*, *Abutilon indicum* showed mild antibacterial activities against *A. hydrophila*, they did show wide-spectrum antibacterial effect on all tested bacteria. Similarly, *Muntingia calabura* and *Camellia sinensis* extracts were considered to have wide-spectrum antibacterial activities since they mildly inhibited *S. agalactiae* but strongly inhibited *E. ictaluri* and *A. hydrophila*. Moreover, *Ageratum conyzoides*, *Eupatorium*

*odotatum*, and *Scoparia dulcis* strongly or moderately inhibited both *E. ictaluri* and *S. agalactiae*. On the other hand, seven plant extracts were inactive to all the tested bacteria, including *Achranthes aspera*, *Areca catechu*, *Tridax procumbens* L., *Vernonia amygdalina*, *Carica papaya*, *Sechium edule*, and *Paederia scandens*.

Out of three tested bacteria, *S. agalactiae* was the most susceptible bacterium, which was sensitive to 32/43 plant extracts. In contrast, the most resistant bacterium was *A. hydrophila* which was unsusceptible to 28/43 screened plant extracts. Besides the plant extracts that exhibited wide-spectrum antibacterial activity mentioned above, six more plant extracts including *Allium fistulosum*, *Elephantopus scaber*, *Vernonia cinerea*, *Elsholtzia cristata*, *Limonia acidissima* and *Murraya koenigii* showed moderate antibacterial activity. Among these *E. scaber* and *M. koenigii* exhibited specific activity towards *S. agalactiae* ( $17.8 \pm 0.83$  mm). Similarly, the growth of *E. ictaluri* was moderately inhibited by *Couroupita guianensis*, *Artemisia vulgaris*, *Kalanchoe pinnata*, *Perilla frutescens*, *Phyllanthus reticulatus* and *Couroupita guianensis* extracts with the inhibition zone from 16 to 20.4 mm. *Artemisia vulgaris* ( $17.4 \pm 1.94$  mm) and *O. corniculata* ( $15.4 \pm 0.58$  mm) extracts revealed the specific activity towards *E. ictaluri*. In the case of *A. hydrophila*, *B. flabellifer*, *K. pinnata*, and *A. galanga* extracts displayed mild inhibitory effects while the rest were inactive.

### MIC and MBC

The crude methanol extracts with strong activity were chosen for MIC and MBC determination. Table 2 shows that the MIC values of the effective extracts against bacterial pathogens ranged from 0.025 to 6.25 mg.mL<sup>-1</sup> and the MBC values were higher than MIC values and ranged from 0.39 to >25 mg.mL<sup>-1</sup>. The MIC and MBC values of plant extracts slightly agreed with their inhibitory zones.

The result showed that the lowest MIC value against *A. hydrophila* was from *L. hyssopifolia* extract (MIC = 0.1 mg.mL<sup>-1</sup>), which could inhibit *E. ictaluri* at the same concentration, lower than *S. agalactiae* (MIC = 0.78 mg.mL<sup>-1</sup>). Moreover, the MBC value of *L. hyssopifolia* extract against *A. hydrophila* was 0.39 mg.mL<sup>-1</sup>, much lower than its value against *E. ictaluri* (6.25 mg.mL<sup>-1</sup>). Similarly, *P. urinaria* extract provided the same MIC and MBC value against *E. ictaluri* (0.1 mg.mL<sup>-1</sup> and 6.25 mg.mL<sup>-1</sup>, respectively). However, its MBC value for *A. hydrophila* (1.56 mg.mL<sup>-1</sup>) was four times lower than the MBC value of *L. hyssopifolia* extract. *Bouea oppositifolia* extract had the same MIC value of *L. hyssopifolia* against *S. agalactiae* (0.78 mg.mL<sup>-1</sup>) and its MBC value was determined as 3.13 mg.mL<sup>-1</sup>, whereas MBC value of *L. hyssopifolia* could not be determined. The lowest MIC value against *E. ictaluri* was recorded for *T. catappa* and *M. calabura* (0.025 mg.mL<sup>-1</sup>) and their MBC was also the same value

Table 1. Antibacterial activity of crude plant extracts against freshwater fish pathogenic bacteria.

No.	Plant extracts	Family	Part used	Mean diameter of inhibition zone (mm ± SD)		
				<i>Aeromonas hydrophila</i>	<i>Edwardsiella ictaluri</i>	<i>Streptococcus agalactiae</i>
1	<i>Abutilon indicum</i>	Malvaceae	Leaf	12.3 ± 2.31	40.6 ± 1.15	18.0 ± 0.00
2	<i>Achranthes aspera</i>	Amaranthaceae	Aerial parts	-	-	11.8 ± 0.83
3	<i>Ageratum conyzoides</i>	Asteraceae	Aerial parts	8.4 ± 0.41	22.6 ± 2.70	20.0 ± 3.46
4	<i>Allium fistulosum</i>	Amaryllidaceae	Whole plant	-	13.3 ± 1.15	17.3 ± 2.89
5	<i>Alpinia galanga</i>	Zingiberaceae	Rhizome	12.7 ± 0.58	40.7 ± 2.40	47.0 ± 1.00
6	<i>Andrographis paniculata</i>	Acanthaceae	Aerial parts	-	-	12.8 ± 0.84
7	<i>Areca catechu</i>	Arecaceae	Fruit	-	-	10.4 ± 0.54
8	<i>Artemisia vulgaris</i>	Asteraceae	Aerial parts	8.6 ± 0.55	17.4 ± 1.94	8.13 ± 0.25
9	<i>Azadirachta indica</i>	Meliaceae	Leaf	-	15.2 ± 1.31	12.6 ± 1.81
10	<i>Bouea oppositifolia</i>	Anacardiaceae	Leaf	31.0 ± 1.00	39.7 ± 0.58	21.7 ± 0.57
11	<i>Borassus flabellifer</i>	Arecaceae	Fruit peel	15.3 ± 2.31	38.7 ± 2.31	17.3 ± 1.53
12	<i>Camellia sinensis</i>	Theaceae	Leaf	17.7 ± 0.58	22.6 ± 0.89	13.4 ± 1.52
13	<i>Carica papaya</i>	Caricaceae	Leaf	-	-	-
14	<i>Couroupita guianensis</i>	Lecythidaceae	Fruit	11.2 ± 0.83	20.4 ± 2.89	14.4 ± 0.55
15	<i>Dracaena cambodiana</i>	Asparagaceae	Bark	10.0 ± 1.15	12.2 ± 0.45	9.6 ± 1.52
16	<i>Eclipta prostrata</i>	Asteraceae	Aerial parts	-	11.6 ± 0.55	13.0 ± 0.82
17	<i>Elephantopus scaber</i>	Asteraceae	Aerial parts	-	11.3 ± 0.58	16.3 ± 1.15
18	<i>Elsholtzia cristata</i>	Lamiaceae	Aerial parts	-	14.8 ± 0.83	16.0 ± 2.65
19	<i>Eupatorium odoratum</i>	Asteraceae	Aerial parts	8.9 ± 0.74	16.8 ± 2.31	17.3 ± 2.08
20	<i>Houttuynia cordata</i>	Saururaceae	Aerial parts	8.7 ± 0.44	11.0 ± 1.87	12.8 ± 1.48
21	<i>Kalanchoe pinnata</i>	Crassulaceae	Leaf	14.0 ± 2.65	19.7 ± 0.58	14.3 ± 1.15
22	<i>Limonia acidissima</i>	Rutaceae	Fruit peel	-	13.7 ± 0.58	17.3 ± 1.15
23	<i>Ludwigia hyssopifolia</i>	Onagraceae	Aerial parts	21.3 ± 1.53	29.3 ± 0.58	20.0 ± 0.00
24	<i>Moringa oleifera</i>	Moringa	Aerial parts	-	9.6 ± 2.31	12.6 ± 1.14
25	<i>Muntingia calabura</i>	Muntingiaceae	Leaf	17.3 ± 1.15	28.4 ± 1.82	15.0 ± 1.73
26	<i>Murraya koenigii</i>	Rutaceae	Leaf	-	-	17.8 ± 0.83
27	<i>Oxalis corniculata</i>	Oxalidaceae	Aerial parts	-	15.4 ± 0.58	8.3 ± 0.29
28	<i>Paederia lanuginosa</i>	Rubiaceae	Aerial parts	-	-	14.3 ± 1.53
29	<i>Paederia scandens</i>	Rubiaceae	Aerial parts	9.4 ± 0.89	8.8 ± 1.79	10.4 ± 1.14
30	<i>Perilla frutescens</i>	Lamiaceae	Leaf	9.2 ± 0.45	16.4 ± 0.89	13.0 ± 0.82
31	<i>Phyllanthus reticulatus</i>	Phyllanthaceae	Aerial parts	-	16.0 ± 0.00	13.0 ± 1.58
32	<i>Phyllanthus urinaria</i>	Phyllanthaceae	Aerial parts	22.0 ± 1.00	30.0 ± 2.35	19.7 ± 0.58
33	<i>Psidium guajava</i>	Myrtaceae	Leaf	9.8 ± 1.15	12.8 ± 2.17	11.3 ± 0.96
34	<i>Punica granatum</i>	Lythraceae	Leaf	18.0 ± 1.00	26.6 ± 3.21	22.0 ± 1.00
35	<i>Scoparia dulcis</i>	Plantaginaceae	Aerial parts	-	16.6 ± 1.14	16.6 ± 1.67
36	<i>Sechium edule</i>	Cucurbitaceae	Leaf	-	-	10.8 ± 0.83
37	<i>Sonneratia caseolaris</i>	Lythraceae	Fruit	19.2 ± 0.45	46.0 ± 4.10	25.3 ± 0.76
38	<i>Sonneratia ovata</i>	Lythraceae	Leaf	18.3 ± 1.48	36.6 ± 1.82	29.9 ± 0.65
39	<i>Terminalia catappa</i>	Combretaceae	Leaf	20.3 ± 1.51	29.4 ± 2.61	18.67 ± 1.15
40	<i>Tridax procumbens</i>	Asteraceae	Aerial parts	-	9.8 ± 1.79	-
41	<i>Vernonia amygdalina</i>	Asteraceae	Leaf	-	-	-
42	<i>Vernonia cinerea</i>	Asteraceae	Aerial parts	-	12.6 ± 0.55	16.6 ± 1.52
43	<i>Wedelia chinensis</i>	Asteraceae	Aerial parts	17.0 ± 2.89	22.7 ± 0.58	18.7 ± 0.58
Antibiotics						
	Doxycycline (30 µg)			20.0 ± 0.58	NT	NT
	Florfenicol (30 µg)			NT	40.7 ± 2.08	NT
	Ampicillin (10 µg)			NT	NT	31.7 ± 0.83
Negative control						
	DMSO			-	-	-

Data are mean ± SD, n = 3; NT: not tested; - : no inhibitory zone.

Strong: ≥20 mm; Moderate: 16-19 mm; Mild: 12-15 mm; Inactive: ≤12 mm.

Table 2. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of effective plant extracts against freshwater fish pathogenic bacteria.

Bacteria	No.	Plant extracts	MIC (mg.mL <sup>-1</sup> )	MBC (mg.mL <sup>-1</sup> )	MBC/MIC ratio
<i>Aeromonas hydrophila</i>	1	<i>Bouea oppositifolia</i>	0.19	0.78	>4
	2	<i>Phyllanthus urinaria</i>	0.10	1.56	>4
	3	<i>Ludwigia. hyssopifolia</i>	0.10	0.39	4
	4	<i>Terminalia catappa</i>	0.39	12.5	>4
<i>Edwardsiella ictaluri</i>	1	<i>Alpinia galanga</i>	0.39	1.56	4
	2	<i>Abutilon indicum</i>	NT	NT	NT
	3	<i>Bouea oppositifolia</i>	0.19	3.13	>4
	4	<i>Borassus flabellifer</i>	1.56	25	>4
	5	<i>Phyllanthus urinaria</i>	0.10	6.25	>4
	6	<i>Ludwigia hyssopifolia</i>	0.10	6.25	>4
	7	<i>Terminalia catappa</i>	0.025	3.13	>4
	8	<i>Muntingia calabura</i>	0.025	3.13	>4
	9	<i>Punica granatum</i>	0.05	6.25	>4
	10	<i>Wedelia chinensis</i>	1.56	ND	ND
	11	<i>Ageratum conyzoides</i>	0.78	3.13	>4
	12	<i>Camellia sinensis</i>	0.39	3.13	>4
	13	<i>Couroupita guianensis</i>	0.39	6.25	>4
<i>Streptococcus agalactiae</i>	1	<i>Alpinia galanga</i>	1.56	12.5	>4
	2	<i>Punica granatum</i>	1.56	ND	ND
	3	<i>Bouea oppositifolia</i>	0.78	3.13	>4
	4	<i>Ageratum conyzoides</i>	6.25	12.5	2
	5	<i>Ludwigia hyssopifolia</i>	0.78	ND	ND

ND: not determined; NT: not tested.

(3.13 mg.mL<sup>-1</sup>). Furthermore, the MIC and MBC values of *P. granatum* (0.05 and 6.25 mg.mL<sup>-1</sup>) were two times higher than the values of *T. catappa* and *M. calabura* against *E. ictaluri*. Although *B. flabellifer* and *W. chinensis* gave the same MIC value as 1.56 mg.mL<sup>-1</sup>, MBC value of *B. flabellifer* was determined at 25 mg.mL<sup>-1</sup> while MBC of *W. chinensis* could not be determined. Based on the MBC/MIC ratio, an antibacterial extract is classified into 2 groups: bactericidal if MBC/MIC ratio  $\leq 4$  or bacteriostatic if the ration was  $>4$  (Canillac and Mourey, 2001). Following this classification, the *L. hyssopifolia*, *A. galanga*, *A. conyzoides* extracts were considered bactericidal to *A. hydrophila*, *E. ictaluri* and *S. agalactiae*, respectively. The rest of the tested extracts were classified as bacteriostatic.

## Discussion

The antibacterial activities of crude methanol extracts derived from 43 plants against common aquatic pathogens in the Mekong Delta were investigated for the potential application in aquaculture to control bacterial diseases. The study showed eight extracts, including *B. oppositifolia*, *W. chinensis*, *T. catappa*, *P. granatum*, *S. caseolaris*, *S. ovata*, *L. hyssopifolia* and *P. urinaria* exhibited strong and wide-spectrum of inhibitory effects on both Gram-negative (*A. hydrophila*, *E. ictaluri*,) and Gram-

positive bacteria (*S. agalactiae*). These extracts may be good candidates for the prevention of concurrent infection of bacterial pathogens in freshwater fish, particularly striped catfish and tilapia. This finding was in agreement with the work of Huynh (2010) on the antibacterial activity of *T. catappa* extracts against both *A. hydrophila* and *E. ictaluri* with the inhibitory zones of 18 and 19 mm, respectively, or *A. hydrophila* and *S. agalactiae* (Caruso et al., 2017).

The extract of *L. hyssopifolia* in methanol expressed similar antibacterial activity against *A. hydrophila* and *E. ictaluri* as in found in the current study (Huynh and Le, 2011). Furthermore, the *A. conyzoides*, *A. galanga*, *B. flabellifer*, *A. indicum*, *E. odotatum*, and *S. dulcis* extracts were found to have strong antibacterial efficacies against *E. ictaluri* and *S. agalactiae*, which are serious problems for tilapia, whereas *M. calabura* and *C. sinensis* extracts can be useful for prevention of co-infection in striped catfish. In addition, the antibacterial activity of *C. sinensis* against *A. hydrophila* was in line with the finding of Akbary (2014), who reported the moderate antibacterial activity of *C. sinensis* against *A. hydrophila* and another Gram-positive bacterium, *Lactococcus graviae*.

Despite the fact that antibacterial activities of *T. catappa*, *P. urinaria*, *M. calabura*, *P. granatum* and *S. caseolaris* extracts against *A. hydrophila* have been

reported (Chitmanat et al., 2005; Zakaria et al., 2006; Britto et al., 2011; Pai et al., 2011; Laith et al., 2012), there is limited information about the wide-spectrum antibacterial activities of the extracts against *E. ictaluri* and *S. agalactiae* used in the current study. The present investigation contrasts Dao et al. (2020) who reported that the ethanol extracts of *W. chinensis* were inactive against *E. ictaluri*. Moreover, *A. galanga*, *A. conyzoides*, *A. indica* and *W. chinensis* showed no inhibition against *A. hydrophila*, which was in agreement with the observation of previous studies (Soma et al., 2014; Hardi et al., 2016; Nguyen et al., 2020; Techaoei, 2022).

In the current study, *A. indicum* expressed a mild antibacterial activity against *A. hydrophila*, which supported the finding of Soma et al. (2014). The results of the present study substantiated the finding of Nair and Hatha (2017), who demonstrated the inactivity of *M. oleifera*, *P. guajava*, *M. koenigii* and *A. indicum* extracts against *A. hydrophila*. Although *A. aspera*, *A. catechu*, *T. procumbens*, *V. amygdalina*, *C. papaya*, *S. edule*, *P. scandens* did not show any antibacterial activity against all tested bacteria, their antibacterial activity against other Gram-negative and positive bacteria was previously documented. *Areca catechu* was shown to be active against *Helicobacter pylori* (Wang and Huang, 2005), *V. amygdalina* against *Streptococcus mutans* and *Staphylococcus aureus* (Anibijuwon et al., 2012), *S. edule* against *Escherichia coli*, *Salmonella typhimurium*, and *Shigella flexneri* (Sibi et al., 2013), *A. aspera* against *E. coli*, *Klebsiella pneumoniae*, *Bacillus subtilis* and *S. aureus* (Ndhlala et al., 2015) and *T. procumbens* against *E. coli*, *S. aureus*, *B. subtilis* and *Proteus mirabilis* (Andriana et al., 2019). The current result differed from the data reported by Korachi et al. (2003), who concluded that *S. edule* gave strong antibacterial activity against *S. agalactiae*. *Carica papaya* methanol extracts revealed a moderate inhibitory effect against *A. hydrophila*, in contrast to the previous result. Variations in the inhibitory effect of plant extracts against tested bacteria might be because of the differences in the plant part used, the age of plants and the local environmental conditions that affected the potency of plants. Furthermore, the extraction method and solvent used could affect the amount of extracted bioactive compounds (Eloff, 1998; Azwanida, 2015).

According to previous antibacterial assay for screening purposes, the plant extracts were generally more effective for Gram-positive than Gram-negative bacteria (Dahiya and Purkayastha, 2012), due to the cell wall structure complexity in Gram-negative bacteria (Silhavy et al., 2010). In our result, the only tested Gram-positive bacterium, *S. agalactiae* was the most sensitive, which was in line with the previous reports. However, Castro et al. (2008) found that *S. agalactiae* was the most resistant bacteria when 46 methanol plant extracts were screened against three fish pathogenic bacteria, including *A. hydrophila*, *F. columnare* and *S. agalactiae*. Only five methanol plant

extracts, including *Calyptanthes clusiifolia*, *Croton floribundus*, *Heisteria silvianii*, *Merremia tomentosa*, *Zanthoxylum riedelianum* exhibited the inhibitory effect on *S. agalactiae* (Castro et al., 2008). In addition, Türker et al. (2009) found *A. hydrophila* to be more sensitive than Gram-positive bacteria, *S. agalactiae*, *Enterococcus faecalis*, and *Lactococcus garvieae* when screening 24 alcoholic and aqueous extracts from 8 Turkish plants.

The data obtained through MIC exhibited the variability in the inhibitory concentration of effective plant extracts for selected bacteria. Previous reports documented a higher MIC value of *T. catappa* methanol and aqueous extracts against *A. hydrophila* at 2 mg.mL<sup>-1</sup> and 0.5 mg.mL<sup>-1</sup>, respectively, in which the MIC value of methanol extract was 5 times higher than the result in our study (0.39 mg.mL<sup>-1</sup>) (Chitmanat et al., 2005; Fakoya et al., 2019). Even though limited information is available for the antibacterial potential of effective plant extracts against three tested bacteria, *A. galanga* methanol extract gave MIC values against other bacteria such as *B. subtilis*, *E. faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *E. aerogene*, *E. cloacae*, *K. pneumoniae*, *P. aeruginosa*, *S. typhimurium* ranging from 0.04 to 0.64 mg.mL<sup>-1</sup> (Rao et al., 2010), which was more similar to the MIC values in the current study.

According to Desrini et al. (2019) the MIC value of *M. calabura* methanol extract against *S. epidermis*, *S. aureus*, and *E. coli* were 0.5, 1, and 2 mg.mL<sup>-1</sup>, respectively and the MBC values were 1, 2, and 4 mg.mL<sup>-1</sup>, respectively. In another report, the methanol extract of *M. calabura* inhibited *S. aureus* and a multi-drug resistant *S. aureus* at the same MIC value of 1250 µg.mL<sup>-1</sup> (Zakaria et al., 2010). Ethanol extract of pomegranate (*P. granatum*) peels inhibited the growth of *Streptococcus mutant* at MIC of 100 mg.mL<sup>-1</sup>, which was much higher than the MIC value of *P. granatum* against *S. agalactiae* in the current study (Abd-El-Aziz and Sallam, 2021). The MIC value of crude extract of green tea *C. sinensis* was found to be at 125 µg.mL<sup>-1</sup> in the case of *E. coli*, *S. aureus*, and MBC at 500 µg.mL<sup>-1</sup> against *S. aureus*, while no MBC value was found against *E. coli* (Khasru et al., 2019). The differences in sensitivity of the bacterial strains and the purity of the plant extracts could be explained in this case. The result obtained in this study exposed some inconsistencies between the antibacterial activity of crude plant extracts performed by using the agar disc diffusion method or by using the broth dilution method. This variation might be related to the different diffusion abilities of the plant extracts in the solid medium, which can affect the diameter of the inhibitory zones (Valgas et al., 2007).

There were limited studies on the antibacterial activity of various medicinal plants against fish pathogens in this study. For example, the ethanol extracts of *Caesalpinia sappan* and *Alpinia galanga* showed strong activity against *A. hydrophila* and

*Streptomyces* sp., reported from Thailand (Techaoei, 2022). *Vibrio harveyi*, *Vibrio alginolyticus*, *Vibrio parahaemolyticus* and *A. hydrophila* were moderately inhibited by some methanol plant extracts of *Pimenta dioica*, *Premna foetida* and *Polygonum chinense*, and aqueous extracts of *P. dioica* and *Syzygium polyanthum* from Malaysia (Razak et al., 2019). Among 12 Mediterranean medicinal-aromatic plants, Greek oregano, savoury and Spanish oregano displayed strong antibacterial activity against *Aeromonas veronii*, *Vibrio anguillarum*, *Vibrio harveyi*, *Vibrio alginolyticus*, *Edwardsiella anguillarum* (Anastasiou et al., 2020). To our knowledge, out of 43 plant extracts examined in the current study, this is the first report of antibacterial activity of *C. guianensis*, *D. cambodiana*, *E. scarber*, *E. cristata*, *L. acidissima*, *O. corniculata*, *P. lanuginosa*, *P. scandens*, *P. reticulatus*, *S. dulcis* and *V. cinerea* against *A. hydrophila*, *E. ictaluri* and *S. agalactiae*.

## Conclusion

The results showed that eight plant extracts *Bouea oppositifolia*, *Wedelia chinensis*, *Terminalia catappa*, *Punica granatum*, *Sonneratia caseolaris*, *Sonneratia ovata*, *Ludwigia hyssopifolia* and *Phyllanthus urinaria* exhibited strong and wide-spectrum antibacterial activity against freshwater fish pathogen, the *Aeromonas hydrophila*, *Edwardsiella ictaluri* and *Streptococcus agalactiae* strains tested. Additionally, a new promising approach to prevent co-infections in tilapia could be from six plant extracts comprising of *Ageratum conyzoides*, *Alpinia galanga*, *Borassus flabellifer*, *Abutilon indicum*, *Eupatorium odoratum*, and *Scoparia dulcis* with the wide-spectrum antibacterial activities to both *E. ictaluri* and *S. agalactiae*. Similarly, two others, *Muntingia calabura* and *Camellia sinensis* extracts were the best candidates to control co-infections in striped catfish due to their wide-spectrum antibacterial activities against both *A. hydrophila* and *E. ictaluri*. However, further safety, stability, and toxicity investigations are necessary to confirm the *in vivo* effectiveness of these plant extracts against tested bacterial pathogens.

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**Author contributions:** Tran Thi My Duyen: Study conception and design, experimental investigation and data analysis, reviewed the results, draft manuscript preparation. Nguyen Trong Tuan: Experimental investigation and data analysis, reviewed the results, draft manuscript preparation. Tran Thi Tuyet Hoa: Study conception and design,

reviewed the results, draft manuscript preparation. All authors approved the final version of the manuscript.

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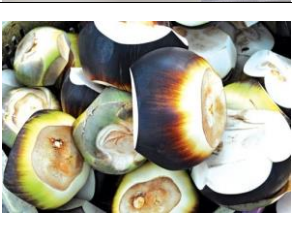

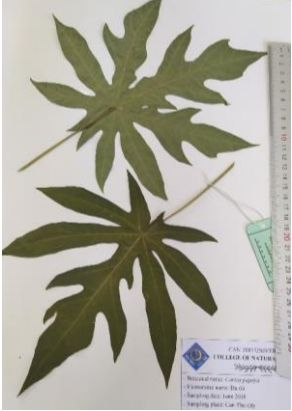








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Supplementary Table 1. Details of the voucher specimens collected from various regions in Vietnam used for antimicrobial activity against freshwater fish pathogens, namely *Aeromonas hydrophila*, *Edwardsiella ictaluri* and *Streptococcus agalactiae*.






No.	Pictures	Information
1.		<ul style="list-style-type: none"> <li>- Botanical name: <i>Abutilon indicum</i> #(Pham, 1999)</li> <li>- Vietnamese name: Cối xay</li> <li>- Collected in: June 2018</li> <li>- Place collected: Dong Thap province -10° 29' 37.676" N 105° 41' 17.444" E</li> <li>- Parts used: Leaf</li> <li>- Code number: Abu062018-ĐT014</li> </ul>
2		<ul style="list-style-type: none"> <li>- Botanical name: <i>Achyranthes aspera</i> #(Pham, 1999)</li> <li>- Vietnamese name: Cỏ xước</li> <li>- Collected in: August 2018</li> <li>- Place collected: An Giang province -10° 31' 17.702" N 105° 8' 33.226" E</li> <li>- Parts used: Aerial parts</li> <li>- Code number: Rad082018-AG(CM)030</li> </ul>
3		<ul style="list-style-type: none"> <li>- Botanical name: <i>Ageratum conyzoides</i> #(Pham, 1999)</li> <li>- Vietnamese name: Hoa ngũ sắc</li> <li>- Collected in: April 2018</li> <li>- Place collected: Can Tho city -10° 2' 42.5832" N 105° 44' 48.6744" E</li> <li>- Parts used: Aerial parts</li> <li>- Code number: Age042018-CT004</li> </ul>
4		<ul style="list-style-type: none"> <li>- Botanical name: <i>Allium fistulosum</i> #(Pham, 1999)</li> <li>- Vietnamese name: Hành hoa</li> <li>- Collected in: June 2018</li> <li>- Place collected: Can Tho city -10° 2' 42.5832" N 105° 44' 48.6744" E</li> <li>- Parts used: Whole plant</li> <li>- Code number: All062018-CT(NK)012</li> </ul>






5		<ul style="list-style-type: none"> <li>- Botanical name: <i>Alpinia galanga</i></li> <li>-(Pham et al., 2000)</li> <li>- Vietnamese name: <b>Riềng nếp</b></li> <li>- Collected in: June 2019</li> <li>- Place collected: Hau Giang province -9° 45' 28.433" N 105° 38' 28.511" E</li> <li>- Parts used: Rhizome</li> <li>- Code number: Alp062019-HG(PH)047</li> </ul>
6		<ul style="list-style-type: none"> <li>- Botanical name: <i>Andrographis paniculata</i></li> <li>-(Pham, 1999)</li> <li>- Vietnamese name: <b>Xuyên tâm liên</b></li> <li>- Collected in: August 2018</li> <li>- Place collected: An Giang province -10° 31' 17.702" N 105° 7' 33.226" E</li> <li>- Parts used: Aerial parts</li> <li>- Code number: And082018-AG(CM)024</li> </ul>
7		<ul style="list-style-type: none"> <li>- Botanical name: <i>Areca catechu</i></li> <li>-(Pham, 1999)</li> <li>- Vietnamese name: <b>Cau</b></li> <li>- Collected in: August 2018</li> <li>- Place collected: Can Tho city -10° 2' 42.5832" N 105° 44' 48.6744" E</li> <li>- Parts used: Fruit</li> <li>- Code number: Are082018-CT(NK)039</li> </ul>
8		<ul style="list-style-type: none"> <li>- Botanical name: <i>Artemisia vulgaris</i></li> <li>-(Pham, 1999)</li> <li>- Vietnamese name: <b>Ngải cứu</b></li> <li>- Collected in: June 2018</li> <li>- Place collected: Can Tho province -10° 2' 42.5832" N 105° 44' 48.6744" E</li> <li>- Parts used: Aerial parts</li> <li>- Code number: Art062018-CT022</li> </ul>
9		<ul style="list-style-type: none"> <li>- Botanical name: <i>Azadirachta indica</i></li> <li>-(Pham, 1999)</li> <li>- Vietnamese name: <b>Sầu đầu</b></li> <li>- Collected in: August 2018</li> <li>- Place collected: An Giang province -10° 31' 17.702" N 105° 7' 33.226" E</li> <li>- Parts used: Leaf</li> <li>- Code number: Aza082018-AG(TT)035</li> </ul>

10		<ul style="list-style-type: none"> <li>- Botanical name: <i>Bouea oppositifolia</i> #(Pham, 1999; Harsono et al., 2018)</li> <li>- Vietnamese name: Thanh trà</li> <li>- Collected in: May 2019</li> <li>- Place collected: Vinh Long province -10° 5' 10.061" N 106° 1' 1.189" E</li> <li>- Parts used: Leaf</li> <li>- Code number: Bou052019-VL(BM)046</li> </ul>
11		<ul style="list-style-type: none"> <li>- Botanical name: <i>Borassus flabellifer</i> #(Pham, 1999)</li> <li>- Vietnamese name: Thốt nốt</li> <li>- Collected in: August 2018</li> <li>- Place collected: An Giang province -10° 31' 17.702" N 105° 7' 33.226" E</li> <li>- Parts used: Fruit peel</li> <li>- Code number: Bor082018-AG(TT)037</li> </ul>
12		<ul style="list-style-type: none"> <li>- Botanical name: <i>Camellia sinensis</i> #(Pham, 1999)</li> <li>- Vietnamese name: Trà xanh</li> <li>- Collected in: March 2018</li> <li>- Place collected: Can Tho city -10° 2' 42.5832" N 105° 44' 48.6744" E</li> <li>- Parts used: Leaf</li> <li>- Code number: Cam032018-CT003</li> </ul>
13		<ul style="list-style-type: none"> <li>- Botanical name: <i>Carica papaya</i> #(Pham, 1999)</li> <li>- Vietnamese name: Đu đủ</li> <li>- Collected in: June 2018</li> <li>- Place collected: Can Tho city -10° 2' 42.5832" N 105° 44' 48.6744" E</li> <li>- Parts used: Leaf</li> <li>- Code number: Car062018-CT019</li> </ul>
14		<ul style="list-style-type: none"> <li>- Botanical name: <i>Couroupita guianensis</i> #(Pham, 1999; NCBI)</li> <li>- Vietnamese name: Dầu lôn</li> <li>- Collected in: April 2018</li> <li>- Place collected: Can Tho city -10° 2' 42.5832" N 105° 44' 48.6744" E</li> <li>- Parts used: Fruit</li> <li>- Code number: Cou042018-CT(NK)006</li> </ul>






15		<ul style="list-style-type: none"> <li>- Botanical name: <i>Dracaena cambodiana</i> #(Pham, 1999)</li> <li>- Vietnamese name: Huyết giác</li> <li>- Collected in: June 2018</li> <li>- Place collected: Hoa Binh province</li> <li>- Parts used: Bark</li> <li>- Code number: Dra062018-HB010</li> </ul>
16		<ul style="list-style-type: none"> <li>- Botanical name: <i>Elipta prostrata</i> #(Pham, 1999)</li> <li>- Vietnamese name: Cỏ mực</li> <li>- Collected in: August 2018</li> <li>- Place collected: An Giang province -10° 31' 17.702" N 105° 7' 33.226" E</li> <li>- Parts used: Aerial parts</li> <li>- Code number: Eli082018-AG(CM)023</li> </ul>
17		<ul style="list-style-type: none"> <li>- Botanical name: <i>Elephantopus scarber</i> #(Pham, 1999)</li> <li>- Vietnamese name: Chi thiên</li> <li>- Collected in: August 2018</li> <li>- Place collected: Da Lat city -11° 56' 57.872" N 108° 28' 43.828" E</li> <li>- Parts used: Aerial parts</li> <li>- Code number: Ele082018-LD(DL)040</li> </ul>
18		<ul style="list-style-type: none"> <li>- Botanical name: <i>Elsholtzia cristata</i> #(Pham, 1999; Pham et al., 2000)</li> <li>- Vietnamese name: Kinh giới</li> <li>- Collected in: April 2018</li> <li>- Place collected: Long An province -10° 41' 44.059" N 106° 14' 35.236" E</li> <li>- Parts used: Aerial parts</li> <li>- Code number: Els042018-LA(MH)007</li> </ul>
19		<ul style="list-style-type: none"> <li>- Botanical name: <i>Eupatorium odoratum</i> #(Pham, 1999; Do et al., 2018)</li> <li>- Vietnamese name: Cỏ lào</li> <li>- Collected in: April 2018</li> <li>- Place collected: Can Tho city -10° 2' 42.5832" N 105° 44' 48.6744" E</li> <li>- Parts used: Aerial parts</li> <li>- Code number: Eup042018-CT005</li> </ul>





20		<ul style="list-style-type: none"> <li>- Botanical name: <i>Houttuynia cordata</i> #(Pham, 1999)</li> <li>- Vietnamese name: Diếp cá</li> <li>- Collected in: June 2018</li> <li>- Place collected: Can Tho city -10° 2' 42.5832" N 105° 44' 48.6744" E</li> <li>- Parts used: Aerial parts</li> <li>- Code number: Hou062018-CT021</li> </ul>
21		<ul style="list-style-type: none"> <li>- Botanical name: <i>Kalanchoe pinnata</i> #(Pham, 1999)</li> <li>- Vietnamese name: Sóng đờ</li> <li>- Collected in: June 2018</li> <li>- Place collected: Hau Giang province -9° 45' 28.433" N 105° 38' 28.511" E</li> <li>- Parts used: Leaf</li> <li>- Code number: Kal062018-HG015</li> </ul>
22		<ul style="list-style-type: none"> <li>- Botanical name: <i>Limonia acidissima</i> #(Pham, 1999)</li> <li>- Vietnamese name: Quách</li> <li>- Collected in: May 2018</li> <li>- Place collected: Tra Vinh province -10° 5' 10.061" N 106° 1' 1.189" E</li> <li>- Parts used: Fruit peel</li> <li>- Code number: Lim052018-TV(DH)009</li> </ul>
23		<ul style="list-style-type: none"> <li>- Botanical name: <i>Ludwigia hyssopifolia</i> #(Pham, 1999)</li> <li>- Vietnamese name: Rau mương</li> <li>- Collected in: June 2018</li> <li>- Place collected: Hau Giang province -9° 48' 45.8676" N 106° 17' 57.4476" E</li> <li>- Parts used: Aerial parts</li> <li>- Code number: Lud062018-HG016</li> </ul>
24		<ul style="list-style-type: none"> <li>- Botanical name: <i>Moringa oleifera</i> #(Pham, 1999)</li> <li>- Vietnamese name: Chùm ngây thân đỏ</li> <li>- Collected in: August 2018</li> <li>- Place collected: An Giang province -10° 31' 17.702" N 105° 7' 33.226" E</li> <li>- Parts used: Aerial parts</li> <li>- Code number: Mor082018-AG(TT)032</li> </ul>

25.		<ul style="list-style-type: none"> <li>- Botanical name: <i>Muntingia calabura</i> #(Pham, 1999)</li> <li>- Vietnamese name: Trúng cá</li> <li>- Collected in: August 2018</li> <li>- Place collected: Can Tho city -10° 2' 42.5832" N 105° 44' 48.6744" E</li> <li>- Parts used: Leaf</li> <li>- Code number: Mun082018-CT(NK)038</li> </ul>
26		<ul style="list-style-type: none"> <li>- Botanical name: <i>Murraya koenigii</i> #(Pham, 1999)</li> <li>- Vietnamese name: Cà ri</li> <li>- Collected in: August 2018</li> <li>- Place collected: An Giang province -10° 31' 17.702" N 105° 7' 33.226" E</li> <li>- Parts used: Leaf</li> <li>- Code number: Mur082018-AG(TT)031</li> </ul>
27.		<ul style="list-style-type: none"> <li>- Botanical name: <i>Oxalis corniculata</i> #(Pham, 1999)</li> <li>- Vietnamese name: Me đất</li> <li>- Collected in: August 2018</li> <li>- Place collected: An Giang province -10° 31' 17.702" N 105° 7' 33.226" E</li> <li>- Parts used: Aerial parts</li> <li>- Code number: Oxa082018-AG(CM)026</li> </ul>
28		<ul style="list-style-type: none"> <li>- Botanical name: <i>Paederia lanuginosa</i> #(Pham, 1999)</li> <li>- Vietnamese name: Mỡ lông</li> <li>- Collected in: May 2018</li> <li>- Place collected: Can Tho city -10° 2' 42.5832" N 105° 44' 48.6744" E</li> <li>- Parts used: Aerial parts</li> <li>- Code number: Pae052018-CT008</li> </ul>
29		<ul style="list-style-type: none"> <li>- Botanical name: <i>Paederia scandens</i> #(Pham, 1999)</li> <li>- Vietnamese name: Mỡ leo</li> <li>- Collected in: June 2018</li> <li>- Place collected: Can Tho city -10° 2' 42.5832" N 105° 44' 48.6744" E</li> <li>- Parts used: Aerial parts</li> <li>- Code number: Pae062018-CT020</li> </ul>

30		<ul style="list-style-type: none"> <li>- Botanical name: <i>Perilla frutescens</i> #(Pham, 1999)</li> <li>- Vietnamese name: Tía tô</li> <li>- Collected in: June 2018</li> <li>- Place collected: Can Tho city -10° 2' 42.5832" N 105° 44' 48.6744" E</li> <li>- Parts used: Leaf</li> <li>- Code number: Per062018-CT018</li> </ul>
31		<ul style="list-style-type: none"> <li>- Botanical name: <i>Phyllanthus reticulatus</i> #(Pham, 1999)</li> <li>- Vietnamese name: Phèn đen</li> <li>- Collected in: August 2018</li> <li>- Place collected: An Giang province -10° 31' 17.702" N 105° 7' 33.226" E</li> <li>- Parts used: Aerial parts</li> <li>- Code number: Phy082018-AG(CM)029</li> </ul>
32		<ul style="list-style-type: none"> <li>- Botanical name: <i>Phyllanthus urinaria</i> #(Pham, 1999)</li> <li>- Vietnamese name: Diệp hạ châu thân đỏ</li> <li>- Collected in: August 2019</li> <li>- Place collected: Hau Giang province -9° 45' 28.433" N 105° 38' 28.511" E</li> <li>- Parts used: Aerial parts</li> <li>- Code number: Phy082019-HG(CT)052</li> </ul>
33		<ul style="list-style-type: none"> <li>- Botanical name: <i>Psidium guajava</i> L. #(Pham, 1999)</li> <li>- Vietnamese name: Ổi</li> <li>- Collected in: March 2018</li> <li>- Place collected: Vinh Long province -10° 5' 10.061" N 106° 1' 1.189" E</li> <li>- Parts used: Leaf</li> <li>- Code number: Psi032018-VL002</li> </ul>
34		<ul style="list-style-type: none"> <li>- Botanical name: <i>Punica granatum</i> #(Pham, 1999)</li> <li>- Vietnamese name: Lựu</li> <li>- Collected in: August 2019</li> <li>- Place collected: Can Tho city -10° 2' 42.5832" N 105° 44' 48.6744" E</li> <li>- Parts used: Leaf</li> <li>- Code number: Pun082019-CT(NK)051</li> </ul>



35		<ul style="list-style-type: none"> <li>- Botanical name: <i>Scoparia dulcis</i> #(Pham, 1999)</li> <li>- Vietnamese name: Cam thảo đất</li> <li>- Collected in: June 2018</li> <li>- Place collected: Can Tho city -10° 2' 42.5832" N 105° 44' 48.6744" E</li> <li>- Parts used: Aerial parts</li> <li>- Code number: Sco062018-CT(HP)011</li> </ul>
36.		<ul style="list-style-type: none"> <li>- Botanical name: <i>Sechium edule</i> #(Pham, 1999)</li> <li>- Vietnamese name: Su su</li> <li>- Collected in: August 2018</li> <li>- Place collected: An Giang province -10° 31' 17.702" N 105° 7' 33.226" E</li> <li>- Parts used: Leaf</li> <li>- Code number: Sec082018-AG(TT)034</li> </ul>
37		<ul style="list-style-type: none"> <li>- Botanical name: <i>Sonneratia caseolaris</i> #(Pham, 1999)</li> <li>- Vietnamese name: Bần chua</li> <li>- Collected in: May 2019</li> <li>- Place collected: Ben Tre province -10° 6' 29.358" N 106° 26' 26.113" E</li> <li>- Parts used: Fruit</li> <li>- Code number: Son052019-BT(BD)044</li> </ul>
38		<ul style="list-style-type: none"> <li>- Botanical name: <i>Sonneratia Ovata</i> Backer #(Pham, 1999; Vo, 2004)</li> <li>- Vietnamese name: Bần ổi</li> <li>- Collected in: May 2019</li> <li>- Place collected: Ben Tre province -10° 6' 29.358" N 106° 26' 26.113" E</li> <li>- Parts used: Leaf</li> <li>- Code number: Son052019-BT(TP)045</li> </ul>
39		<ul style="list-style-type: none"> <li>- Botanical name: <i>Terminalia catappa</i> #(Pham, 1999)</li> <li>- Vietnamese name: Bàng</li> <li>- Collected in: August 2018</li> <li>- Place collected: Can Tho city -10° 2' 42.5832" N 105° 44' 48.6744" E</li> <li>- Parts used: Leaf</li> <li>- Code number: Ter082019-CT(NK)050</li> </ul>

40		<ul style="list-style-type: none"> <li>- Botanical name: <i>Tridax procumbens</i> #(Pham, 1999)</li> <li>- Vietnamese name: <b>Cỏ mui</b></li> <li>- Collected in: August 2018</li> <li>- Place collected: An Giang province -10° 31' 17.702" N 105° 7' 33.226" E</li> <li>- Parts used: Aerial parts</li> <li>- Code number: Tri082018-AG(CM)025</li> </ul>
41		<ul style="list-style-type: none"> <li>- Botanical name: <i>Vernonia amygdalina</i> Del #(Audu et al., 2012)</li> <li>- Vietnamese name: <b>Mật gấu</b></li> <li>- Collected in: August 2018</li> <li>- Place collected: An Giang province -10° 31' 17.702" N 105° 7' 33.226" E</li> <li>- Parts used: Leaf</li> <li>- Code number: Ver082018-AG(CM)028</li> </ul>
42		<ul style="list-style-type: none"> <li>- Botanical name: <i>Vernonia cinerea</i> #(Pham, 1999)</li> <li>- Vietnamese name: <b>Bạch đầu ông</b></li> <li>- Collected in: August 2018</li> <li>- Place collected: An Giang province -10° 31' 17.702" N 105° 7' 33.226" E</li> <li>- Parts used: Aerial parts</li> <li>- Code number: Ver082018-AG(CM)027</li> </ul>
43		<ul style="list-style-type: none"> <li>- Botanical name: <i>Wedelia chinensis</i> #(Pham, 1999)</li> <li>- Vietnamese name: <b>Cúc sài đất</b></li> <li>- Collected in: June 2018</li> <li>- Place collected: Vinh Long province -10° 5' 10.061" N 106° 1' 1.189" E</li> <li>- Parts used: Aerial parts</li> <li>- Code number: Wed062018-VL017</li> </ul>

#The plants were identified based on the guide published by author(s) name that appears in parenthesis after the botanical name.

Supplementary Table 2. Antibiogram of commonly used antibiotics tested against freshwater fish pathogens, namely *Aeromonas hydrophila*, *Edwardsiella ictaluri* and *Streptococcus agalactiae*.

Bacteria	No.	Plant extracts/ Antibiotics	Mean diameter of inhibitory zone (mm ± SD)
<i>Aeromonas hydrophila</i>	1	Erythromycin	20.3 ± 0.58
	2	Gentamycin	21.0 ± 1.00
	3	Ciprofloxacin	28.3 ± 1.15
	4	Cefotaxime	37.0 ± 1.00
	5	Doxycycline	20.5 ± 0.58
<i>Edwardsiella ictaluri</i>	1	Florfenicol	45.3 ± 2.08
	2	Amoxicillin	21.0 ± 1.15
	3	Cefotaxime	29.0 ± 1.53
	4	Levofloxacin	37.0 ± 2.08
	5	Doxycycline	17.0 ± 0.58
<i>Streptococcus agalactiae</i>	1	Florfenicol	18.3 ± 0.58
	2	Cefotaxime	37.3 ± 1.15
	3	Ampicillin	31.3 ± 1.15
	4	Kanamycin	10.7 ± 1.15
	5	Ceftazidime	30.7 ± 1.53