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The Vulnerary Potential of Malaysian Traditional Vegetables as Antibacterial Agents of Fish Pathogens: A Preliminary Study

Rashidah Abdul Razak[1,2]*, Mohd Firdaus Nawi[1,2], Nur Izzati Farhanah Mohd Nasir[1], Nor Farhana Ayuni Abidin[1] and Nur Ajierah Jamaludin[1]

[1]Department of Marine Science, Kulliyyah of Science, International Islamic University Malaysia, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia
[2]Institute of Oceanography and Maritime Studies, Kulliyyah of Science, International Islamic University Malaysia, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia

ABSTRACT

Controlling antibiotic use in aquaculture demands the development of more sustainable alternative treatments for bacterial diseases. Therefore, the present study aims to evaluate the *in vitro* antibacterial effects of ethanolic extracts derived from ten popular and commonly consumed Malaysian traditional vegetables against *Aeromonas hydrophila*, *Aeromonas jandaei*, *Aeromonas sobria*, and *Edwardsiella tarda*. Various parts of plants were assessed for their inhibitory activity using disc diffusion, minimum inhibitory concentration, and minimum bactericidal concentration (MBC) methods. The *Persicaria odorata* and *Garcinia atroviridis* extracts extracted using the maceration method showed a wide range of inhibitory effects, but others showed less activity. *Aeromonas hydrophila* was the most susceptible bacterial strain, with all plant extracts suppressing its growth, while *A. sobria* is the most resistant strain. The minimum inhibition concentration (MIC) value ranged from 0.39 to 100 mg/ml, and all tested bacteria's MBC/MIC ratio was demonstrated to be bactericidal (MBC/MIC ratio <4). The findings of this study reveal the potential of *P. odorata* and *G. atroviridis* extracts as natural antibacterial agents that could be a safer and more effective alternative treatment in controlling bacterial infections in freshwater fish.

Keywords: Antibacterial activity, fish pathogenic bacteria, minimum bactericidal concentration, minimum inhibition concentration, traditional vegetable

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E-mail addresses:
rashidahrazak@iium.edu.my (Rashidah Abdul Razak)
firdausn@iium.edu.my (Mohd Firdaus Nawi)
izzatihana18@gmail.com (Nur Izzati Farhanah Mohd Nasir)
farhanaayuni08@gmail.com (Nor Farhana Ayuni Abidin)
ajierah98@gmail.com (Nur Ajierah Jamaludin)
* Corresponding author

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INTRODUCTION

The evolution and spread of antibiotic resistance in fish pathogens pose substantial difficulties to the aquaculture sector and public health. The indiscriminate use of antibiotics in aquaculture can result in the growth of antibiotic-resistant bacteria and an excessive buildup of antibiotic residues in aquaculture products, especially fish (Okon et al., 2022). The use of antibiotics in aquaculture is not strictly controlled in many developing countries. Even in cases where rules are in place, strict adherence to these regulations is often absent. Fish producers in many countries unintentionally administer antibiotics without accurately determining the underlying causes of fish infection (Rahman et al., 2022). These practices jeopardise antibiotic effectiveness in treating bacterial infections in animals and humans and raise worries about the possible transmission of antibiotic-resistant bacteria from aquaculture products to consumers (Pepi & Focardi, 2021).

As a result, different strategies for controlling fish infections in aquaculture are urgently needed. Natural compounds such as polyphenols, alkaloids, terpenoids, and saponins (Silva & Fernandes Júnior, 2010) obtained from herbs or plants, which have been utilised for centuries in traditional medicine for their medicinal value, are one potential option. Malaysian traditional vegetables, with their diverse phytochemical profiles and abundant diversity (Ghasemzadeh et al., 2018; Maran et al., 2022; Mohd Noor et al., 2020), have the potential to provide natural antibacterial properties against fish infections. Moreover, it has been proven that plant extracts are less expensive, more readily available, simple to administer, and safer than commercial antibiotics (Vaou et al., 2021). Most importantly, they are effective, safer and have fewer side effects on the treated aquatic animals (Guo et al., 2023; Zhang et al., 2022).

Plant extracts have been shown to have antibacterial activity against various bacterial strains that are important in aquaculture productivity. A recent study by Tkachenko et al. (2023) determined the antibacterial properties in the leaf extracts of *Ficus elastica* and its cultivars, which inhibited the growth of *A. sobria*, *A. hydrophila*, and *Aeromonas salmonicida* subsp. *salmonicida*. The leaf extracts of various *Ficus* spp. also demonstrated antimicrobial action against other fish bacterial pathogens, including *Serratia liquefaciens*, *Yersinia ruckeri*, *Pseudomonas fluorescens*, and *Shewanella putrefaciens* (Tkachenko et al., 2022). Essential oils derived from natural plants such as *Eucalyptus camaldulensis* (Bektaş & Özdal, 2022), *Gaultheria procumbens*, *Litsea cubeba* (Klūga et al., 2021), *Cinnamomum cassia* (Junior et al., 2022), *Origanum vulgare* subsp. *hirtum*, *Thymbra capitata*, and *Satureja thymbra* (Anastasiou et al., 2020) exhibited strong inhibitory activity against the growth of aquaculture bacterial pathogens.

Little research has been done on the antibacterial properties of popular and commonly consumed Malaysian traditional

vegetables against aquaculture pathogens *in vitro* and *in vivo*. Therefore, this study aimed to explore the *in vitro* antibacterial efficiency of ethanol extracts derived from ten Malaysian traditional vegetables against the common fish bacterial pathogens, *A. hydrophila*, *A. sobria*, *A. jandaei*, and *E. tarda* that affect freshwater fish aquaculture productions.

MATERIALS AND METHODS

Plant Materials and Extract Preparation

Fresh part of ten Malaysian traditional vegetables were sourced from Kuantan, Pahang, Malaysia, with details shown in Table 1 and Figure 1. The specimens were identified by a botanist of the Department of Plant Science, Kulliyyah of Science (KOS), International Islamic University Malaysia (IIUM), Dr. Rozilawati Shahari, and voucher specimens were deposited at the herbarium, KOS, IIUM. Each plant material was cleaned and dried at 40ºC for 2–3 days. The dry materials were crushed into powder and stored in dark, sealed bags. Each plant's powdered materials were weighed into a 500 ml conical flask, and 80% ethanol (HmbG Chemicals, Malaysia) was added with a ratio of 1:10 (w/v) before macerating for 48 hr. After centrifuging the mixtures 300 × *g* for 10 min, they were filtered using Whatman No. 1 filter paper. The collected filtrates were subjected to rotary evaporation (BUCHI R-100 equipped with vacuum pump V-100 and Interface I-100, Germany) at 40ºC, aiming to eliminate the solvent from the extract.

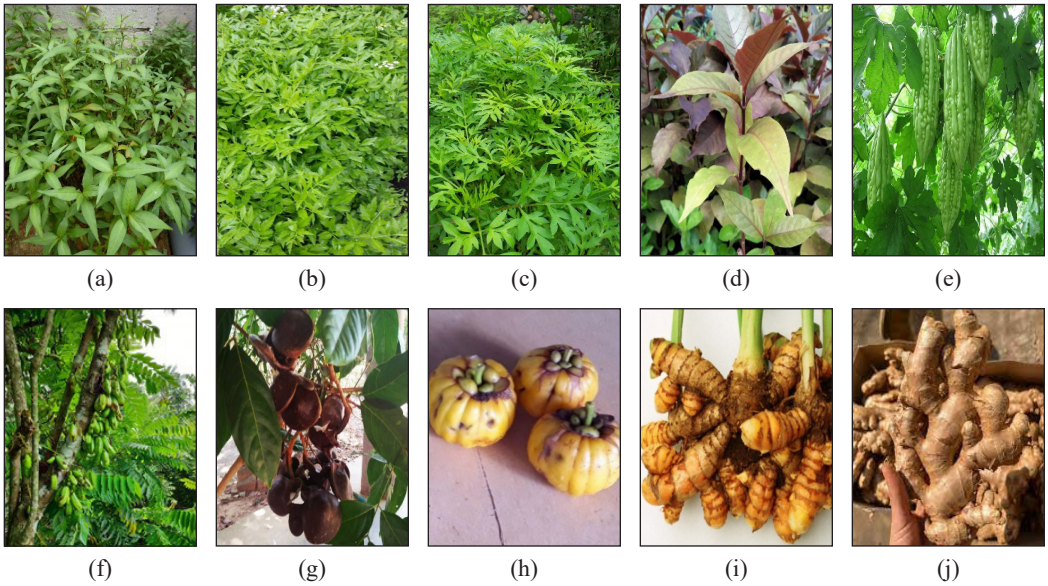

Figure 1. Plant parts used in this study: (a) leaf of *Persicaria odorata*; (b) leaf of *Oenanthe javanica*; (c) leaf of *Cosmos caudatus;* (d) leaf of *Gynura bicolour*; (e) leaf and fruit of *Momordica charantia*; (f) leaf and fruit of *Averrhoa bilimbi*; (g) leaf and legume of *Pithecellobium jiringa*; (g) the fruit of *Garcinia atroviridis*; (i) rhizome of *Curcuma longa*; and (j) rhizome of *Zingiber officinale*

Table 1
Description of plant extracts used in this study

Scientific name	Common name	Botanical family	Part used
Persicaria odorata	Vietnamese coriander	Polygonaceae	Leaf
Oenanthe javanica	Chinese celery, Japanese parsley	Apiaceae	Leaf
Cosmos caudatus	King's salad	Asteraceae	Leaf
Gynura bicolor	Okinawan spinach	Asteraceae	Leaf
Momordica charantia	bitter melon	Cucurbitaceae	Leaf and fruit
Averrhoa bilimbi	Bilimbi	Oxalidaceae	Leaf and fruit
Pithecellobium jiringa	Jering	Fabaceae	Leaf and legume
Garcinia atroviridis	Asam Gelugur, asam keping	Clusiaceae	Fruit
Zingiber officinale	Ginger	Zingiberaceae	Rhizome
Curcuma longa	Turmeric	Zingiberaceae	Rhizome

The leftover solvent was evaporated further in the oven at 40ºC, and the crude extracts were kept in dark containers at -20ºC before use (Eruygur et al., 2019).

Test Organisms and Preparation of Inoculum

Aeromonas hydrophila K3T8, *A. sobria* K1P2, *A. jandaei* K2P2, and *E. tarda* K3P2 were isolated from kidneys of diseased Pangasius catfish (*Pangasius hypophthalmus*) and red hybrid tilapis (*Oreochromis* spp.) and identified using 16S rRNA and specific genes (Hussain et al., 2014; Sakai et al., 2009) polymerase chain reaction (PCR). The cultures were maintained in tryptic soy agar (TSA, Merck, Germany). A loopful of 24-hr-old cultures were inoculated in 9 ml sterile 0.9% saline water (Fisher Scientific, USA). Each inoculum was prepared with a cell concentration of 1.5×10^8 CFU/ml by comparing with 0.5 McFarland standard (ThermoFisher Scientific, USA) to obtain a standardised inoculum for the tests.

Antibacterial Activity

Disc Diffusion Assay

The disc diffusion method was used to assess the antibacterial activity of the extracts on fish pathogenic bacteria. The experiment complied with the Clinical and Laboratory Standards Institute (CLSI) recommendations (Clinical and Laboratory Standards Institute, 2018). Briefly, on the surface of sterile Mueller Hinton agar (MHA, Merck, Germany), 100 µl of fresh bacterial inoculum (approximately 1-2 $\times 10^6$ CFU/ml according to McFarland standards) was swabbed uniformly and left to dry. Then, sterilised filter paper discs (6 mm diameter, Whatman, Merck, Germany) were placed on the surface of the MHA after being impregnated with 20 µl of each extract (60 µg per disc) dissolved in 2% dimethyl sulfoxide (DMSO, Fisher Scientific, USA) and left to dry for 15 min. Negative control was disc embedded with 2% DMSO, whereas positive controls were tetracycline, chloramphenicol, erythromycin, and

oxytetracycline (50 µg per disc, Calbiochem, USA). The bacteria-inoculated plates were incubated inverted at 37ºC for 24 hr. After incubation, the diameters (mm) of the inhibition zones around the discs were measured and performed in triplicate.

The Determination of MIC

A subset of antibacterial action extracts was evaluated for the MIC and MBC. The broth microdilution method determined the MIC of plant extracts against the bacterial strains according to the CLSI guidelines (CLSI, 2018). In 96-well microtiter plates, the extracts were serially diluted twofold in Mueller Hinton broth (MHB, Merck, Germany) to generate final concentrations ranging from 0.20 to 200 mg/ml. Subsequently, a comparable 50 µl bacterial suspension volume was introduced into each well with an estimated 10^6 CFU/ml concentration. Following incubation at 37ºC for 16–18 hr, the bacterial growth was detected by assessing medium turbidity. The MIC refers to the lowest concentration of the extracts necessary to prevent bacterial growth without causing turbidity in the medium, as shown in the negative control (wells contain media only). The positive control wells contain bacterial suspension without any extracts. The experiments were conducted three times for each assay.

The Determination of the MBC

The wells that exhibited bacterial inhibition in MIC, including the positive (wells contain bacterial suspension without any extracts) and negative (wells contain media only) controls, were subjected to plating on MHA using 10 µl aliquots of the contents. The plated samples were then incubated at 37ºC for 16–24 hr, as described by Junior et al. (2019). The MBC was determined as the concentration of the extracts at which no visible growth of bacteria was evidenced on MHA. The experiments were conducted in triplicate.

Statistical Analysis

The data were presented as means ± standard errors (SE). The statistical analysis utilised a one-way analysis of variance (ANOVA) and a post hoc Duncan's test, all conducted using SPSS Statistical 26 software. A significance level of $p < 0.05$ was deemed significant for all statistical tests.

RESULTS

Zone of Inhibition

Table 2 shows the results of the antibacterial screening assays as determined by the disc diffusion method. The antibacterial potential of plant extracts varied significantly ($p = 0.00$) depending on the type of plant and bacterial strain. The plant extracts displayed inhibitory zones with diameters ranging from 6.77 to 26.33 mm, with the most remarkable outcomes observed from *P. odorata* and *G. atroviridis*.

Persicaria odorata and *G. atroviridis* extracts exhibited a broad spectrum of inhibitory effects due to their significant antibacterial activity (inhibition zones of ≥ 15.00 mm) against the four tested bacterial strains, whereas the other extracts exhibited

Table 2
Antibacterial activity of the plant extracts as measured by disc diffusion assay

		The mean diameter of the inhibition zones (mm ± SE)			
		Aeromonas hydrophila	***Aeromonas sobria***	***Aeromonas jandaei***	***Edwardsiella tarda***
Plant species	Part				
Persicaria odorata	Leaves	$20.53 \pm 0.09^{f,C}$	$26.33 \pm 0.33^{d,D}$	$17.33 \pm 0.33^{,B}$	$15.00 \pm 0.12^{c,A}$
Oenanthe javanica	Leaves	$8.47 \pm 0.03^{bc,A}$	NA	ND	$10.03 \pm 0.03^{b,B}$
Cosmos caudatus	Leaves	$10.03 \pm 0.09^{d,B}$	NA	ND	$9.47 \pm 0.03^{b,A}$
Gynura bicolor	Leaves	$10.07 \pm 0.07^{d,A}$	NA	ND	NA
Momordica charantia	Leaves	$7.77 \pm 0.14^{ab,B}$	NA	$8.97 \pm 0.09^{b,C}$	$6.77 \pm 0.15^{a,A}$
Momordica charantia	Fruit	$7.37 \pm 0.03^{ab,B}$	NA	$7.20 \pm 0.17^{a,AB}$	$6.87 \pm 0.20^{a,A}$
Averrhoa bilimbi	Leaves	$7.53 \pm 0.20^{ab,B}$	NA	$7.10 \pm 0.09^{a,A}$	NA
Averrhoa bilimbi	Fruit	$9.13 \pm 0.07^{cd,B}$	$8.00 \pm 0.17^{a,A}$	$7.53 \pm 0.20^{ab,A}$	$9.27 \pm 0.26^{b,B}$
Pithecellobium jiringa	Leaves	$7.00 \pm 0.00^{a,A}$	NA	$7.10 \pm 0.23^{a,A}$	$8.53 \pm 0.37^{b,B}$
Pithecellobium jiringa	Fruit	$7.17 \pm 0.20^{ab,A}$	$8.50 \pm 0.17^{a,B}$	$7.43 \pm 0.24^{ab,A}$	NA
Garcinia atroviridis	Fruit	$16.33 \pm 0.88^{e,AB}$	$26.33 \pm 0.33^{d,C}$	$17.33 \pm 1.33^{d,B}$	$13.50 \pm 0.76^{e,A}$
Zingiber officinale	Rhizome	NA	$12.00 \pm 0.58^{b,A}$	NA	NA
Curcuma longa	Rhizome	NA	$13.67 \pm 0.88^{c,A}$	NA	NA
Controls					
Erythromycin*		$20.00 \pm 1.15^{f,A}$	$25.67 \pm 0.88^{d,B}$	$25.00 \pm 1.15^{e,B}$	$32.00 \pm 1.73^{e,C}$
Tetracycline*		$28.03 \pm 0.20^{g,B}$	$13.40 \pm 0.17^{c,A}$	$13.10 \pm 0.06^{c,A}$	$27.90 \pm 0.47^{d,B}$
Chloramphenicol*		$29.13 \pm 0.35^{g,AB}$	$29.90 \pm 0.21^{e,B}$	$32.00 \pm 0.58^{f,C}$	$28.33 \pm 0.29^{d,A}$
Oxytetracycline*		$44.33 \pm 0.88^{h,C}$	$31.67 \pm 0.67^{f,A}$	$35.00 \pm 1.15^{g,B}$	$33.33 \pm 0.88^{e,AB}$
2% DMSO**		NA	NA	NA	NA

Note. Different lowercase letters indicate differences between plant extracts within each pathogen, and different capital letters indicate differences between pathogens within each plant extract ($p < 0.05$). Inhibition zones > 15 mm were categorised as strong activity, 8 to 15 mm as moderate activity, and 1 to 8 mm as weak activity; SE = Standard error; NA = No growth inhibition; ND = Not tested; DMSO = Dimethyl sulfoxide; * = Positive control; ** = Negative control

limited antibacterial activity. However, the antibacterial potential of *Z. officinale* and *C. longa* extracts against *A. sobria*, *C. caudatus*, and *G. bicolor* extracts against *A. hydrophila* was observed since their inhibition zones were more than 10.00 mm.

Aeromonas hydrophila was this study's most susceptible bacterial strain, with all plant extracts inhibiting its growth. This pathogen is significantly suppressed by *P. odorata* and *G. atorividis* extracts, moderately inhibited by *O. javanica*, *C. caudatus*, *G. bicolor*, and *A. bilimbi* extracts, and poorly inhibited by the other five extracts. Meanwhile, *A. sobria* is the most resistant strain, with no inhibition zone observed in seven plant extracts and only faintly inhibited (8.00 ± 0.17 mm) by *A. bilimbi* extract.

The negative control (2% DMSO) had no inhibition zone. In contrast, the three positive controls (erythromycin, chloramphenicol, and oxytetracycline) had strong antibacterial action against all pathogens tested (inhibition zones ranging from 20.0 to 44.33 mm). Meanwhile, one reference antibiotic (tetracycline) showed significant antibacterial action against *A. hydrophila* (28.03 ± 0.20 mm) and *E. tarda* (27.90 ± 0.47 mm), but only moderate activity against *A. sobria* (13.40 ± 0.17 mm) and *A. jandaei* (13.10 ± 0.06 mm).

MIC

The MIC values of the crude extracts that showed positive results in the antibacterial activity tests are presented in Tables 3 to 6, ranging from 0.39 to 100.00 mg/ml. *Persicaria odorata* extract demonstrated the lowest MIC against all tested bacteria (MIC 0.39 mg/ml), followed by *G. atroviridis* extract, which had MICs of 1.56 mg/ml against *A. sobria*, *A. jandaei*, and *E. tarda*, and MIC of 6.25 mg/ml against *A. hydrophila*.

A weak inhibition action was observed in fruit and leaf extracts of *M. charantia* (MIC of 50.00 and 100.00 mg/ml, respectively) and fruit and leaf extracts of *A. bilimbi* against *A. hydrophila* (MIC = 50.00 mg/ml), rhizome extract of *C. longa* and fruit extracts of *A. bilimbi* and *P. jiringa* against *A. sobria* (MIC = 50.00 mg/ml), leaf extract of *M. charantia* and fruit extract of *A. bilimbi* against *A. jandaei* and *E. tarda* (MIC = 50.00 mg/ml, respectively). However, no inhibitory action was identified in *P. jiringa* fruit and leaf extracts against *A. hydrophila* and *A. jandaei* and *M. charantia* fruit and leaf extracts against *A. jandaei* and *E. tarda*.

Mode of Action and MBC

The MBC values ranged from identical to the MIC to substantially lower, as shown in Tables 3 to 6. Similar to the MIC result, *P. odorata* ethanolic extract showed significantly higher bactericidal activity against all tested bacteria (MBC value of 0.20 mg/ml). Based on the CLSI (2018), a drug is considered to exhibit bactericidal activity when the MBC/MIC ratio is ≤ 4. In contrast, the drug is bacteriostatic when the corresponding MBC/MIC ratio ≥ 8. According to the MBC/MIC ratio results, all extracts with positive MIC findings were bactericidal against all tested bacteria

Table 3
Minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) values of plant extracts on Aeromonas hydrophila

Plant	Part	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC
Persicaria odorata	Leaves	0.39	0.39	1
Oenanthe javanica	Leaves	12.50	12.50	1
Cosmos caudatus	Leaves	1.56	1.56	1
Gynura bicolor	Leaves	0.78	0.78	1
Momordica charantia	Leaves	50.00	100.00	2
Momordica charantia	Fruit	100.00	100.00	1
Averrhoa bilimbi	Leaves	50.00	100.00	2
Averrhoa bilimbi	Fruit	50.00	50.00	1
Pithecellobium jiringa	Leaves	NA	ND	ND
Pithecellobium jiringa	Fruit	NA	ND	ND
Garcinia atroviridis	Fruit	6.25	6.25	1

Note. NA = No growth inhibition; ND = Not tested

Table 4
Minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) values of plant extracts on Aeromonas sobria

Plant	Part	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC
Persicaria odorata	Leaves	0.39	0.39	1
Averrhoa bilimbi	Fruit	50.00	50.00	1
Pithecellobium jiringa	Fruit	50.00	50.00	1
Garcinia atroviridis	Fruit	1.56	1.56	1
Zingiber officinale	Rhizome	12.50	12.50	1
Curcuma longa	Rhizome	50.00	100.00	2

Note. NA = No growth inhibition; ND = Not tested

Table 5
Minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) values of plant extracts on Aeromonas jandaei

Plant	Part	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC
Persicaria odorata	Leaves	0.39	0.39	1
Momordica charantia	Leaves	50.00	100.00	1
Momordica charantia	Fruit	NA	ND	ND
Averrhoa bilimbi	Leaves	NA	ND	ND
Averrhoa bilimbi	Fruit	50.00	100.00	2
Pithecellobium jiringa	Leaves	NA	ND	ND
Pithecellobium jiringa	Fruit	NA	ND	ND
Garcinia atroviridis	Fruit	1.56	1.56	1

Note. NA = No growth inhibition; ND = Not tested

Table 6
Minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) values of plant extracts on Edwardsiella tarda

Plant	Part	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC
Persicaria odorata	Leaves	0.39	0.39	1
Oenanthe javanica	Leaves	1.56	1.56	1
Cosmos caudatus	Leaves	0.78	1.56	2
Momordica charantia	Leaves	NA	ND	ND
Momordica charantia	Fruit	NA	ND	ND
Averrhoa bilimbi	Fruit	50.00	50.00	1
Pithecellobium jiringa	Leaves	25.00	50.00	2
Garcinia atroviridis	Fruit	1.560	1.56	1

Note. NA = No growth inhibition; ND = Not tested

(MBC/MIC ratio < 4). Most extracts had a comparable bactericidal effect, as evidenced by the MBC being equivalent to the MIC (MBC = MIC). In contrast, six extracts demonstrated a bactericidal mechanism of action when administered at concentrations beyond the MIC (MBC > MIC). The results indicate that the MBC values for *A. hydrophila* were slightly higher (100.00 mg/ml; MIC 50.00 mg/ml) when using the leaf extracts from both *M. charantia* and *A. bilimbi* (Table 3). Similarly, the MBC values showed minimal variations for *A. sobria* (100.00 mg/ml; MIC 50.00 mg/ml) when treated with *C. longa* and *A. jandaei* (100.00 mg/ml; MIC 50.00 mg/ml) using the fruit extract of *A bilimbi* (Tables 4 and 5). As for *E. tarda*, the MBC values were slightly elevated in *C. caudatus* (1.56 mg/ml; MIC 0.78 mg/ml) and *P. jiringa* (50.00 mg/ml; MIC 25.00 mg/ml) (Table 6).

DISCUSSION

The aquaculture sector has long relied on antibiotics as a strategy to control infectious bacterial diseases. However, in light of the rise of antibiotic resistance and the consequential environmental impacts associated with their utilisation, there is a need for alternative approaches. Plants and their derivatives have exhibited considerable potential as viable alternatives to antibiotics in the treatment of bacterial infections. Their application is gaining popularity because of its convenient preparation process, low cost, less adverse effects, and environmental impact (Bondad-Reantaso et al., 2023).

This study presents the findings on the antibacterial properties of some Malaysian traditional vegetables, highlighting their potential as a viable substitute for antibiotics in managing the proliferation of fish pathogens, including *A. hydrohila*, *A. sobria*, *A. jandaei*, and *E. tarda*. Among them, *P. odorata* and *G. atroviridis* extracts had the highest activity against the tested pathogens. *Aeromonas hydrophila* and *A. sobria* showed susceptibility (IZ ≥ 20 mm), whereas *A. jandaei* and *E. tarda* exhibited moderate susceptibility to *P.*

odorata extract in the present study at 300 mg/ml concentration. In a study conducted by Ridzuan et al. (2013), the effectiveness of *P. odorata* extract at different concentrations (ranging from 50 to 400 mg/ml) was compared against eight bacterial strains. The results showed that both American Type Culture Collection (ATCC) and wild strains of *Streptococcus aureus* and *Streptococcus epidermis* were susceptible to the extract. However, both strains of *Streptococcus pyogenes* showed intermediate susceptibility to *P. odorata* at the highest concentration of 400 mg/ml. These results indicate that plant extract concentration plays a crucial role in determining its effectiveness in combating specific bacteria. Based on phytochemical analysis by Nguyen et al. (2020), *P. odorata* leaves contained various phytochemical compounds such as alkaloids, tannins, anthraquinone, flavonoids, terpenoids, coumarins, saponin, and other reducing compounds, which indicated its potential as antibacterial agents. However, the concentration of these bioactive compounds in the extract varies according to multiple factors, including selection of solvents, extraction techniques, plant materials and extraction time (AL Ubeed et al., 2022).

Persicaria odorata, commonly used in cuisines and has various medical properties, has also been reported to contain pharmacological activities such as antioxidants, anti-inflammatory, antibacterial, and anticancer effects (Azmi et al., 2021). The disc diffusion and time-kill assays revealed that silver nanoparticles synthesised from *P. odorata* inhibited methicillin-resistant *Staphylococcus aureus*, *S. aureus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa*. These findings support the notion that *P. odorata* has antibacterial characteristics (Aminullah et al., 2021). Similarly, Řebíčková et al. (2020) demonstrated the antimicrobial efficiency of *P. odorata* essential oils against various bacteria, including both Gram-negative and Gram-positive strains. Specifically, these essential oils exhibited effectiveness against *Escherichia coli*, *S. aureus*, *P. aeruginosa*, *Enterococcus faecalis*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Seratia marcescense*, and *Bacillus subtilis*. These findings are similar to and further support the results of this study. Our antibacterial study of *P. odorata* extract yielded remarkably similar results of 15.00 to 26.33 mm inhibition zones, showcasing the consistent effectiveness against four common fish bacterial pathogens.

Our investigation into the antibacterial potential of *G. atroviridis* extract revealed results similar to those of *P. odorata* extract. Organic acids and flavonoids have been identified as the primary components of *G. atroviridis* extracts (Shahid et al., 2022), and some of these were reported to have biological activities (Reang et al., 2021; Schobert & Biersack, 2019; Tan et al., 2016). A variety of studies undertaken by several research groups have proved *G. atroviridis*' antibacterial ability. A recent study by Thongkham et al. (2021) investigated the antimicrobial activity of the ethanolic extract derived from *G.*

atroviridis fruit. The study demonstrated the effectiveness of this extract against various microorganisms, including *E. coli* TISTR 073, *Streptococcus agalactiae* ATCC 27956, *B. subtilis* DMST 3763, *Streptococcus intermedius* DMST 5024, *S. epidermidis* DMST 12853, *S. aureus* DMST 4745, *Candida albicans* ATCC 10, 231, and *Malassez pachydermatis*. Later, Niyomdecha et al. (2022) also synthesised *G. atroviridis* fruit ethanolic extract and discovered that it exhibited inhibitory effects against *E. coli* and *S. enterica* with inhibition zones of 33.11 and 31.58 mm, respectively, at a dosage of 200 mg/ml. Tan et al. (2019) conducted a similar study and discovered that stem bark extracts of *G. atroviridis* have inhibitory activities against food-borne bacteria, *Proteus mirabilis*, *S. epidermidis*, *Bacillus cereus*, *B. subtilis*, methicillin-resistant *S. aureus*, *E. coli*, *Citrobacter freundii*, *P. aeruginosa*, *K. pneumonia*, *Shigella boydii*, and anti-yeast capabilities against *Candida utilis*.

The MBC results in this study corresponded to the low MIC value. *Persicaria odorata* extract exhibited potentially bactericidal activity against all pathogenic bacteria tested (*A. hydrophila*, *A. sobria*, *A. jandaei*, and *E. tarda*) with MBC of 0.20 mg/ml, whereas *G. atroviridis* extract exhibited MBC of 0.78 mg/ml, except for *A. hydrophila*, which was less sensitive and had MBC of 3.13 mg/ml. The MIC and MBC outcomes obtained from evaluating plant extracts with antimicrobial properties indicate that *P. odorata* and *G. atroviridis* can be utilised to control and prevent fish bacterial infections. These results are in accordance with those of Bacayo et al. (2018), Niyomdecha et al. (2022), Řebíčková et al. (2020), Tan et al. (2019). The wide range in MIC of *P. odorata* and *G. atroviridis* extracts seen in many studies could be attributed to differences in the extraction process, solvents, and bacterial strains utilised. Variations in MIC of different plant extracts may also result from differences in their chemical compositions and the volatile nature of those components (Mostafa et al., 2018).

As far as the authors are aware, there have been no earlier findings on *G. atroviridis* extract as an antibacterial agent against fish pathogenic bacteria; thus, further comparison with other publications is not conceivable. However, there is one report about testing the extract of *P. odorata* against fish bacterial pathogenic by Najiah et al. (2011). Their study found that the methanolic and aqueous extract of *P. odorata* had a mild level of antibacterial effectiveness against *S. aureus* with an inhibition zone of 8.00 mm but no antibacterial activity against *E. coli*, *E. tarda*, *A. hydrophila*, *C. freundii*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *S. agalactiae*, and *Streptococcus aginosus*. Meanwhile, the present study showed that the ethanolic extract of the plant has significant inhibitory effects on the growth of *A. hydrophila*, *A. jandaei*, *A. sobria*, and *E. tarda*. This discrepancy could be attributed to the extraction process and solvent used, which affect the concentration of active components in extracts and species variation (Farahmandfar et al., 2019).

CONCLUSION

Due to the rapid rise of antibiotic-resistant organisms, there is an ongoing need to explore new antimicrobial compounds, especially from natural sources. The present study's findings demonstrated the potential of Malaysian traditional vegetables in inhibiting the growth of fish pathogenic bacteria, albeit to varying degrees. Due to their high antibacterial activity, *P. odorata* and *G. atroviridis* extracts are considered to have significant potential for future use as antibacterial agents in aquaculture. Moreover, additional investigation is required to identify these plants' specific active chemical compounds and their impact on fish metabolism. Another effective method for implementing extracts should also be considered.

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