Metabolite Composition and Bioactivity of *Murdannia loriformis* against Marine Parasitic Leech and *Vibrio* Species

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Abstract

Vibriosis and parasitic infestation pose significant challenges in aquaculture. The parasitic leech Pterobdella arugamensis harms fish and makes them more susceptible to Vibrio secondary infections. While toxic chemical treatments are commonly used, there is a growing demand for safer, eco-friendly alternatives. Consequently, research has increasingly focused on natural products derived from plants. In the current study, we investigated the anti-Vibrio and antiparasitic potentials of the aqueous extract of Murdannia loriformis (Hassk.) Rolla Rao et Kammathy, a medicinal plant belonging to the Commelinaceae family. The extract was tested against five Vibrio species and the parasitic leech P. arugamensis. Additionally, its metabolite composition was analysed using mass spectrometry techniques, including liquid chromatography quadrupole orbitrap mass spectrometry (LC-q-Orbitrap-MS). The extract of M. loriformis *exhibited bactericidal activities against Vibrio species at concentrations of 100 and 50 mg.mL^{-1} with inhibition zones ranging from 7.33 ± 0.47 mm to 17.33 ± 0.45 mm, in a dose-dependent manner. The extract also demonstrated dose-dependent antiparasitic effects against P. arugamensis, with mortality time ranging from 10.37 ± 1.77 to 67.02 ± 5.38 minutes at concentrations of 100 to 12.5 mg.mL^{-1}. The LC-q-Orbitrap-MS analysis detected 39 metabolites in both positive and negative modes, including numerous important metabolites such as orientin, corchorifatty acid F, pyridoxine, nicotinic acid, schaftoside, corymboside, vicenin-2, hexadecanamide, pinolenic acid, eleutherazine B, dimethylfraxetin, paprazine, and trigonelline. These findings highlight the significant anti-Vibrio and antiparasitic potential of M.* loriformis *extract, making it a promising natural substitute for controlling Vibrio infections and parasitic leech infestations in aquaculture systems.*

Keywords: *Anti-Vibrio, Seafood Security, Aquaculture, Marine Leech, Mass Spectrometry*

Introduction

Aquaculture plays a vital role in addressing global food security and ecological sustainability. The industry is badly affected by vibriosis and parasitic leech infestations. Vibriosis is caused by pathogenic *Vibrio* species, including *V. owensii, V. parahaemolyticus, V. harveyi, V. anguillarum, and V. alginolyticus.* It leads to high mortality rates in cultured fish, resulting in substantial economic losses. The severity of the disease is often exacerbated by poor management practices and environmental stressors (Pradeepkiran, 2019; de Souza Valente and Wan, 2021; Maran *et al.,* 2023).

In addition to vibriosis, the marine parasitic leech, including *Pterobdella arugamensis*; previously named *Zeylanicobdella arugamensis* (Hirudinea, Piscicolidae), also causes the mortality of the host fish, such as grouper, hybrid grouper, seabass, etc. (Azmey *et al.,* 2022). The parasitic leech attaches to its host and feeds on its blood, potentially causing severe health issues such as reduced growth rates and, finally, mortality in a short time. The parasitic leech infestations also contribute to the development of vibriosis.(Cruz-Lacierda *et al.,* 2000; Kua *et al.,* 2006, 2014; Ravi and Yahaya, 2017; Rimmer and Glamuzina, 2019).

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The control of vibriosis and parasites often involves chemicals that are harmful to both aquatic life and the environment, necessitating effective treatment strategies (Leal *et al.,* 2018; Mohamad *et al.,* 2019). Natural control agents, including plant-derived extracts, provide a sustainable solution for managing pests and diseases in aquaculture due to the presence of various bioactive metabolites (Reverter *et al.,* 2014). One such potential candidate is *Murdannia loriformis* (Hassk.) Rolla Rao et Kammathy (*M. loriformis*) belongs to the family Commelinaceae. In English, it is known as "*Beijing grass,"* or *"Angel grass"* while in Bahasa Malay it is known as "*Rumput Siti Khatijah"* or *"Rumput Cina"*. The plant is distributed in Asia's forests and grassy slopes, including Malaysia. The plant has attracted attention for its medicinal potential, such as for psoriasis and eczema (Kunnaja *et al.,* 2014). Further, it has been reported to exhibit antioxidant (Kittipongpittaya *et al*., 2015), anti-inflammatory (Kunnaja *et al*., 2014), and antibacterial properties against *Staphylococcus aureus*, *S. epidermidis,* and *Escherichia coli* (Kosuwin *et al*., 2024).

However, anti-*Vibrio* and antiparasitic properties of *M. loriformis* extract remain unexplored. Therefore, the current study aims to investigate the anti-*Vibrio* and antiparasitic potential of the aqueous extract of the plant and to highlight the various antipathogenic metabolites using liquid chromatography quadrupole orbitrap mass spectrometry (LC-q-Orbitrap-MS). By exploring the anti-pathogenic potential of *M. loriformis* as a natural disease management tool, this research contributes to the development of sustainable aquaculture practices that prioritise environmental health and safety.

Material and Methods

Extraction

The plant sample was cleaned with distilled water and air-dried at room temperature (25°C). A high-capacity mill was used to grind the dried plant separately. Around 21 g of the dry plant powder was boiled with distilled water in a ratio of 1:10 for 10 minutes on a stir plate. The decoctions were drawn off and allowed to cool at room temperature for an hour. The extracts were filtered through a strainer to remove coarse residues, and the filtrate was then filtered again using Whatman No. 1 filter paper. The filtrate was lyophilized using a freeze dryer after being held at -80°C for 24 hours.

Vibrio strains and stock preparation

The anti-*Vibrio* activities of the extract were tested against five different species of gram-negative bacteria of the genus *Vibrio*, including *V. alginolyticus* (ATCC17749), *V. anguillarum* (ATCC19264), *V. harveyi* (ATCC35084), *V. owensii* (isolated from *Kappaphycus alvarezii)*, (Mangun *et al.*, 2025) and *V. parahaemolyticus* (ATCC17802). The bacterial stock was obtained from the Fish Disease Laboratory, Borneo Marine Research Institute, Universiti Malaysia Sabah. The bacterial strains were thawed and cultured. Approximately 50 µl of the bacteria were cultured in 5 mL tryptone soy broth (TSB) containing 2 % sodium chloride and incubated for 24 h at 25°C in a shaking incubator.

Anti-Vibrio activity

The anti-*Vibrio* activity of the extract was evaluated using the disc diffusion method with slight modifications (Mostafa *et al.,* 2018). Tryptone soy agar plates were used in a biosafety chamber to simulate aseptic conditions and prevent contamination. The agar plates were divided into two sections to test the same extract at the same concentration. Whatman filter discs were inserted into nutrient agar plates using a sterile cork borer (6 mm), and the bacteria were spread on the solid plates using a sterile swab moistened with the bacterial suspension. Then, 100 µl of the aqueous extract (100 $mg.mL^{-1}$) was added to the discs prepared in the inoculated plates. As a positive control, filter paper discs containing oxytetracycline (10 µg) were utilised. The plates were incubated at 37°C for 24 h, and the zone of inhibition, if any, around the wells was measured in mm.

Parasitic leech collection

The adult parasitic leeches, *P. arugamensis,* were obtained from the aquaculture facilities of the Borneo Marine Research Institute, Universiti Malaysia Sabah. The hybrid grouper (*Epinephelus fuscoguttatus x E. lanceolatus*) (150–350 g), infested with parasitic leeches, was placed in a small tank containing seawater from the cage, and leeches were removed individually by hand. The separated leeches were placed into a container containing filtered seawater.

Antiparasitic activity

Healthy adult parasitic leeches were divided into 6 groups. Each group was provided with 6 parasitic leeches in a Petri dish. Group 1 was treated with seawater only (negative control), while Group 2 was treated with a 0.25% formalin solution (positive control). Groups 3,4,5, and 6 were treated with 100, 50, 25, and 12.5 $mg.mL^{-1}$ of extract of *M. loriformis*, respectively. The aqueous extract was dissolved in filtered seawater and applied to the parasitic leeches in a petri dish. During the experiment, inactivity,

mortality time, and the percentage mortality of parasite leeches were recorded (Shah *et al.,* 2021). Parasitic Leech mortality was assessed by gently probing the organisms with a needle. Individuals with no movement or response to physical stimulation for up to 10 min were considered dead.

Qualitative metabolites screening

The qualitative metabolite screening of the aqueous crude extract of *M. loriformis* was conducted to assess the presence of different metabolites, including flavonoids, tannins, phenolics, alkaloids, anthocyanins, saponins, terpenoids, anthraquinones, and steroids (Harborne, 1998).

Liquid Chromatography Quadrupole Orbitrap Mass Spectrometry (LC-q-Orbitrap-MS) analysis

The extract of *M. loriformis* was reconstituted with methanol, and the concentration was adjusted before the LC-q-Orbitrap-MS analysis. A 1.0 µl reconstituted sample was chromatographically separated with a Thermo Scientific Synchronis C18 column (1.7 µm x 100 mm x 2.1 mm). The column was maintained at 25 °C, and a flow rate of 500 µl.min^{-1} during analysis. The mobile phases were 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The LC gradient followed the previously established program (Shah *et al.,* 2020). Briefly, the gradient started at 5% solvent B for 5 min and progressed to 100% solvent B in 15 min. and was kept for 5 min. Later, the column was conditioned as an initial for 5 minutes before the subsequent injection. The acquired MS and MS/MS data were analysed, following the previous methodology with minor modifications (Ling *et al.,* 2018).

Statistical analysis

The IBM SPSS Statistics 25 Windows package (IBM, Armonk, NY, US) was used to analyse the data. Significant differences between groups were determined using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests (Chong *et al.,* 2021; Zaki *et al.,* 2022). The means and standard error of the means (S.D.) were used to present all results. P-values of less than 0.05 were considered significant (Safuan *et al.,* 2018).

Results and Discussion

Anti-Vibrio assay

Various concentrations of the extract were used to test the anti-*Vibrio* effects of *M. loriformis* against *V. owensii, V. parahaemolyticus, V. harveyi, V. anguillarum,* and *V. alginolyticus.* The extract showed inhibition at 100 and 50 mg.mL^{-1}, while no inhibition was noted at 25 and 12.5 mg.mL^{-1} (Table 1). The aqueous extract of *M. loriformis* demonstrated bactericidal activities against *Vibrio* species, with notable inhibition observed at higher concentrations (100 and 50 mg.mL^{-1}). The finding aligns with broader research on plant-derived antimicrobial agents (Srikacha and Ratananikom, 2020; Pedrosa *et al.,* 2020; Maran *et al.,* 2023). At 100 mg.mL^{-1}, a larger inhibition zone was noticed against *V. anguillarum* (17.33 ± 0.58 mm, *V. harveyi* (17.00 ± 2.0 mm), and *V. alginolyticus* (15.33 ± 1.53 mm), compared to *V. owensii* (12.00± 1.0 mm) and *V. parahaemolyticus* (11.00 ± 1.0 mm). This variability may arise from differences in cell composition or the efficacy of the efflux pump among *Vibrio* species (critical membrane-bound transporters that expel antimicrobial agents) (Stephen *et al.,* 2022). The larger inhibition zone at 100 mg.mL^{-1} versus 50 mg.mL^{-1} also suggests that metabolites in *M. loriformis* aqueous extracts require a high concentration to disrupt bacterial membranes or critical metabolic pathways. Similar results were also noticed in a study by Alenazy (2023), where the methanol extract of *Trigonella foenumgraecum* exhibited a larger zone of inhibition against *Staphylococcus aureus* and *Escherichia coli* at higher concentrations compared to lower ones.

Antiparasitic assay

Table 2 shows the mortality time and percentage of parasitic leeches exposed to seawater, formalin, and various concentrations of *M. loriformis* extract.

Behaviour of P. arugamensis after being treated with controls and extract concentrations.

Upon exposure to the control treatment in seawater, the parasite leech exhibited unaltered behaviour. When exposed to a 0.25% formalin treatment, the leech became active and aggressive before dying. Across the concentration group treatment, exposure to 100 and 50 mg.mL^{-1} concentrations of the extract induced high activity and aggression in the leeches, leading to their death within a rapid timeframe of 10.37 minutes to 17.21 minutes. Conversely, when subjected to lower concentrations of 25 and 12 mg.mL^{-1}, the leeches displayed no aggressive or active behaviour, maintaining only a normal swimming pattern until their gradual demise within 21.72 min to 67.02 minutes. While still alive, the leeches used their suckers to attach to the petri dish, but exposure to the treatment prompted a slowdown in their activity, marked by body retraction movements. After the leeches die, they slowly detach their suckers from the petri dish.

Table 1. The anti-*Vibrio* effect of *M. loriformis* extract

Conc. (mg.mL^{-1})	Zone of Inhibition (mm)				
	Vibrio owensii Mean ± S.D	*Vibrio parahaemolyticus* Mean ± S.D	*Vibrio harveyi* Mean ± S.D	*Vibrio anguillarum* Mean ± S.D	*Vibrio alginolyticus* Mean ± S.D
Control OTC (10 mg.mL^{-1})	32.33 ± 2.08	34.33 ± 0.58	27.00 ± 2.00	31.33 ± 5.50	42.00 ± 1.73
M. loriformis (100 mg.mL^{-1})	12.00 ± 1.00	11.00 ± 1.00	17.00 ± 2.00	17.33 ± 0.58	15.33 ± 1.53
M. loriformis (50 mg.mL^{-1})	10.00 ± 1.00	7.33 ± 0.58	14.00 ± 3.00	11.67 ± 2.08	8.00 ± 1.00
M. loriformis (25 mg.mL^{-1})	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
M. loriformis (12.5 mg.mL^{-1})	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Each value represents the mean ± S.D of triplicates per group.
All data showed significant differences between groups with *P*-value <0.05

Table 2. Mortality time and percentage of all parasitic leeches at different concentrations of *M. loriformis* extract

Group	Concentration (mg.mL^{-1}) Mean ± S.D	Mortality time (min) Mean ± S.D	Mortality %
Seawater (Negative Control)		>720	0
Formalin (Positive Control)	0.25%	3.08 ± 0.40	100
M. loriformis	100	10.37 ± 1.77	100
M. loriformis	50	17.21 ± 2.18	100
M. loriformis	25	21.72 ± 2.15	100
M. loriformis	12.5	67.02 ± 5.38	100

Each value represents the mean ± S.D. of 6 parasitic leeches per group. All data showed significant differences between groups with a *P*-value <0.05

The findings of this study demonstrate the potential antiparasitic activity of the aqueous extract of *M. loriformis* against the marine parasitic leech *Pterobdella arugamensis.* Complete mortality of the parasitic leeches was observed across all the tested concentrations, with mortality times showing a clear dose-dependent response. Higher concentrations (100 and 50 mg.mL^{-1}) resulted in significantly shorter times (10.37 ± 1.77 min and 17.21 ± 2.18 min, respectively), while lower concentrations (25 and 12.5 mg.mL^{-1}) required longer exposure times (21.72 ± 2.15 min and 67.02 ± 5.38 min, respectively). This dose-dependent trend aligns with the typical plant extract treatment, where high concentrations generally show stronger bioactivity due to increased availability of bioactive metabolites. The efficacy of *M. loriformis* extract in achieving complete mortality highlights its potential as a natural antiparasitic agent, particularly for aquaculture applications, where infestation of *P. arugamensis* can cause significant economic losses. Compared to other plant and seaweed extracts previously reported, such as *Melastoma malabathricum*, *Piper betle, Nephrolepis biserrata,* and *Sargassum* sp., which demonstrate antiparasitic activity in a very short time, *M. loriformis* appears similarly effective but requires slightly longer exposure times depending on the concentration (Shah *et al.,* 2020; Wan Norhana *et al.,* 2021; Haron *et al.,* 2022). The result also underscores the importance of optimizing exact concentration for practical use in aquaculture. Lower concentration may be less effective in rapidly controlling infestation, whereas high concentration could provide immediate relief but may require further evaluation for cost effectiveness and environmental safety.

Physicochemical parameters

The water quality parameters of the solution applied for anti-*Vibrio* and antiparasitic analyses are displayed in Table 3. These parameters include temperature (°C), pH value, and dissolved oxygen (mg.L^{-1}).

Qualitative screening

The qualitative metabolite screening of aqueous *M. loriformis* showed the presence of different classes, including flavonoids, tannins, phenolics, alkaloids, saponins, terpenoids, and steroids, while showing the absence of anthocyanins and anthraquinone. Among these, tests of alkaloids, saponins, and steroids indicated a strong presence (Table 4).

Table 3. Parameter Measurement of Treatment Solution

Treatment	Temperature (°C)	pH	Dissolved Oxygen (mg.L^{-1})
Seawater (Normal control)	27.6	7.89	5.01
Formalin (*0.25%,* Positive *control)*	27.8	7.04	4.75
M. loriformis 100 mg.mL^{-1}	27.8	6.84	4.14
M. loriformis 50 mg.mL^{-1}	27.7	6.83	4.51
M. *loriformis 25 mg.mL^{-1}*	27.8	6.84	4.71
M. *loriformis 12.5 mg.mL^{-1}*	27.6	6.91	5.32

Table 4. Qualitative metabolite screening of aqueous extract of *M. loriformis*

Class of metabolites	Presence /absence
Flavonoids	(+)
Tannins	(+)
Phenolic	(+)
Alkaloids	(++)
Anthocyanins	(-)
Saponin	(++)
Terpenoids	(+)
Anthraquinones	(-)
Steroids	(++)

+ = present, ++ = strong present, - = absent.

The anti-leech and anti-*Vibrio* properties of *M. loriformis* might be attributed to its natural metabolites. Preliminary screening of the aqueous extract of *M. loriformis* revealed a relatively higher proportion of fatty acids and flavonoid compounds. The composition aligns with findings by Che Sulaiman *et al*., 2021, who also reported the presence of phenolics, flavonoids, tannins, chlorophylls, alkaloids, and steroids in the plant. Furthermore, the solvent extract of *M. loriformis* has been shown to contain alkaloids and steroids with known antimicrobial properties (Phatthalung *et al.,* 2012).

LC-q-Orbitrap-MS analysis of M. loriformis aqueous extract

The aqueous extract of *M. loriformis* was analysed using LC-q-Orbitrap-MS in both positive and negative ionisation modes to achieve maximum metabolite coverage. The combined use of both modes ensures that all basic and acidic functional groups in *M. loriformis* are detected by the sensor to improve the compound identification accuracy for comprehensive characterisation.

Positive Mode of LC-q-Orbitrap-MS

A total of twenty-eight (28) compounds from different functional groups are detected in the positive mode of LC-q-Orbitrap-MS (Figure 1). The identified compounds are tabulated along with their retention time, compound name, molecular formula, molecular weight, and mass error (Table 5).

Twenty-eight compounds were identified through LC-q-Orbitrap-MS analysis for *M. loriformis* aqueous extracts in the positive mode (Table 5), with mostly flavonoid compounds (13-19); followed by fatty derivatives (9-12); three compounds individually in aromatic (3-5) and purine classes (23-25); two compounds in classes coumarin (6-7); indole (20-21) and vitamin B (27-28). The rest are amide (2), diketopiperazine (8), monocarboxylic acid (22), and quinolone (26) (Figure 2).

Negative Mode of LC-q-Orbitrap-MS

A total of eleven (11) compounds are detected in the negative mode of LC-q-Orbitrap-MS (Figure 3). The identified compounds are tabulated along with their retention time, compound name, class, molecular formula, and molecular weight mass error (Table 6).

Eleven compounds were identified through LC-q-Orbitrap-MS analysis for *M. loriformis* aqueous extracts in the negative mode (Table 6), consisting of five fatty acids (32-36); an aromatic (29); a carotenoid (30); an ester (31); a flavonoid (37); a phenolic (38), and a terpenoid (39) (Figure 4). Among the detected metabolites, trigonelline (1) is identified as a natural polar hydrophilic alkaloid known for its antioxidant, anti-inflammatory, antimicrobial, and antiparasitic activities (Mohamadi *et al.,* 2018; Esmaeili *et al.,* 2024; Nguyen *et al.,* 2024). Trigonelline has demonstrated antibacterial activity against multidrug-resistant strains of *Staphylococcus*

Table 5. Metabolite profile in aqueous extract of *M. loriformis* analysed via the positive mode of LC-q-Orbitrap-MS analysis

Class	Name	Formula	Mass Error [ppm]	*m/z*	RT [min]	Reference Ion
Alkaloid	Trigonelline	$C_7 H_7 N O_2$	0.27	138.0550	0.615	$[M+H]^{+1}$
Amide	Paprazine	$C_{17} H_{17} N O_3$	0.19	284.1282	6.767	$[M+H]^{+1}$
Aromatic	N-Acetyl-DL-tryptophan	$C_{13} H_{14} N_2 O_3$	0.02	247.1077	5.836	$[M+H]^{+1}$
Aromatic	2,3,4,9-Tetrahydro-1H-beta-carboline-3-carboxylic acid	$C_{12} H_{12} N_2 O_2$	0.91	217.0974	4.325	$[M+H]^{+1}$
Aromatic	α-Pyrrolidinopropiophenone	$C_{13} H_{17} N O$	0.15	204.1383	4.918	$[M+H]^{+1}$
Coumarin	Dimethylfraxetin	$C_{12} H_{12} O_5$	0.17	237.0758	6.023	$[M+H]^{+1}$
Coumarin	5,7-Dihydroxy-4-methylcoumarin	$C_{10} H_8 O_4$	0.13	193.0496	4.734	$[M+H]^{+1}$
Diketopiperazine	Eleutherazine B	$C_{22} H_{36} N_4 O_6$	1.10	453.2711	4.558	$[M+H]^{+1}$
Fatty acid	13-Hydroxy-9,11,15-octadecatrienoic acid	$C_{18} H_{30} O_3$	0.62	295.2270	8.554	$[M+H]^{+1}$
Fatty acid	Pinolenic acid	$C_{18} H_{30} O_2$	0.61	279.2320	8.434	$[M+H]^{+1}$
Fatty amide	Hexadecanamide	$C_{16} H_{33} N O$	1.07	256.2638	16.153	$[M+H]^{+1}$
Fatty amide	Stearamide	$C_{18} H_{37} N O$	1.11	284.2951	17.118	$[M+H]^{+1}$
Flavonoid	Orientin	$C_{21} H_{20} O_{11}$	1.48	449.1085	5.232	$[M+H]^{+1}$
Flavonoid	1,5-Anhydro-1-[5,7-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromen-6-yl]hexitol	$C_{21} H_{20} O_{10}$	0.73	433.1133	5.648	$[M+H]^{+1}$
Flavonoid	Vicenin-2	$C_{27} H_{30} O_{15}$	1.42	595.1666	4.826	$[M+H]^{+1}$
Flavonoid	Corymboside	$C_{26} H_{28} O_{14}$	1.81	565.1562	5.309	$[M+H]^{+1}$
Flavonoid	Kaempferol 3-neohesperidoside	$C_{27} H_{30} O_{15}$	1.42	595.1666	5.096	$[M+H]^{+1}$
Flavonoid	3'-hydroxy Puerarin	$C_{21} H_{20} O_{10}$	1.39	433.1135	5.481	$[M+H]^{+1}$
Flavonoid	Schaftoside	$C_{26} H_{28} O_{14}$	1.00	565.1557	5.067	$[M+H]^{+1}$
Indole	4-Indolecarbaldehyde	$C_9 H_7 N O$	0.65	146.0601	6.060	$[M+H]^{+1}$
Indole	trans-3-Indoleacrylic acid	$C_{11} H_9 N O_2$	0.33	188.0707	5.754	$[M+H]^{+1}$
Monocarboxylic acid	Indoleacetic acid	$C_{10} H_9 N O_2$	0.9	176.0708	5.418	$[M+H]^{+1}$
Purine	Adenine	$C_5 H_5 N_5$	0.76	136.0619	0.817	$[M+H]^{+1}$
Purine	Guanine	$C_5 H_5 N_5 O$	0.53	152.0568	0.842	$[M+H]^{+1}$
Purine	Hypoxanthine	$C_5 H_4 N_4 O$	1.34	137.0459	1.017	$[M+H]^{+1}$
Quinolone	8-Hydroxyquinoline	$C_9 H_7 N O$	0.13	146.0601	3.949	$[M+H]^{+1}$
Vitamin B	Nicotinic acid	$C_6 H_5 N O_2$	1.50	124.0395	0.916	$[M+H]^{+1}$
Vitamin B	Pyridoxine	$C_8 H_{11} N O_3$	0.93	170.0814	0.956	$[M+H]^{+1}$

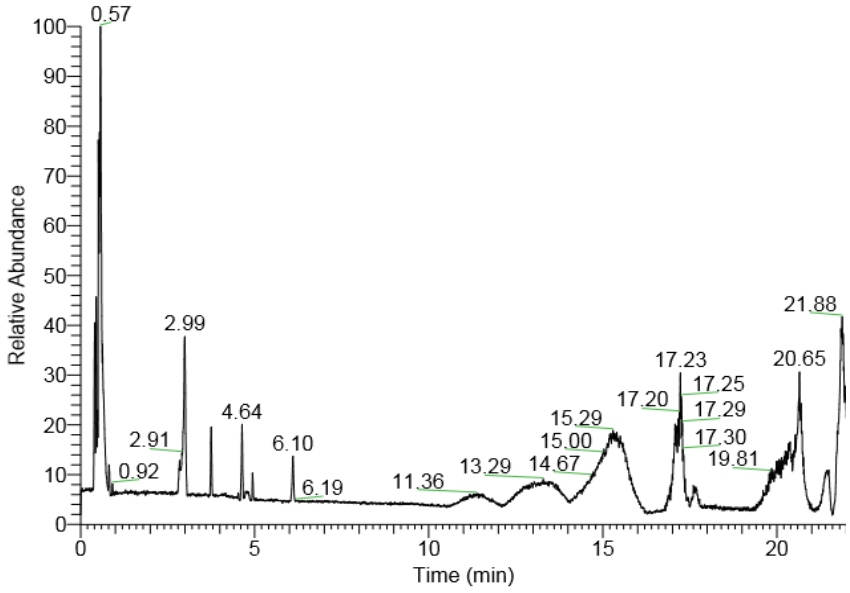

Figure 1. Chromatogram of *M. loriformis* aqueous extract via positive mode of LC-q-Orbitrap-MS analysis.

aureus and *Escherichia coli*, including inhibition of biofilm formation, which is crucial for controlling resistant infections (Alenazy, 2023). It has also demonstrated antiparasitic properties against *Leishmania major* (Esmaeili *et al.,* 2024). Other compounds that have been reported with antioxidant properties include Paprazine (2), dimethylfraxetin (6), eleutherazine B (8), and hexadecanamide (11) (Zayed *et al.,* 2014; Suryavanshi and Rathore 2017; Munaeni *et al.,* 2019; Porras-Dávila *et al.,* 2022). Pinolenic acid (10), also detected in *M. loriformis,* has been reported with significant antioxidant and anti-inflammatory properties (Takala *et al.,* 2022a, b). Orientin (13) has demonstrated broad-spectrum antiviral effects against viruses such as Parainfluenza virus type 3 and Herpes Simplex Virus Type 2 (HSV-2), as well as antibacterial activity against *E coli, S. aureus*, *Klebsiella pneumoniae*, and others. (Lam *et al.,* 2016). Furthermore, it has shown dose-dependent inhibition of six strains of *Mycobacterium*

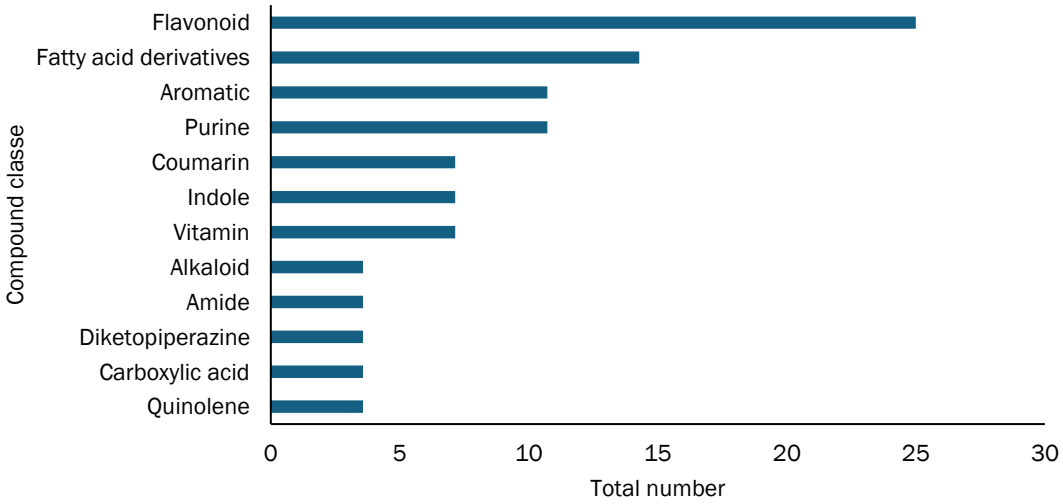

Figure 2. The summary of the compound classes detected in the positive mode of LC-q-Orbitrap-MS analysis.

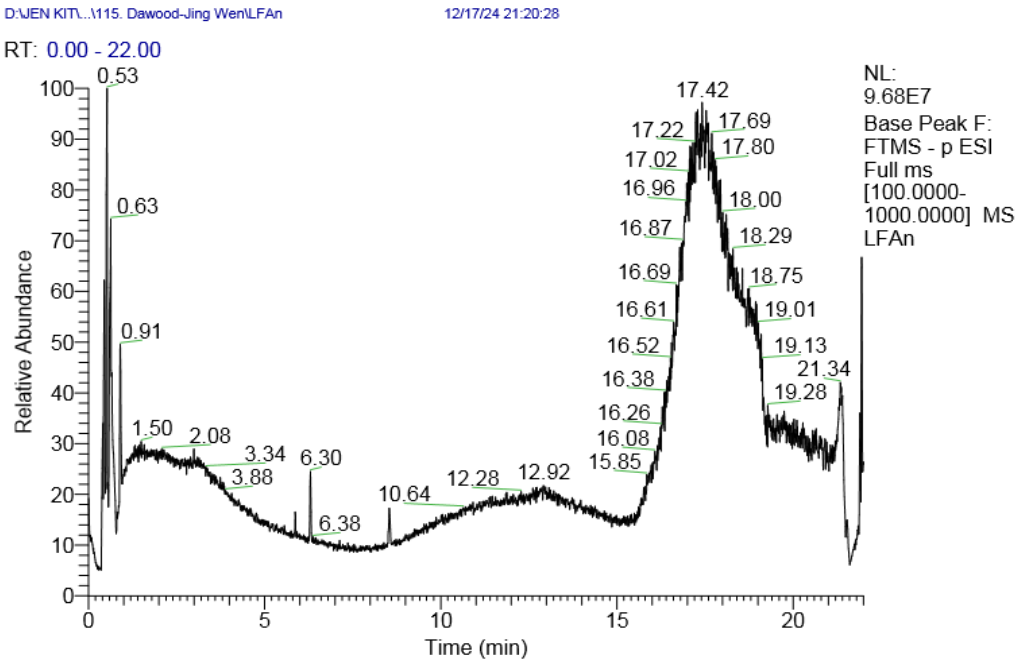

Figure 3. Chromatogram of *M. loriformis* aqueous extract via the negative mode of LC-q-Orbitrap-MS analysis

Table 6. Metabolite profile in aqueous extract of *M. loriformis* analysed via the negative mode of LC-q-Orbitrap-MS analysis

Class	Name	Formula	Mass Error (ppm)	*m/z*	RT [min]	Reference Ion
Aromatic	N-Acetyl-DL-tryptophan	$C_{13}H_{14}N_2O_3$	-1.53	245.0928	5.840	$[M-H]^{-1}$
Carotenoid	Epidihydrophaseic acid	$C_{15}H_{22}O_5$	-1.56	281.139	5.046	$[M-H]^{-1}$
Ester	Isopropyl 6-O-(4-carboxy-3-hydroxy-3-methylbutanoyl) -β-D-glucopyranoside	$C_{15}H_{26}O_{10}$	-0.57	365.1451	4.718	$[M-H]^{-1}$
Fatty acid	(+/-)9,10-dihydroxy-12Z-octadecenoic acid	$C_{18}H_{34}O_4$	-0.39	313.2383	10.945	$[M-H]^{-1}$
Fatty acid	(15Z)-9,12,13-Trihydroxy-15-octadecenoic acid	$C_{18}H_{34}O_5$	-0.40	329.2332	9.110	$[M-H]^{-1}$
Fatty acid	13-Hydroxy-9,11,15-octadecatrienoic acid	$C_{18}H_{30}O_3$	-1.14	293.2119	11.492	$[M-H]^{-1}$
Fatty acid	16-Hydroxyhexadecanoic acid	$C_{16}H_{32}O_3$	-1.21	271.2275	14.435	$[M-H]^{-1}$
Fatty acid	Corchorifatty acid F	$C_{18}H_{32}O_5$	-0.42	327.2176	8.111	$[M-H]^{-1}$
Flavonoid	Orientin	$C_{21}H_{20}O_{11}$	-0.94	447.0929	5.133	$[M-H]^{-1}$
Phenolic	5-(beta-D-glucopyranosyloxy)-2-hydroxybenzoic acid	$C_{13}H_{16}O_9$	-0.81	315.0719	3.387	$[M-H]^{-1}$
Terpenoid	2-[(2S,4aR,8aS)-2-hydroxy-4a-methyl-8-methylidene-3,4,5,6,7,8a-hexahydro-1H-naphthalen-2-yl]prop-2-enoic acid	$C_{15}H_{22}O_3$	-1.55	249.1492	13.164	$[M-H]^{-1}$

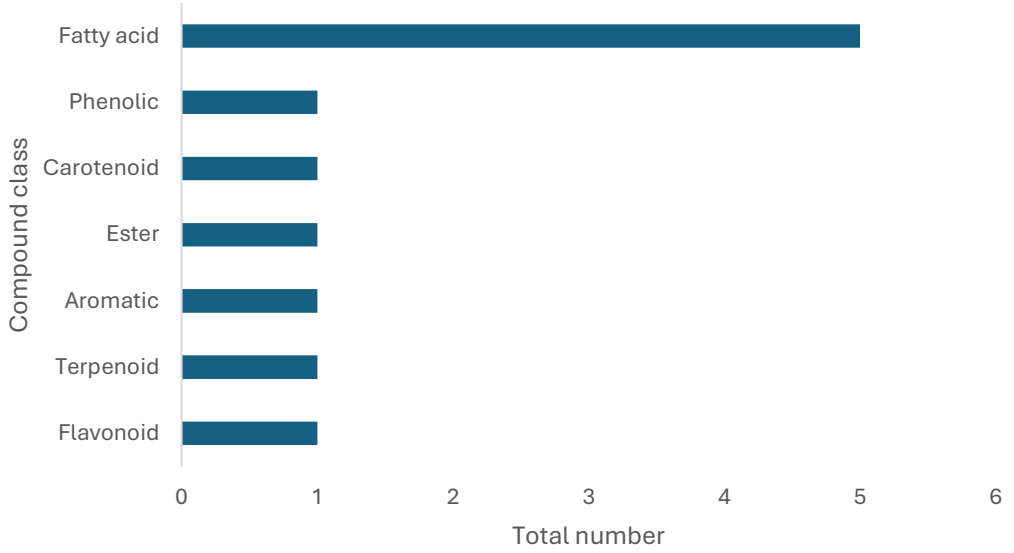

Figure 4. The summary of the compound class detected in the negative mode of LC-q-Orbitrap-MS analysis

tuberculosis (Askun, 2023). Additionally, metabolites such as vicenin-2 (15), corymboside (16), and schaftoside (19) have been reported to exhibit a wide range of biological activities, including antioxidant, anti-inflammatory, and wound-healing properties (Nagaprashantha *et al.,* 2011; Islam *et al.,* 2014; Kang *et al.,* 2015; Kim *et al.,* 2018, 2024; Povetyeva *et al.,* 2023).

Overall, the extract of *M. loriformis* exhibits promising dual bioactivity against both *Vibrio* species and the parasitic marine leech *P. arugamensis,* indicating its potential application in aquaculture for disease management. The extracts' bactericidal and antiparasitic effects were notably concentration dependent, with higher doses producing greater inhibition zones and faster mortality rates. Metabolite analysis indicated diverse groups of metabolites, known for their antimicrobial, antiparasitic, antioxidant, and anti-inflammatory activities.

Conclusion

The present study highlights the potential of *M. loriformis* aqueous extract as a dual-function natural agent exhibiting both bactericidal activity against *Vibrio* species and antiparasitic effects against the marine parasitic leech *P. arugamensis.* The bioactivities observed were clearly concentration dependent, with high concentrations producing significantly larger inhibition zones and faster parasitic mortality rates. These effects might be attributed to the rich profile of bioactive metabolites identified through LC-q-Orbitrap-MS analysis,

including alkaloids, terpenoids, and flavonoids such as trigonelline and orientin metabolites, previously reported for their antibacterial, antioxidant, and antiparasitic properties. These findings support the ethnopharmacological relevance of *M. loriformis* extract and propose potential use as a natural, eco-friendly alternative to synthetic chemicals in aquaculture, particularly for the management of bacterial infection and parasitic infestation. Additionally, for its practical application, further investigations are needed to determine optimal dosage formulation, evaluate long-term safety in the aquatic environment, and assess cost-effectiveness and environmental sustainability.

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