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Phytochemical compositions and potential application of *Zingiber officinale* extract against *Vibrio parahaemolyticus* causing AHPND in *Liptopenaeus vannamei*

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ABSTRACT:

This study investigated the phytochemical composition and antibacterial activity of *Zingiber officinale* rhizome extracts obtained using solvents of different polarities (methanol, ethyl acetate, chloroform, dichloromethane, and n-hexane). GC-MS identified 65 volatile and semi-volatile compounds across the five solvent extracts, with methanol providing the broadest chemical coverage. Phenolics and sesquiterpenoid-related compounds were the dominant classes, and gingerol was consistently detected as a major constituent across solvent systems. The antibacterial activity of *Z. officinale* methanol extracts was evaluated against *Vibrio parahaemolyticus* using agar well diffusion and resazurin-based microdilution assays. The methanolic extract showed a clear concentration-dependent antibacterial effect against *V. parahaemolyticus*, with inhibition zones increasing to 19.87 ± 0.29 mm at 500 mg/mL after 48 h. Both MIC and MBC were 7.81 mg/mL, indicating bactericidal activity under the tested conditions. The above data illustrate that the potential of ginger-derived extracts as candidates for developing herbal preparations for aquaculture biosecurity, especially, controlling the Vibrio diseases in shrimps as well as contributing to reduce antibiotic reliance and improving sustainability in shrimp aquaculture in Vietnam and worldwide.

KEY WORDS: Zingiber officinale, Vibrio parahaemolyticus, AHPND, Litopenaeus vannamei, antimicrobial activity

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INTRODUCTION

Aquaculture, particularly shrimp farming, is a key contributor to Vietnam's economic growth, providing employment opportunities and generating substantial export revenues (Van Nguyen et al., 2021). Among cultured shrimp species, white-leg shrimp (*Litopenaeus vannamei*) has become the dominant farmed species. White shrimp (*Litopenaeus vannamei*) were introduced into Vietnam in the early 2000s, after which their cultivation rapidly expanded, particularly in the central provinces. This species is predominantly farmed under intensive culture systems characterized by small pond sizes, high stocking densities, and the use of formulated pelleted feeds. The popularity of white shrimp farming is attributed to its short culture cycle, high productivity, and relatively lower risks of disease outbreaks and economic losses compared with traditional shrimp species (Lan, 2013, Hai et al., 2015).

According to the Vietnam Fisheries Surveillance Department, nearly 711,000 ha of brackish-water shrimp ponds were farmed nationwide during the first eight months of 2025, of which approximately 106,500 ha were devoted to white-leg shrimp (VASEP,

2025c). In 2025, Vietnam's shrimp export value exceeded USD 4.6 billion, with white-leg shrimp accounting for more than USD 2.98 billion, representing approximately 65% of total shrimp export revenue (VASEP, 2025b). Despite this rapid expansion, the shrimp farming sector continues to face significant challenges, particularly disease outbreaks that threaten production efficiency and long-term sustainability.

Aquatic diseases remain a persistent and complex risk in shrimp aquaculture. Since its first report in China in 2009 (ASIA, 2020), acute hepatopancreatic necrosis disease (AHPND) has rapidly spread to major shrimp-producing countries, including Vietnam, AHPND remains one of the most prevalent and destructive shrimp diseases, affecting thousands of hectares of farming areas annually and causing substantial economic losses to farmers (FAO, 2013). Alongside AHPND, other diseases such as white spot syndrome disease, enterocytozoon hepatopenaei (EHP), and white feces syndrome continue to occur sporadically in many farming regions (VASEP, 2025c). Among these, AHPND is considered one of the most severe, primarily caused by *Vibrio parahaemolyticus*, which induces extensive hepatopancreatic tissue damage and mass

In Vietnam, one of the world's leading shrimp producers, the intensification of aquaculture has been accompanied by widespread use of antimicrobial agents to control bacterial diseases, particularly vibriosis (Vo et al., 2025). Previous studies have documented the frequent application of several antibiotic classes in shrimp farming, including tetracyclines (e.g., oxytetracycline), sulfonamides (e.g., sulfamethoxazole), and fluoroquinolones (e.g., ciprofloxacin and enrofloxacin), often without veterinary supervision (Thuy et al., 2011, Chi et al., 2017, Carrique-Mas et al., 2023). Such extensive and sometimes unregulated use of antibiotics exerts strong selective pressure, promoting the emergence and dissemination of antimicrobial resistance genes in aquaculture environments, while also leading to repeated rejections of exported shrimp consignments due to antibiotic residues exceeding permissible limits (Loc, 2003, Khuu, 2019, Quyen et al., 2020). Under mounting pressures from environmental degradation, disease outbreaks, rising production costs, and strict export regulations, the adoption of sustainable and antibiotic-free farming strategies has become a critical requirement for maintaining the competitiveness and long-term growth of Vietnam's shrimp industry (VASEP, 2025a).

Consequently, herbal extracts and natural bioactive compounds have attracted increasing attention as potential antibacterial agents in Vietnamese aquaculture (Linh et al., 2025). Plant-derived extracts are rich in biologically active constituents, including alkaloids, phenolics, flavonoids, and terpenoids (Awuchi, 2019). These compounds not only inhibit pathogenic microorganisms but also serve as growth promoters and immunostimulants, thereby improving the survival rates of aquatic organisms (Chang, 2000, Citarasu, 2010). Moreover, medicinal plants are generally low-cost, locally available, easy to process, biodegradable, and environmentally friendly (Punitha et al., 2008).

Among these potential botanicals, ginger (*Zingiber officinale* Roscoe) is a widely cultivated tropical species, including in Vietnam, and has long been used in traditional medicine. Ginger rhizomes are rich in bioactive compounds such as monoterpenes, sesquiterpenes, phenolic derivatives, aldehydes, and ketones, which confer diverse biological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer effects (Alfuraydi et al., 2024). Several in vitro studies have reported the inhibitory effects of ginger extracts against various Vibrio species (Aminzare et al., 2018, Ekesiobi et al., 2025). However, in Vietnam, studies focusing on the chemical composition and antibacterial efficacy of *Z. officinale* extracts against *V. parahaemolyticus*, the causative agent of AHPND in shrimp, remain limited, and existing research has largely focused on ginger essential oils rather than solvent-based extracts (Huong et al., 2022, Tran et al., 2023, Phuong et al., 2025, Chen et al., 2025). Therefore, this study was conducted to characterize the phytochemical profiles of *Z. officinale* extracts via Gas Chromatography-Mass Spectrometry (GC-MS) and to evaluate their antibacterial activity against *V. parahaemolyticus* strains isolated from cultured white-leg shrimp. The findings aim to provide a scientific foundation for the application of ginger extracts as a safe and sustainable biological solution for disease control in shrimp aquaculture in Vietnam.

MATERIALS AND METHODS

Sample preparation

Zingiber officinale rhizomes were purchased from Ngoc Chi Market, Vinh Thanh Commune, Hanoi City, Vietnam, in June 2025. The samples were thoroughly washed with tap water and then oven-dried at 40°C until a constant mass was reached, ensuring the moisture content remained below 10%. The dried samples were ground into a fine powder using a QE-500 blender (Vietnam) to a particle size of < 100 mesh. The resulting powder was stored in polyethylene zip-lock bags and kept in a sealed plastic container at room temperature under dry, dark conditions until use.

Phytochemical determination

The extraction was performed using solvents of different polarities, including methanol, chloroform, dichloromethane, n-hexane, and ethyl acetate. Approximately 10 g of *Zingiber officinale* rhizome powder was soaked separately in 100 mL of each solvent for 48 h. The mixture was then processed in an ultrasonic bath at a frequency of 20 kHz for 60 minutes, maintaining the temperature below 50 °C. The resulting extract was cooled to room temperature and centrifuged to separate the liquid phase. The

supernatant was filtered through Whatman No. 1 filter paper, followed by filtration through a 0.22 µm syringe membrane prior to GC-MS analysis.

Chemical profiling was conducted using a Gas Chromatography system (Agilent GC 7890B) coupled with Mass Spectrometry detector (Agilent MS 5977A). Chromatographic separation was performed on an Agilent DB-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The injector temperature was set at 280 ºC with a split ratio of 100:1. High-purity helium served as the carrier gas at a constant flow rate of 1 mL/min. The oven temperature program was initiated at 40 °C, increased to 100 °C at 5 ºC/min, then ramped to 300 °C at 8 °C/min, and held isothermally for 2 min. The mass spectrometer was operated in electron impact (EI) mode at 70 eV. The ion source temperature was set to 230 °C, and the mass-to-charge (m/z) scan range was 35-450 amu. Compound identification was conducted by comparing the obtained mass spectra with the NIST11 standard reference database.

Anti-bacterial activity determination

Preparation of Zingiber officinale extracts

Approximately 200 g of *Zingiber officinale* powder was macerated in 2 L of methanol for 48 h at 28 °C with intermittent stirring (2-3 times/day). The mixture was then subjected to ultrasonication for 60 min and filtered through 11 µm Whatman filter paper (UK). The extraction procedure was repeated three times (n = 3) under identical conditions. The combined filtrates were concentrated under reduced pressure using a rotary evaporator (R-210, Büchi, Switzerland) to remove the solvent. The resulting crude extract was further dried in an oven at 40 °C (Memmert UN110, Germany) for 48 h to obtain the final extract, which was used for antibacterial activity assays.

Preparation of Vibrio parahaemolyticus

V. parahaemolyticus used in this study was isolated from AHPND-infected *Litopenaeus vannamei* collected from a shrimp farm in Giao Long commune, Giao Thuy district, Nam Dinh province, Vietnam. The isolate was cultured on tryptic soy broth agar (TSB) supplemented with 1.5% NaCl and on thiosulfate citrate bile salts sucrose (TCBS) agar, followed by incubation at 28-30 °C for 24 h. Colony morphology, color characteristics, and Gram staining were examined to confirm bacterial purity. A single typical colony was inoculated into 5 mL of TCBS broth containing 1.5% NaCl and incubated at 28-30 °C for 6 h, then transferred to 100 mL of the same medium and further incubated for 24 h. Bacterial cells were harvested by centrifugation at 4.500 rpm for 5 min at 5 °C and resuspended in sterile 1.5% NaCl solution. Bacterial density was adjusted to approximately 10^9 CFU/mL based on optical density at 600 nm (OD_{600} = 1.0 ± 0.02) and confirmed by plate counting on TCBS agar.

Anti-bacterial zone diameter determination

Approximately 500 mg of *Zingiber officinale* extract (Section 2.3.1) was dissolved in 1 mL of DMSO to obtain a stock solution at 500 mg/mL. The stock was subsequently serially diluted with DMSO to prepare concentrations of 400, 300, 200, 100, 50, 25, 10, 5, and 1 mg/mL. A bacterial suspension (10^9 CFU/mL) was serially diluted with sterile 0.85% NaCl to obtain a final concentration of 10^6 CFU/mL. An aliquot of 100 µL of the standardized suspension was spread onto TCBS agar plates. After 10-15 min, wells (8 mm diameter) were punched aseptically and filled with 100 µL of *Zingiber officinale* extracts at concentrations ranging from 1 to 500 mg/mL. Doxycycline (30 µg/mL) and dimethyl sulfoxide (DMSO) were used as positive and negative controls, respectively. Plates were incubated at 28-30 °C for 24 h and 48 h, and inhibition zones were measured in millimeters to evaluate antibacterial activity against *V. parahaemolyticus*.

Determination of Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC and MBC of *Zingiber officinale* extract were determined using a broth microdilution assay in 96-well microplates, following the method described by Anja Klančnik et al. (2010) with minor modifications (Klančnik et al., 2010). The materials used in this assay included *Z. officinale* extract (500 mg/mL), doxycycline (30 µg/mL) as a positive control, dimethyl sulfoxide (DMSO), sterile distilled water as negative controls, *V. parahaemolyticus* suspension (10^6 CFU/mL), tryptic soy broth (TSB), and resazurin indicator solution. Initially, 100 µL of sterile TSB was dispensed into each well of the microplate. Next, 100 µL of *Z. officinale* extract, doxycycline, DMSO, or sterile water was added to the first well of each row (A-H) and mixed thoroughly. Then, 100 µL of the mixture from the first well was transferred to the next well containing 100 µL TSB to achive a twofold dilution, mixed, and the procedure was repeated sequentially until the twelfth well. Finally, 100 µL was discarded from the twelfth well so that each well contained 100 µL prior to inoculation. Thereafter, 100 µL of *V. parahaemolyticus* suspension (10^6 CFU/mL) was added to each well, and the plates were incubated at 28-30 °C for 16-18 h. Following incubation, 40 µL of 0.01% (w/v) resazurin solution was added to each well to assess bacterial viability. The MIC was defined as the lowest concentration at which no color change of the resazurin indicator (blue color retained) was observed, indicating inhibition of bacterial growth. To determine the MBC, 100 µL from wells showing no color change was spread onto Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar plates and incubated at 28-30 °C. The MBC was identified as the lowest concentration at which no bacterial colonies were observed on the agar plates. All experiments were conducted in triplicate, and mean values were calculated to evaluate the antibacterial activity of the *Z. officinale* extract.

Statistical analysis

All experiments were conducted in triplicate, and data were expressed as mean ± standard deviation (SD). Statistical differences among treatments were analyzed using one-way analysis of variance (ANOVA), followed by the least significant difference (LSD) post hoc test. Differences were considered statistically significant at $p \leq 0.05$.

RESULTS

Phytochemical Profile by GC-MS Analysis of Z. officinale Extracts

In this study, solvents with different polarities were employed to extract bioactive compounds from *Zingiber officinale*. The extraction polarity followed the order: methanol > ethyl acetate > chloroform ≈ dichloromethane > n-hexane, and the number of detected compounds tended to increase with solvent polarity. This trend is expected because solvent polarity governs solubilization of oxygenated phenolics and related semi-volatile constituents in ginger, thereby enriching extracts for GC-MS components (Jaapar et al., 2017, Gonzalez-Gonzalez et al., 2023). In addition, ultrasound-assisted extraction is known to enchance mass transfer and improve recovery of key ginger bioactive, which explain the higher chemical diversity observed in the more polar extracts.

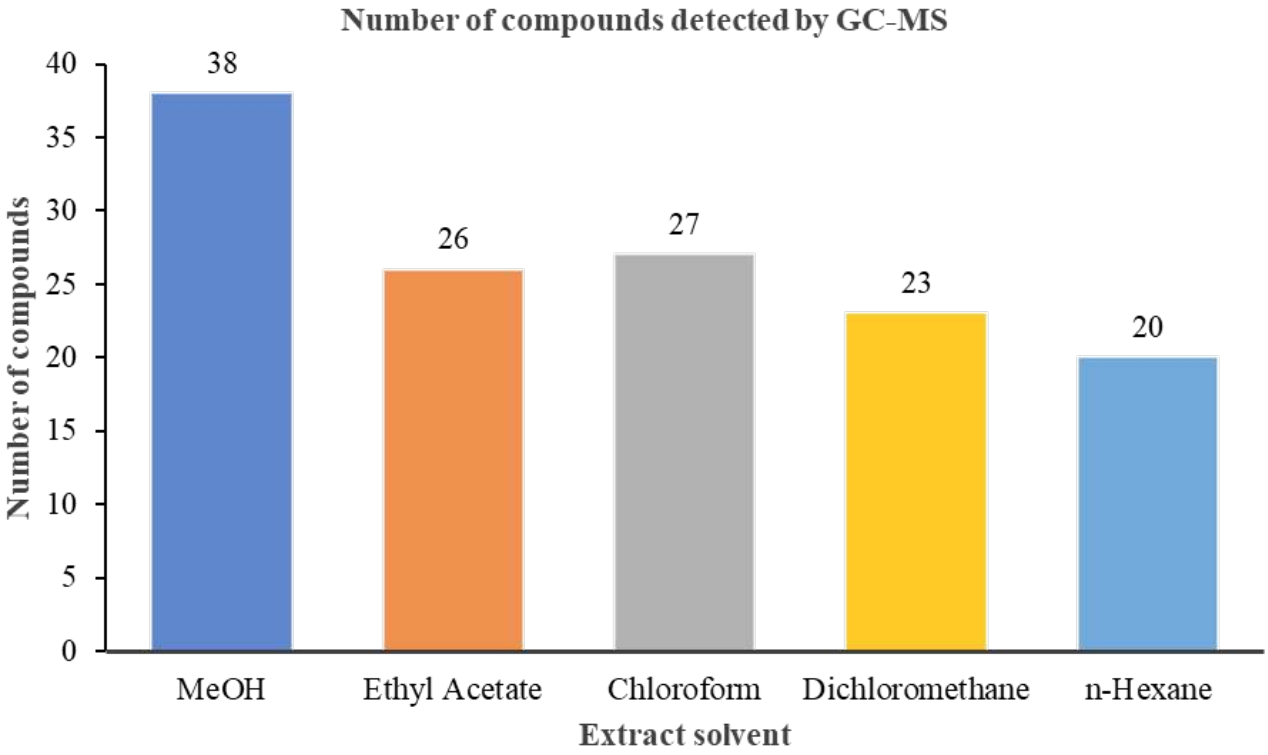

Fig. 1. Total number of chemical constituents identified in *Z. officinale* extracts using various extraction solvents

As shown in Fig. 1, volatile and semi-volatile compounds were tentatively identified in all solvent extracts. Methanol exhibited the highest extraction chemical coverage, with a total of 38 compounds detected, while chloroform ranked second with 27 compounds. These were followed by ethyl acetate, dichloromethane, and *n*-hexane, with 26, 23, and 20 compounds detected, respectively. Overall, these findings indicate that solvent polarity strongly influenced the phytochemical yield and chemical diversity of *Z. officinale* extracts.

Bioactive Constituents of Z. officinale Extracts

The identified compounds were classified into multiple groups, including aldehydes, alkaloids, aminophenols, diterpenoids, esters, fatty acids, hydrocarbons, ketones, monoterpenes, monoterpenoids, phenolics, sesquiterpenes, sesquiterpenoids, terpenes, and terpenoids. Overall, the profile of *Z. officinale* extracts was dominated by phenolic compounds, sesquiterpenoids, aldehydes, and terpenes (Table 1, Fig. 2), although their relative abundances varied across solvents.

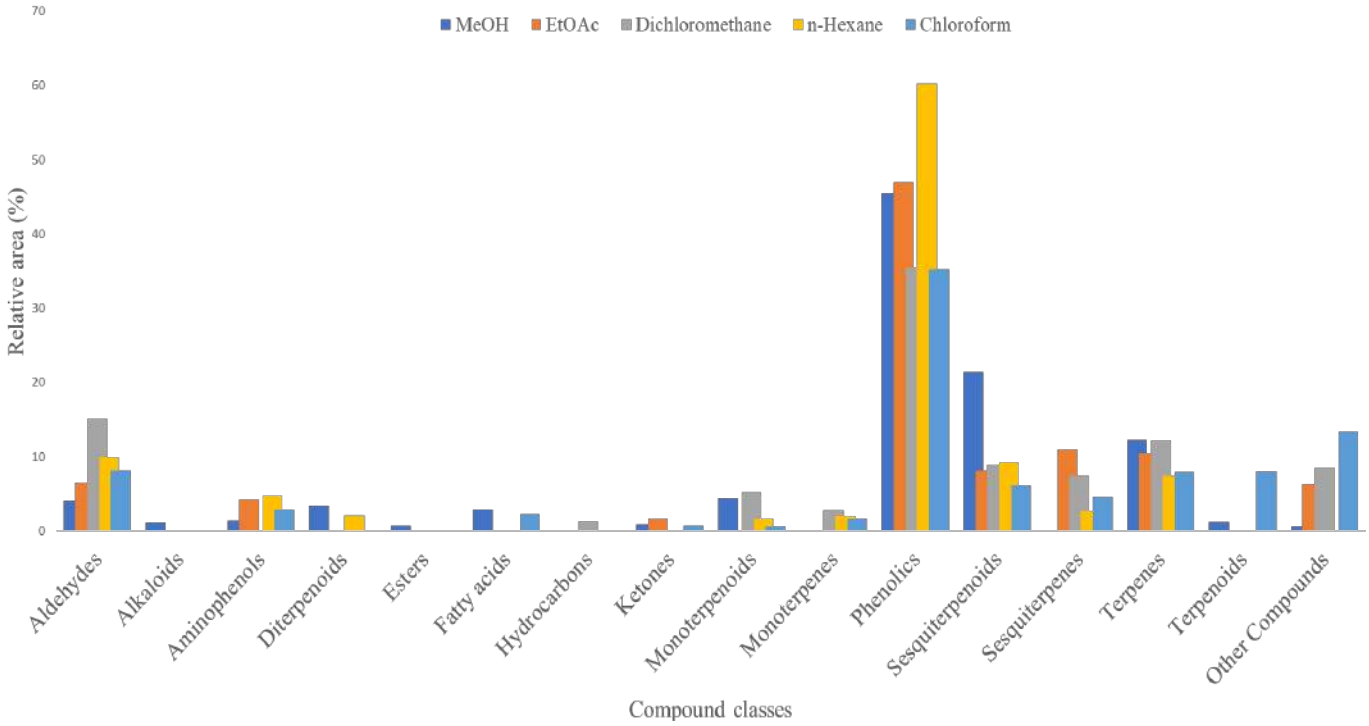

Fig. 2. Relative area (%) of compound classes in *Zingiber officinale* extracts obtained using different solvents

GC-MS analysis identified a total of 65 compounds across five solvents of different polarities, indicating a solvent-dependent phytochemical composition. These compounds were classified into major classes including phenolics, sesquiterpenoids/sesquiterpenes, aldehydes, terpenes, monoterpenoids, diterpenoids, fatty acids, aminophenols, and alkaloids. The observed variation in both composition and abundance reflects the influence of solvent polarity on extraction selectivity.

Phenolic compounds and sesquiterpenoid-related constituents were the dominant groups in the extracts. The phenolic class was particularly prominent, exhibiting both a broad distribution across solvents and high relative abundance. Major phenolics included eugenol; butan-2-one, 4-(3-hydroxy-2-methoxyphenyl)-; 2-butanone, 4-(4-hydroxy-3-methoxyphenyl)-; phenol, 4-ethyl-2-methoxy-; propan-2-one, 1-(4-isopropoxy-3-methoxyphenyl)-; and gingerol. Notably, gingerol exhibited the highest relative abundance across all extracts, reaching 22.14% in methanol, 20.06% in *n*-hexane, and 15.76% in dichloromethane. In addition, 2-butanone, 4-(4-hydroxy-3-methoxyphenyl)- also showed relatively high area percentages across multiple solvents.

Several sesquiterpenoid and terpene-related compounds were consistently detected across all solvent systems, including hexanal, decanal, 1,3-cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-, and cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-. Their consistent presence and moderate-to-high relative abundance suggest that these compounds are structurally stable constituents of ginger rhizomes.

The distribution of minor phytochemical classes was strongly solvent-specific. Hydrocarbons were detected exclusively in the dichloromethane extract, whereas esters and alkaloids were only observed in methanolic extract. Sesquiterpenes were not detected from the methanol extract, while monoterpenoids were not detected in the ethyl acetate extract. Other classes, including aminophenols, diterpenoids, fatty acids, ketones, monoterpenes, and terpenoids, were unevenly distributed among the five solvents, further highlighting the selective nature of solvent extraction.

Overall, the phytochemical profile of *Z. officinale* was dominated by phenolics and sesquiterpenoid-related constituents, with gingerol being the most abundant compound across the extraction systems.

Table 1. GC-MS analysis of compounds identified in *Zingiber officinale* extracts using different solvents

No.	Compound name	Mol Weight (amu)	Formula	Area (%)					Classification
				MeOH	Ethyl acetate	DCM	Hexane	Chloroform	
1	Hexanal	100.089	$C_6H_{12}O$	2.45	2.51	6.99	3.72	3.15	Aldehyde
2	Camphene	136.125	$C_{10}H_{16}$	0.36	-	1.02	-	-	Monoterpenoid
3	Octanal	128.12	$C_8H_{16}O$	0.49	-	1.35	0.92	0.89	Aldehyde
4	β-Phellandrene	136.23	$C_{10}H_{16}$	-	0.85	1.64	-	0.81	Monoterpene
5	Eucalyptol	154.24	$C_{10}H_{18}O$	0.14	-	-	-	-	Terpenoid
6	Decanal	156.27	$C_{10}H_{20}O$	0.63	4.01	6.72	5.23	4.1	Aldehyde
7	endo-Borneol	154.25	$C_{10}H_{18}O$	0.46	-	-	-	-	Monoterpenoid
8	α-Terpineol	154.24	$C_{10}H_{18}O$	0.3	-	-	-	-	Monoterpenoid
9	Citronellol	156.26	$C_{10}H_{20}O$	0.16	-	-	-	-	Monoterpenoid
10	2-Undecanone	170.296	$C_{11}H_{22}O$	-	-	-	-	0.74	Ketone
11	Geraniol	154.25	$C_{10}H_{18}O$	1.08	-	-	1.71	-	Monoterpenoid
12	Citral	152.23	$C_{10}H_{16}O$	0.16	-	-	-	-	Monoterpenoid
13	β-ylangene	204.35	$C_{15}H_{24}$	0.26	-	-	-	-	Sesquiterpenoids
14	Eugenol	164.2	$C_{10}H_{12}O_2$	-	-	-	8.26	-	Phenolic
15	Geranyl acetate	196.28	$C_{12}H_{20}O_2$	1.06	-	-	-		Terpenoid
16	γ-Elemene	204.35	$C_{15}H_{24}$	0.44	-	-	-	-	Sesquiterpenoids
17	cis-β-Farnesene	204.35	$C_{15}H_{24}$	0.62	-	-	-	-	Sesquiterpenoids
18	Alloaromadendrene	204.35	$C_{15}H_{24}$	0.16	-	-	-	-	Sesquiterpenoids
19	α-Farnesene	204.35	$C_{15}H_{24}$	-	-	1.22	-	-	Sesquiterpenoids
20	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-	204.35	$C_{15}H_{24}$	17.26	4.64	6.33	4.45	4.49	Sesquiterpenoids
21	Cyclohexanemethanol, 4-ethenyl-α,α,4-trimethyl-3-(1-methylethenyl)-, [1R-(1α,3α,4β)]-	222.36	$C_{15}H_{26}O$	0.51	-	-	-	-	Sesquiterpenoids
22	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	202.172	$C_{15}H_{22}$	5.52	3.3	4.24	3.5	2.29	Terpenes
23	7-epi-cis-sesquisabinene hydrate	222.36	$C_{15}H_{26}O$	-	1.12	-	1.7	-	Sesquiterpenoids
24	3-Cyclohexene-1-methanol, .alpha.,4-dimethyl-.alpha.-(4-methyl-3-pentenyl)-, [R-(R*,R*)]-	222.37	$C_{15}H_{26}O$	-	0.79	-	1.01	1.01	Sesquiterpenoids
25	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane	222.37	$C_{15}H_{26}O$	0.88	-	-	-	-	Sesquiterpenoids
26	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	204.35	$C_{15}H_{24}$	6.25	4.06	4.93	3.95	3.32	Terpenes
27	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	222.37	$C_{15}H_{26}O$	0.57	-	-	-	-	Sesquiterpenoids
28	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	222.36	$C_{15}H_{26}O$	0.57	-	-	-	-	Monoterpenoid
29	Cyclopropanemethanol, .alpha.,2-dimethyl-2-(4-methyl-3-pentenyl)-, [1.alpha.(R*),2.alpha.]-	182.167	$C_{12}H_{26}O$	-	-	1.34	2.03	0.7	Sesquiterpenoids

30	3-Cyclohexene-1-methanol, α,4-dimethyl-α-(4-methyl-3-pentenyl)-, [R-(R*,R*)]-	222.36	$C_{15}H_{26}O$	0.48	-	-	-	-	Monoterpenoid
31	2-Butenoic acid, 3,7-dimethyl-6-octenyl ester	224.33	$C_{14}H_{24}O_2$	0.72	-	-	-	-	Esters
32	1,6,10,14-Hexadecatetraen-3-ol, 3,7,11,15-tetramethyl-, (E,E)-	290.261	$C_{20}H_{34}O$	-	1.55	-	-	-	Sesquiterpenoids
33	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	192.29	$C_{13}H_{20}O$	0.79	-	-	-	-	Sesquiterpenoids
34	α-Copaene	204.35	$C_{15}H_{24}$	-	7.4	-	-	-	Sesquiterpene
35	3,6-Dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran	152.23	$C_{10}H_{16}O$	0.87	-	-	-	0.62	Monoterpenoid
36	γ-Muurolene	204.35	$C_{15}H_{24}$	-	-	2.47	-	0.63	Sesquiterpene
37	Butan-2-one, 4-(3-hydroxy-2-methoxyphenyl)-	194.23	$C_{11}H_{14}O_3$	-	1.52	13.27	-	-	Phenolic
38	Squalene	410.391	$C_{30}H_{50}$	-	2.24	-	-	1.43	Terpenes
39	Spiro[4.5]dec-6-en-8-one, 1,7-dimethyl-4-(1-methylethyl)-	220.35	$C_{15}H_{24}O$	0.92	1.68	-	-	-	Ketone
40	2-Naphthalenemethanol, decahydro-α,α,4a-trimethyl-8-methylene-, [2R-(2α,4a.α,8a.β.)]-	222.36	$C_{15}H_{26}O$	0.76	-	-	-	-	Diterpenoid
41	β-Bisabolene	204.35	$C_{15}H_{24}$	-	1.31	2.1	1.34	2.21	Sesquiterpene
42	n-Hexadecanoic acid	256.42	$C_{16}H_{32}O_2$	1.57	-	-	-	2.3	Fatty acid
43	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	204.35	$C_{15}H_{24}$	-	-	1.26	-	0.72	Sesquiterpene
44	4-(2,2-Dimethyl-6-methylenecyclohexyl)butanal	194.31	$C_{13}H_{22}O$	0.48	-	-	-	-	Aldehyde
45	geranyl-p-cymene	270.235	$C_{18}H_{26}$	-	-	1.11	1.08	0.88	Monoterpene
46	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,3a.beta.,4.alpha.,7.beta.)]-	204.35	$C_{15}H_{24}$	-	1.2	1.66	1.31	1.11	Sesquiterpene
47	Geranyl-α-terpinene	272.25	$C_{20}H_{32}$	-	-	-	0.91	-	Monoterpene
48	(E,E,E)-3,7,11,15-Tetramethylhexadeca-1,3,6,10,14-pentaene	272.46	$C_{20}H_{32}$	2.57	-	-	2.13	-	Diterpenoid
49	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-	426.386	$C_{30}H_{50}O$	-	1.08	-	-	-	Terpenoid
50	Gingerol	294.38	$C_{17}H_{26}O_4$	22.14	9.61	15.76	20.06	1.69	Phenolic
51	1-Formyl-2,2,6-trimethyl-3-cis-(3-methylbut-2-enyl)-5-cyclohexene	220.183	$C_{15}H_{24}O$	-	-	4.2	-	-	Monoterpenoid

52	trans-Geranylgeraniol	290.261	$C_{20}H_{34}O$	-	2.74	-	-	-	Diterpenoid
53	2-Butanone, 4-(4-hydroxy-3-methoxyphenyl)-	194.22	$C_{11}H_{14}O_3$	23.4	15.45	-	20.31	16.69	Phenolic
54	Ketone, 1-cyclohexen-1-yl methyl, semicarbazone	181.122	$C_9H_{15}N_3O$	-	4.88	6.36		12.69	-
55	Ethanone, 2-(1H-imidazo[4,5-b]pyridin-2-yl)-1-(4-morpholyl)-	278.084	$C_{12}H_{14}N_4O_2S$	0.66	1.36	2.08	-	0.71	-
56	Phenol, 4-ethyl-2-methoxy-	152.084	$C_9H_{12}O_2$	-	12.6	-	-	13.69	Phenolic
57	Cyclohexane, 3,4-bis(1-methylethenyl)-1,1-dimethyl-	192.34	$C_{14}H_{24}$	-	1.04	-	-	-	Sesquiterpene
58	6-Amino-2,4-dimethylphenol	137.18	$C_8H_{11}NO$	1.44	4.24	-	4.72	2.89	Aminophenol
59	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	354.52	$C_{21}H_{38}O_4$	1.33	-	-	-	-	Fatty acid
60	Propan-2-one, 1-(4-isopropoxy-3-methoxyphenyl)-	222.126	$C_{13}H_{18}O_3$	-	7.84	6.55	11.66	3.13	Phenolic
61	17-Norcorynan, 16-(2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)-, [16(R),20β]-	438.27	$C_{29}H_{34}N_4$	1.09	-	-	-	-	Alkaloid
62	6-(3,5-Dimethyl-furan-2-yl)-6-methyl-hept-3-en-2-one	220.146	$C_{14}H_{20}O_2$	-	-	-	-	8.01	Terpenoid
63	Campesterol	400.68	$C_{28}H_{48}O$	0.44	-	-	-	-	Terpenes
64	1,19-Eicosadiene	278.297	$C_{20}H_{38}$	-	-	1.34	-	-	Hydrocarbons
65	β-Sitosterol	414.386	$C_{29}H_{50}O$	-	0.85	2.95	-	0.87	Terpenes

Antibacterial properties of Z. officinale

The antibacterial activity of the methanolic *Z. officinale* extract against AHPND-associated *V. parahaemolyticus* was assessed using agar well-diffusion inhibition zones and broth microdilution-derived MIC/MBC endpoints. In AHPND, virulence of *V. parahaemolyticus* is closely linked to plasmid-encoded PirA/PirB toxins; therefore, identifying non-antibiotic strategies that reduce pathogen burden is of direct relevance to shrimp health management (Xiao et al., 2017).

Table 2. Antibacterial inhibition zone of *Z. officinale* methanol extracts against *V. parahaemolyticus*

Concentration (mg/mL)		Antibacterial inhibition zone (mm)	
		24 h	48 h
C1	500	$20.22^{a} \pm 0.32$	$19.87^{a} \pm 0.29$
C2	400	$18.63^{b} \pm 0.30$	$18.34^{b} \pm 0.37$
C3	300	$18.01^{b,c} \pm 0.42$	$17.90^{b,c} \pm 0.37$
C4	200	$12.39^{d} \pm 0.35$	$12.08^{d} \pm 0.42$
C5	100	$11.23^{e} \pm 0.38$	$11.12^{e} \pm 0.38$
C6	50	$10.01^{f} \pm 0.45$	$9.82^{f} \pm 0.54$
C7	25	$9.05^{g} \pm 0.43$	$8.91^{g} \pm 0.41$
C8	10	$6.44^{h} \pm 0.47$	$6.38^{h} \pm 0.43$

C9	5	$3.51^{i} \pm 0.48$	$3.30^{i} \pm 0.36$
C10	1	0	0
C(-)	Negative control	0	0
C(+)	Positive control	$17.88^{c} \pm 0.18$	$17.36^{c} \pm 0.11$

Note: Values within the same column followed by different letters (a-i) indicate statistically significant differences at $p \leq 0.05$ according to the LSD test.

Overall, inhibition zone diameters increased with extract concentration, indicating a dose-dependent effect. At 48 h, zones were slightly smaller than at 24 h for most treatments, although the rank order remained unchanged. Fig. 3 shows the antibacterial inhibition zone of *Z. officinale* on the agar well diffusion at concentrations (5-500 mg/mL) whereas no inhibition on the growth of bacterial were observed at 1 mg/mL concentrations or in the negative control (DMSO).

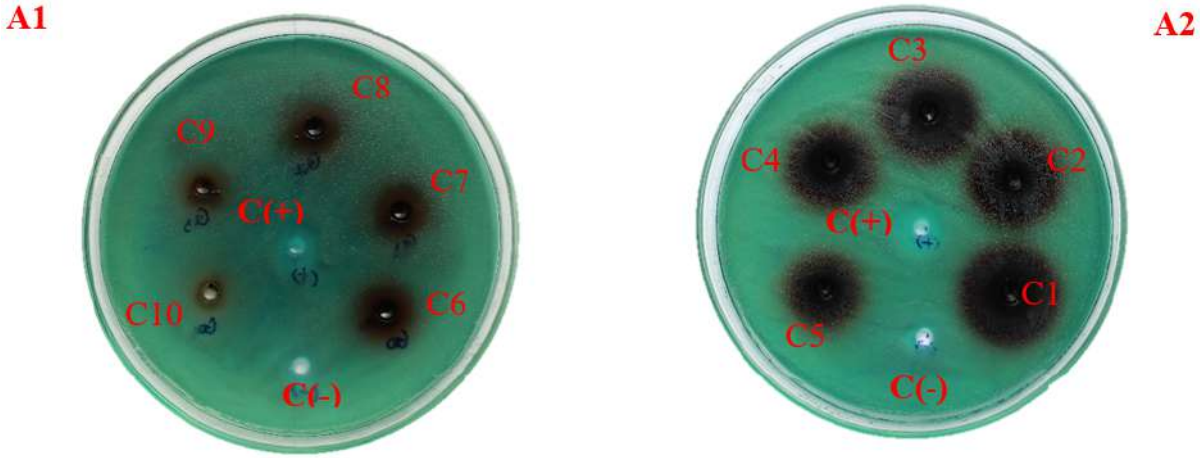

Fig. 3. Antibacterial inhibition zones of *Zingiber officinale* methanol extracts against *V. parahaemolyticus* after 24 h of incubation (A1, A2). Labels C1-C10 denote increasing extract concentrations ranging from 1 to 500 mg/mL; C(-) represents the negative control (DMSO), and C(+) represents the positive control (doxycycline, 30 µg/mL).

At 24 h, 500 mg/mL (C1) exhibited the largest inhibition zone (20.22 ± 0.32 mm), significantly greater than all other treatments ($p < 0.05$). Concentrations of 400 and 300 mg/mL (C2 and C3) also showed strong antibacterial activity (18.63 ± 0.30 mm and 18.01 ± 0.42 mm, respectively), with no significant difference between them ($p > 0.05$), both were lower than C1 but comparable to the positive control. A marked reduction in inhibition zones was observed at concentrations ≤ 200 mg/mL, supporting a concentration-dependent antibacterial effect of the extract.

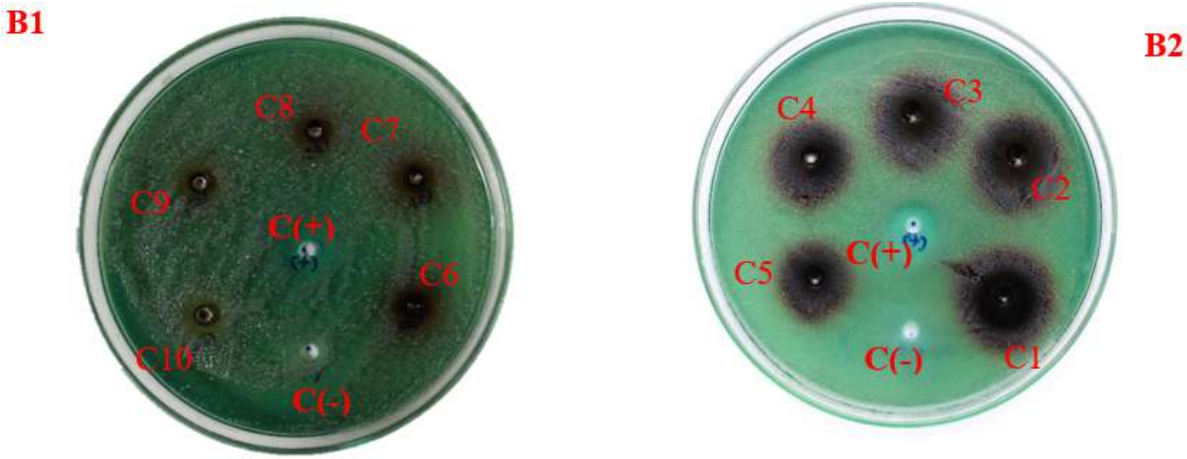

Fig. 4. Antibacterial inhibition zones of *Zingiber officinale* methanol extracts against *V. parahaemolyticus* after 48 h of incubation (B1, B2). Labels C1-C10 denote increasing extract concentrations ranging from 1 to 500 mg/mL; C(-) represents the negative control (DMSO), and C(+) represents the positive control (doxycycline, 30 µg/mL).

A similar trend was observed at 48 h, where the inhibition zones slightly decreased compared with those at 24 h; however, the ranking of treatments remained consistent. The extract at 500 mg/mL maintained the strongest inhibitory effect (19.87 ± 0.29 mm,

$p < 0.05$), significantly higher than all lower concentrations. Notably, the 300 mg/mL treatment showed inhibition comparable to the positive control ($p > 0.05$). Overall, the antibacterial activity of the methanolic *Z. officinale* extract against V. parahaemolyticus was primarily influenced by extract concentration rather than incubation duration.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Z. officinale extract

Fig. 5 illustrates the MIC assay results for methanolic *Zingiber officinale* extracts against *V. parahaemolyticus*, with sterile water, DMSO (negative control), and doxycycline (positive control). As shown in Fig. 5, the resazurin indicator displayed distinct color responses among treatments, reflecting differences in antibacterial activity. In wells containing sterile water (A1-A12 and B1-B12), the resazurin dye changed from blue to pink, indicating active bacterial metabolism and no inhibitory effect. Similar color changes were observed in the DMSO control wells (E2-E12 and F2-F12), indicating that DMSO at the tested concentration did not inhibit bacterial growth. Notably, wells E1 and F1 (undiluted/highest DMSO concentration) retainded the blue color, suggesting inhibitory effects at a high DMSO level. This interpretation is supported by reports showing that DMSO can modulate bacterial phenotypes (including biofilm formation) in a concentration-dependent manner, underscoring the importance of reporting and interpreting solvent controls based on working (diluted) solvent levels (Summer et al., 2022). Therefore, solvent-control interpretation was based on the diluted wells (E2-E12 and F2-F12). In contrast, wells treated with doxycycline (C8-C12 and D8-D12) retained the blue color up to defined dilution levels, indicating effective inhibition of *V. parahaemolyticus*.

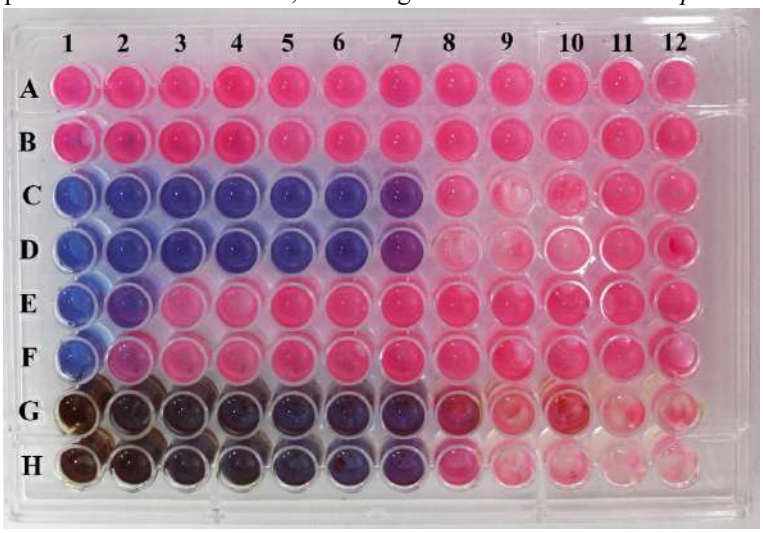

Fig. 5. The MIC of *Z. officinale* extracts against *Vibrio parahaemolyticus*, in comparison with sterile water, doxycycline, and DMSO. Wells A1-A12 and B1-B12 represent sterile water (negative control); wells C1-C12 and D1-D12 represent doxycycline (positive control); wells E1-E12 and F1-F12 represent DMSO (solvent control); wells G1-G12 and H1-H12 represent serial dilutions of the methanolic *Z. officinale* extract.

Notably, wells treated with *Z. officinale* extracts (G8-G12 and H8-H12) also retained the blue color at lower dilution levels, indicating a clear inhibitory effect on bacterial growth. The MIC was defined as the lowest concentration at which no color change of resazurin was observed. The MIC and MBC of *Z. officinale* extract against *V. parahaemolyticus* were both 7.81 mg/mL, whereas the MIC and MBC values of doxycycline against *V. parahaemolyticus* were 0.47 µg/mL. Resazurin serves as a metabolic-viability indicator, and MBC confirmation by plating from non-growth wells strengthens the inference that growth inhibition translated into bactericidal activity under the tested conditions (Elshikh et al., 2016). These findings indicate that the methanolic *Z. officinale* extract exhibited bactericidal activity against AHPND-associated *V. parahaemolyticus* under the tested conditions.

DISCUSSION

Zingiber officinale has been used as spice and as natural additives, flavoring agent and herbal remedy for cold, throat and chest infections and cough, weight loss, cold treatment, antiemetic effects, phlegm elimination, coughing relief and other health benefits properties (Sharma et al., 2016, Li et al., 2019, Raharjo et al., 2025). Previous studies have reported that ginger contains diverse phytochemical constituents, including terpenoids, phenolic compounds, carbohydrates, and lipids, which contribute to its biological activities (Sharma et al., 2016). In particular, ginger-derived compounds have been associated with antioxidant, anti-inflammatory, antimicrobial, antiemetic, and other health-related effects (Li et al., 2019). These properties support the potential use of ginger as a source of natural bioactive compounds for disease control applications.

The present study demonstrated that the phytochemical composition of *Z. officinale* extracts was strongly influenced by extraction solvent polarity. Among the tested solvents, methanol provided the broadest chemical coverage, followed by chloroform, ethyl acetate, dichloromethane, and *n*-hexane. This pattern suggests that polar organic solvents are more effective in extracting

oxygenated and semi-polar bioactive constituents from ginger. The greater chemical diversity observed in the methanolic extract may also be related to the use of ultrasound-assisted extraction, which can improve mass transfer and enhance the recovery of gingerols, shogaols, and other phytochemicals from ginger matrices (Jaapar et al., 2017, Gonzalez-Gonzalez et al., 2023).

Our findings are consistent with previous reports describing the rich phytochemical diversity of ginger. Bhargava et al. (2012) identified 40 and 32 compounds in methanolic and ethanolic extracts of *Z. officinale*, respectively, and reported the presence of alkaloids, saponins, tannins, and flavonoids (Bhargava et al., 2012). Similarly, Amir et al. (2011) reported that *Z. officinale* is a potent source of flavonoids and phenolic compounds (Amir et al., 2011). Chinonye et al. (2016) identified major compounds in the rhizome of *Z. officinale* using GC-MS analysis (Chinonye et al., 2016), whereas Sharma and Kumar reported phytochemical constituents and antioxidant activity in ginger rhizomes (Sharma and Kumar, 2018). Sanusi et al. (2019) and Karpagapandi & Sultana (2021) further confirmed the phytochemical richness of ginger extracts obtained under different extraction conditions (Sanusi et al., 2019, Karpagapandi and Sultana, 2021). More recent studies by El-Gohary et al. (2024) and Sulieman et al. (2024) also showed that the number and type of compounds detected in *Z. officinale* extracts can vary depending on extraction method, solvent type, sample origin, and analytical conditions (El-Gohary et al., 2024, Sulieman et al., 2024). Therefore, the differences between the present results and previous studies may be attributed to solvent polarity, extraction conditions, geographical origin of the rhizomes, post-harvest handling, drying temperature, matrix moisture, and GC-MS sensitivity.

In the present study, phenolic compounds and sesquiterpenoid-related constituents were the dominant groups in *Z. officinale* extracts, with gingerol being one of the most abundant compounds across extraction systems. Gingerols are recognized as major bioactive constituents of ginger and have been widely associated with the medicinal value of this plant (Li et al., 2019). Zingerone and other phenolic derivatives identified in the extracts may also contribute to the overall biological activity of ginger (Raharjo et al., 2025). In addition, terpene- and sesquiterpenoid-related compounds detected in this study are consistent with previous reports on the chemical composition and antimicrobial potential of ginger rhizomes and ginger essential oils (Sharma et al., 2016). These findings suggest that the antibacterial activity observed in the present study may be associated with the combined presence of phenolics, terpenoids, and sesquiterpenoid-related compounds rather than a single compound alone.

The agar well diffusion assay showed that the methanolic *Z. officinale* extract exerted a concentration-dependent antibacterial effect against AHPND-associated *V. parahaemolyticus*. Higher extract concentrations produced larger inhibition zones, while the negative control showed no inhibition. Although the inhibition zones at 48 h were slightly smaller than those at 24 h, the overall treatment ranking remained unchanged, suggesting that the antibacterial effect was mainly concentration-dependent. However, inhibition zones from agar diffusion assays are influenced by diffusion kinetics, compound solubility, and matrix effects. Therefore, similar inhibition zone diameters between crude plant extracts and conventional antibiotics do not necessarily indicate equivalent intrinsic antibacterial potency (Klančnik et al., 2010, Othman et al., 2011, Bubonja-Šonje et al., 2020).

The antibacterial activity observed in this study is generally consistent with previous studies on ginger extracts and related plant-derived antimicrobial agents. Baskaran Subramani reported concentration-dependent antibacterial activity of ethanolic *Z. officinale* extracts, including activity against *V. parahaemolyticus* (Subramani and Baradwaj, 2016). Evbuomwan Lucky et al. showed that *Z. officinale* extracts exhibited antibacterial activity against multidrug-resistant microbial isolates, with differences in activity depending on solvent type and tested microorganisms (Lucky et al., 2017). Elmowalid et al. reported that ginger extracts modulated immune responses and enhanced clearance of multidrug-resistant *Escherichia coli* in broiler chicks, further supporting the antibacterial relevance of ginger-derived compounds (Elmowalid et al., 2019). Sanusi et al. also documented antibacterial activities of ginger extracts collected from different locations, emphasizing the influence of extraction and sample conditions on antimicrobial performance (Sanusi et al., 2019). In addition, Anthony Ekesiobi et al. demonstrated that *Z. officinale* extract showed inhibitory activity against *Vibrio cholerae* strains, supporting the broader anti-*Vibrio* potential of ginger-derived preparations (Ekesiobi et al., 2025).

The MIC and MBC values obtained in the present study further confirmed the antibacterial effect of the methanolic *Z. officinale* extract against *V. parahaemolyticus*. The MIC and MBC were both 7.81 mg/mL, indicating bactericidal activity under the tested conditions. The identical MIC and MBC values suggest that the extract not only inhibited bacterial growth but also killed the bacteria at the same concentration. This finding is consistent with the concentration-dependent inhibition observed in the agar well diffusion assay. Previous studies have also reported MIC and MBC values for ginger extracts against different bacterial species, although values vary considerably depending on solvent type, extraction method, bacterial strain, inoculum density, and assay conditions (Subramani and Baradwaj, 2016, Lucky et al., 2017, Elmowalid et al., 2019, Hossain et al., 2020, Dharmapala and Amarakoon, 2024). These variations highlight the need for standardized antimicrobial testing and careful reporting of assay conditions when comparing plant extract activities across studies.

The interpretation of MIC and MBC results also requires consideration of solvent effects. In this study, DMSO was used as the solvent control. The observation that the highest DMSO concentration retained the blue color suggests that concentrated DMSO may exert inhibitory effects, whereas diluted DMSO wells did not inhibit bacterial growth. This is consistent with reports showing that DMSO can influence bacterial phenotypes in a concentration-dependent manner (Summer et al., 2022). Therefore, interpretation

of solvent controls should be based on the final working concentration of DMSO in assay wells, and future studies should clearly report the final DMSO percentage used in antibacterial assays.

The relevance of this study is strengthened by the role of *V. parahaemolyticus* in AHPND. AHPND-associated virulence is closely related to plasmid-encoded PirAB toxins in *Vibrio* species (Xiao et al., 2017). Therefore, identifying natural agents capable of reducing *V. parahaemolyticus* burden may contribute to more sustainable disease management strategies in shrimp aquaculture. Previous research has shown that ginger and its component shogaol can inhibit *Vibrio* biofilm formation and orally protect shrimp against AHPND, supporting the translational relevance of ginger-based interventions in shrimp health management (Soowannayan et al., 2019). Together with the present findings, this evidence suggests that *Z. officinale* extract may be a promising candidate for further development as a natural antibacterial preparation for controlling *Vibrio*-associated diseases in *Litopenaeus vannamei*.

Overall, the present study shows that methanolic extraction yielded a chemically rich *Z. officinale* extract with notable antibacterial activity against AHPND-associated *V. parahaemolyticus*. The results highlight the importance of solvent polarity in shaping phytochemical composition and antibacterial potency. However, this study was conducted under in vitro conditions; therefore, further studies are required to evaluate safety, optimize formulation and dosing, determine effective delivery methods, and validate efficacy in vivo in shrimp challenged with AHPND-associated *V. parahaemolyticus*. Such work would support the development of ginger-based strategies to reduce antibiotic reliance and improve the sustainability of shrimp aquaculture in Vietnam.

CONCLUSION

This study demonstrates that solvent polarity strongly influences the GC-MS phytochemical profile of *Zingiber officinale* extracts. Methanol yielded the broadest chemical coverage (38 detected compounds), followed by chloroform (27 compounds). Ethyl acetate, dichloromethane, and *n*-hexane yielded 26, 23, and 20 detected compounds, respectively. Across all solvent systems, the identified constituents were assigned to diverse chemical classes, with phenolics and sesquiterpenoid-related compounds as dominant groups. Notably, gingerol was consistently detected as the most abundant compound across extraction solvents, providing a plausible chemical basis for downstream antibacterial effects. In vitro antibacterial assays showed that the methanolic *Z. officinale* extract exerted a concentration-dependent inhibitory effect against AHPND-associated *Vibrio parahaemolyticus*. The highest activity was observed at 500 mg/mL, producing an inhibition zone of 19.87 ± 0.29 mm after 48 h. The MIC and MBC were both 7.81 mg/mL, indicating bactericidal activity under the assay conditions. Given that AHPND outbreaks are linked to virulent *V. parahaemolyticus* strains, these findings support the potential of ginger-derived extracts as candidates for developing herbal preparations for aquaculture biosecurity. However, further studies are required to standardize solvent exposure (including reporting final DMSO % v/v in assay wells), evaluate safety, optimize formulation and dosing, and validate efficacy in vivo in *Litopenaeus vannamei* challenged with AHPND-associated *V. parahaemolyticus*. Such efforts may ultimately contribute to reducing antibiotic reliance and improving sustainability in shrimp aquaculture in Vietnam.

Statements and Declarations: The authors declare no conflicts of interest

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