PHYTOCHEMICAL COMPONENTS AND ANTIMICROBIAL ACTIVITY OF METHANOL EXTRACTS FROM GARLIC (*Allium sativum* L.) AGAINST *Vibrio parahaemolyticus*

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TÓM TẮT

THÀNH PHẦN HÓA HỌC VÀ KHẢ NĂNG KHÁNG KHUẨN CỦA CAO CHIẾT METHANOL TỪ TỎI (*Allium sativum* L.) ĐỐI VỚI *Vibrio parahaemolyticus*

Dịch chiết methanol từ tỏi (Allium sativum L.) được phân tích bằng GC-MS cho thấy sự hiện diện của 30 hợp chất sinh học, bao gồm aldehyde, phenol, acid béo và các hợp chất organosulfur. Nghiên cứu nhằm đánh giá hoạt tính kháng khuẩn của chiết xuất tỏi đối với Vibrio parahaemolyticus, một tác nhân gây bệnh quan trọng trong nuôi trồng thủy sản. Kết quả khuếch tán thạch cho thấy dịch chiết tỏi ức chế rõ rệt vi khuẩn ở nồng độ 50-500 mg/mL. Sau 24 giờ, đường kính vòng ức chế dao động từ 12,31 đến 20,28 mm, tăng dần theo nồng độ. Hoạt tính giảm dần sau 48-72 giờ, nhưng ở nồng độ cao nhất (500 mg/mL) sau 72 giờ vẫn duy trì hiệu quả ức chế đáng kể (17,51 ± 0,37 mm). DMSO (đối chứng âm, nồng độ 1%) không tạo vòng ức chế, khẳng định hoạt tính kháng khuẩn xuất phát từ dịch chiết tỏi. Nồng độ ức chế tối thiểu (MIC) và nồng độ diệt khuẩn tối thiểu (MBC) được xác định lần lượt là 125 mg/mL và 250 mg/mL. Những kết quả này bước đầu cho thấy chiết xuất tỏi có tiềm năng sinh học đáng chú ý đối với V. parahaemolyticus.

Keywords: *Phytochemical, Allium sativum* L., GC-MS, *Antibacterial, Vibrio parahaemolyticus*.

1. INTRODUCTION

According to the Ministry of Agriculture and Rural Development (now the Ministry of Agriculture and Environment), the brackish water shrimp farming area in Vietnam in 2024 would reach 737,000 hectares, with an output of 1.26 million tons, an increase of 5.3% compared to 2023. However, the expansion of farming area increased the risk of environmental pollution, disease outbreaks, widespread use of drugs, chemicals and antibiotics leading to residues in the product. In 2024, the total shrimp farming area was damaged by 21,590 hectares, of which 4,121 hectares (19%) were due to diseases, mainly white spot, acute hepatopancreatic necrosis, red body, white feces and microsporidia [1, 2].

To control diseases, many farmers tended to overuse antibiotics. Le Cong Tuan's (2021) survey recorded that 96.5% of farming households used antibiotics, of which 85.5% combined multiple types in the same treatment and 68.4% used higher doses than recommended [3]. Nguyen Quoc Thinh et al. (2020) also showed that shrimp farmers in Tra Vinh used banned antibiotics [4]. As a result, many strains of *Vibrio* spp. were increasingly resistant to antibiotics. Specifically, *V. parahaemolyticus* isolated in the Southern provinces had an antibiotic resistance rate of 15.3-31.8% [5]. Ho Khanh Duy et al. (2019) reported that 240 strains of *Vibrio* spp. from Long An, Ben Tre, Bac Lieu, and Ninh Thuan were all resistant to 24 tested antibiotics [6]. Ngo Thi Huyen et al. (2022) also recorded that *Vibrio* strains isolated in the North were 100% resistant to 4-8 antibiotics [7]. These figures showed that the overuse of antibiotics in shrimp farming increased drug resistance, making it difficult to treat diseases and potentially posing a risk to food safety.

In this context, finding alternatives to antibiotics was necessary. The use of herbs in the prevention and treatment of aquatic diseases was becoming increasingly popular due to its advantages: readily available raw materials, low cost, and containing many antioxidant and antibacterial compounds [8]. A survey by Hong Mong Huyen et al. (2020) showed that more than 50% of shrimp farming households in Ca Mau and Soc Trang used herbs [9], while Le Nguyen Thien Phuc et al. (2024) demonstrated that herbal preparations from betel, pennywort, and basil significantly improved the survival rate of shrimp after *V. parahaemolyticus* infection, even more effective than some commonly used antibiotics [10].

Among herbs, garlic (*Allium sativum* L.) is known not only as food but also as a medicine with many biological effects such as cardiovascular protection, anti-oxidation and especially antibacterial thanks to the main active ingredient allicin and many other sulfur compounds [11-13]. Many studies demonstrated that methanol extract from garlic (*Allium sativum* L.) exhibits a broad antibacterial spectrum, inhibiting many Gram-positive and Gram-negative bacteria [14, 15]. In Vietnam, new studies on garlic mainly focused on qualitatively determining the main active ingredient groups, while in-depth studies analyzing chemical composition by GC-MS were limited, and if any, most of them only focused on the chemical composition as well as the biological activity of garlic essential oil [16-19]. This showed that study on the composition and biological activity of *Allium sativum* L. extracts, especially methanol extract, still had a lot of potential to exploit. Therefore, this study was conducted to analyze the chemical composition of methanol extract of *Allium sativum* L. by GC-MS method, and at the same time evaluated the antibacterial ability against *Vibrio parahaemolyticus*, thereby providing more scientific basis for the application of garlic (*Allium sativum* L.) as an alternative to antibiotics in sustainable shrimp farming.

2. EXPERIMENTAL

2.1 Sample preparation and GC-MS analysis

Fresh garlic bulbs were purchased from Ngoc Chi Market, Vinh Ngoc Commune, Dong Anh District, Hanoi, Vietnam, in June 2023. The plant species was identified by Prof. Dr. Tran The Bach, an ecologist at the Institute of Biology, Vietnam Academy of Science and Technology. A voucher specimen was deposited in the laboratory.

The garlic bulbs were peeled, washed, sliced, and dried at 40-50°C to a constant mass. The dried garlic bulbs were extracted with methanol at a ratio of 1:10 (mass/volume) at room temperature for 48 hours. The extract was filtered through Whatman paper, then evaporated under vacuum at 40°C and dried to obtain the dry extract. 100 mg of the extract was diluted with 10 mL of MeOH, filtered through a 0.22 µm PTFE filter into a 2 mL vial for chemical composition analysis. The analysis was performed on a GC-MS system (Agilent 7890B GC/5977A MSD, USA) with a DB-5MS column (30 m × 0.25 mm × 0.25 µm), He carrier gas (1 mL/min), injection volume 1 µL, injection chamber temperature 280 °C. The temperature program was increased from 40ºC to 100ºC (rate 5ºC/min), continued to increase to 300°C (rate 8°C) and held for 2 min. The MS start time was 4 min, and end time was 45 min with solvent cut time was of 4 min. Using computer searches on a NIST Ver. 11 MS data library and comparing the spectrum obtained through GC-MS, the volatile compounds present in the plant samples were identified.

2.2 Antibacterial activity assay using agar disk-diffusion testing

The extract was dissolved in DMSO at concentrations ranging from 1-500 mg/mL. *Vibrio parahaemolyticus* was cultured in TSB medium supplemented with NaCl (15 g/L). The bacterial density was determined by measuring OD at 600 nm and adjusted to 10^6 CFU/mL through dilution.

On agar plates, 100 µL of bacterial suspension was spread evenly, and after 10–15 minutes, wells of 6 mm diameter were made. Each well was filled with 100 µL of the extract at the respective concentrations. DMSO (1%) served as the negative control, while doxycycline (30 µg/mL) was used as the positive control. Plates were incubated at 28-30°C for 24 hours, and the diameter of the inhibition zones (mm) surrounding the wells was measured [20]. Each experiment was repeated 3 times.

2.3 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC and MBC of *Allium sativum* L. extracts were determined based on the color change of resazurin reagent. The experiment was performed in a 96-well plate. TSB medium was added 100 µL to the wells of the 96-well plate using an 8-channel pipette. Each herbal solution/Doxycycline/sterile distilled water/DMSO was added 100 µL were added to two rows of the first wells in rows A1 to H1 to reach a final volume of 200 µL mixture. Serial dilutions were made between the wells by mixing and 100 µL of the mixture was transferred to the adjacent column. The procedure was repeated up to the twelfth well of the row, discard 100 µL of the mixed solution in the last well. *V. parahaemolyticus* bacterial suspension (10^6 CFU/mL) was added 100 µL to the wells and incubated at 28-30 ºC for 16 to 18 hours. Then, 40 µL of 0.01% Resazurin reagent solution was added to all wells to evaluate the survival rate of bacteria in each well. The lowest extract concentration at which resazurin remained blue was recorded as the minimum inhibitory concentration (MIC) (mg/mL). From the wells that remained colorless (blue), 100 µL of the test solution was taken and spread onto TSA medium plates, then incubated at 28-30°C for 24 hours. The lowest extract concentration, at which no colonies appeared at the drop site was determined as the minimum bactericidal concentration (MBC) (mg/mL) [20].

3. RESULTS AND DISCUSSION

3.1. Chemical Composition of Garlic (*Allium sativum* L.) Extract

The GC-MS analysis of methanol extract of garlic (*Allium sativum* L.) revealed the presence of 30 compounds, including aldehydes, phenols, fatty acids, and organosulfur compounds. These compounds were identified with a match quality greater than 80% compared to the NIST11 library. Detailed information, including retention time (RT), molecular formula, molecular weight, and relative concentration (peak area %), was presented in Table 1, while the GC-MS chromatogram of the methanol extract was shown in Figure 1.

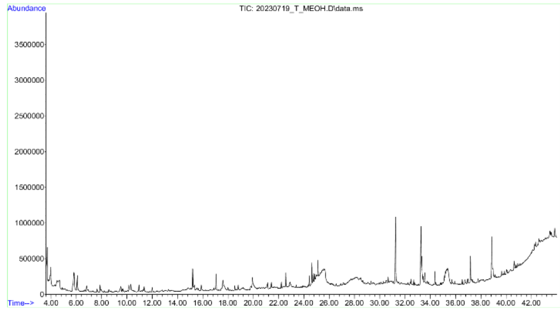

Figure 1. *Phytochemical analysis peaks of the methanolic extract of garlic (Allium sativum L.) by GC-MS.*

Table 1. *Recapitulation of Chemical Content Profile of Allium sativum L. Extract*

S/No	R. Time (mins)	Chemical component	Formula	Molecular weight	Concentrations (%)	Qual. (%)
1	6,082	2,3-Butanediol	$(CH_3CHOH)_2$	90,12	5,61	86
2	6,809	Furfural	C_4H_3OCHO	96,08	1,18	93
3	7,875	2-Furanmethanol	$C_5H_6O_2$	98,10	1,40	97
4	9,518	1,3-Dithiane	$CH_2S_2C_3H_6$	120,24	1,39	83
5	10,308	4,5-Dihydro-2-methylimidazole-4-one	$C_4H_8N_2O$	110,16	2,51	84
6	10,96	Pentanoic acid, 2-phenylethyl ester	$C_{13}H_{18}O_2$	206,28	0,93	80
7	11,361	2-Furancarboxaldehyde, 5-methyl-	$C_6H_6O_2$	110,11	0,90	86
8	13,305	3-Piperidinol, 1,4-dimethyl-, trans-	$C_7H_{15}NO$	115,17	0,77	82
9	14,86	Piperazine, 1,4-dimethyl-	$C_6H_{14}N_2$	114,19	0,65	87
10	15,198	Diallyl disulphide	$C_6H_{10}S_2$	146,27	2,00	83
11	15,875	6-Deoxy-D-mannono-4-lactone	$C_6H_{10}O_5$	162,142	0,65	87
12	17,054	(Methylthio)-acetonitrile	C_3H_5NS	87,14	1,58	87
13	17,593	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144,12	2,52	82
14	18,797	Hydrazinecarbodithioic acid, 1-methyl-, methyl ester	$C_3H_8N_2S_2$	136,23	1,33	80
15	19,198	Piperidine	$C_5H_{11}N$	85,15	1,04	84
16	19,925	5-Hydroxymethylfurfural	$C_6H_6O_3$	126,11	3,24	89
17	21,129	Trisulfide, di-2-propenyl	$C_6H_{10}S_3$	178,33	0,68	81
18	21,417	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150,17	0,67	93
19	22,571	2,4,6,(1H,3H,5H)-Pyrimidinetrione, 5-acetyl-	$C_6H_6N_2O_4$	170,12	2,02	82
20	22,884	DL-Proline, 5-oxo-, methyl ester	C_6H_9NO	143,14	1,22	82
21	25,116	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	$C_{15}H_{24}$	204,35	12,20	96
22	25,555	N1-(4-hydroxybutyl)-N3-methylguanidine acetate	$C_8H_{17}N_3O_3$	203,24	12,98	83
23	28,214	3-Deoxy-d-mannoic lactone	$C_6H_{10}O_5$	162,14	8,58	80
24	31,261	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256,42	6,82	99

S/No	R. Time (mins)	Chemical component	Formula	Molecular weight	Concentrations (%)	Qual. (%)
25	32,477	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282,5	0,62	90
26	33,267	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280,24	9,91	99
27	34,358	Isopropyl linoleate	$C_{21}H_{38}O_2$	322,5	0,97	80
28	35,361	.gamma.-Sitosterol	$C_{29}H_{50}O$	414,39	8,83	99
29	37,166	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330,5	2,18	90
30	38,847	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{38}O_4$	354,52	4,62	95

This study used GC-MS to analyze the chemical composition of methanol extract from *Allium sativum* L. A total of 30 phytochemical constituents with diverse biological activities were identified. Among them, N1-(4-hydroxybutyl)-N3-methylguanidine acetate (12.98%) was found to be the predominant compound, recognized for its antibacterial activity [21]. Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R,S)] (12.20%), classified as a sesquiphellandrene, was reported to disrupt membrane fluidity and integrity, leading to cell lysis and inhibition of bacterial enzyme activity [22], and was also associated with anticancer potential [23]. In addition, 3-Deoxy-D-mannoic lactone (8.58%) was previously reported by Shobana (2009) to exhibit antibacterial properties in ethanol extracts of *sativum* [24].

Fatty acids represented 17.35% of the methanolic *Allium sativum* L. extract, confirming their role as important plant-derived bioactive compounds with antibacterial properties [25]. The major fatty acids detected included 9,12-octadecadienoic acid (Z,Z) (9.91%), n-hexadecanoic acid (6.82%), and octadec-9-enoic acid (0.62%), which was also consistently reported in previous GC-MS studies [26-28]. Fatty acids were tested for their antibacterial activity against the against Gram-positive bacteria (*S. aureus*, *S. pyogenes*) and Gram-negative species (*E. coli*, *P. aeruginosa*) [29]. Beyond their antimicrobial potential, these fatty acids were widely recognized for anti-inflammatory and antioxidant activities [30, 31].

5-Hydroxymethylfurfural (3.24%) was also detected, in agreement with the findings of Cun Chen et al. (2019), who reported its predominance in three garlic varieties at concentrations of 26.78%, 47.10%, and 4.88%, respectively. This compound was demonstrated to exhibit antitumor [32], antioxidant [33], and cytoprotective [34] activities.

In addition, characteristic organosulfur compounds such as diallyl disulphide (DADS, 2.00%) and trisulfide, di-2-propenyl (DATS, 0.68%) were identified [35]. These compounds were well-documented for their wide-ranging biological activities, including antifungal, antiviral, antioxidant, and anticancer properties [28, 36, 37]. *In vitro* studies showed that DADS and DATS exhibited strong antibacterial activity against *Escherichia coli*, *Helicobacter pylori*, *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* [35, 38].

The observed variations in the number and relative abundance of secondary metabolites in comparison with the previous reports might be attributed to differences in extraction solvents, analytical procedures, or GC-MS instrumentation. Collectively, these findings indicated that the methanolic *Allium sativum* L. extract contained a broad spectrum of bioactive phytochemicals with potential antioxidant, anti-inflammatory, anticancer, antifungal, and antibacterial activities.

3.2. Antibacterial activity against *V. parahaemolyticus*

The antibacterial activity of *Allium sativum* L. extract, determined by the agar well diffusion method, was presented in Figures 2-4 and Table 2. The results showed that natural compounds extracted from garlic exhibited antibacterial activity in the concentration range of 50-500 mg/mL. The positive control (Doxycycline 30 µg/mL) produced an inhibition zone of 16.99 ± 0.39 mm at 24 h, but completely lost its activity at 48 h and 72 h.

Table 2. *Zone of inhibition of methanol extract of Garlic (Allium sativum L.) against V. parahaemolyticus*

Different concentrations of methanol extract (mg/mL)	Diameter of Inhibition Zone (mm) ± SD		
	24h	48h	72h
500	20,28 ± 0,42	20,01 ± 0,35	17,51 ± 0,37
400	19,71 ± 0,35	19,11 ± 0,39	16,49 ± 0,30
300	19,31 ± 0,38	18,23 ± 0,32	16,05 ± 0,31
200	16,73 ± 0,31	15,35 ± 0,37	14,64 ± 0,33
100	14,62 ± 0,37	13,50 ± 0,40	12,04 ± 0,35
50	12,31 ± 0,30	10,23 ± 0,33	9,26 ± 0,39
25	0	0	0
10	0	0	0
5	0	0	0
1	0	0	0
Doxycycline (30 µg/mL)	16,99 ± 0,39	0	0

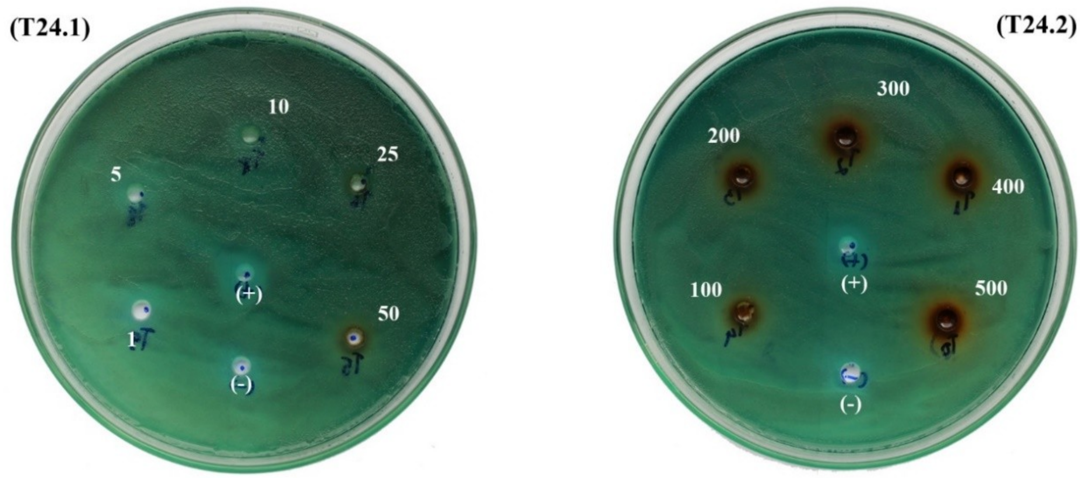

Figure 2. *Diameter of inhibition zones of methanolic Allium sativum L. extract after 24h (T24.1, T24.2). (+) Doxycycline (30 µg/mL); (-) DMSO (1%)*

The diameter of the antibacterial zone ranged from 12.31-20.28 mm at concentrations of 50-500 mg/mL after 24 hours. The inhibitory effect increased gradually with concentration, with the highest value at 500 mg/mL (20.28 ± 0.42 mm). DMSO (1%, negative control) did not form antibacterial zones, confirming that the antibacterial effect obtained was due to the active ingredient in the *Allium sativum* L. extract.

Figure 3. *Diameter of inhibition zones of methanolic Allium sativum L. extract after 48h (T48.1, T48.2). (+) Doxycycline (30 µg/mL); (-) DMSO (1%)*

At 48 h, the diameter of the antibacterial zone tended to decrease slightly, to 20.01 ± 0.35 mm at a concentration of 500 mg/mL, and from 15.35-19.11 mm at a concentration of 200-400 mg/mL. Meanwhile, Doxycycline completely lost its effect, indicating that the antibacterial activity of *Allium sativum* L. was more stable than that of commercial antibiotics after 48 h.

At 72h, the antibacterial activity continued to decline significantly, especially at low concentrations (≤100 mg/mL, only 9.26-12.04 mm). However, at a high concentration of 500 mg/mL, the inhibition zone still reached 17.51 ± 0.37 mm, indicating a relatively stable inhibitory effect over time. This showed that the prolonged effect of *Allium sativum* L. extract against *Vibrio parahaemolyticus*.

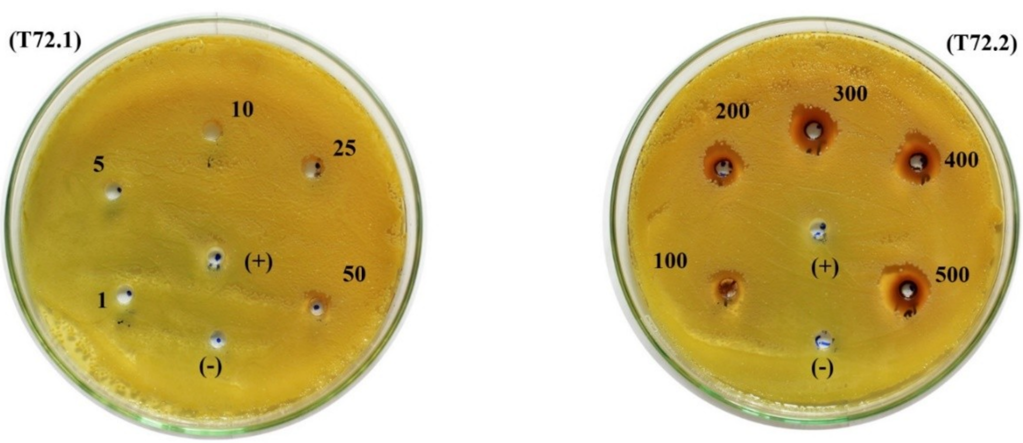

Figure 4. *Diameter of inhibition zones of methanolic Allium sativum L. extract after 72h (T72.1, T72.2). (+) Doxycycline (30 µg/mL); (-) DMSO (1%)*

The decline in antibacterial efficacy after 48-72 h might be attributed to the natural degradation of organosulfur compounds (e.g., allicin, diallyl disulfide, diallyl trisulfide), which were inherently unstable and prone to volatilization, thereby reducing inhibitory activity over extended exposure. In addition, the rapid growth rate and adaptive capacity of *V. parahaemolyticus* might further contribute to the diminished antibacterial effect observed over time. Nevertheless, the results clearly demonstrated that at higher concentrations, methanolic *Allium sativum* L. extract was able to maintain stable and sustained antibacterial activity, outperforming doxycycline in long-term efficacy.

These findings were consistent with previous reports. Angela Mercy et al. (2018) demonstrated that the fresh garlic juice exhibited inhibitory activity against *Vibrio* spp. [39]. Similarly, Raja Zouari Chekki et al. (2014) reported that ethanol extracts of garlic (*Allium sativum*) produced inhibition zones of up to 18 mm against *Staphylococcus aureus*, closely aligning with the 24 h results of the present study [36]. In contrast, Ramadhaniah et al. (2023) observed that a 96% ethanol extract of garlic (*Allium sativum* L.) produced inhibition zones of only 4.9-11.9 mm against *V. parahaemolyticus*, markedly lower than the maximum value of 20.28 mm observed here with the methanol extract [40]. Notably, Thi-Phuong-Mai Cao et al. (2025) reported that the aqueous garlic extract (10 g/mL) achieved an inhibition zone of 21.6 ± 4.3 mm against *V. parahaemolyticus*, which was comparable to the current result obtained at 500 mg/mL [8]. Such differences might arise from various factors, including the solvent type and concentration used for extraction, as well as the origin, cultivar, and growth conditions of the garlic.

Overall, the findings strongly suggested that methanolic *Allium sativum* L. extract contained a diverse spectrum of bioactive compounds capable of exerting sustained antibacterial effects. Although a decline in activity over 48-72 h reflected the intrinsic instability and gradual degradation of organosulfur compounds, the extract still demonstrated superior long-term efficacy compared to doxycycline. These results highlighted the potential of methanolic *Allium sativum* L. extract as a promising alternative or complementary agent to conventional antibiotics in aquaculture, particularly for controlling *V. parahaemolyticus*, where the antimicrobial resistance was an escalating concern.

3.3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Methanolic Extracts of *Allium sativum* L. against *Vibrio parahaemolyticus*

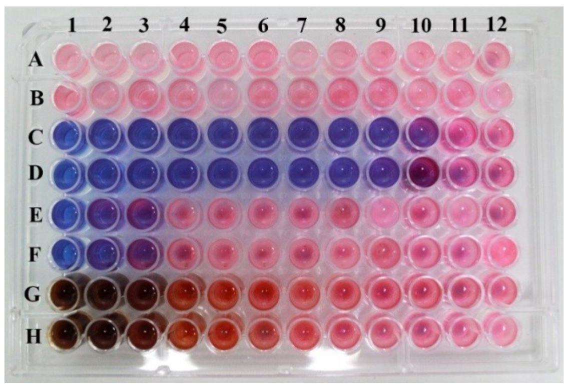

Figure 5. *Minimum inhibitory concentration (MIC, mg/mL) of methanolic Allium sativum L. extract against Vibrio parahaemolyticus, in comparison with sterile water, DMSO, and doxycycline. Wells A1-A12 and B1-B12 represented sterile water (negative control); wells C1-C12 and D1-D12 represented doxycycline (positive control); wells E1-E12 and F1-F12 represented DMSO (solvent control); wells G1-G12 and H1-H12 represented serial dilutions of the methanolic Allium sativum L. extract.*

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the methanolic *Allium sativum* L. extract against *Vibrio parahaemolyticus* were presented in Figure 5. The MIC was determined at the third dilution, corresponding to 125 mg/mL, while the MBC was established at 250 mg/mL. These findings were further corroborated by the resazurin assay, which showed complete inhibition of bacterial growth and no color change at concentrations equal to or exceeding the MBC.

The results of this study were generally consistent with previous reports, although notable differences were observed in the absolute values of MIC and MBC. Agnieszka Magryś et al. (2021) reported that 70% ethanolic *Allium sativum* L. extracts could inhibit a wide range of bacteria, including multidrug-resistant strains, exhibiting both bactericidal and bacteriostatic effects [41]. Similarly, Tran Thi Phuong Dung et al. (2022) demonstrated that *Allium sativum* L. extract in 70% ethanol exhibited MIC and MBC values of 0.16 mg/mL and 0.63 mg/mL, respectively, while the 70% methanol extract displayed even stronger activity with MIC and MBC values of 0.01 mg/mL and 0.04 mg/mL, indicating a highly potent effect against *Edwardsiella ictaluri* [8]. In contrast, Thi-Phuong-Mai Cao et al. (2025) reported MIC and MBC values of 0.15625 g/mL and 0.625 g/mL, respectively, for garlic extract against *V. parahaemolyticus*, which were lower than those observed in the present study [42].

In this study, the methanolic extract of garlic (*Allium sativum* L. exhibited an MIC of 125 mg/mL and an MBC of 250 mg/mL against *V. parahaemolyticus*. The MIC was defined as the lowest concentration of *Allium sativum* L. extract that inhibited visible bacterial growth, whereas the MBC represented the minimum concentration required to achieve the complete bacterial eradication. According to Canillac and Mourey (2001), an MBC/MIC ratio $\leq$ 4 indicated bactericidal activity, while a ratio > 4 suggested bacteriostatic effects. With an MBC/MIC ratio of 2, the present results confirmed that *Allium sativum* L. extract possessed bactericidal activity against *V. parahaemolyticus*.

These findings highlighted the variability in antibacterial potency of garlic (*Allium sativum* L.) depending on the extraction solvent, bacterial species tested, and methodological conditions. Nevertheless, the consistent demonstration of bactericidal properties across studies reinforced the potential application of *Allium sativum* L. extract as a natural antimicrobial agent in aquaculture disease management.

4. CONCLUSION

Methanol extract of garlic (*Allium sativum* L.) contained 30 bioactive compounds, including aldehydes, phenols, fatty acids, and organosulfur compounds. The extract demonstrated strong antibacterial activity against *Vibrio parahaemolyticus*, with inhibition zones of 12.31-20.28 mm at 50-500 mg/mL and MIC and MBC values of 125 mg/mL and 250 mg/mL, respectively. These results provided preliminary evidence of the biological potential of *Allium sativum* L. extract against *Vibrio parahaemolyticus*.

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