MAJOR COMPONENT AND ANTIMICROBIAL ACTIVITY OF THE EXTRACT OF BITTER LEAF (*Vernonia amydalina*) AGAINST *Vibrio parahaemolyticus*

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ABSTRACT

It is an undeniable fact that natural bioactive compounds extracted from diverse plants and herbs have attracted significant public attention and scientific interest due to their health-promoting benefits as antioxidants and antimicrobial agents. In this research, the main phytochemical compositions of *V. amygdalina* leaves were determined by Gas chromatography–mass spectometry (GC-MS). There were 18 groups detected from the methanol extract of *V. amygdalina* leaves, in which, fatty acids were the major component (55.29%), followed by the ketone group at 10.44%. Phenol and terpenoid compounds comprised 6.19% and 5.13% respectively, ranking third and fourth in abundance. The antibacterial properties of the extract from *V. amygdalina* leaves were evaluated against *V. parahaemolyticus* which caused Acute hepatopancreatic necrosis disease (AHPND) in Whiteleg shrimp (*Liptopenaeus vannamei)*. The antibacterial zone diameters of the leaf extract ranged from 9.5 mm to 19.0 mm during 72 hours of the inhibition, corresponding to the concentration of *V. amygdalina* changed from 200 mg.mL^{-1} to 500 mg.mL^{-1}. This illustrates the potential application of *V. amygdalina* leaf extracts as the supply food or used to produce bio-preparations for preventing and controlling the growth of *V. parahaemolyticus* in shrimp culture industry.

Keywords: *Vernonia amygdalina*, *Vibrio parahaemolyticus*, AHPND, *Liptopenaeus vannamei*, antimicrobial activity.

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20NTRODUCTION

Research on natural compounds and biologically active substances in plants is a highly regarded area of study that has captured the attention of scientists worldwide. This is due to the wide diversity and potential medical and antimicrobial applications of these compounds. Previous studies have shown that secondary metabolites exhibit a diverse range of biological activities, including anti-inflammatory, antimicrobial, and antioxidant properties, as well as anticancer effects. This underscores the significant potential of phenolic compounds for various applications in the pharmaceutical industry, functional foods, cosmetics, food industry, nutraceuticals, and packaging industry (Oksana et al., 2018; Hamad, 2021; Pandya et al., 2023). They were detected in various fruit trees such as bananas, apples, mangos, oranges, papayas, peaches, pomegranates, strawberries, pineapples, and watermelons (Hamad, 2021). The change in structure of phenolic compounds generates flavonoids in tea, wine, cereals, roots, flowers, fruits, and vegetables that are considered as cruicials for numerous applications in pharmacology, cosmetics, nutraceuticals, and medicine. This is

attributed to their ability to alter the activity of crucial cellular enzymes, as well as their antioxidative, anti-inflammatory, antimutagenic, and antimicrobial properties (Panche et al. (2016). Terpenes and terpenoids are bioactive compounds found in essential oils. They have a wide range of biological effects, including anti-inflammatory, antibacterial, anticancer, and antiallergic properties. Previous studies have shown that these compounds also possess food preservative and antibacterial qualities, making them a promising option for industrial applications. (Ayu et al., 2022). In addition, diterpenoids in plants were reported with various bioactivities such as antibiotic, antimicrobial, antiviral, and antitumor properties (Ahmad and Zahia, 2008), while plant steroids contain various bioactivities such as antibacterial, anti-tumor, antihelminthic, cardiotonic activity, cytotoxic, hepatoprotective, immunosuppressive, and plant growth hormone regulator, which play an important role in agrochemical, medical, and pharmacological properties (Snehal and Jignasha, 2015). It is interesting to note that vitamin E in plants is known as a lipid-soluble antioxidant that can convert free radicals into less reactive compounds, which is necessary for human diets as well as for plant environment adaptation (Yue et al., 2022). Moreover, fatty acids are components found in both animals and plants which were previously proposed to play a pivotal role in signal transduction and biological effects, controlling cellular processes, contributing to prohibit numerous diseases in humans and animals (Philip, 2015; Carla et al., 2018; Edgar et al., 2020; Xiangzhou et al., 2023). The above data illustrate that natural bioactive compounds in plants possess potential applications in a variety of sectors, including agrochemicals, cosmetics, the food industry, pharmacology, plant science, and geomedicine, which need to be considered for further research (Refaz et al., 2023; Monika and Amar, 2023).

V. amygdalina is a herbal plant belonging to the *Asterales* order, *Asteraceae* family, and *Vernonia* genus, which distributes and develops in tropical areas of Asia and Africa with diverse weather conditions (Alara et al., 2017). Since the leaves have a bitter taste, it is commonly referred to as bitter leaf, containing numerous phytochemicals and bioactive compounds such as alkaloids, cardiac glycosides, and flavonoids, which are considered to exhibit potential antioxidant and antimicrobial activities (Alara et al., 2017; Dinh and Vu, 2021; Ugbogu et al., 2021). This plant has been widely utilized as traditional medicines for the treatment of diseases in human and domestic animals in Vietnam as well as many countries worldwide. In the previous studies, *V. amygdalina* was used to treat typhoid, kidney diseases, inlammatory disease, suppressing the growth of cancer and malaria (Atangwho et al., 2012; Fadimu et al., 2014; Okpe et al., 2016; Yedjou et al., 2018; Asante et al., 2019). In addition, *V. amygdalina* extracts could be used to treat coughs, constipation, fevers, hypertension and other infections (Amira and Okubadejo, 2007; Michael et al., 2010; Fadimu et al., 2014). Furthermore, a variety of nutrients was found in *V. amygdalina* roots, stems, and leaves, including proteins, amino acids, minerals, vitamins, fats, fibres, and carbohydrates (Alara et al., 2017; Oyeyemi et al., 2018).

In this research, we determined the major component in the *V. amygdalina* leaf extracts through GC-MS system and evaluated the inhibitory effects of *V. amygdalina* extracts against pathogenic of *V. parahaemolyticus*, isolated from farmed Pacific white shrimp in Vietnam. This bacterial species has caused Acute Hepatopancreatic Necrosis Disease (AHPND), which contributed to mass mortality within the first 35 day-old shrimp, since this disease causes dysfunction and destruction of the hepatopancreatic tissues of shrimp. The inhibited test was conducted during 72 hours via evaluation of the antibacterial zone diameter, the minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC). The finding of this research provides a potential possibility of utilizing this natural antibacterial agent in shrimp aquaculture.

MATERIALS AND METHODS

Sample preparation: The mature leaves of *V. amygdalina* were collected at the Hung Tien commune, Nam Dan district, Nghe An province, Vietnam, in March 2023. The samples were washed with tap water and then dried in an oven at 40 ºC to a constant mass, with the moisture content less than 10%. The finely ground *V. amygdalina* powder, achieved by using a QE-500 blender (Vietnam) and sieved to less than 100 mesh sizes, was carefully stored in polyethylene zip bags. These bags were then placed in a sealed plastic container and stored at room temperature in a dry, sunlight-free environment until ready for use.

Phytochemical determinatio: The extraction process involved using methanol as the solvent. Approximately 5 grams of *V. amygdalina* leaf powder was soaked in 50 mL of MeOH for 48 hours. The extraction was then carried out using an ultrasonicator bath operating at a frequency of 20 KHz for 60 minutes at a temperature below 50 °C. The extracted solution was cooled to room temperature, and then centrifuged to separate different layers. The aqueous part part initially filtered via Whatman No.1, and then purified through a 0.22 µm syringe membrane before analysis using a gas chromatography system (Agilent GC 7890A) coupled with a mass spectrometry detector (Agilent MS 5977).

An Agilent DB-5MS analytical column, with a length of 30 m, an inner diameter of 0.25 mm, and a thickness of 0.25 µm was used for the GC conditions. The liquid autosampler was set up for splitless mode with a 1 µL injection volume, 1-minute injection time, and a 250 °C injection temperature. The separation process used helium with 99.999%

purity as the carrier gas, maintaining a consistent flow rate of 1.0 mL/min. The column oven's temperature program was initiated at 40°C and held for 5 minutes before being increased by two heating stages. The temperature was initially raised from 40 °C to 100 °C at a consistent rate of 1 °C per minute between the 5th and 17th minute. Following this, in the second stage, the temperature was further increased from 100 °C to 300 °C at a rate of 8 °C/min from the 17th to the 42nd minute, and then maintained at 300 °C for 2 minutes. The mass spectrometer's electron impacted ionization source was kept at 230 °C, while the interface temperature was 300 °C and the quadrupole was kept at 150 °C. Volatile compounds were detected using positive electron impact ionization tandem mass spectrometry (EI-MS/MS) in the full scan analysis mode, with a total analysis time of 44 minutes per sample and a solvent cutting time of 3 minutes. The device was controlled using the Enhanced MassHunter software, and sample analysis data was processed using the Xcarlibur software.

Antibacterial activity determination

Preparation of V. amygdalina extracts: About 200 g dried powder of *V. amygdalina* leaf was soaked in 2-liter methanol for 48 hours at 28°C and stirred thoroughly 2-3 times per day. Then, the mixture underwent a 60-minute ultrasonication before being filtered through 11µm filter paper (Whatman, UK). The procedure was performed in triplicate (n = 3) to re-collect the powder for another round of extraction and ultrasonication under the same conditions. To eliminate the methanol solvent, the combined filtrate was initially evaporated using a vacuum rotavapor (R-100, Buchi, Switzerland) and then dried at 40°C in a Memmert oven (model number 1, Germany) for an additional 48 hours to obtain the final extract. This extract was utilized to assess its antibacterial effectiveness against *V. parahaemolyticus* which was isolated from the infectious whiteleg shimps.

Preparation of V. parahaemolyticus: *V. parahaemolyticus* used in this study was isolated from the AHPND-affected shrimp collected from a shrimp farm in the Giao Long commune, Giao Thuy district, Nam Dinh province, Vietnam. This bacterium was cultured on tryptic soy broth agar (TSB) supplemented with 1.5% NaCl and thiosulfate citrate bile salts sucrose (TCBS) plate and incubated at a temperature of 28 °C to 30 °C for 24 hours. Then the color and shape of the colony was observed. Gram staining was done to determine the purity of the bacteria. A single colony on the plate was inoculated into a 5 mL TCBS solution combined with 1.5% NaCl and incubated at 28-30 °C. After 6 hours of incubation, one mL of the medium containing bacteria was transferred to 100 mL of TCBS supplemented with 1.5% NaCl and incubated at 28-30 °C for 24 h. The centrifugation procedure was performed at 4,500 × g for 5 minutes at 5 °C to collect bacterial cells, which were resuspended in a sterile 1.5% NaCl solution. The optical density (OD) of bacterial concentration (colony-forming unit; CFU) was estimated by at a wavelength of 600 nm (OD = 1.0±0.02) to achieve a density that is equivalent to 10^9 CFU.mL^{-1} combined with the colony counting method on TCBS medium.

Antibacterial zone diameter determination: The dilution procedure of about 500 mg of the extracts in 5 mL of DMSO solution to create a stock with a concentration of 500 mg/mL. This stock was then diluted with DMSO solution to obtain lower concentrations of 400, 300, 200, 100, 50, 25, 10, 5, and 1 mg/mL, respectively. The bacterial suspension solution with a density of 10^9 CFU.mL^{-1} was diluted with 0.85% NaCl in a gradually decreasing ratio of 1:9 to obtain the bacterial concentration of 10^6 CFU.mL^{-1} used in the infection experiment. A pipet was used to draw 100 µL of the bacterial suspension solution drop into the center of the TCBS agar plate and rinse with a glass rod until the agar surface was dry. After 10-15 minutes, several holes in the agar plate were punched with a diameter of 8mm, which wase then filled with 100 µl *V. amygdalina* extracts with different concentrations (1-500 mg.mL^{-1}). In addition, 100 µl of doxycycline (30 µg/mL) was used as the positive control, while 100 µL of DMSO solution was the negative control. Subsequently, the agar plates were incubated at 28-30 ºC for 24 hours before the inhibition zones against the bacteria growth were measured (in mm).

Determination of Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC): *V. amygdalina* extract (500 mg.mL^{-1}), doxycycline antibiotic (30 µg.mL^{-1}), DMSO, sterile water, *V. parahaemolyticus* bacteria (10^6 CFU.mL^{-1}), TSB bacterial culture medium, and Resazurin solution were prepared to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *V. amygdalina* extract. The MIC and MBC of the extract were determined using a micro-well method as previously described by Kongchum et al. (2016) with some adjustments. Firstly, 100 µL of *V. parahaemolyticus* bacterial solution (with a density of 10^6 CFU.mL^{-1}) was added to all 96 wells. Then, two sets of each 100 µL of the aqueous extract obtained by the methanol method as previously described (at a concentration of 500 mg.mL^{-1}), doxycycline, DMSO, and sterile water, were added to two rows of the first wells in rows A to H to reach a final volume of 200 µL mixture. Subsequently, 100 µL of the solutions in these wells were transferred to the next well in each row. The quadratic dilution procedure was similarly repeated up to the twelfth well of each row, and 100 µL of the well-mixed solution from the last well was discarded. The mixture was then incubated at a temperature of 28-30 ºC for 16-18 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 MIC was determined by the concentration of the well where the blue color of the reagent was unchanged, meaning the bacteria were not present in the mixtures. The MBC was determined using 100 µL of sample in wells that contained the blue color of the reagent, which was spread evenly on a TCBS agar plate and incubated at a temperature of 28-30 ºC. The appearance of bacteria was observed on experimental agar plates to determine the MBC of the *V. amygdalina* extract. Three replications were performed in all of the experiments, and average values were used to evaluate the antibacterial activity of *V. amygdalina* extract.

RESULTS

Phytochemical compositions of *V. amygdalina* leaves: As can be seen from Fig. 1, a total of 18 major phytochemicals such as ketone, glyceride, phenol, fatty acid, pentitol, flavonoid, peptide, sesquiterpene, terpenoid, alkaloid, alcohol, trehalose, diterpenoid, steroid, vitamin E, triterpene, polyphenol and essential oil were extracted by methanol and determined in *V. amygdalina* leaf extract using GC-MS equipment.

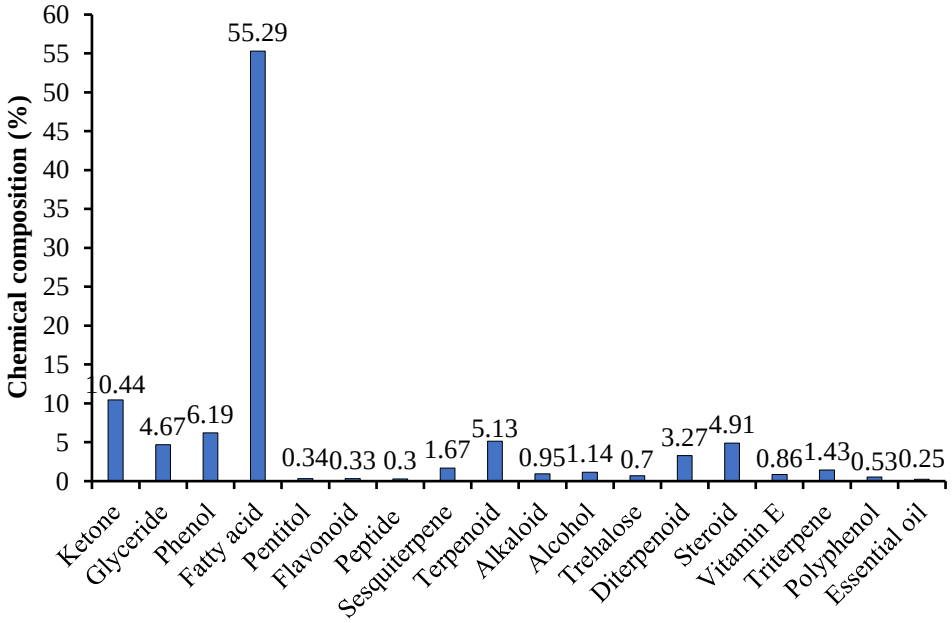

Fig. 1. The major phytochemical compositions of *V. amygdalina* leaves

It's worth noting that the *V. amygdalina* extract exhibited a significant variance in the detected bioactive compound groups. Notably, fatty acids made up the highest percentage at 55.29%, followed by the ketone group at 10.44%. Phenol and terpenoid compounds comprised 6.19% and 5.13% respectively, placing them in the third and fourth positions. Glyceride and diterpenoid were detected in lower concentrations, accounting for 4.67% and 3.27% respectively. The third group had a concentration between 1% and 2%, including sesquiterpene (1.67%), steroid (1.63%), triterpene (1.43%), and alcohol (1.14%). Other biologically active substances in the extract had negligible concentrations, such as alkaloid (0.95%), vitamin E (0.86%), fatty alcohol (0.83%), trehalose (0.70%), polyphenol (0.53%), pentitol (0.34%), flavonoid (0.33%), peptide (0.30%), and essential oil (0.25%) (Fig. 1).

Antibacterial properties of *V. amygdalina* extract: The antibacterial activity of *V. amygdalina* leaf extracts was tested against *V. parahaemolyticus* growth, the causative agent of AHPND in Pacific whiteleg shrimp (*L. vannamei)*. The study focused on determining parameters such as antibacterial inhibition zone diameter, minimum inhibition concentration, and minimum bactericidal concentration.

In general, antibacterial inhibition zone diameters substantially increased with the increasing concentrations of *V. amygdalina* extracts, while the growth of *V. parahaemolyticus* was enhanced with the longer experimental time. Particularly, the results from Fig. 2 exhibited a much clearer antibacterial inhibition zone in agar well diffusion at high concentrations (50-500 mg/mL), with no observed effects on bacterial growth at low concentrations (1-25 mg/mL). Strikingly, the inhibition zones at 500 mg/mL and 400 mg/mL were 24.0 ± 0.7 mm and 22.0 ± 1.0 mm, respectively,

surpassing the doxycycline inhibition zone (21.0 mm) after 24 hours in the inhibition test. However, the lower values of the inhibition zone diameters against *V. Parahaemolyticus* were lower at 19.5 ± 2.1 mm, 17.5 ± 0.7 mm, 15.5 ± 0.7 mm, and 12.0 ± 0.5 mm for the *V. amygdalina* extracted concentrations of 300, 200, 100, and 50 mg.mL^{-1}, respectively (Fig. 2A1, A2).

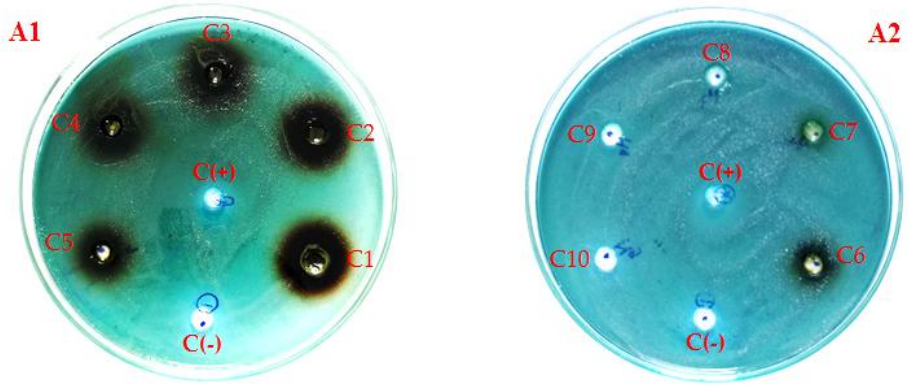

Fig. 2. Antibacterial inhibition zone diameter of *V. amygdalina* leaf extracts after 24h (A1, A2), where C1-C10 represents the concentrations of *V. amygdalina* leaf extracts ranging from 1-500 mg.mL^{-1}; C(-) is DMSO, the negative control

After 48 hours, as shown in Fig. 3B1, B2, *V. parahaemolyticus* appeared a rapid growth on the agar plates, leading to a reduction in the antibacterial inhibition zones of *V. amygdalina* extracts, while doxycycline (30 µg.mL^{-1}) had no effect on the growth of *V. parahaemolyticus* after 48 hours. Meanwhile, the antibacterial inhibition zone diameters of *V. amygdalina* extracts were measured at 21.5 ± 0.7 mm, 19.0 ± 1.4 mm, 16.0 ± 1.4 mm, 12.5 ± 0.7 mm, and 9.5 ± 0.7 mm, corresponding to their concentrations of 500, 400, 300, 200, and 100 mg.mL-1, respectively (Fig. 3B1, B2).

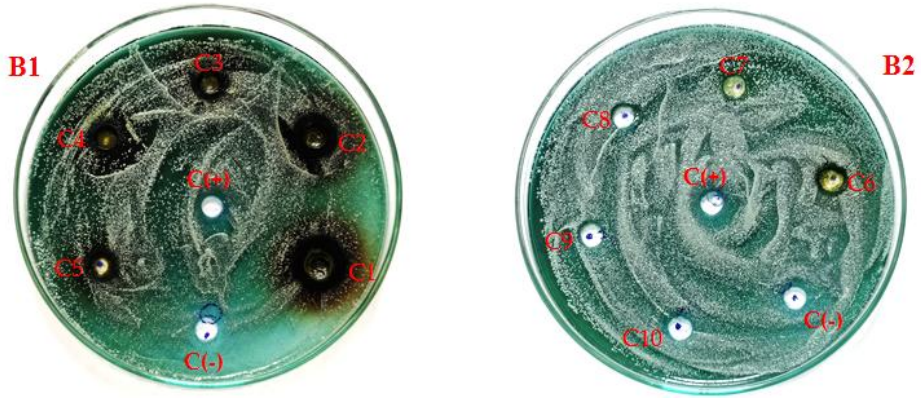

Fig. 3. Antibacterial inhibition zone diameter of *V. amygdalina* leaf extracts after 48h (B1-2), where C1-C10 represents the concentrations of *V. amygdalina* leaf extracts ranging from 1-500 mg.mL^{-1}; C(-) is DMSO, the negative control

Moreover, the antibacterial inhibition zones icreased significantly from 9.5 ± 0.7 mm to 19.0 ± 1.4 mm, corresponding to the increasing concentrations of *V. amygdalina* extract of 200, 300, 400, and 500 mg/mL-1 respectively after 72 hours of inhibition (Fig. 4D1, D2).

There was no evidence of DMSO affecting the growth of *V. parahaemolyticus* during the 72-hour treatment. On the other hand, the impact of doxycycline on the bacteria became evident after 24 hours of inhibition, and high concentrations of the extract displayed consistent effects throughout the duration of the experiments.

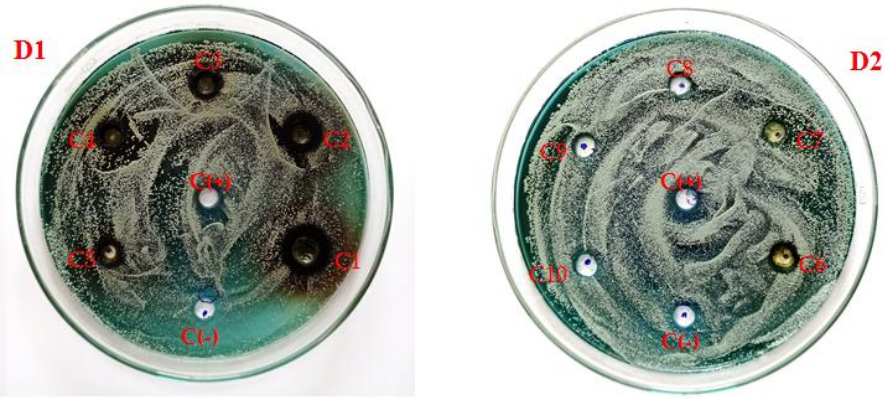

Fig. 4. Antibacterial inhibition zone diameter of *V. amygdalina* leaf extracts after 72h (D1-2), where: C1-C10 represents different concentrations of the extracts ranging from 1-500 mg.mL^{-1}; C(-) is DMSO, the negative control

Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *V. amydgalina* extract: In Fig. 5, a notable color variation was observed when comparing the MIC values across different extracted concentrations, sterile water, DMSO, and doxycycline. The wells (A1-12 and B1-12) containing sterile water showed a color change from blue to pink when the resazurine reagent was added, indicating no inhibition. Similar results were observed in wells E2-12 and F2-12 (DMSO control), wells C4-12 and D4-12 (doxycycline), and wells G5-12 and H5-12 (*V. amygdalina extracts*). Notably, the MIC and MBC values of *V. amygdalina* extracts for *V. parahaemolyticus* were determined at 62.5 mg.mL^{-1}. In contrast, the MIC and MBC values of doxycycline against *V. parahaemolyticus* were found to be 3.75 µg.mL^{-1} and 30 µg.mL^{-1}, respectively. Fig. 5 shows that the extracts have the minimum inhibition on *V. parahaemolyticus* growth compared to sterile water, negative control (DMSO), and positive control (doxycycline).

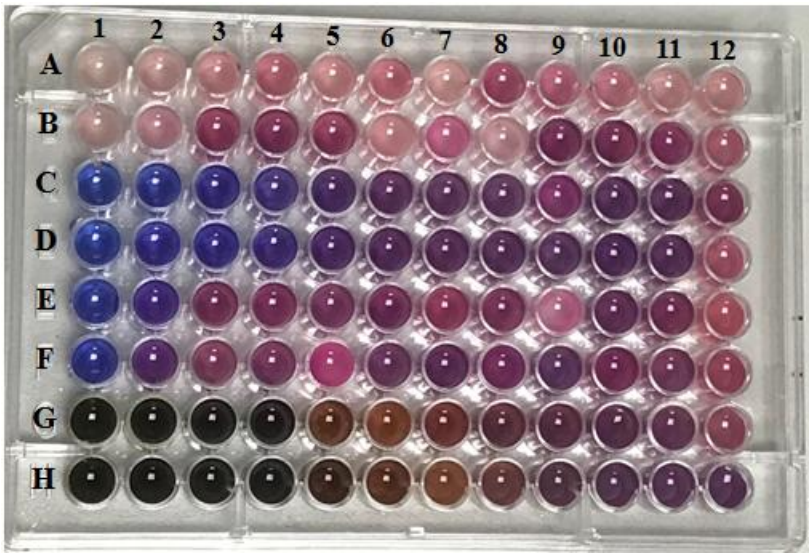

Fig. 5. The MIC of *V. amygdalina* leaf extracts in comparison with those of sterile water, DMSO, and doxycycline. The wells A1-12 and B1-12 were the MICs of sterile water; the wells C1-12 and D1-12 were the MICs of doxycycline; the wells E1-12 and F1-12).

DISCUSSION

In the present study, combining GC-MS analysis method with ultrasonic extraction allowed for the identification of phytochemical groups in the methanol extraction of *V. amygdalina* leaves. 18 groups were detected including ketone, glyceride, phenol, fatty acid, pentitol, flavonoid, peptide, sesquiterpene, terpenoid, alkaloid, alcohol, trehalose, diterpenoid, steroid, vitamin E, triterpene, polyphenol, and essential oil. Notably, fatty acid was by far the highest component,

constituting 55.29% of the extract. In the previous studies, although several analytical methods have been proposed for the separation and determination of bioactive compounds from ***V. amygdalina*** leaves, different groups of compounds and its concentrations were recorded due to disimilar conditions. Particularly, Alara et al., (2019) have detected only 11 major compounds from the shade-dried ethanolic extract, in which, acyclic diterpene alcohol (43.69%), unsaturated fatty acid (28.86%), In the other study, Olusola-Makinde et al., (2021) determined 23 and 20 bioactive compounds in the aqueous and ethanol extracts of *V. amygdalina* leaves. The extract also revealed the presence of coumarin and oleic acid. Similarly, phytochemical analysis of ethanol extract of *V. amygdalina* revealed to possess several main components, such as oxalate, phylate, tannins, saponins, flavonoid, cyanogenic glycosides, and alkaloids (Udochukwu et al.,2015). Aparted from ethanol, methanol is widely used as a solvent for extracting secondary metabolites from plant materials due to its ability to efficiently extract a wide range of compounds such as phenolic compounds, flavonoids, alkaloids, terpenoids, and other bioactive substances. Methanol is preferred for its ability to extract a diverse range of phytochemicals and its effectiveness in extracting compounds with antimicrobial and antioxidant properties. *in plant ta*. High concentrations of total phenols and flavonoids were detected in the methanol extract of Kunth leaves (*Phoenix loureiroi)* (Rajan et al.,2020). The findings also showed higher antioxidant activity in DPPH• scavenging (67.47%), lipid peroxidation inhibition (55.10%). In other studies, 18 compounds extracted from *R. stricta* were determined, in which the major compound groups were alkaloids (Ahmed et al., 2018).

Plant extracts contain bioactive constituents that have been previously evaluated their antimicrobial effects on infectious diseases brouht by resistant pathogens. The extracts of *V.amygdalina* leaves were determined to serve as antimicrobial agents against diverse microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, with MIC values of between 12.5 and 50 mg.mL^{-1} (Ghamba et al.,2014). In our research, we observed the most significant antibacterial inhibition zones against *V. Parahaemolyticus* at 24.0 mm, 21.5 mm, and 19.0 mm at the concentration of *V. amygdalina* extracts of 500 mg.mL^{-1} after 24, 48, and 72 hours, respectively. It's worth noting that there was no observed impact of DMSO on the growth of *V. parahaemolyticus* during the 72-hour experiment. Furthermore, MIC and MBC of *V. amygdalina* extract were measured at 62.5 mg.mL^{-1}. In another study, the higher values of MIC inhibitions on *Streptococcus mutans* were identified under the treatment of ethanolic and aqueous extracts of *V.amygdalina* leaves at 25 and 55 mg/ml, respectively (Akinpelu, 1999; Anibijuwon et al.,2012). Evaluations of the antibacterial properties of *V. amygdalina* leaf extracts were carried out on *Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus, Corynebacterium diphtheriae, Escherichia coli, Enterococcus faecali, Streptococcus pyogenes* with the inhibition zone diameters ranged between 21-30 mm (Olusola-Makinde et al., 2021). Similarly, the antibacterial activity of *V. amygdalina* extract prohibited development of *Salmonella typhi* and *Salmonella paratyphi*, with inhibition zones ranging from 8.1-22.5 mm and 7.3-23.3 mm, respectively, as the concentration of *V. amygdalina* extract increased from 7.3 mg/mL to 9.3 mg/mL (Femi et al., 2021). While Lucky et al. (2018) found that ethanol extracts of *V. amygdalina* against *Escherichia coli* with inhibition zones ranging from 7.0±0.0 mm at 25 mg.mL^{-1} to 14.5±2.5 mm at 200 mg.mL^{-1}. Furthermore, the high concentration of fatty acids and their derivatives might contribute to bactericidal activities. These were previously discovered to be highly effective against *Clostridium perfringens* (Nanshan et al., 2023; Luis-Miguel et al., 2021) and *Streptococcus pyogenes* (Yff et al., 2002). Duru and Onuh (2018) noted that the fatty acids in the extract of *V. amygdalina* leaves showed potentially inhibition against *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella* sp. and moderate antifungal activity against *Aspergillus nomius*. The above data demonstrated that fatty acids in the extracts of *V. amygdalina* leaves play a pivotal role as antibacterial agents against various types of bacterias and fungies that caused diseases on different animals, humans, and the environment. It is necessary to further studies to produce bio-preparations or drugs to prevent and control bacterial diseases in aquaculture, especially the *V. parahaemolyticus* caused by AHPND in *Liptopenaeus vannamei*, contributing to minimize the use of antibiotics for disease prevention and treatment in shrimp farming, promoting the growth of farmed shrimp, increasing product quality, improving economic efficiency, and sustainable development of shrimp farming in Vietnam as well as in countries around the world.

Conclusions: In summary, there were 18 phytochemical groups found in the methanol extract of *V. amygdalina* leaf, in which, fatty acid was detected as the major component with 55.29%. The diameters of the antibacterial zones for *V. amygdalina* leaf extract ranged from 9.5 mm (200 mg/mL) to 19.0 mm (500 mg/mL) after 72 hours of inhibition. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *V. amygdalina* leaf extracts were determined to be 62.5 mg/mL^{-1}. This suggests the potential application of *V. amygdalina* leaf extracts to prevent and control bacterial diseases in aquaculture, especially *V. parahaemolyticus* caused by AHPND in *Liptopenaeus vannamei*.

Author Contributions: Van Nhan Le conceived and designed the experiments; Viet Anh Le, Hai Anh Tran, Thi Oanh Doan, Thi Quynh Bui, performed the experiments; Hung Manh Nguyen, Cong Thuong Phi, Van Tien Tran, Tobor Janda, Gabriella Szalai, analyzed the data; Van Diep Le, Thi Thanh Mai Nguyen, Nguyen Khang La contributed reagents/materials/analysis tools; Van Nhan Le, Quang Minh Bui, Ngoc Minh Truong wrote the paper.

Conflicts of Interest: The authors declare no conflicts of interest.

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