Universal Journal of Agricultural Research 11(6): 948-954, 2023 http://www.hrpub.org
DOI: 10.13189/ujar.2023.110603

Karamunting (*Rhodomyrtus tomentosa*) Leaf Extract Inhibits the Growth of *Vibrio* sp. Isolated from Pacific White-leg Shrimp (*Litopenaeus vannamei*)

Ni Putu Sinta Puspa Dewi[1], Retno Kawuri[2], Made Pharmawati[3], Pande Gde Sasmita Julyantoro[4,*]

[1]Master Programme of Biology, Faculty of Mathematics and Natural Science, Udayana University, Indonesia
[2]Lab of Microbiology, Biology Study Programme, Faculty of Mathematics and Natural Science, Udayana University, Indonesia
[3]Lab of Genetics, Biology Study Programme, Faculty of Mathematics and Natural Science, Udayana University, Indonesia
[4]Faculty of Marine Science and Fisheries, Udayana University, Indonesia

Received August 7, 2023; Revised October 24, 2023; Accepted November 14, 2023

Cite This Paper in the Following Citation Styles

(a): *[1] Ni Putu Sinta Puspa Dewi, Retno Kawuri, Made Pharmawati, Pande Gde Sasmita Julyantoro , "Karamunting (Rhodomyrtus tomentosa) Leaf Extract Inhibits the Growth of Vibrio sp. Isolated from Pacific White leg Shrimp (Litopenaeus vannamei)," Universal Journal of Agricultural Research, Vol. 11, No. 6, pp. 948 - 954, 2023. DOI: 10.13189/ujar.2023.110603.*

(b): *Ni Putu Sinta Puspa Dewi, Retno Kawuri, Made Pharmawati, Pande Gde Sasmita Julyantoro (2023). Karamunting (Rhodomyrtus tomentosa) Leaf Extract Inhibits the Growth of Vibrio sp. Isolated from Pacific White leg Shrimp (Litopenaeus vannamei). Universal Journal of Agricultural Research, 11(6), 948 - 954. DOI: 10.13189/ujar.2023.110603.*

Abstract The Pacific White Leg Shrimp (*Litopenaeus vannamei*) is an introduced shrimp species in Indonesia that faces some cultivation obstacles, such as infection of vibriosis disease caused by *Vibrio* spp. This study aimed to isolate *Vibrio* spp. from *L. vannamei* shrimp, then test the capability of Karamunting (*Rhodomyrtus tomentosa*) leaf extract for inhibiting the *Vibrio* isolate by minimum inhibitory concentration (MIC) assays and challenge test toward *L. vannamei* shrimp. Vibrio identification was carried out by sequencing using the 16S rDNA gene, and MIC tests were performed using ethanol extract of Karamunting leaves through the diffusion well method. The extract was also applied to the shrimp feed for *in vivo* experiment. The molecular identification showed that the *Vibrio* isolate has a similarity to *Vibrio azureus* B12-3, and *in vitro* experiment resulted in the MIC value of the extract against *V. azureus* was 3%. Finally, the addition of 100 ppm Karamunting extract to the shrimp feed increases the shrimp survival up to 80% and is significantly different ($p<0,05$) compared to the control when challenged with *V. azureus*. This study indicated that the Karamunting (*R. tomentosa*) has the potency to be a promising natural product to protect *L. vannamei* shrimp from Vibrio infection.

Keywords Extract, Karamunting, Shrimp, Vibrio

1. Introduction

Litopenaeus vannamei shrimp is one of the aquaculture species with high economic value in Indonesia [1]. However, the culture of vannamei shrimp also has various problems, including disease infection. Vibriosis caused by Vibrio bacteria is one of the important shrimp diseases that can cause mass mortality and huge economic loss in the shrimp aquaculture industry. Some species of Vibrio are the main cause of the diseases, such as *V. harveyi, V. campbellii, V. alginolyticus, V. mimicus, V. penaicida, V. vulnificus, V. anguillarum, V. owensii, V. splendidus, V. fluvialis, V. atlanticus, V. crassostreae, V. cyclitrophicus, V. gigantis, V. toranzoniae, V. nigripulchritudo,* and *V. parahaemolyticus* [2]. Diseases of decapod crustaceans caused by or associated with those *Vibrio* species have been reported by Valente and Wan [2], namely luminescent vibriosis, zoea II syndrome, septic hepatopancreatic necrosis disease, shell disease, tail necrosis, red body disease, *Vibrio*-caused bacteremia, summer syndrome,

white feces disease (WFD) and the newly emergent disease that causes significant mortality in penaeid shrimp, the acute hepatopancreatic necrosis disease (AHPND).

Efforts to control vibriosis in shrimp farming have been made in various ways, such as by administering antibiotics, probiotics, and herbal products. The misuse of either a single or combination of antibiotics often resulted in the spread and development of antibiotic-resistant bacteria. Piamsomboon and Han [3] investigated the WFD outbreak in vannamei shrimp and reported that isolated *V. harveyi* was resistant to tetracycline and fluoroquinolone, which shows that the isolate is resistant to more than one type of antibiotic. Therefore, the use of antibiotics is not recommended anymore for aquaculture activities. Studies related to restriction on the use of antibiotics in aquaculture from several countries have reported chloramphenicol and enrofloxacin have been banned for *P. monodon* and marine fish culture, as well as fluoroquinolones and enrofloxacin in Vietnam [4]. The list of antibiotics includes amoxicillin, chloramphenicol, chlortetracycline, ciprofloxacin, erythromycin, furazolidone, gentamycin S, oxytetracycline, penicillin G, streptomycin, sulphamerazine S and sulphisoxazole were banned in China [5], while chloramphenicol, penicillin, nitrofurans, and beta-lactams except for amoxicillin were banned in Thailand [6]. Furthermore, the study from Lulijiwa *et al.* [7] shows a strong reduction in antibiotic use due to technological improvements and stricter regulations in some aquaculture-producer countries.

Recently, natural herbal products have been considered as alternatives to antibiotics to protect aquaculture animals from bacterial disease problems. One natural product that has the potential as an antibacterial is the Karamunting (*Rhodomyrtus tomentosa*) plant, originally found in Central Kalimantan, Borneo Island [8]. The leaves of *R. tomentosa* contain phenolic compounds, saponins, flavonoids, and tannins, which act as antibacterials. Ethanol extract of *R. tomentosa* leaf displayed promising antibacterial activity against 47 clinical isolates of *Streptococcus pyogenes* [9], while the ethyl acetate extract of *R. tomentosa* leaf also showed antimicrobial activity against *Escherichia coli* and *Pseudomonas aeruginosa* [10]. In this study, we investigated the capability of Karamunting *R. tomentosa* leaf extract to inhibit Vibrio isolated from the Pacific white leg shrimp *L. vannamei*.

2. Materials and Methods

2.1. Isolation of *Vibrio* spp.

Vibrio bacteria were isolated from *L. vannamei* shrimp cultured in Serangan Village, Denpasar, Bali Province, Indonesia. The shrimp were collected using a purposive sampling method, with the criteria of necrosis and redness on the swimming legs of the shrimp samples. The sample was put into a 10 mL tube with Alkaline Peptone Water (APW) media and then incubated for 8 hours at 30ºC. 1 sample loop was taken from APW medium and streaked for a single colony on Thiosulfate Citrate Bile Salt Sucrose (TCBS) Agar medium, then incubated for 24 hours at 30ºC.

2.2. Molecular identification of *Vibrio* spp.

a. DNA Extraction

DNA extraction was done using ZR Fungal/Bacterial DNAKit. 50 – 100 mg of pure *Vibrio* spp. bacteria was added with 200 µL of isotonic buffer (PBS) to the ZR BashingBead lysis tube. 2.0 mL supernatant was transferred to a new BashingBead lysis tube, processed at a maximum speed of 5 minutes, then centrifuged at 10,000 rpm for 1 minute. The supernatant obtained was transferred to a new Zymo-Spin IV Spin Filter tube in a Collection Tube of 400 µL, and 1,200 µL of bacterial DNA binding buffer was added, then centrifuged at 7000 rpm for 1 minute. 800 µL of the top layer (aqueous phase) was taken and transferred to Column IIC Zym-Spin, which was placed in a collection tube and then centrifuged at 10,000 rpm for 1 minute. The remaining buffer was discarded, and the top layer (aqueous phase) was taken and transferred to Column IIC Zymo-Spin as much as 800 µL mixed with buffer in a collection tube, then centrifuged at 10,000 rpm for 1 minute [11].

The DNA pellet was transferred to a new Zymo-Spin IIC Column, and 200 µL Pre-Wash Buffer was added, then centrifuged at 10,000 rpm for 1 minute. The dried DNA pellet was added 500 µL of bacterial DNA washing buffer to the Zymo-Spin IIC column and centrifuged at 10,000 rpm for 1 minute. The Zymo-Spin IIC column containing the DNA pellet was transferred to a clean 1.5 ml microcentrifuge tube, and 100 µl of DNA Elution Buffer was added directly to the column matrix. The pellet was centrifuged at 10,000 rpm for 30 seconds to elute the DNA [11].

b. PCR (Polymerase Chain Reaction)

PCR was carried out in cycles: pre-denaturation 1 cycle for 1 minute at 95ºC and continued to 35 cycles with denaturation for 15 seconds at 96ºC, annealing for 30 seconds at 52ºC, and extension for 45 seconds at 72ºC. After 35 cycles, it is continued with a final hold at 4ºC for infinity. The total PCR reaction was 25 µL consisting of 9.5 dd H_2O, 1 µL DNA template, 1 µL of each primer, and 12.5 µL MyTaq Red Mix 2x. The primers used were 27F: AGAGTTTTGATCMTGGTCAG and 1492R: TACGGYTACCTTGTTACGACTT [12]. Observation of PCR products was carried out by gel electrophoresis using 0.8% agarose in TBE buffer. Electrophoresis was carried out at 100 volts for 45 minutes. DNA visualization was carried out on a UV transilluminator [13]. PCR product purification using the Zymoclean^TM Gel DNA Recovery Kit [11].

c. DNA Sequencing and Analysis

DNA sequencing was carried out at PT. Genetics Science Indonesia Jakarta. The DNA sequences obtained were searched for homology to the Bank gene using BLAST. The determination of kinship with several Vibrio isolates was carried out by aligning the nucleotide sequences of the samples with the sequences in the Gene Bank using the Mega X program [14].

d. Preparation of *Vibrio* spp. test bacteria suspension

The test bacterial suspension was prepared in the nutrient broth (NB) media. 1 loop of *Vibrio* spp. test bacterial colony was taken from the NA and kept in an incubator at 37ºC for 24 hours. The $1x10^8$ cells/mL bacterial cell density was obtained using the McFarland standard of 5% [15].

e. Preparation of Karamunting Leaf Ethanol Extract

Karamunting leaves were collected from Pangkalan Bun, Central Kalimantan. A total of one kilogram of fresh leaves was weighed, then air-dried for 3 weeks and re-weighed to obtain the dry weight. The sample was blended using Oxone Eco Hand Blender & Chopper type OX-161 until it became powder. The maceration stage was performed by mixing 10 g of Karamunting leaf powder and 1 liter of ethanol 100%. Then, stir until well blended and store at room temperature for 3 days. The leaf powder extract was filtered using filter paper and then evaporated using a vacuum rotary evaporator at 35-45ºC and a speed of 75 rpm to obtain a thick extract [16]. Furthermore, a 100% concentration stock solution was prepared by weighing 10 g of the thickest extract for the highest concentration and then dissolving it using ethanol to obtain a stock solution volume of 10 mL. The stock solution was then diluted to obtain a concentration of 90%, 70%, 50%, 30%, and 10% (w/v).

f. Resistance Test and MIC

The inhibition test used the diffusion well method by taking 200 µL of the *Vibrio* spp. bacteria suspension. The ethanol extract of the leaves was inoculated into the wells as much as 20 µL according to the concentration of 90%, 70%, 50%, 30%, 10%, and 0%, then incubated at 37ºC for 24 hours. All treatments were repeated 4 times. After 24 hours, the inhibition zone was observed and measured using a caliper [17]. The MIC test was carried out using the diffusion well method.

2.3. Experimental Setup

An aquarium with a diameter of 30 cm and a height of 20 cm was used as the test container. The shrimp body size used in this study was 5 – 6.5 cm long and 6 – 10 g in body weight, with 10 shrimps in each experimental container. The shrimp acclimatized for 7 days and fed with a dose of 5% of body weight. The feed sank in crumble form, containing 30% crude protein. Shrimp were fed ad libitum 3 times a day.

The leaf powder extract per concentration of 50 g, 100 g, and 200 g, which had obtained a thick extract, was then dissolved using 100 mL of sterile water (w/v). The test feed was made by spraying the extract with different doses of 100 mL into each 1 kg vannamei shrimp commercial feed. Then, it was mixed and air-dried.

2.4. Challenge Test Treatment

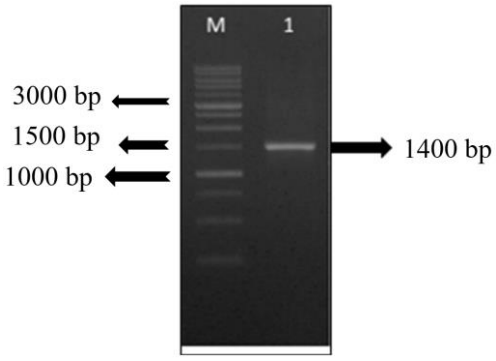
Molecularly identified *Vibrio* spp. were taken 1 loop of the bacterial colony from oblique NA, then put into an APW medium and incubated for 8 hours at 30ºC. 1 sample loop was taken from APW media and harvested by centrifugation at 5000 rpm for 5 minutes. The pellets obtained were resuspended in 10 mL of 0.9% (w/v) NaCl. Cultures were standardized according to McFarland 5% with a colony density of $1x10^8$ cells/mL [15]. Furthermore, a challenge test was carried out by administering *Vibrio* spp. bacterial culture was injected into the shrimp's carapace, and in all shrimp treatments, as much as 0.1 mL/head and then observed for 1-96 hours until the shrimp experienced clinical symptoms. Furthermore, maintenance of test animals for 10-14 days by giving the same feed according to the treatment dose The tested shrimp's maintenance was controlled at optimum water quality [18].

3. Results

3.1. Isolation and Molecular Identification of *Vibrio* sp.

The macroscopic observations showed that the colony shape was green round, the flat edge was clear green, and the colony surface was smooth with a flat embossed elevation. The microscopic form of *Vibrio* sp1 bacteria on Gram staining was bent rod cells (1.6 – 2.2 µm) and red bacterial cells, which belong to Gram-negative bacteria based on Gram staining.

Molecular identification was carried out using PCR with 16S rRNA and produced a product with a size of 1400 bp (Figure 1). Sequencing of PCR products yielded 1367 bp long DNA sequences. The similarity of other *Vibrio* species can be seen from the maximum-likelihood (ML phylogenetic tree of *Vibrio* sp1. (Figure 2).



M: 100 bp ladder molecular marker, 1: *Vibrio azureus* bacteria samples

Figure 1. 16S rRNA gene amplification of *Vibrio* sp.1

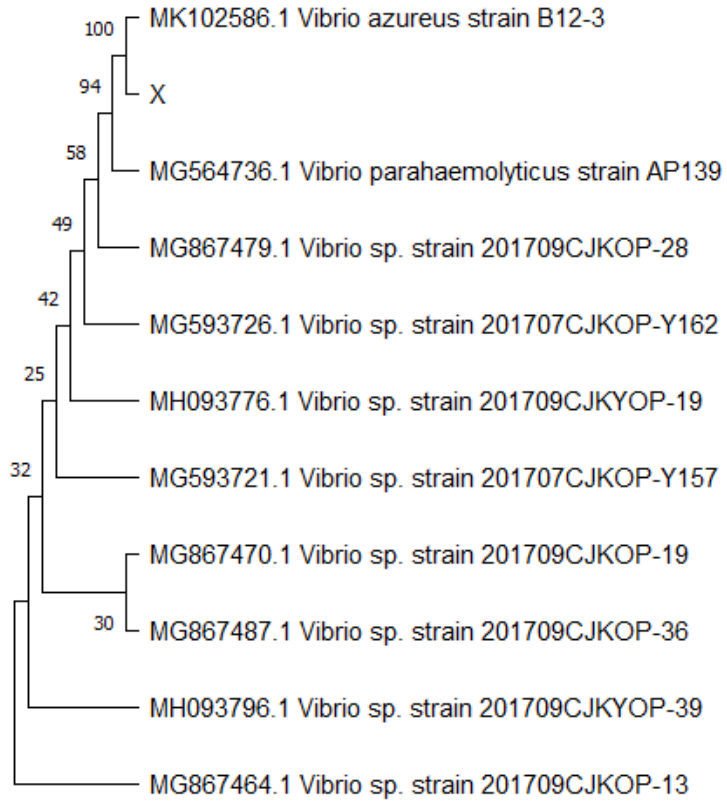


Figure 2. Maximum-likelihood (ML) phylogenetic tree resulted from the analysis of the 16S rRNA gene

The DNA sequence of the sample isolates is compared with the DNA sequences with the same similarities in the gene bank. Figure 2 shows that Vibrio 1 isolate is very close to *V. azureus* strain B12-3.

3.2. Inhibition of Karamunting Leaf Ethanol Extract and MIC against *Vibrio azureus*

The inhibition of the ethanol extract of karamunting leaves was observed through the diameter of the inhibition zone formed. The bacterial suspension used was $1x10^8$ cells/mL. The results of extract inhibition within 24 hours are shown in Table 1.

The largest inhibition zone was found in the 90% treatment, which was 9.38 cm, while the 10% treatment and control had inhibition zone diameters of 6.55 cm and 0.00. Based on statistical tests, the 90% and 70% concentration treatments were not significantly different, the 30% and 10% treatments were not significantly different, but the 50% treatment was the only treatment significantly different from all treatments. In contrast, the control does not show an inhibition zone. The diameter of the inhibition zone (MIC) is shown in Table 2.

Table 1. Inhibition of ethanol extract of Karamunting leaves against *V. azureus*

No	Treatment (%)	N	Inhibition zone (mm)*
1	control	4	0.00 ± 0.00^a
2	10%	4	6.55 ± 0.41^b
3	30%	4	7.05 ± 0.33^b
4	50%	4	7.98 ± 0.53^c
5	70%	4	8.90 ± 0.66^d
6	90%	4	9.38 ± 0.59^d

*Numbers followed by different letter notations in the table indicate significantly different values ($p \leq 0.05$ based on the F test at the 5% level. If the results of the F Test show significantly different results, then proceed with Duncan's test at the 5% level).

Table 2. Minimum Inhibitory Concentration of Karamunting leaf ethanol extract *against V. azureus.*

No	Treatment (%)	Inhibition zone (mm)
1	Control	0.0
2	4	8.0
3	3	6.2
4	2	0.0

The MIC of the ethanol extract of Karamunting leaves was 3%, with an inhibition zone of 6.2 mm. This MIC value is important information on using Karamunting leaf extract to combat the Vibriosis disease in aquaculture.

3.3. *In vivo* Study of Adding Karamunting Leaf Extract on the Shrimp Feed

Karamunting leaf ethanol extract was used through vannamei shrimp feed with 4 different extract concentrations, namely 0 ppm, 50 ppm, 100 ppm, and 200 ppm.

Clinical symptoms of vannamei shrimp after a challenge test were shown by changes in the body (Figure 3).

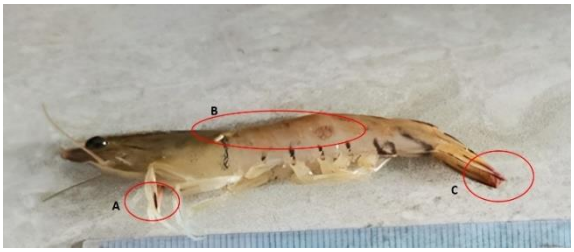


Figure 3. Clinical symptoms of vannamei shrimp injected with *Vibrio azureus* after 14 days of treatment. (a) reddened swimming legs, (b) melanosis on the body segments of the shrimp, and (c) necrosis on the tail of the shrimp

On the first and the second day, the condition of the shrimp was still normal with the control treatment, 50 ppm, 100 ppm, and 200 ppm. Then on the third and fourth day, all shrimp legs and tails experienced redness in all treatments (Table 3). The survival rate of shrimp is presented in Table 4.

Table 3. Clinical symptoms of post-infection *vannamei* shrimp

No	Karamunting Leaf Extract Feed Treatment			
	0 ppm	50 ppm	100 ppm	200 ppm
1	-	-	-	-
2	-	-	-	-
3	+	+	+	+
4	+	+	+	+
5	++	+	+	+
6	++	+	+	+
7	+++	++	+	+
8	+++	++	+	+
9	+++	++	++	+
10	+++	++	++	++
11	+++	+++	++	++
12	+++	+++	++	++
13	+++	+++	++	++
14	+++	+++	++	++

Information:
-: normal shrimp
+: swimming legs (pleopod), reddened telson, reddened tail (uropod).
++: necrosis of the tail
+++: melanosis of the body segments of the shrimp

Table 4. Percentage of Survival Rate (SR) in *vannamei* shrimp

Treatment (ppm)	Survival Rate (%)*
0 (control)	20 ± 7.0^{a}
50	22 ± 8.3^{a}
100	80 ± 10.0^{b}
200	66 ± 8.9^{c}

*Different letters in the same column indicate significantly different results ($p \leq 0.05$) based on Duncan's test results

4. Discussion

The sequencing results obtained for *Vibrio* sp. isolates were 1397 bp. BLAST results of *Vibrio* sp1 isolate identified as *V. azureus* strain B12-3, with 100% sequence identity and an E-value of 0.0. The identity sequence of the *Vibrio* spp. isolate is 100%, which means that the level of similarity in the isolates found is comparable to the comparison specimens in GenBank [19]. The similarity of other *Vibrio* species can be seen in the results of the dendrogram of *Vibrio* sp. (Figure 2). *Vibrio* sp 1 isolate has similarities with *V. azureus* strain B12-3, indicating higher branching accuracy. *Vibrio* sp1 bacteria isolated from vannamei shrimp were identified molecularly using 16S rRNA as *V. azureus* strain B12-3. This bacterium resembles *V. parahaemolyticus*, is luminous, and has a positive oxidation state. Wullur et al. [20] grouped three species of bacteria in the *harveyi* clade of the genus Vibrio, namely *V. harveyi*, *V. parahaemolyticus*, and *V. azureus.*

The results of the inhibition of the ethanol extract of Karamunting leaves in the 90% treatment showed the largest zone, namely 9.38 mm, and the 10% treatment produced the smallest inhibition zone, namely 6.5 mm, and the control treatment had an inhibition zone of 0.00 mm. Antibacterial activity will be further inhibited if the concentration of the extract used is higher, which indicates that the content of antibacterial compounds is greater [21]. Karamunting leaves are reported to contain flavonoids and triterpenoid compounds, which have antimicrobial activity [22].

The study on the Minimum Inhibitory Concentration (MIC) of the ethanol extract of Karamunting leaves against *V. azureus* has never been done. This study showed a MIC value of 6.2 mm at 3% ethanol extract of Karamunting leaves against *V. azureus*. Sinulingga et al. [23] reported the MIC value of Karamunting leaves against *Pseudomonas aeruginosa* bacteria was 14.56 at a concentration of 200 mg/mL.

Effect of feed adding Karamunting leaf extract on vannamei shrimp showing clinical symptoms after infection with *V. azureus*. The highest effect of infection was necrosis on almost the entire shrimp body, indicating a *Vibrio* infection. Aziz [24] reported the challenge test results in which *V. harveyi* was given a density of 10^7 CFU/mL and experienced clinical symptoms from day 1 to day 3. Based on the results of statistical tests on survival

rate (SR) values in vannamei shrimp, which were significantly different ($p \leq 0.05$) compared to other concentrations, the most effective average SR value was 80% at a concentration of 100 ppm (Table 4). The survival rate increased with the use of higher doses related to the content of the extract. This means that the ability of the Karamunting leaf extract feed to reduce vibriosis infection is quite small. Alloul et al. [25] stated that there were no deaths after being challenge-tested with *V. campbellii* for 62 hours. However, shrimp mortality happened after this period.

The vannamei shrimp survival rate is influenced by several environmental factors: temperature, salinity, and pH. The study by Dayalan et al. [26] reveals that shrimp *L. vannamei* can self-regulate temperature variation in the environment to a certain extent. In addition, Jeffer et al. [27] suggested that low salinity does not seem to affect osmotic regulation to the extent that growth and survival rates will be affected in vannamei shrimp. If the acclimation process is followed properly, the species has excellent potential in inland saline waters at salinity as low as 1 ppt. A low salinity value can affect the survival rate of vannamei shrimp, while a high salinity value will affect the level of retention and osmoregulation of nutrients in the shrimp's body. Yu et al. [28] suggested that long-term lower or higher pH stress could adversely affect gut microbiota functions, thus reducing shrimp's growth, immunity, antioxidant capacity, and even intestine histology damage. The average pH parameter value in rearing media is around 8, which is good for the growth of the shrimp.

5. Conclusions

Based on this research, it can be concluded that the isolated bacterial identified molecularly as *Vibrio azureus* B12-3 with a 100% similarity value confirmed that it could cause vibriosis disease in the Pacific white leg shrimp *Litopenaeus vannamei*. Using 100 ppm ethanol extract of Karamunting leaf extract increased shrimp survival by up to 80% when challenged with *V. azureus*. The study showed that Karamunting leaf extract can potentially prevent vibriosis disease in vannamei shrimp cultivation. This will contribute scientifically to the addition of alternative natural ingredients that can be used to prevent bacterial diseases in shrimp culture. Economically, this can be a low-cost alternative to expensive antibiotics and chemical compounds. Besides the cost benefits, using natural extracts such as Karamunting leaves will be a more environmentally friendly alternative, and the product is safe for consumption. Further research regarding the mode of action of Karamunting leaf extract is needed to find out in more detail whether the extract affects reducing pathogen virulence or increasing the immunity of vannamei shrimp.

REFERENCES

[1] Wijayanto D., Nursanto D.B., Kurohman F., Nugroho R.A., "Profit maximization of whiteleg shrimp (*Litopenaeus vannamei*) intensive culture in Situbondo Regency, Indonesia," AACL Bioflux Vol 10, no 6, pp. 1436-1444, 2017. http://www.bioflux.com.ro/aacl

[2] Valente C.D.Z., Wan A.H.L., "Vibrio and major commercially important vibriosis diseases in decapod crustaceans," Journal of Invertebrate Pathology Vol 181, p. 107527, 2021. doi: https://doi.org/10.1016/j.jip.2020.107527

[3] Piamsomboon P., Han J. E., "White Feces Syndrome, A Multifactorial Syndrome of Cultured Shrimp: A Mini Review," Fishes, Vol. 7, no. 6, p. 339, 2022. https://doi.org/10.3390/fishes7060339

[4] Chi T.T.K., Clausen J.H., Van P.T., Tersbøl B., Dalsgaard A., "Use practices of antimicrobials and other compounds by shrimp and fish farmers in Northern Vietnam," Aquaculture Reports, Vol 7, pp. 40-47, 2017. https://doi.org/10.1016/j.aqrep.2017.05.003

[5] Liu X., Steele J.C., Meng X-Z., "Usage, residue, and human health risk of antibiotics in Chinese aquaculture: a review," Environmental Pollution, Vol. 223, pp. 161–169, 2017. https://doi.org/10.1016/j.envpol.2017.01.003

[6] Baoprasertkul P., Somsiri T., Boonyawiwat V., "Use of veterinary medicines in Thai aquaculture: current status. In: Bondad-Reantaso M.G., Arthur J.R., Subasinghe R.P., "(eds) Improving Biosecurity through Prudent and Responsible Use of Veterinary Medicines in Aquatic Food Production," FAO, Rome, Italy, p. 83, 2012.

[7] Lulijwa R., Rupia E.J., Alfaro A.C., "Antibiotic use in aquaculture, policies and regulation, health and environmental risks: a review of the top 15 major producers," Reviews in Aquaculture Vol 12, no 2, pp. 1–24, 2019. doi: 10.1111/raq.12344

[8] Ramadhan G., Krisridwany A., Wibowo A.E., Kurniawan M.F., Damarwati V.L., "A Literature Review: The Potential of Karamunting Plant (*Rhodomyrtus tomentosa*) As Antibacterial Agent," Jurnal Farmasi Sains dan Praktis Vol 9, No. 2, p 96-103, 2023. https://doi.org/10.31603/pharmacy.v9i1.6415

[9] Limsuwan S., Kayser O., Voravuthikunchai S.P,. "Antibacterial Activity of *Rhodomyrtus tomentosa* (Aiton) Hassk. Leaf Extract against Clinical Isolates of *Streptococcus pyogenes*," Evid Based Complement Alternat Med, Vol. 2012, p. 697183, 2012. doi: 10.1155/2012/697183

[10] Zeyohanness S.S., Hamid H.A., Zulkifli F.H., "Poly(vinyl alcohol) Electrospun Nanofibers Containing Antimicrobial *Rhodomyrtus tomentosa* Extract," Journal of Bioactive and Compatible Polymers, Vol. 33, no. 6, pp. 585-596, 2018. https://doi.org/10.1177/0883911518801040

[11] Zymo Research. "Microbiomics," Zymo Research Corp. USA. P. 54, 2019. https://zymoresearch.eu/pages/microbiomics

[12] Said I.G., Abdelwahed N.A.M., Awad H.M., Shallan M.A., El-Shahed K.Y.I., Abdel-Rahim E.A., "Enhancement of Clavulanic Acid Production by Streptomyces sp. MU-

NRC77 via Mutation and Medium Optimization," Tropical Journal of Pharmaceutical Research, Vol. 16, No.1, Pp. 31-42, 2017. http://dx.doi.org/10.4314/tjpr.v16i1.5

[13] Green M.R., Sambrook J., "Molecular cloning: A Laboratory Manual (fourth edition)," Cold Spring Harbor Lab Press, 2014.

[14] Kumar S., Stecher G., Li M., Knyaz C., Tamura K.," MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms," Molecular Biology and Evolution, Vol. 35, No. 6, pp.1547-1549, 2018. DOI: 10.1093/molbev/msy096

[15] Zapata A., Ramirez-Arcos S., "A Comparative Study of McFarland Turbidity Standards and the Densimat Photometer to Determine Bacterial Cell Density," Current Microbiology, Vol. 70, pp. 907–909, 2015. https://doi.org/10.1007/s00284-015-0801-2

[16] Athaillah A., Lestari U., "Antibacterial Activity Test of Etanol Extract From Dried Simplicia Garlic (Allium sativum L.) Towards *Bacillus cereus* Bacteria," Journal of Pharmaceutical and Science, Vol. 3, No. 2, pp. 93-99. 2020. https://doi.org/10.36490/journal-jps.com.v3i2.5

[17] Rahayu E., Lahay N., Jamilah, "Antibacterial Inhibition Test Against the Combination Extract of Moringa Leaf (Moringa oliefera) and Basil Leaf (Ocimum basilicum) as a Substitute for Feed Additive," Hasanuddin J. Anim. Sci., Vol. 3, No. 2, pp. 85-94, 2021. Doi:10.20956/hajas.V3i2.20074

[18] Pratiwi R., Sudiarsa I.N., Amalo P., Utomo Y.W.W, "Production Performance of Super Intensive Vannamei Shrimp *Litopenaeus vannamei* at PT. Sumbawa Sukses Lestari Aquaculture, West Nusa Tenggara," Journal of Aquaculture and Fish Health, Vol. 11, no. 1, 2022. DOI: 10.20473/jafh.v11i1.21143

[19] Culot A., Grosset N., Bruey Q., Auzou M., Giard J.C.,, Favard B., Wakatsuki A., Baron S., Frouel S., Techer C., Gautier M., "Isolation of Harveyi clade Vibrio spp. collected in aquaculture farms: How can the identification issue be addressed?," Journal of Microbiological Methods, Vol. 180, 2021. https://doi.org/10.1016/j.mimet.2020.106106

[20] Wullur S., Napitupulu H., Wantania L.L., Ginting E.L, Warouw V., Tilaar S., Tallei T.E., Rumengan I.F.M., "Molecular Identification of Bacteria Isolated From Culture Medium of Rotifer Fed on Fishery Waste Diet," Biodiversitas, Vol. 21, No. 6, pp. 2735-2740, 2020. DOI:10.13057/biodiv/d210649

[21] Srikacha N., Ratananikom K., "Antibacterial activity of plant extracts in different solvents against pathogenic bacteria: An in vitro experiment." J. Acute Dis., Vol. 9, No. 5, pp. 223-226. 2020. DOI https://doi.org/10.4103/2221-6189.291288

[22] Septiani I., Ichrom M.Y., Rasyid N.I., " The effect of Karamunting (*Rhodomyrtus tomentosa*) leaf extract on the number of macrophages in pulp inflammation," Dentino Jurnal Kedokteran Gigi, Vol. 6 no. 2, pp. 131-135, 2021. http://dx.doi.org/10.20527/dentino.v6i2.11994

[23] Sinunglingga S.E., Hasibuan P.A.Z., Suryanto D., "Antibacterial Activity of Karamunting (*Rhodomyrtus Tomentosa* Aiton Hassk) Leaf Extract and Fractions," Asian Journal of Pharmaceutical and Clinical Research. Vol. 11, No. 3, pp.163-165. 2018. DOI:10.22159/ajpcr.2018.v11i3.23505

[24] Aziz, Cahyadi J., "Benefits of Tiwai Onion (*Eleutherine americana*) Extract as Phytopharmaceutical Plant to Inhibit the Growth of Bacteria *Vibrio harveyi* Through in-Vitro and in-Vivo." Jurnal Ilmiah Perikanan dan Kelautan, Vol. 12, No. 1, pp. 105-112, 2020. http://doi.org/10.20473/jipk.v12i1.12826

[25] Alloul A., Muys M., Hertoghs N., Kerckhof F., Vlaeminck S.E., "Cocultivating Aerobic heterotrophs and Purple Bacteria for Microbial Protein in Sequential Photo- and Chemotrophic Reactors," Bioresource Technology, Vol. 319, p. 124192, 2020. https://doi.org/10.1016/j.biortech.2020.124192

[26] Dayalan V., Kasivelu G., Raguraman V., Sharma A.N., "Studies on temperature impact (sudden and gradual) of the white-leg shrimp *Litopenaeus vannamei*," Environ. Sci. Pollut. Res. Int., 2022. doi: 10.1007/s11356-022-20963-y

[27] Jaffer Y.D., Saraswathy R., Ishfaq M., Antony J., Bundela D.S., Sharma P.C., "Effect of low salinity on the growth and survival of juvenile pacific white shrimp, *Penaeus vannamei*: A revival," Aquaculture, Vol. 515, 2020. https://doi.org/10.1016/j.aquaculture.2019.734561

[28] Yu Q., Xie J., Huang M., Chen C., Qian D., Jian G. Qin, Chen L., Jia Y., Li E., "Growth and health responses to a https://doi.org/10.1016/j.aqrep.2020.100280