

EFFECTS OF DIETARY OF ROSELLA (*Hibiscus sabdariffa*) SUPPLEMENTATION ON WHITE LEG SHRIMP (*Litopenaeus vannamei*) AGAINST ACUTE HEPATOPANCREATIC DISEASES CAUSED BY *Vibrio parahaemolyticus*

Nguyen Thi Truc Linh*, Phan Thi Thanh Truc

Tra Vinh University

ARTICLE INFO	ABSTRACT
Received: 22/9/2023	This study evaluated the effects of rosella (<i>Hibiscus sabdariffa</i>) extracts supplied in commercial feed on the survival and the density of <i>Vibrio</i> spp. in shrimp hepatopancreas following the challenge with <i>Vibrio parahaemolyticus</i> , causing acute hepatopancreatic disease (AHPND). The shrimp in each treatment was prepared by feeding with herbal feed 1, 1.5, and 2% for 30 days, then challenged with <i>V. parahaemolyticus</i> and further cultured for 14 days. Each treatment was designed with 3 replicates. Results showed that the shrimps fed on diets with rosella extract have improved the survival of shrimps and inhibited the harmful <i>Vibrio</i> spp. density significantly in comparison with the controls ($P < 0.05$). Through the hepatopancreatic histopathology analysis, it was also shown that using rosella extract can reduce the effects of AHPND on the hepatopancreas structure of white leg shrimp. This study demonstrated the potential of using rosella extracts to improve the resistance of white-leg shrimp against <i>V. parahaemolyticus</i> .
Revised: 24/10/2023	
Published: 31/10/2023	
KEYWORDS	
<i>Hibiscus sabdariffa</i>	
<i>V. parahaemolyticus</i>	
AHPND	
White leg shrimp	
<i>Vibrio</i> spp.	

ẢNH HƯỞNG CỦA VIỆC BỔ SUNG CHẤT CHIẾT LÁ GIẤM (*Hibiscus sabdariffa*) LÊN KHẢ NĂNG KHÁNG BỆNH HOẠI TỬ GAN TỤY CẤP TÍNH DO VI KHUẨN *Vibrio parahaemolyticus* GÂY RA TRÊN TÔM THẺ CHÂN TRẮNG (*Litopenaeus vannamei*)

Nguyễn Thị Trúc Linh*, Phan Thị Thanh Trúc

Trường Đại học Trà Vinh

THÔNG TIN BÀI BÁO	TÓM TẮT
Ngày nhận bài: 22/9/2023	Nghiên cứu được thực hiện nhằm đánh giá hiệu quả của chất chiết lá giấm (<i>Hibiscus sabdariffa</i>) bổ sung trong khẩu phần ăn lên tỉ lệ sống và mật độ của vi khuẩn <i>Vibrio</i> spp. trên gan tụy tôm thẻ chân trắng (<i>Litopenaeus vannamei</i>) trọng lượng từ 10 - 12 gram cảm nhiễm với vi khuẩn <i>Vibrio parahaemolyticus</i> gây bệnh hoại tử gan tụy cấp tính. Tôm được cho ăn với thức ăn bổ sung chất chiết lá giấm ở các nồng độ 1; 1,5; 2% trong 30 ngày, sau đó cảm nhiễm với vi khuẩn <i>V. parahaemolyticus</i> và tiếp tục quan sát trong 14 ngày. Mỗi nghiệm thức được bố trí lặp lại 3 lần. Kết quả nghiên cứu cho thấy bổ sung chất chiết lá giấm có khả năng cải thiện tỉ lệ sống và ức chế sự phát triển vi khuẩn <i>Vibrio</i> spp. trên gan tụy tôm, khác biệt có ý nghĩa ($p < 0,05$) so với nghiệm thức đối chứng. Thông qua phân tích mô bệnh học gan tụy tôm chứng tỏ chất chiết lá giấm còn giúp làm giảm những ảnh hưởng của bệnh hoại tử gan tụy cấp tính lên cấu trúc gan tụy của tôm thẻ chân trắng. Nghiên cứu đã chứng minh tiềm năng của việc sử dụng chất chiết lá giấm trong nâng cao khả năng đề kháng AHPND trên tôm thẻ chân trắng.
Ngày hoàn thiện: 24/10/2023	
Ngày đăng: 31/10/2023	
TỪ KHÓA	
Bệnh hoại tử gan tụy cấp tính	
<i>Hibiscus sabdariffa</i>	
<i>V. parahaemolyticus</i>	
Tôm thẻ chân trắng	
<i>Vibrio</i> spp.	

DOI: <https://doi.org/10.34238/tnu-jst.8803>

* Corresponding author. Email: truclinh@tvu.edu.vn

1. Introduction

Acute hepatopancreatic necrosis disease (AHPND) has caused severe losses in farmed populations of marine shrimp *P. vannamei* and *P. monodon*. The disease emerged in China and Vietnam in 2010 and spread throughout Southeast Asia. It was later reported in countries in both North and South America. The disease has had significant economic impacts on the shrimp aquaculture industry. From 2010 to 2016, the combined losses from China, Malaysia, Mexico, Thailand, and Vietnam due primarily to outbreaks of AHPND, including losses at the farm gate and those resulting from a drop in feed sales and exports, were estimated at over US\$ 44 billion [1]. In Viet Nam, AHPND is identified as the cause of the biggest damage on shrimp with more than 2,000 ha infected areas per year [2].

Chemotherapeutics are extensively used in the aquaculture industry as a prophylactic and control measure for a variety of infections, including AHPND. Farmers commonly use antibiotics and chemicals to treat the pathogen in aquaculture. However, using chemicals and antibiotics might cause environmental contamination and antibiotic resistance of the pathogenic bacteria. Moreover, chemical or antibiotic residuals are one of the criteria that plays a vital role in exporting agricultural products. Other undesirable side-effects and impacts on human health have resulted in and affect non-target organisms [3]-[5]. In several studies, the pathogenic *V. parahaemolyticus* was resistant to many antibiotics [6]-[7]. Therefore, natural products from medicinal plants and marine seaweeds are considered potential alternatives for the prevention and treatment of AHPND in shrimp [8]. Extracts from some herbs have the ability to antagonize and prevent AHPND, such as two brown seaweeds *Padina tetrastrum* and *Sargassum ilicifolium* [9] betel (*Piper betle*), Gotu kola (*Centella asiatica*) and garlic (*Allium sativum*) [10], *Polygonum chinense* L. [11] the *P. urinaria* and the *T. catappa* [12].

Roselle plant (*H. sabdariffa* L.) belongs to the kingdom Plantae, division Magnoliophyta, class Magnoliopsida, order Malvaceae, family Malvaceae, genus *Hibiscus*. This plant has long been used as herbal medicine and cultivated throughout the world, especially in tropical and subtropical areas such as Africa and Asia. The health benefits of rosella are attributed to its polyphenol content (flavonoids, tannins, quercetin, anthocyanins, saponins terpenoids, phenolics) [13]. Previous study showed that it had significant antibacterial activity against many veterinary and human bacterial pathogens such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella enterica*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris* and *Bacillus cereus*, *Acinetobacter baumannii*; *Staphylococcus aureus*, *E. coli* [14].

In aquaculture, researching on rosella is still limited. In order to provide more information on the effect of using rosella to increase the resistance to AHPND on white leg shrimp, the study "Effects of dietary of rosella (*H. sabdariffa*) supplementation on white leg shrimp (*L. vannamei*) against acute hepatopancreatic diseases caused by *V. parahaemolyticus*" was carried out.

2. Materials and Methods

2.1. Bacterial preparation

The isolation of *V. parahaemolyticus* causing acute hepatopancreatic necrosis disease belongs to the bacterial collection of the Faculty of Agriculture, Tra Vinh University [15]. The bacteria were recovered in Tryptic Soy Broth (TSB, Merck) supplemented with 1.5% NaCl (TSB+), incubated at 28°C for 18 hours, then streaked onto tryptic soy agar (Merck) supplemented with 1.5% NaCl (TSA+) and continued to incubate at 28°C for 18 hours. Checking the purity of bacteria via colony morphology and Gram staining method. The pure bacteria were grown in TSB+ medium at 28°C for 18 hours, determined the density by spectrophotometer at 610 nm, and adjusted to obtain an OD value = 0.1 ± 0.02 (equivalent to a density of 10^8 CFU/ml).

2.2. The dietary supplementation experiments

Experimental shrimps: The healthy white leg shrimp weighting from 8-10 grams was obtained from a shrimp farm at Tra Vinh University. Upon arrival at our lab, shrimp were randomly selected for screening against the typical diseases, including WSSV, AHPND, EHP, and YHV, and proved to be negative for infection. The shrimps were acclimatized with sterilized and continuously aerated seawater for three days. They were fed four times a day on commercial shrimp feed. After the acclimatization period, shrimps were randomly distributed into 15 composite tanks (500L of water volume) contained 60 individuals/tank.



Figure 1. The healthy white leg shrimp (*Litopenaeus vannamei*) using in the experiment

Feed diet preparation: The extract of *H. sabdariffa* was mixed with feed at final concentrations of 1, 1.5, and 2%. A control diet was prepared without extract.

The experimental setup: There were five treatments in triplication, including two control groups (without extract) and three experimental groups, that mixed at 1, 1.5, and 2% of the extracts. Shrimps were fed four times daily at 07:00, 11:00, 15:00, and 19:00 hour. The ratio was initially 7 - 10% of body weight and it adjusted according to the shrimps feeding response.

2.3. Determination of the effect of *H. sabdariffa* supplementation on white leg shrimp against acute hepatopancreatic diseases caused by *V. parahaemolyticus*

After 30 days of feeding, 30 shrimps from each tank were chosen to perform the bacterial challenge. There were five treatments in triplication and the details of the experimental design were described in Table 1. The challenge method was done following the protocol described by Tran et al., (2013) [16] with AHPND *V. parahaemolyticus* at 10^6 CFU/mL.

Table 1. The experiment-designed description

No	Treatment	Feed with <i>H. sabdariffa</i>	<i>V. parahaemolyticus</i> challenged (10^6 CFU/ml)
1	Positive control (PC)	No	Yes
2	Negative control (NC)	No	No
3	1% <i>H. sabdariffa</i>	Yes	Yes
4	1.5% <i>H. sabdariffa</i>	Yes	Yes
5	2% <i>H. sabdariffa</i>	Yes	Yes

Shrimp were fed four times daily and water was exchanged at 30% after three days of challenge and further exchanged at 30% daily. The temperature was kept at 27 °C - 30, pH at 7.5 - 8.5, and alkalinity at 110 - 120 mg CaCO₃/L.

Observation and sampling:

Shrimps were observed daily for clinical signs and mortality after the challenge. The mortality of shrimps after 14 days of the challenge was calculated using the formula given: Mortality (%) = (died shrimp after a challenge/total shrimp in the tank)*100%.

Water quality parameters were checked daily using Sera test kits (Sera, Germany) to maintain suitable conditions for shrimp culture.

The density of *Vibrio* in shrimp hepatopancreas (HP) was determined based on a counting method described by Far et al., (2019) [17] on the days 0, 3, 7, and 14 after the challenge. Briefly, three shrimp individuals were collected randomly from each tank. Samples of shrimp HP were collected in sterile conditions, weighed, and milled in 0.85% NaCl solution. Thereafter, the suspension was diluted to different concentrations and 100 µL of each dilution was spread on Thiosulfate Citrate Bile Salts Sucrose (TCBS, Merck) to enumerate *Vibrio* spp. The agar plates

were further incubated at 28°C for 24 and 48 h, respectively. The densities of the bacteria were determined by counting the colonies growing on agar plates using this formula: Density of bacteria (CFU/g) = (number of colonies x dilution)/V/M, where V is the volume of the suspension placed on the TCBS agar plate (mL) and M is the weight of the shrimp HP(g).

Three shrimps per tank were randomly collected to examine histopathology changes in hepatopancreas on the 3rd day after the challenge. Histological analysis was performed following the protocol described in the previous study [18]. Shrimp hepatopancreas were fixed in Davidson's solution for 48 h and placed in 70% ethanol solution. Subsequently, samples were passed through ethyl alcohol 70%, 80%, 95%, 100%, and xylene solution. Then, samples were cut into pieces with 5 µm thickness. Subsequently, the slide samples were stained with Hematoxylin and Eosin (H and E), and the infected and healthy shrimp tissues were identified under the microscope at 10X and 40X magnification and labeled for further data analysis.

2.4. Data analysis

SPSS software V.22 was used to compare the mean between different treatments and one-way analysis of variance (ANOVA) was used to analyze the data. Duncan's multiple-comparison test was used to determine the significant difference among treatments at a 0.05 significance level.

3. Results and discussion

3.1. Water quality parameters

During the culture experiment, NH₃ and NO₂ concentrations, temperature, alkalinity, and pH of the tank water ranged from 0 to 0.13 mg/L; 0 - 4 mg/L, 27 - 28°C, 80 - 120 mg CaCO₃/L, and pH 7.0 - 8.5, respectively. In general, the water quality parameters were well managed and did not negatively affect the shrimp's average growth and development during the experimental period.

3.2. The density of *Vibrio* spp. in shrimp hepatopancreas

Before the challenge, the total *Vibrio* spp. in shrimp hepatopancreas (HP) ranged from 1.5 - 2 × 10² CFU/g, had no significant (p>0.05) between all experiential treatments. In the negative control, *Vibrio* density in the shrimp HP did not significantly change during the experiment (ranging from 2 to 5 × 10² CFU/g). However, in all the treatment challenges with AHPND, *Vibrio* density in the shrimp HP increased significantly (p<0.05) on day 3, then decreased followed by the treatment and sampling time. The results are shown in Table 2.

Table 2. *Vibrio* spp. density in white leg shrimps HP

Treatment	<i>Vibrio</i> spp. density (× 10 ⁴ CFU/g)			
	0	3	7	14
Negative control	0.02 ± 0.01 ^{Aa}	0.02 ± 0.02 ^{Aa}	0.05 ± 0.03 ^{Aa}	0.04 ± 0.02 ^{Aa}
Positive control	0.02 ± 0.015 ^{Aa}	520.67 ± 97.17 ^{Bb}	32.03 ± 16.1 ^{bC}	4.11 ± 2.68 ^{aD}
1% <i>H. sabdariffa</i>	0.02 ± 0.01 ^{Aa}	0.25 ± 0.04 ^{aB}	0.18 ± 0.08 ^{aB}	0.04 ± 0.02 ^{aC}
1.5% <i>H. sabdariffa</i>	0.015 ± 0.01 ^{Aa}	0.23 ± 0.08 ^{aB}	0.07 ± 0.01 ^{aB}	0.03 ± 0.02 ^{aC}
2% <i>H. sabdariffa</i>	0.02 ± 0.012 ^{Aa}	0.10 ± 0.05 ^{aB}	0.07 ± 0.05 ^{aB}	0.07 ± 0.02 ^{aB}

Note: Within a column/row, treatments with the same small/big letters are not significantly different (p<0.05)

The *Vibrio* spp. density in shrimps HP at positive control treatment was 5.2 ± 0.97 × 10⁶ CFU/g on day 3rd then reduced significantly (p<0.05) on day 7th (3.2 ± 1.6 × 10⁵) and day 14 (4.11 ± 2.68 × 10⁴ CFU/g) post-infection. While among the extract supplement, the lower density was recorded in all of the treatments, different significantly (p<0.05) in comparison with positive control as the density ranged from 0.4 - 2.5 × 10³ CFU/g, 0.3 - 2.3 × 10³ and 0.7 - 1 × 10³ CFU/g, followed by the supplemented concentration of the extract was 1, 1.5 and 2%, respectively. However, there was no significant between the supplement groups at all the sampling times (p>0.05).

Currently, the studies on the effects of herbs on shrimp's pathogenic bacteria are still limited. The reduction of *Vibrio* spp. was shown by Phuc and Thanh (2022) [10], in supplied three herbal extracts of betel (*P. belle*), Gotu kola (*C. asiatica*), and garlic (*A. sativuni*). The result also significantly reduced *Vibrio* density in shrimp after being challenged with 5.27×10^4 - 1.15×10^5 ; 5.27×10^4 - 1.14×10^5 and 5.17×10^4 - 2.61×10^4 CFU/g respectively, compared with the ranging from 5.29×10^5 - 4.97×10^6 in the positive control. According to Cowan (1999) [19], the antibacterial effect of herbal extracts is due to antibacterial compounds such as phenols, quinones, flavonoids, flavones, tannins, and coumarins, and through mechanisms such as cell wall disruption, inhibits protein synthesis and DNA synthesis, inhibits bacterial enzyme secretion. In the rosella plant, the antimicrobial compounds are polyphenolic flavonoids including quercetin, kaempferol, hibiscetine, and sabdaretine [13]. The previous study showed that it has significant antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella enterica*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris* and *Bacillus cereus*, *Acinetobacter baumannii* [14]. In this study, we have also proved that the use of *H. sabdariffa* extract can reduce and well-controlled the density of *Vibrio* spp. in the hepatopancreas of shrimp with a density lower than 100 - 500 times than that of the supplemented treatment after being challenged with the pathogenic *V. parahaemolyticus*.

3.3. Effect of dietary of *H. sabdariffa* on the survival of white-leg shrimps challenged with AHPND

The mortality of the positive control (PC), negative control (NC), and the three different concentrations of rosella extract incorporated in diets-fed shrimps are presented in Figure 2.

All the challenged group died within two days and stopped dying 7 days after infection. After 14 days of challenge, the positive control group of shrimps fed on a diet devoid of extract acceded to death (46.67%) within seven days after challenging with *V. parahaemolyticus*, while the lower rate was recorded on the rosella extract treatment with 17.3%, 16.7%, and 22.7% followed by 1%, 1.5%, and 2% of concentration, respectively. The results revealed that variation in the survival rate of white-leg shrimp fed with positive control and other groups was statistically significant ($p < 0.05$). However, there was no significant difference between the rosella extract-supplemented treatments ($p > 0.05$).

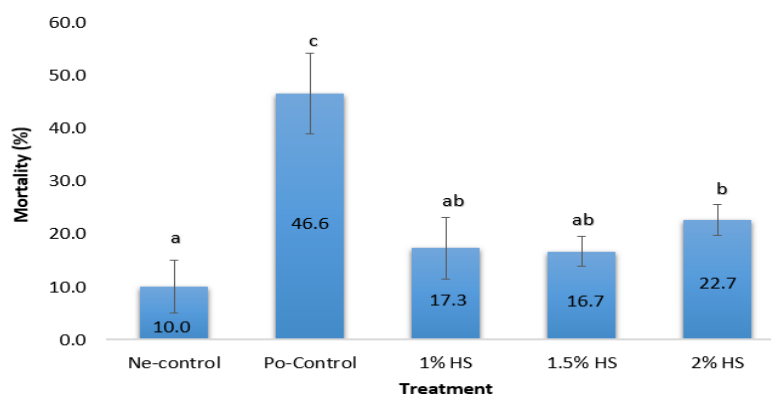


Figure 2. The accumulated mortality (%) of white leg shrimp challenged with *V. parahaemolyticus*

The challenge test indicated the ability of the rosella to protect white-leg shrimp from *V. parahaemolyticus*. A similar effect was also recorded in several previous studies with the other herb extracts. Aftabudin et al. (2021) [9] used the crude extract of *P. chinense* L. added to the shrimp culture water at 30 g/m^3 to improve the shrimp survival rate by 60% compared with 0% in positive control. Phuc and Thanh (2022) [10] observed the lowest mortality levels using 1% and 2% during 30 days of the *P. urinaria* and *T. catappa* exposed to white leg shrimp infected with *V.*

parahaemolyticus. Moreover, high concentrations of extracts (20,000 & 40,000 mg/L) were found to be associated with lower mortality levels for *L. vannamei* [20]. According to Kumar et al. (2021) [8] the extracts of betel (*Piper belle*), Gotu kola (*Centella asiatica*), and garlic (*Allium sativuni*) either incorporated in commercial feed or directly applied in cultured water increased the survival rate and controlled the *Vibrio* spp. the density of the white-leg shrimp following the challenge with *V. parahaemolyticus*.

Generally, using herb extract is considered one of the potential phytochemicals that can replace the synthetic chemicals or antibiotics used in aquaculture, and the protective effects of herbs on shrimp are established through improved growth and nutrient absorption, antimicrobial mechanisms, and non-specific immune system enhancement [21].

3.4. The hepatopancreatic histopathology

The histopathology of shrimp hepatopancreas in the negative control showed a normal structure of hepatopancreas with present and a high number of B, R, and F cells (Figure 3 A and B). Meanwhile, the histological changes were recorded in all AHPND-challenged treatments with the number and level of these changes differed among treatments, especially in the supplementary rosella extract. In particular, the ratio of 100% shrimp's HP in PC treatment showed typical signs of AHPND histopathology as described by Tran et al., (2013) [16] such as (1) the atrophied HP tubules, lack of B, F, and E cells; (2) sloughing of HP cells to the lumens and (3) showed secondary infection with a mass of hemocytes infiltration and melanization (Figure 3 C and D). While all of the treatment groups record a lack of B, R, and F cells with the prevalence as 55.6%, 55.6%, and 44.4% followed by 1%, 1.5%, and 2% of the extract respectively. There was only 11.1% in the 1% extract treatment shown hemocyte infiltration (Figure 3 E and F) (Table 3).

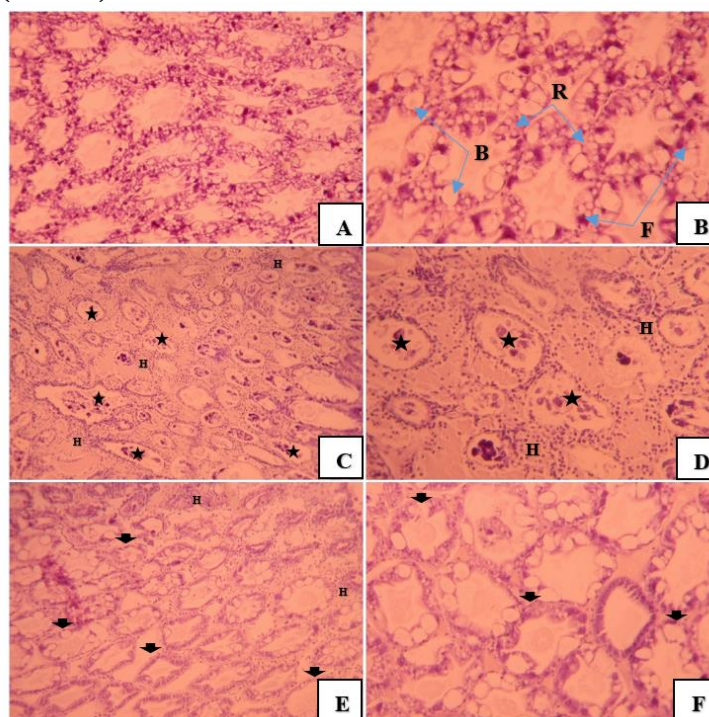


Figure 3. The hepatopancreatic histopathology at day 3 after challenge with AHPND. (A,B) Negative control. A present with a numerous number of B cells (B), R cells (R) and F cells (F). (C, D) Positive control. The lack of B, R, F cells and sloughing of the epithelial cells into HP tubule lumen (star), hemocyte infiltration (H). (E, F). 1% rosella extract supplement treatment. The lack of B, R, F cells (arrow) and hemocyte infiltration (H). (A, C, E: 10X; B, D, F: 40X)

Table 3. Histopathological changes of shrimp's HP infected with *V. parahaemolyticus* after 3 days

Histopathology	Prevalent (%)				
	Negative control	Positive control	1.0% HS	1.5% HS	2.0% HS
Lack of B, R, and F cells	0	100	55.56	55.6	44.4%
Sloughing of the epithelial cells	0	100	11.1%	0	0
Hemocyte infiltration/Melanization	0	100	0	0	0

On the 14th day of infection, white leg shrimp showed recovery in treatments supplemented with the extract, determined through histopathological characteristics of these treatments had an almost normal structure, however, the positive control treatment recovered at a lower rate. In summary, based on histopathological records, it was confirmed that the supplement of the extract had reduced the effect of acute hepatopancreatic necrosis disease in white-leg shrimp.

4. Conclusion

The using of rosella extracts by continuous feeding for 30 days was able to reduce the concentration of *Vibrio* spp. in hepatopancreas, reducing the impact of the disease and improving the survival rate of white leg shrimp challenged with pathogenic *V. parahaemolyticus*. From the above results, further studies need to perform pond experiments to confirm the effectiveness of using rosella in the prevention of AHPND on white leg shrimp in a field and continue to study of the effect rosella on some other pathogenic bacteria on aquatic animals.

REFERENCES

- [1] K. F. J. Tang and M. G. Bondad-Reantaso, "Impacts of acute hepatopancreatic necrosis disease on commercial shrimp aquaculture," *Rev Science Technology*, vol. 38, no. 2, pp. 477-490, 2019, doi: 10.20506/rst.38.2.2999.
- [2] Department of Animal Health, *Report on Aquatic disease prevention in 2021 and plan in 2022*, Hanoi, 6th of December, 2021.
- [3] S. Aftabuddin, A. Kader, A. M. Kamal, and M. Zafar, "Present status on the use of antibiotics and chemicals in shrimphatcheries and grow-out ponds and their environmental implications in Bangladesh," *Aquaculture, Aquarium, Conservation & Legislation International Journal of the Bioflux Society*, vol. 2, no. 4, pp. 369-379, 2009.
- [4] B. Vaseeharan and R. Thaya, "Medicinal plant derivatives as immunostimulants: an alternative to chemotherapeutics and antibiotics in aquaculture," *Journal of Aquaculture International*, vol. 22, no. 3, pp. 1079-1091, 2014.
- [5] P. Grenni, V. Ancona, and A. B. Caracciolo, "Ecological effects of antibiotics on natural ecosystems: a review," *Microchemical Journal*, vol. 136, pp. 25-39, 2018.
- [6] T. H. O. Dang, Q. P. Truong, and T. P. Nguyen, "Antibacterial resistance of *Vibrio parahaemolyticus* isolated from shrimp farms located in east coastal region of the Mekong Delta, Vietnam," *CTU Journal of Innovation and Sustainable Development*, vol. 14, no. 2, pp. 1-7, 2022.
- [7] P. T. H. Ha, Q. V. C. Thi, N. P. Thuy, and N. T. Luan, "Multi-antibiotics resistance phenotype of pathogenic *Vibrio parahaemolyticus* isolated from acute hepatopancreatic necrosis disease in *Litopenaeus vannamei* farmed in the Mekong Delta," *Journal of the World Aquaculture Society*, vol. 54, no. 4, pp. 1070-1087, 2023.
- [8] V. Kumar, S. Roy, B. K. Behera, P. Bossier, and B. K. Das, "Acute Hepatopancreatic Necrosis Disease (AHPND): Virulence," *Pathogenesis and Mitigation Strategies in Shrimp Aquaculture*, vol. 13, no. 8, 2021, Art. no. 524, doi: 10.3390/toxins13080524.
- [9] S. AftabUddin, M. A. M. Siddique, A. Habib, S. Akter, S. Hossen, P. Tanchangya, and M. A. Al, "Effects of seaweeds extract on growth, survival, antibacterial activities, and immune responses of *Penaeus monodon* against *Vibrio parahaemolyticus*," *Italian Journal of Animal Science*, vol. 20, no. 1, pp. 243-255, 2021.

-
- [10] L. N. T. Phuc and N. M. Thanh, "Effects of herbal extracts on the resistance of the white leg shrimp (*Litopenaeus vannamei*) juveniles to *Vibrio parahaemolyticus*," *Journal of Vietnam Agricultural Science and Technology*, vol. 68, no. 14, pp. 54-60, 2022.
- [11] M. H. Truong, T. Y. Pham, T. H. Pham, T. M. L. Huynh, T. H. M. Pham, T. L. Do., T. H. V. Tran, and T. V. Phan, "Antibacterial effect of *Polygonum chinense* L. extract on pathogen bacteria of acute hepatopancreatic necrosis disease in brackish shrimps," *Journal of Vietnam Agriculture Science and technology*, vol. 59, no. 6, pp. 19-24, 2017.
- [12] M. H. Hong, V. T. Nguyen, V. H. Huynh, and T. T. H. Tran, "Current status of using herbal plants in shrimp farming in the Mekong Delta, Vietnam," *Can Tho University Journal of Science*, vol. 56, no. 1, pp. 137-148, 2020.
- [13] E. Ramadhanti and Marlina, "A Review: Antibacterial Activity of Roselle (*Hibiscus sabdariffa*) Calyx Extract against Pathogenic Bacteria," *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, vol. 5, no. 5, pp. 55-60, 2020.
- [14] E. M. Abdallah, "Antibacterial efficiency of the Sudanese Roselle (*Hibiscus sabdariffa* L.), a famous beverage from Sudanese folk medicine," *Journal of intercultural ethnopharmacology*, vol. 5, no. 2, pp. 186-190, 2016.
- [15] T. T. L. Nguyen, N. A. Trinh, T. H. T. Tran, T. T. Nguyen, K. H. Huynh, K. L. Pham, T. N. T. NguyenTinh, and Q. P. Truong, "Selection of lactic acid bacteria (LAB) antagonizing *Vibrio Parahaemolyticus*: The pathogen of acute hepatopancreatic necrosis disease (AHPND) in whiteleg shrimp (*Penaeus Vannamei*)," *Journal of Biology*, vol. 8, no. 4, 2019, doi: 10.3390/biology8040091.
- [16] L. Tran, L. Numan, R. M. Redman, L. L. Mohny, C. R. Pantoja, K. Fitzsimmons, and D. V. Lightner, "Determination of the infectious nature of the agent of early mortality syndrome (EMS) affecting Penaeid shrimp," *Diseases of Aquatic Organisms*, vol. 105, no. 1, pp. 45-55, 2013, doi: 10.3354/dao02621.
- [17] H. Z. Far, C. R. B. Saad, H. M. Daud, S. A. Harmin, and S. Shakibazadeh, "Effect of *Bacillus subtilis* on the growth and survival rate of shrimp (*Litopenaeus vannamei*)," *African Journal Biotechnol*, vol. 8, pp. 3369-3376, 2019.
- [18] D. V. Lightner, *A handbook of shrimp pathology and diagnostic procedure for disease of shrimp*, World Aquaculture Society, Baton Rouge, LA. pp. 1-72, 1996.
- [19] M. M. Cowan, "Plant products as antimicrobial agents," *Clin Microbiol Rev.*, vol. 12, no. 4, pp. 564-582, 1999, doi: 10.1128/CMR.12.4.564.
- [20] T. H. N. Nguyen, T. T. L. Nguyen, and T. T. T. Phan, "Effects of moringa leaf extracts on the survival rate and the resistant ability to acute hepatopancreatic necrosis disease on white leg shrimp," *Journal of Vietnam Agriculture Science and technology*, vol. 112, no. 3, pp. 138-143, 2022.
- [21] I. Effendi, D. Yoswaty, H. Syawal, B. Austin, A. R. Lyndon, R. Kurniawan, S. Wahyuni, and A. Al-Harbi, "The Use of Medicinal Herbs in Aquaculture Industry: A Review," *Current Aspects in Pharmaceutical Research and Development*, vol. 7, pp. 7-20, 2022.