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Antimicrobial activity of herbal extracts against *Vibrio* spp. bacteria isolated from white feces syndrome on white leg shrimp (*Litopenaeus vannamei*) in some provinces in the mekong delta

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ABSTRACT

The study was conducted to determine the antibacterial activity of three herbal extracts: guava (Psidium guajava), leafflower (Phyllanthus urinaria L), beach daisy (Wedelia biflora (L.) DC) against Vibrio spp. isolated from white feces syndrome infected shrimp in some provinces in the Mekong Delta. The antibacterial activity of the three herbal extracts was evaluated by the well diffusion method, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The results indicated that there were 102 isolates in total of 378 shrimp samples which were clarified into 7 groups. Among these group, Vibrio alginolyticus group were the most abundant species with the percentage of 50 %, followed by Vibrio cholerae group with the percentage of 17.7%. The herbal extracts of P. guajava and P. urinaria L showed a broad - spectrum antibacterial activity against ten isolates which were selected for MIC and MBC test. The extract of P. urinaria L had the strongest antibacterial activity against V. harveyi CM3HPA2 and V. alginolyticus CM3IB2 (MIC of 0.2 mg/ml, MBC of 0.39 mg/ml). The obtained results indicated that the herbal extract of P. urinaria L will be a good candidate for reducing opportunistic pathogens Vibrio spp. abundant in gastrointestinal tract of shrimp.

1. INTRODUCTION

The Mekong Delta is the most productive area for both brackish and freshwater aquacultures in Viet Nam with high potential development. White leg shrimp (*Liptopennaeus vannamei*) is a type of shrimp that is widely cultivated in Viet Nam and also in some countries of Southern Asia as a source of rich nutrient food. The more intensive farming shrimps are grown, the more disease outbreaks occur. To improve production, farmers try to increase stocking density; therefore, it leads to disease outbreak easily. Aquatic animal diseases can be caused by bacteria, parasites, viruses, and fungi (FAO, 2018).

The white feces syndrome (WFS) which refers to the presence of a floating white fecal string in ponds rearing shrimp (*Penaeus monodon & L. vannamei*) has caused significant economic damage in the cultured shrimp industry in China, Indonesia, Malaysia, Thailand, Viet Nam, and other countries in Southeast Asia (Mires & Board, 2010). The syndrome is found to be associated with the opportunistic pathogens *Vibrio* spp in combination with other possible unknown pathogens (Aranguren et al., 2019).

The growth of several bacterial diseases was initially controlled by the use of antibiotics. It increases the risks of antibiotic residues in shrimp tissues and shrimp products. It is pointed out that the antimicrobial resistant agents of the *Vibrio* species isolated from the shrimps farming is high (Jeyasanta et al., 2017). In that case, herbal extracts gradually become the priority as good alternative prevention for aquatic diseases due to its natural product availability.

There is a possibility for developing new antiviral drugs against white spot syndrome virus (WSSV) infection from *P. guajava* (Velmurugan et al., 2012). Other studies have proved that *Phyllanthus urinaria L* has pharmacological effect such as hepatoprotective (Hau et al., 2009), anticancer (Huang et al., 2006) based on that it includes flavonoids, tannins, and other benzenoid constituents (Liu et al., 1999). Several medical plants used as shrimp feed supplement include bael fruit (*Aegle marmelos)*, garlic *(Allium sativum)*, Indian birthwort *(Aristolochia indica)*, Indian lilac *(Azadirachta indica)*, golden shower tree *(Cassia fistula)*, madagasca periwinkle *(Catharanthus roseus)*, turmeric *(Curcuma longa)* (Balasubramanian et al., 2007). Therefore, this study was conducted to evaluate the antimicrobial activity of *Psidium guajava* L., *Phyllanthus urinaria* L. and *Wedelia biflora (L.) DC.* for reducing opportunistic pathogens *Vibrio* spp. in the gastrointestinal tract and improving disease resistance of shrimp with highly promising to the aquaculture industry.

2. METHODOLOGY

2.1. Sample collection

Shrimp samples were collected from 63 ponds of farms in Dam Doi, Cai Nuoc, Phu Tan districts - Ca Mau province, Thanh Phu district - Ben Tre province, My Xuyen, Vinh Chau districts - Soc Trang province. From each pond, 3 diseased shrimps (the presence of gross signs such as reduced feeding, retarded growth, floating white fecal string in ponds and in the feeding tray, etc.) and 3 healthy shrimps were collected. The shrimp samples were collected at shrimp farm. Shrimp samples were put into plastic bags with oxygen supplied and transported to the laboratory.

2.2. Bacteria isolation and storage

First of all, shrimp samples were put on clean trays for observing and recorded the external and internal clinical signs. Secondly, the inoculating loop was used to get samples of bacterial from the intestine, hepatopancreatic of shrimp specimens and then streaking onto the Thiosulfate Citrate Bile Salts Sucrose agar (Merck) plate. After that, the plates were incubated for 24 hours at 28^{o}C. The dominant bacterial colonies were subcultured onto Trypto-casein Soy Agar supplemented with 1.5% NaCl (TSA+1.5% NaCl) media and incubated for 24 hours at 28^{0}C. A pure well-isolated colony was selected and incubated into Brain Heart Infusion Broth supplemented with 1.5% NaCl (BHIB+1.5% NaCl) for 24 hours at 28^{0}C. These served as stock cultures by mixing with glycerol (20%). The stock cultures were stored in the lowest compartment of the freezer (-80^{0}C) (Lightner, 1996).

2.3. Bacterial identification

Bacterial identification was done by primary tests including (Gram staining, motility, oxidase, and catalase). The commercially available kit API 20E (BioMerieux, France) was used for determining the biochemical profiles of isolates. The 16S rRNA gene of these isolates was sequenced and compared to GenBank database - NCBI BLAST for species identification. According to information on clinical signs recorded from farmers in different locations, 22 isolates with typical clinical signs were randomly selected for identification.

2.4. Herbal extraction

P. guajava, P. urinaria L and *W. biflora (L.) DC* extracts were supplied by Vibo Company. Firstly, the collected of whole plant samples were cleaned to get rid of dust particles, then were cut into small pieces and ground into fine powder by an electrical blender. The powder samples were macerated in methanol 96% (the ratio 1:10) for 3 days at room temperature. After that, the extracted solutions were filtered through Whatman no. 1 filter paper and released the solvent by a rotary vacuum evaporator at 40^{o}C.

2.5. Antibacterial activity

Ten isolates selected randomly from 22 identified isolates were used to evaluate the antibacterial activities of herbal extracts by the standard well diffusion method (Magaldi et al., 2004). A cotton swab was dipped into the bacterial suspension of approximately 1 x 10^{8} CFU/mL tube and streaked on TSA+1.5% NaCl discs. The wells were made by a 6 mm diameter standard sterile cork. The herbal extracts were diluted with dimethyl sulfoxide (DMSO) solution to reach the final concentration at 100, 50 and 25 mg/mL. These wells were filled up with 50 µL of herbal extracts. The plates were in-

cubated at 28°C for 24 hours. DMSO was used as a negative control. Antibacterial activity was determined by measuring the diameter of the inhibition zone (Oonmetta-aree et al., 2006).

2.6. Minimal inhibitory concentration (MIC)

Two herbal extracts **(***P. guajava, P. urinaria L)* were selected for the Minimum Inhibitory Concentration (MIC) test. In the broth dilution method, 3 mL of herbal extract in the BHIB+1.5% NaCl solutions were prepared by two fold dilutions from 25 to 25/1024 (0.02) mg/mL (10 times) in the test tubes. The standardized bacterial suspension of 10 isolates ($1x10^6$ CFU/mL) was inoculated in the series of broth dilution at 28°C for 24 hours. MIC was determined as the herbal extracts that completely inhibit the growth of bacteria on the lowest concentration (Oonmetta-aree et al., 2006).

2.7. Minimum bactericidal concentration (MBC)

Thirty µl of ten isolates from MIC asays were streaked onto TCBS plate, and counted colonies after 24-hour incubation for 28°C. MBC was determined as the lowest concentration of the herbal extracts in which no bacteria growth (Oonmetta-aree et al., 2006).

2.8. Data analysis

The average value, percentage and standard deviation were analyzed by using Microsoft Office Excel 2010 software and the 16S-RNA gene sequenced results of isolates were compared to GenBank database - NCBI Blast.

3. RESULTS AND DISCUSSIONS

3.1. Bacterial isolation and identification

A total of 63 shrimp ponds including 39 farms in Ca Mau province (located in Dam Doi, Cai Nuoc, Phu Tan districts), 10 farms in Soc Trang province (located in My Xuyen, Vinh Chau districts), and 14 farms in Ben Tre province (located in Thanh Phu district) from October 2019 to August 2020. The clinical signs included an empty and discontinuous gut, a paler and white hepatopancreas, retarded growth, reduced feeding, some ponds showed white feces strings in the feeding trays according to information obtained from the farmers. There were 102 isolates in total 378 shrimp samples collected from shrimp ponds. According to phenotypic characteristics, colony morphology on TCBS and TSA+1.5% NaCl media, these isolated strains were clarified into 7 groups (Table 1).

Table 1. Colonial morphology characteristics of 7 isolated groups on TCBS and TSA + medium

CODE	TCBS medium	TSA medium
A1	Green colonies, typical 2–3 mm diameter, round, opaque, green, or bluish colonies, convex	Light yellow, round, smooth colonies, 1- 2mm, convex, slip
A2	Green colonies, small, 1-2mm	Small, round, lucent, transparency, smooth colonies, 1mm
A3	Green colonies, 3-4 mm in diameter	Round, yellow, opaque color 2mm
A4	Blue green colonies	Round, sticky, opaque color 1mm
A5	Green colonies, round	Round, filamentous 1-2mm
B1	Large, flat, smooth yellow colonies, 2-3 mm in diameter	Round, flat, 2mm
B2	Large yellow colonies	Large yellow, round, convex colonies, 2mm

Five dominant groups B2 (50%), B1 (17.7 %), A4 (10.8%), A2 (6.9%) and A1 (5.8%) (Table 2) consisting of 22 isolates were chosen for bacterial identification.

Table 2. The results of sampling and proportion of *Vibrio* spp. groups isolated in white leg shrimp

Location	No. shrimp sampling	No. isolates from white leg shrimp							Sampling time
		A1	A2	A3	A4	A5	B1	B2	
Phu Tan[1]	42	1	0	0	6	3	1	5	10/2019
Dam Doi[1]	102	0	2	1	2	0	0	13	12/2019
Cai Nuoc[1]	90	2	2	1	1	0	6	16	06/2020
Thanh Phu[2]	84	3	1	0	2	1	6	9	07/2020
Vinh Chau, My Xuyen[3]	60	0	2	3	0	0	5	8	08/2020
Total	378	6	7	5	11	4	18	51	
Percentage (%)		5.8	6.9	4.9	10.8	3.9	17.7	50	

Notes: 1. Ca Mau province; 2. Ben Tre province; 3. Soc Trang province.

Table 3. Morphological, physiological and biochemical characteristics of 22 isolates belonging to 5 groups

	A1 (n=2)	**A2 (n=2)**	**A4 (n=4)**	**B1 (n=6)**	**B2 (n=8)**
Gram - staining	-	-	-	-	-
Morphology	curved-rod (comma)	curved-rod (comma)	curved-rod (comma)	curved-rod (comma)	curved-rod (comma)
TCBS	Green colony	Green colony	Green colony	Yellow colony	Yellow colony
Motility	+	+	+	+	+
Catalase	+	+	+	+	+
Oxidase	+	+	+	+	+
Facultative anaerobes	+	+	+	+	+
Facultative aerobes	+	+	+	+	+
β- galactosidase	+	+	+	+	+
Arginine	-	-	+	-	-
Lysine	+	+	-	+	+
Ornithine	+	+	-	+	-
Citrate	+	+	+	+	+
H2S	-	-	-	-	-
Urease	-	-	-	-	-
Tryptophan deaminase	+	+	+	+	+
Indole	+	+	+	+	+
Voges - Proskauer	+	+	+	+	+
Gelatinase	+	+	-	+	-
Glucose	+	+	+	+	+
Mannose	+	+	+	+	+
Inositol	-	-	-	-	-
Sorbitol	-	-	-	-	-
Rhamnose	-	-	-	-	-
Sucrose	+	-	-	+	+
Melibiose	-	-	-	-	-
Amygdalin	+	+	+	-	+
Arabinose	+	-	-	-	+

The results indicated that five dominant groups were identified as *V. parahaemolyticus* (A1), *V. haveryi* (A2), *V. vulnificus* (A4), *V. cholerae* (B1) and *V. alginolyticus* (B2) by secondary kit test API 20E (Table 3). The 16s - rRNA gene sequence of five dominant groups (A1, A2, A4, B1, B2) was also submitted to GenBank database with an accession no. NR119058.1, KT982474.1, NR117906.1, NR044853.1, NR118258.1 and the similarity between its 16S rRNA gene sequence and other isolates in the GenBank database is 99.78%, 100%, 99.5%, 100%, 99.85%, respectively.

Another study in Thailand also showed large amounts of *Vibrio* bacteria including *V. vulnificus, V. fluvialis, V. parahaemolyticus, V. alginolyticus, V. damselae, V. minicus* and *V. cholerae* at different proportions of 80, 44, 26, 20, 28, 8 and 6% in haemolymph and intestine with two - fold higher than that of healthy ones, respectively (Somboon et al., 2012). Interestingly, *V. parahaemolyticus, V. vulnificus, V. cholerae* and *V. anguillarum* have been recovered in the *L. vannamei* infected white feces syndrome in Jawa Tengah province, Indonesia (Jayadi et al., 2016). Supono et al. (2019) showed that *V. vulnificus, V. parahaemolyticus* and *V. alginolyticus* were suspected of triggering white feces syndrome in East Lampung Regency, Indonesia. In addition, five species including *V. parahaemolyticus, V. fluvialis, V. mimicus* and *V. alginolyticus* had been reported from infected white feces syndrome of *L. vannamei* shrimps in Andhra Pradesh (Mastan, 2015).

Presently, the causative agents of white feces syndrome in shrimp remains unknown. This syndrome

has been associated with a microsporidian - EHP (*Enterocytozoon hepatopenaei*) (Rajendran et al., 2016). In addition, EHP also was proposed as the causative agent in *P. monodon* (Ha et al., 2013) and *L. vannamei* (Tang et al., 2016). However, it was failed to illustrate white feces syndrome in the experiment infected EHP (Tangprasittipap et al., 2013). Woraprayote et al. (2020) reported that *Vibrio* abundances increase in shrimp haemolymph and intestine has been also related to white feces syndrome.

Among these groups in this study, *V. alginolyticus* (B2 group) was the most abundant species with the percentage of 50 % and *V. cholerae* (B1 group) was the second high abundant species with the percentage of 17.7%. Similarly, *V. alginolyticus* and *V. fluvialis* have been recorded as the major pathogen contribute the occurrence of white feces syndrome in grow-out ponds of *P. monodon* in Sri Lanka (Sandaruwan Kumara & Hettiarachchi, 2017). Moreover, Cao et al. (2015) found that *V. cholerae* isolate was a pathogen for white feces syndrome on *L. vannamei* in China.

In general, based on the percentage of isolated bacterial groups, total of 10 isolates including 5 isolates of *V. alginolyticus* with the highest percentage (50%); 2 isolates of *V. cholerae* (17.7%); 1 isolate of *V. vulnificus* (10.8%), 1 isolate of *V. haveryi* (6.9%), 1 isolate of *V. parahaemolyticus* (5.8%) were chosen for screening of antimicrobial activity of different herbal extracts.

3.2. Screening of antimicrobial activity of different herbal extracts on ten isolated *Vibrio* spp. bacteria

The antibacterial activity of herbal extracts using standard well diffusion method (Magaldi et al., 2004) with the results shown in Figure 3 and Table 4. The results indicated that three tested herbal extracts exhibited different antibacterial activities against ten isolates.

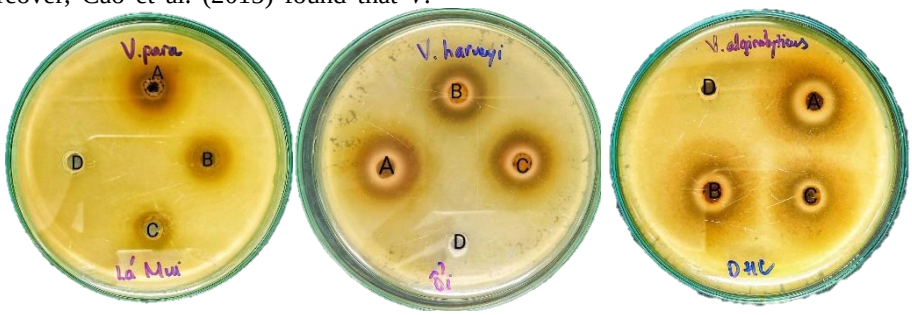

Figure 3. Antimicrobial activity of tested herbal extracts in *Vibrio* spp. bacteria

(A) 100 mg/ mL, (B) 50 mg/ mL, (C) 25 mg/ mL, (D) DMSO

Noticeably, among three tested herbal extracts, P. urinaria L showed the highest antibacterial activity against all ten isolates with the growth inhibition zone fluctuated from 12.7 to 24.85 mm. In detail, the results pointed that the highest antibacterial activity against V. alginolyticus CM3IB2 with the inhibition zone was 24.85 mm, 23.4 mm, 20.45mm at three concentrations.

Besides, DMSO played a role as the negative control of all tests showed the zone at 0 mm (Table 4).

In this study, *P. guajava* showed the intermediate antibacterial activity with the highest inhibition zone were 22.95, 20.7, 17.7 mm on *V. alginolyticus* CM3IB2 (Table 4). The extract of *W. biflora (L.) DC* showed the lowest antibacterial activity than the other two herbal extracts with the highest inhibition zone were 16.45, 14.35, 11.45 mm on *V. parahaemolyticus* BTIA1 and the lowest inhibition zone on *V. cholerae* CM1HPB1 (Table 4).

In addition, mangrove leaf extract (*Rhizophora apiculata*) had the best effect on *V. parahaemolyticus* with the inhibition zone of 5.61 mm at 700 mg/L (Supono et al., 2019). According to Widowati et al. (2018), microalgae *Dunaliella salina* and *Tetraselmis chui* were applied as biocontrol agents for 21 days showing a decrease of bacteria amount and its capable against *Vibrio*. Moreover, supplementation of hen egg white lysozome (HEWL) at 0.125 g/kg into feed was demonstrated as an effective method not only to avoid antibiotic treatment in aquaculture but also stimulated resistance to *Vibrio* infection as well as white feces syndrome (Woraprayote et al., 2020).

Table 4. Mean of inhibition zone diameter of three herbal extracts in ten isolates

Isolates	Mean of inhibition zone diameter (mm)								
	P. urinaria L			*P. guajava*			*W. biflora (L.) DC*		
	100 mg/mL	50 mg/mL	25 mg/mL	100 mg/mL	50 mg/mL	25 mg/mL	100 mg/mL	50 mg/mL	25 mg/mL
V.parahaemolyticus BTIA1	18.9±0.4	17.25±0.25	15.75±0.45	18.3±0.9	15.8±0.2	14.2±0.2	16.45±0.75	14.35±0.75	11.45±0.05
V. harveyi CM3HPA2	24.65±0.45	23.9±0.3	22.65±0.45	22.7± 0.4	21.8±0.4	20.6±0.6	16.2±0.2	13.85±0.45	11.45±0.25
V. vulnificus CM2HPA4	17.15±0.15	15.1±0.3	13.45±0.25	11.85±0.55	10.9±0.4	9±0	12.15±0.15	11±0.3	7.05±0.05
V. cholerae CM3HPTB1	19.7±0.3	18.15±0.15	16.75±0.35	17.35±0.25	16.2±0.2	13.75±0.25	14.9±0.3	13.4±0.2	11.7±0.3
V. cholerae CM1HPB1	17.95±0.35	16.4±0.2	15.15±0.15	14.3±0.3	12.25± 0.05	10.6±0.4	9.85±0.25	8.8±0.3	8±0.2
V. alginolyticus CM2HPB2	16.7±0.3	15.85±0.15	14±0	15.4±0.2	13.15± 0.85	11.9±0.4	13.8±0.2	11.6±0.4	10.95±0.25
V. alginolyticus STIB2	18.1±0.2	16.65±0.15	15.5±0.2	17.65±0.45	13.85±0.35	12.6±0.4	12.15±0.15	11.1±0.1	9.65±0.35
V. alginolyticus CM2IB2	18.2±0.2	16.2±0.6	14.95±0.15	15.95±0.35	14.65±0.35	13±0	14.8±0.2	13.4±0.2	11.25±0.15
V.alginolyticus CM3HPB2	15.9±0.1	14.25±0.15	12.7±0.4	14±0	11.55±0.55	10.15±0.15	14.95±0.15	13.4±0.6	10.75±0.25
V. alginolyticus CM3IB2	24.85±0.15	23.4±0.6	20.45±0.15	22.95±0.15	20.7±0.3	17.7±0.3	12.4±0.2	11±0	8.05±0.05

Notes: Growth inhibition zone: Resistant ≤ 9 mm; Intermediate: ≥ 10 - 13 mm; Susceptible: ≥ 15 mm (Chaweepack et al., 2015)

3.3. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC and MBC were determined based on the results of the antibacterial activity test. Amongst tested herbal extracts, *P. guajava* and *P. urinaria L* with higher antibacterial activity were continuously tested to determine MIC and MBC. The result is shown in Table 5.

The results indicated that the highest MIC values of *P. urinaria L* extracts were defined at 0.2 mg/mL on *V.parahaemolyticus* BTIA1, *V. harveyi* CM3HPA2, *V. alginolyticus* CM3IB2 and *V. alginolyticus* CM3HPB2, MBC values were 0.78, 0.39, 0.39, 1.56 mg/mL, respectively. In detail, the highest ratio of MBC/MIC of *P. urinaria L* extract were 2 on *V. harveyi* CM3HPA2, *V. alginolyticus* CM3IB2 while the ratio values were 4 on *V.parahaemolyticus BTIA1*, *V. cholerae* CM3HPTB1, *V. cholerae* CM1HPB1, *V. alginolyticus* STIB2, *V. alginolyticus* CM2IB2 and the values were 8 on the remaining isolates.

As reported by Canillac and Mourey. (2001), the ratio of MBC/MIC is equal to or less than 4 means that the extraction considered bactericidal ability. On the other hand, this ratio is larger than 4 that shows the bacteriostatic ability. In this study, *P. urinaria L* extract showed higher values of the ratio of MBC/MIC compared to *P. guajava* on all ten isolates with the MBC/MIC ratio values was 4 on *V. parahaemolyticus* BTIA1, *V. harveyi* CM3HPA2, *V. alginolyticus* CM3IB2 while it was 16 only on *V. vulnificus* CM2HPA4, and the ratio values were 8 on 6 isolates other.

Phyllanthus amarus had antibacterial activity against *V. parahaemolyticus* causing AHPND on whiteleg shrimp *(L. vannamei)* in both kinds of fresh plants (250 mg/mL) and dried plants (125 mg/mL) (Phuong et al., 2019). Guava leaf extract (*P. guajava*) was proved that it had the resistance ability to 21 bacterial strains: *L. monocytogenes* (5 strains), *S. aureus* (4 strains), *E. coli* (6 strains) *S. enteritidis* (4 strains) , *V. parahaemolyticus*, *B. cereus* and 5 bacteria that spoil food (Mahfuzul Hoque et al., 2007). In addition, 11 tested herbal extracts showed antibacterial activity among sixteen, *P. guajava* and *M. charantia* displayed the highest activity against *Vibrio harveyi* and *V. parahaemolyticus* with the MIC of *P. guajava* was found to be 0.625 mg/mL, while the MIC of *M. charantia* was 1.25 mg/mL (Direkbusarakom et al, 1998).

Table 5. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of tested herbal extracts

Isolates	MIC (mg/ml)		MBC (mg/ml)		MBC/MIC ratio	
Herbal extracts	***P. guajava***	***P. urinaria L***	***P. guajava***	***P. urinaria L***	***P. guajava***	***P. urinaria L***
V. parahaemolyticus BTIA1	0.2	0.2	0.78	0.78	4	4
V. harveyi CM3HPA2	0.2	0.2	0.78	0.39	4	2
V. vulnificus CM2HPA4	0.78	0.39	12.5	3.13	16	8
V. cholerae CM3HPTB1	0.39	0.39	3.13	1.56	8	4
V. cholerae CM1HPB1	0.78	0.39	6.25	1.56	8	4
V. alginolyticus CM2HPB2	0.39	0.39	3.13	3.13	8	8
V. alginolyticus STIB2	0.39	0.39	3.13	1.56	8	4
V. alginolyticus CM2IB2	0.39	0.39	3.13	1.56	8	4
V. alginolyticus CM3HPB2	0.78	0.2	6.25	1.56	8	8
V. alginolyticus CM3IB2	0.2	0.2	0.78	0.39	4	2

4. CONCLUSIONS AND RECOMMENDATIONS

4.1. Conclusions

There were 102 isolates in total shrimp samples which had clinical signs of white feces syndrome. Among these groups, *V. alginolyticus* group was the most abundant species with the percentage of 50 %, followed by *V. cholerae* group with a percentage of 17.7%. The herbal extracts of *P. guajava*, *P. urinaria L* showed a broad spectrum antibacterial activity against 10 isolates in diseased shrimp.

The extract of *P. urinaria L* had the strongest antibacterial activity against *V. harveyi* CM3HPA2 and *V. alginolyticus* CM3IB2 (MIC of 0.2 mg/mL, MBC of 0.39 mg/mL).

4.2. Recommendations

Among isolated bacterial strains, *V. alginolyticus* group was the most abundant species with a percentage of 50%. It should be carried out in the challenge test for the confirmation of the possible causative agents and the recurrence of white feces syndrome. The herbal extracts of *P. guajava*, *P. urinaria L* should be further studied in *in vivo* studes.

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