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RESEARCH ARTICLE

Antimicrobial of nyirih (*Xylocarpus granatum*) against pathogens of tiger shrimp post-larvae

[version 1; peer review: 2 approved with reservations]

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Abstract

Background: *Xylocarpus granatum* has been used as a medicinal plant by coastal communities, which may indicate that this plant is a potential source of pharmaceuticals.

Methods: *Xylocarpus granatum* leaf extract was tested as an antimicrobial agent for pathogens infecting tiger shrimp post-larvae. Of the treatments applied to the post-larvae, 25 were crudely extracted with ethanol, distilled water, and seawater solvent given by immersion. *Vibrio harveyi* and *Saprolegnia* sp. were microbial species used for the test.

Results: *X. granatum* extract had the potential to inhibit *V. harveyi* and *Saprolegnia* sp., reducing infection and improving the survival of shrimp. Shrimp soaked with *X. granatum* extract had a total *Vibrio* count ranging from 14.67×10^3 to 22.67×10^3 CFU/ml. The survival rate of shrimp was recorded as 53.33% to 78.67% and 54.67% to 76.00% due to *V. harveyi*, and *Saprolegnia* sp infection, respectively. The relative percentage of the survival of shrimp protected from *V. harveyi* and *Saprolegnia* sp infection in treatments compared to negative controls ranged from 40.61% to 72.89% and 35.84% to 66.12%, respectively.

Conclusions: Leaf extracts of *X. granatum*, which might have better antimicrobial activities to prevent tiger shrimp from pathogenetic infection, were consecutively extracted ethanol at 800-1,000 ppm, distilled water at 800-1,000 ppm, and seawater at 1,000 ppm.

Keywords

antimicrobial, pathogen, tiger shrimp, *Xylocarpus granatum*

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Introduction

Shrimp pond culture in East Kalimantan, Indonesia, is generally conducted in a traditionally extensive system, and the filling and draining of ponds is fully dependent upon tides. Wild biota transported into ponds along with the tide water during filling might carry pathogens. Vibriosis and fungal often affect larvae and post-larvae of tiger shrimp in hatcheries and ponds in East Kalimantan¹. The use of antibiotics to prevent diseases is a problem, since uncontrolled use may bring about resistance and toxicity². As an alternative, to minimize the use of antibiotics, various plant extracts have been reported to have the ability to reduce microbial attacks in aquaculture thus reducing the risk of death³⁻⁵.

Various species of mangrove are found in the Mahakam Delta⁶ and are from six important genera: Pedada (*Sonneratia*), Api-api (*Avicennia*), Bakau (*Rhizophora*), Tancang (*Bruguiera*), Nyirih (*Xylocarpus*), and Nipah (*Nypa*)⁷. Mangroves have long been used for traditional medicine by coastal communities. Several studies have been published concerning mangrove activity on pathogens in humans, animals, and plants⁸.

Research using mangrove extract as a source of pharmaceutical ingredients and drugs, or as an antibacterial for tackling diseases in shrimp culture, has shown positive developments³. The use of plant extracts has been reported by many authors, proving that they are able to be utilized as an antibacterial and antifungal or as immunostimulants without causing resistance^{1,4,9}. In this experiment, leaf extract of *Xylocarpus granatum* was examined as an antibacterial and antifungal material to maintain the health and survival of tiger shrimp post-larvae in captivity.

Methods

Leaf extract of *X. granatum*

Leaves of *X. granatum* were obtained from a shrimp pond area in the Muara Badak Subdistrict of the Kutai Kartanegara District in East Kalimantan, Indonesia. Leaves were washed and drained until there was no water and, after being dried to around 40% of their original weight, were chopped and wind-dried in a room not exposed to direct sunlight. After about 50 days, the leaves were then macerated with three different solvents namely 80% ethanol, distilled water, and 22‰ seawater for 24 hours. The ratio between leaves and solvents was 300 g of leaves in 2,100 ml solvent. The maceration product was extracted by evaporation, and the extraction products were heated over the bath until the ethanol solvent was evaporated to obtain crude. Extractions with distilled water and seawater were stopped when the solvent reached 10% of the initial volume.

Microbes

Microbes used for the challenge tests were *Vibrio harveyi* and the fungi *Saprolegnia* sp., supplied by the Laboratory of Aquatic Microbiology, Faculty of Fisheries and Marine Science, Mulawarman University, Samarinda. Before use, the pathogenicity of *V. harveyi* was tested by intramuscularly injecting 0.05 ml of the bacteria at a dose of 12.4×10^4 CFU/ml into five 3-g tiger shrimp. After 5 days, when the shrimp showing signs of redness *V. harveyi* was then isolated from the hepatopancreas. Furthermore, *V. harveyi* was isolated and cultured on

Thiosulfate Citrate Bile Salt Sucrose Agar medium (Merck KGaA. 1.10263.0500) and incubated for 24 hours at 33°C. *Saprolegnia* sp. was rejuvenated by culturing it on Potato Dextro Agar medium (Himedia REF M096-500G) incubated at 33°C for 24 hours.

Water and aquarium

Seawater with 22‰ salinity, and 28°C temperature was used as the culture medium for shrimp, and confirmed to be free from pathogens by isolating and identifying *Vibrio* sp and *Saprolegnia* sp. The seawater was deposited in a tank for 2 days and then flowed to another tank and aerated. Each aquarium was filled with 5 l sea water and aerated.

Tiger shrimp

The PL-25 tiger shrimp came from a brood stock and controlled hatchery which never applies chemicals or drugs and were confirmed to be *Vibrio*-free after sampling for isolation and bacterial culture in the medium. Each aquarium was stocked with 25 shrimp.

Treatment

The treatments were leaf extract, extracted with ethanol solvent, distilled water or seawater solvent, and each leaf extract treated to the shrimp had concentrations of 1,000, 800, 600, or 400 ppm (where 1,000 ppm is 1 ml of extract in 1,000 ml of water). Control treatments consisted of a positive control, the antibiotic erythromycin 500 mg/1,000 ml, and a negative control, NaCl 0.85%. Assessments were carried out every 6 hours.

As many as 25 shrimp of PL-25 were soaked in each extract solution at each indicated concentration in each aquarium, and each treatment was replicated three times. The challenge test with each of *V. harveyi* and *Saprolegnia* sp. in each aquarium was performed 6 days after extract-soaking by immersing a concentration of 10.6×10^5 CFU/ml microbes into the shrimp culture medium. The shrimp was reared for 28 days for the challenge test. Clinical symptoms, including changes in activity, appetite, and reflexes, were observed every day. Clinical symptoms were analysed by calculating the percentage of shrimp in an aquarium showing inactive (passive) motion, decreasing appetite, and weakening response of reflex. Observation of pathological anatomy (PA) was performed on dead shrimp and at the end of experiment based on changes in colour, shape, and other abnormalities in the shrimps' bodies and organs. PA observation included the body condition, carapace, legs, uropod, hepatopancreas, and abdomen (Table 1).

Dead shrimp were recorded each day in order to calculate shrimp survival rate. In addition, the total content of *V. harveyi* proliferating in each shrimp was calculated using total plate count (TPC) method. The shrimp bacteria were isolated and cultured on Thiosulfate Citrate Bile Salt Sucrose Agar medium, after incubation for 24 hours at 33°C the number of growing *V. harveyi* colonies was counted. The relative percentage of survival (RPS) was also applied to know the effectiveness of leaf extract *X. granatum* in preventing shrimp from *V. harveyi* infection using the following formula:

$$RPS = (1 - (\text{mortality of treatment shrimp}) \times (\text{mortality of control shrimp})^{-1}) \times 100\%$$

Table 1. Criteria of pathological anatomy of shrimp.

Condition of body	Carapace	Legs	Uropod/tail	Hepatopankreas	Stomach	Value	Criteria
Completed	Normal	Normal	Normal	Normal	Normal	1	Normal
Change in color	Change in color	Change in color	Change in color	Change in color	Change in color	2	Minor damaged
Deformed	Deformed <50 %	Deformed <50 %	Deformed <50%	Swollen	Swollen	3	Moderate damaged
Incompleted	Deformed >50 %	Deformed 50%	Deformed >50%	Shrink	Shrink	4	Mayor damaged

Results and discussion

Effect of extract treatment

The body colour of shrimp changed darker and shifted back to normal colour again, after 24 to 36 hours of extract soaking. The change of colour indicated the intrusion of extract into the body fluids of the shrimp as osmoregulatory processes try to balance the osmotic pressure of body fluids with its surrounding environment. The extract-penetrating body fluids will stimulate the immunity mechanism of shrimp. Discolouration in the shrimp is a sign that foreign substances are penetrating the body fluids, influencing the chromatophore, which is part of a shrimp's immunity system^{1,4}. Colour change in crustaceans can be stimulated by many factors as a behavioural form for adaptation and protection¹⁰⁻¹².

Effects of challenge

At 2 days after the challenge test with *V. harveyi*, the activities and appetites of the shrimp were decreasing. Clinical symptoms on day 14 were increasingly apparent in negative controls. Clinical symptoms of the shrimp incubated with seawater extract were more apparent than other treatments, especially at concentrations of 400 ppm and 600 ppm. Those incubated with ethanol and distilled water extract appeared to be better than the positive control. Ethanol extract 800 ppm and 1,000 ppm and distilled water extract 1,000 ppm resulted in a better average activity of shrimp. Better appetite was also shown by shrimp in the culture medium with ethanol and distilled water extract 1,000 ppm, and the reflex response of shrimp was found better in the ethanol extract 1,000 ppm. However, all clinical symptoms in the shrimp were showing improvement until day 28, except for negative controls.

Clinical symptoms on shrimp challenged with *Saprolegnia* sp. began to appear on day 6, and shrimp appeared healthier than shrimp challenged with *V. harveyi*. In the negative control, legs were visibly dirty, a symptom that did not appear in treatments challenged with *V. harveyi*. Clinical symptoms on shrimp on days 14 and 28 in all extract treatments appeared better than antibiotic-added positive controls. Clinical symptoms that appeared on shrimp for all treatments are presented in Table 2. Shrimp infected with *V. harveyi* exhibited symptoms of decreasing activity, weakening reflex responses and loss of appetite^{1,13}. Raw data behind each table is available on OSF¹⁴.

Pathological effects of challenge

Better anatomical pathology was shown by shrimp in all extract treatments when compared to negative controls. Some individual shrimp in negative controls challenged with *V. harveyi* experienced anatomical pathology issues, such as: incomplete organs; reddish carapace; broken rostrum; reddish and broken legs; swollen and gripped uropod; brownish, reduced, and disturbed hepatopancreas; and brownish, hard abdomen. Shrimp in negative controls challenged with *Saprolegnia* sp., showed a lighter anatomical pathology than those challenged with *V. harveyi*. The anatomical pathology of shrimp was indicated with reddish appearance in the body, legs, and uropod or with incomplete carapace, legs, and uropod. The hepatopancreas softened, and both it and the stomach changed brownish. *Vibrio* sp. causes gills to turn dull pale and reddish yellow and carapace dark reddish, pleopods and uropod to break, and hepatopancreas slightly reddish to dark red¹⁵. Vibriosis brings reddish black colour on shrimp, red spots on legs and uropod, haemorrhage in the body, and deformity and moulting failure¹³.

Based on clinical symptoms and anatomical pathology, shrimp culture in a medium with extract treatments showed a better physiological condition and resistance against for *V. harveyi* and *Saprolegnia* sp. infection, compared to negative controls. The positive control gave almost the same result as the ethanol and distilled water extract treatments. This evidence indicated that *X. granatum* leaf extract is supposed to be effective at preventing tiger shrimp from both *V. harveyi* and *Saprolegnia* sp. infection. *Vibrio* infection may occur in all phases of shrimp development, from egg to broodstock¹⁶. Vibriosis, especially caused by the luminous *Vibrio*, often brings about serious losses in shrimp hatcheries¹⁷. *Vibrio* species are abundant in a seawater environment, and some opportunistic pathogenic strains are associated with the immunity of cultured shrimp in unfavourable environmental conditions¹⁸.

Number of infectious colonies

The colonial density of *V. harveyi* (TVC) in shrimp soaked with leaf extract on the last day of the experiment ranged from 14.67×10^3 to 22.67×10^3 CFU/ml. This bacterial count indicated that leaf extract for all solvents at concentrations ranging from 400 to 1,000 ppm could inhibit the proliferation of *V. harveyi* better than negative controls with TVC of 25.67×10^3 CFU/ml.

Table 2. The average of clinical symptoms of tiger shrimp given the extract of *X. granatum* and challenge tested with microbes.

<i>V. harveyi</i>													
Extract	Day	Low activity (%)				Decreased appetite (%)				Low reflex (%)			
		1,000 ppm	800 ppm	600 ppm	400 ppm	1,000 ppm	800 ppm	600 ppm	400 ppm	1,000 ppm	800 ppm	600 ppm	400 ppm
Ethanol	14	53.33	53.33	64.00	78.67	50.67	52.00	64.00	70.67	52.00	53.33	61.33	78.67
	28	36.00	32.00	36.00	46.67	34.67	37.33	36.00	37.33	28.00	24.00	28.00	26.67
Distilled water	14	53.33	57.33	72.00	77.33	50.67	60.00	69.33	72.00	60.00	69.33	72.00	73.33
	28	34.67	40.00	49.33	48.00	34.67	40.00	44.00	44.00	33.33	36.00	34.67	34.67
Sea water	14	56.00	68.00	69.33	78.67	53.33	62.67	69.33	69.33	62.67	65.33	76.00	73.33
	28	36.00	42.67	49.33	50.67	38.67	40.00	42.67	42.67	28.00	34.67	32.00	36.00
Positive control	14	57.33				53.33				49.33			
	28	48.00				42.67				32.00			
Negative control	14	78.67				78.67				76.00			
	28	90.67				85.33				90.67			
<i>Saprolegnia</i> sp.													
Extract	Day	Low activity (%)				Decreased appetite (%)				Low reflex (%)			
		1,000 ppm	800 ppm	600 ppm	400 ppm	1,000 ppm	800 ppm	600 ppm	400 ppm	1,000 ppm	800 ppm	600 ppm	400 ppm
Ethanol	14	41.33	40.00	45.33	65.33	46.67	46.67	57.33	64.00	48.00	49.33	54.67	70.67
	28	24.00	21.33	24.00	33.33	28.00	29.33	30.67	29.33	22.67	20.00	22.67	21.33
Distilled water	14	60.00	57.33	60.00	60.00	49.33	60.00	64.00	65.33	64.00	66.67	66.67	68.00
	28	32.00	33.33	36.00	38.67	26.67	32.00	36.00	37.33	26.67	26.67	28.00	29.33
Sea water	14	48.00	61.33	64.00	65.33	53.33	61.33	62.67	62.67	57.33	61.33	70.67	69.33
	28	26.67	29.33	34.67	34.67	34.67	36.00	34.67	34.67	25.33	29.33	25.33	29.33
Positive control	14	46.67				50.67				49.33			
	28	40.00				37.33				28.00			
Negative control	14	78.67				78.67				76.00			
	28	76.00				78.67				84.00			

The TVC value in the positive controls included in the value range of leaf extract treatments, which was 16.00×10^3 CFU/ml. The strongest inhibition of extract against *V. harveyi*, consecutively, was distilled water extract at 1,000 ppm, distilled water extract at 800 ppm, ethanol extract of 800 ppm, and ethanol extract of 1,000 ppm, as presented in Table 3. The bacterial density of *V. harveyi* in water, sediment, and shrimp samples varied between 6.0×10^3 to 88×10^3 CFU/ml, $1,200 \times 10^3$ to $10,400 \times 10^3$ CFU/g, and 5.0×10^3 to 73×10^3 CFU/ml, respectively¹⁹. Bacterial density of *V. harveyi* in hepatopancreas of 1.5-month-old tiger shrimp, 14 days after the challenge test with 10^5 CFU/ml, was about 14.67×10^3 CFU/ml.

The above facts indicated that *X. granatum* leaves extracted with distilled water and ethanol solvents, at concentrations between 800 to 1,000 ppm, were potentially antibacterial and able to inhibit

V. harveyi growth better than antibiotics. All *Vibrio* isolates were found to be resistant to ampicillin, gentamycin, oxytetracyclin, chloramphenicol, trimethoprim, and kanamycin—the antibiotics commonly used in aquaculture²⁰. Crude ethanolic extract of *X. granatum*, *in vitro* at 400 ppm, could inhibit the growth of *Staphylococcus epidermis*, *Staphylococcus aureus*, *Shigella boydii*, *Proteus* spp., *Escherichia coli*, and *Streptococcus pyogenes*²¹.

Survival rates

The survival rate of shrimp incubated with extracts of *X. granatum* was better than the negative control, ranging from 53.33% to 78.67% following to *V. harveyi* infection, and 54.67% to 76.00% following to *Saprolegnia* sp. infection. The survival rates in the positive control was recorded as 78.67% and 77.33%, and in the negative control were 21.33%–29.33% (Table 4). The RPS of shrimp soaked with extract, in the negative controls, and in the

positive controls ranged from 40.61% to 72.89%, from 35.84% to 66.12%, and from 67.97% to 72.98%, respectively. The highest RPS against *V. harveyi* infection was 72.89% obtained in treatments of ethanol extract at 1,000 ppm, distilled water extract of 1,000 ppm, and positive control, followed consecutively, by distilled water extract of 800 ppm 71.14%, ethanol extract of 800 ppm 69.39%, and seawater extract of 1,000 ppm 67.81%.

The highest RPS against *Saprolegnia* sp. infection was in ethanol extract of 1,000 ppm (66.12%), followed by ethanol extract of 800 ppm (66.01%), distilled water extract of 1,000 ppm (65.90%), and seawater extract (64.27%) (Table 5).

The above results indicated that *X. granatum* extract had the potential to protect shrimp from both *V. harveyi* and *Sapro-*

Table 3. The average total content of *V. harveyi* in shrimp incubated with extracts of *X. granatum* leaves.

Extract	Concentration	Total vibrio count (× 10 ³ CFU/ml)			
		Shrimp		Water	
		Day 14	Day 28	Day 14	Day 28
Ethanol	1,000 ppm	15.33	15.00	13.33	12.00
	800 ppm	15.67	15.00	13.33	12.33
	600 ppm	16.33	15.67	16.00	15.67
	400 ppm	18.33	19.00	17.67	17.00
Distilled water	1,000 ppm	15.67	14.67	13.67	13.33
	800 ppm	16.00	15.00	14.00	14.00
	600 ppm	17.67	17.67	18.33	17.00
	400 ppm	19.67	20.33	20.00	16.33
Seawater	1,000 ppm	16.33	16.67	14.33	14.67
	800 ppm	17.00	17.67	14.33	15.00
	600 ppm	19.67	20.00	16.33	16.00
	400 ppm	22.00	22.67	18.33	16.00
Positive control		15.67	16.00	12.67	12.33
Negative control		24.00	25.67	21.67	22.33

Table 4. The survival rate at shrimps given the extract of *X. granatum* leaves and challenge tested with microbes.

Extract	Day	Survival Rate (%)							
		<i>V. harveyi</i>				<i>Saprolegnia</i> sp.			
		1,000 ppm	800 ppm	600 ppm	400 ppm	1,000 ppm	800 ppm	600 ppm	400 ppm
Ethanol	14	82.67	84.00	82.67	70.67	84.00	84.00	81.33	80.00
	28	78.67	76.00	73.33	62.67	76.00	76.00	76.00	65.33
Distilled water	14	84.00	78.67	76.00	66.67	82.67	84.00	76.00	72.00
	28	78.67	77.33	72.00	61.33	76.00	74.67	69.33	62.67
Sea water	14	82.67	76.00	74.67	64.00	81.33	81.33	77.33	66.67
	28	74.67	73.33	64.00	53.33	74.67	72.00	58.67	54.67
Positive control	14	84.00				84.00			
	28	78.67				77.33			
Negative control	14	40.00				58.67			
	28	21.33				29.33			

Table 5. The average of relative percentage of survival (RPS) on extract of *X. granatum* leaves given to tiger shrimp to protect from microbe infection.

Extract	Day	Relative percentage of survival (%)							
		<i>V. harveyi</i>				<i>Saprolegnia</i> sp.			
		1,000 ppm	800 ppm	600 ppm	400 ppm	1,000 ppm	800 ppm	600 ppm	400 ppm
Ethanol	14	70.79	72.75	70.45	50.81	61.67	60.19	54.63	50.00
	28	72.89	69.39	66.14	52.46	66.12	66.01	66.01	50.98
Distilled water	14	72.67	64.30	59.18	43.46	57.78	59.44	41.67	32.22
	28	72.89	71.14	64.39	50.79	65.90	64.05	56.64	47.28
Sea water	14	70.45	59.78	57.56	38.93	54.81	53.33	42.96	18.70
	28	67.81	66.14	54.30	40.61	64.27	60.35	41.50	35.84
Positive Control	14	72.67				60.37			
	28	72.98				67.97			

legnia sp. infection, and, thus, may be applied to improve the survival rate of shrimp in captivity. *Vibrio* attacks may cause shrimp death from the larva to adult stage if reared in ponds²². The causative vibriosis generally is *V. harveyi* leading to mortality range of around 40–65%, while the causative agent for the failure of larval development in hatcheries is commonly *Saprolegnia* sp.

X. granatum has been utilized by the local community for food, animal feed, food preservatives, and traditional medicine. Mangroves are the best choice to isolate natural or bioactive products to challenge against bacteria and fungi²³. Substances extracted from mangrove may function as herbal remedies to treat various biological dysfunctions with minimal side effects but with maximum healing potential²⁴. Mangroves provide rich secondary metabolites, such as alkaloids, flavonoids, phenolics, steroids, and terpenoids. Natural phenols, alkaloids, and flavonoids have antioxidant, antibacterial, anti-tumour, and anti-viral properties^{25–27}. Flavonoids are synthesized by plants to respond to microbial infections, and, *in vitro*, these metabolites are effective antimicrobial substances against microorganisms extensively²⁸. Flavonoid and phenolic compounds from natural sources are known to be associated with a variety of biological activities, such as antioxidant properties, anti-inflammatory actions, and anticancer activities²³.

Conclusions

Leaf extracts of *X. granatum* had the antimicrobial potential to inhibit the infection of tiger shrimp by *V. harveyi* and *Saprolegnia* sp. The ethanol and distilled water leaf extract at concentrations of 800 and 1,000 ppm exhibited higher activity

in inhibiting and reducing the infection of *V. harveyi* and *Saprolegnia* sp. than antibiotics.

Data availability

Raw data for tables can be accessed on OSF, DOI: <https://doi.org/10.17605/OSF.IO/349SK>¹⁴.

Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain dedication).

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References

1. Saptiani G, Asikin AN, Ardhani F, *et al.*: **Tanaman bakau api-api putih (*Avicennia marina*) berpotensi menghambat mikrob patogen dan melindungi post larva udang windu (The potential of *Avicennia marina* to inhibits pathogen microbes and protects the post larva of tiger prawn).** *J Veteriner*. 2018; 19(1): 45–54.
[Publisher Full Text](#)
2. Saptiani G, Hardi EH, Pebrianto CA, *et al.*: ***Alpinia galanga* extracts for improving egg hatchability and larval viability of catfish.** In: *AIP Conference Proceedings*. 2016; 1755: 140002-1–140002-5.
[Publisher Full Text](#)
3. Ramesh K, Natarajan M, Sridhar H, *et al.*: **Anti-Vibrio activity of mangrove and mangrove associates on shrimp pathogen, *Vibrio harveyi* VSH5.** *Global Veterinaria*. 2014; 12(2): 270–276.
[Reference Source](#)
4. Saptiani G, Prayitno SB, Anggoro S: **The effectiveness of *Acanthus ilicifolius* in protecting tiger prawn (*Penaeus monodon* F.) from *Vibrio harveyi* infection.** *J Coast Dev*. 2012; 15(2): 217–224.
[Reference Source](#)
5. Saptiani G, Hardi EH, Pebrianto CA, *et al.*: **Ekstrak Daun Pepaya dan Kangkung untuk meningkatkan daya tetas telur dan kelangsungan hidup larva lele (Extracts of *Carica papaya* and *Ipomoea Aquatica* for improving egg hatchability and larval viability of Catfish).** *J Veteriner*. 2016; 17(2): 285–291.
[Publisher Full Text](#)
6. Saptiani G, Asikin AN, Ardhani F, *et al.*: **Mangrove plants species from Delta Mahakam, Indonesia with antimicrobial potency.** *Biodiversitas*. 2018; 19(2): 516–521.
[Publisher Full Text](#)
7. Sidik AS: **The changes of mangrove ecosystem in Mahakam Delta, Indonesia: A complex social environmental pattern of linkages in resources utilization.** *Paper presented at the South China Sea Conference 2008.* The South China Sea: Sustaining ocean productivities, Maritime Communities and the Climate. Kuantan, Malaysia 25-29th November. 2008.
[Reference Source](#)
8. Sahoo G, Mulla NSS, Ansari ZA, *et al.*: **Antibacterial Activity of Mangrove Leaf Extracts against Human Pathogens.** *Indian J Pharm Sci*. 2012; 74(4): 348–351.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
9. Saptiani G, Handayani Hardi E, Agus Pebrianto C, *et al.*: **Antimicrobial potential of *Carica papaya*, *Ipomoea aquatica*, *Alpinia galanga* and *Piper betle* against the aquatic microbials.** *Nusantara Bioscience*. 2016; 8(2): 252–257.
[Publisher Full Text](#)
10. Wade NM, Budd A, Irvin S, *et al.*: **The combined effects of diet, environment and genetics on pigmentation in the Giant Tiger Prawn, *Penaeus monodon*.** *Aquaculture*. 2015; 449(1): 78–86.
[Publisher Full Text](#)
11. Wade NM, Paulo C, Goodall J, *et al.*: **Quantitative methods to measure pigmentation variation in farmed Giant Tiger Prawns, *Penaeus monodon*, and the effects of different harvest methods on cooked colour.** *Aquaculture*. 2014; 433: 513–519.
[Publisher Full Text](#)
12. Bernal Rodríguez CE, Carvajal García A, Ponce-Palafox JT: **The color of marine shrimps and its role in the aquaculture.** *Int J Aquac Fish Sci*. 2017; 3: 62–65.
[Publisher Full Text](#)
13. Saptiani G, Prayitno SB, Anggoro S, *et al.*: **The influence of *Acanthus ilicifolius* extracts to histopathological on hepatopancreas of tiger shrimp (*Penaeus monodon* F.).** *IUMARCC*. 2017; 2(10): 1–6.
[Reference Source](#)
14. Saptiani G: **Antimicrobial of Nyirih (*Xylocarpus Granatum*) against Pathogens of Tiger Shrimp Post-Larvae.** *OSF*. 2018.
<http://www.doi.org/10.17605/OSF.IO/349SK>
15. El Far SAH, Khalil RH, Saad TT, *et al.*: **Occurrence, characterization and antibiotic resistance patterns of bacterial communities encountered in mass kills of pond cultured Indian prawn (*Fenneropenaeus indicus*) at Damietta governorate, Egypt.** *Int J Fish Aquat Stud*. 2015; 2(4): 271–276.
[Reference Source](#)
16. Lomeli-Ortega CO, Martínez-Díaz SF: **Phage therapy against *Vibrio parahaemolyticus* infection in the whiteleg shrimp (*Litopenaeus vannamei*) larvae.** *Aquaculture*. 2014; 434: 208–211.
[Publisher Full Text](#)
17. Mirbakhsh M, Akhavan Sepahy A, Afsharnasab M, *et al.*: **Molecular identification of *Vibrio harveyi* from larval stage of Pacific White Shrimp (*Litopenaeus vannamei*) Boone (Crustacea:Decapoda)By polymerase chain reaction and 16S rDNA sequencing.** *Iran J Fish Sci*. 2014; 13(2): 384–393.
[Reference Source](#)
18. Cadiz RE, Traifalgar RFM, Sanares RC, *et al.*: **Comparative efficacies of tilapia green water and biofloc technology (BFT) in suppressing population growth of green vibrios and *vibrio parahaemolyticus* in the intensive tank culture of *Penaeus vannamei*.** *AAAL Bioflux*. 2016; 9(2): 195–203.
[Reference Source](#)
19. Kannapiran E, Ravindran J, Chandrasekar R, *et al.*: **Studies on luminous, *Vibrio harveyi* associated with shrimp culture system rearing *Penaeus monodon*.** *J Environ Biol*. 2009; 30(5 suppl): 791–795.
[PubMed Abstract](#)
20. Heenatigala PPM, Fernando MUL: **Occurrence of bacteria species responsible for vibriosis in shrimp pond culture systems in Sri Lanka and assessment of the suitable control measures.** *Sri Lanka J Aquat Sci*. 2016; 21(1): 1–17.
[Publisher Full Text](#)
21. Alam MA, Sarder M, Awal MA, *et al.*: **Antibacterial activity of the crude ethanolic extract of *Xylocarpus granatum* stem barks.** *Bangl J Vet Med*. 2006; 4(1): 69–72.
[Publisher Full Text](#)
22. Kumaravel K, Ravichandran S, Sritama S: ***In vitro* antimicrobial activity of shrimps haemolymph on clinical pathogens.** *African J Microbiol Res*. 2010; 4(23): 2592–2596.
[Reference Source](#)
23. Veni PS, Sunita S, Srinivasulu A: **Antibacterial and phytochemical screening of *Xylocarpus moluccensis* leaf and stem on selected drug resistant and sensitive bacteria.** *Int J Microbiol Res*. 2014; 5(1): 30–34.
[Reference Source](#)
24. Prabhu VV, Guruvayoorappan C: **Phytochemical screening of methanolic extract of mangrove *Avicennia marina* (Forssk.) Vierh.** *Pelagia Res Libr Des Pharmacia Sin*. 2012; 3(1): 64–70.
[Reference Source](#)
25. Baba S, Chan HT, Kainuma M, *et al.*: **Botany, uses, chemistry and bioactivities of mangrove plants III: *Xylocarpus granatum*.** 2016; 14(1): 1–4.
[Reference Source](#)
26. Soonthornchareonnon N, Wiwat C, Chuakul W: **Biological activities of medicinal plants from mangrove and beach forests.** *Mahidol Univ J Pharm Sci*. 2012; 39(1): 9–18.
[Reference Source](#)
27. Sharief Md N, Srinivasulu A, Satya Veni P, *et al.*: **Quantification of phytochemicals and antibacterial activity of fruit extract of *Avicennia officinalis*.** *Asian J Pharm Clin Res*. 2014; 7(2): 127–130.
[Reference Source](#)
28. Kumar S, Pandey AK: **Chemistry and biological activities of flavonoids: an overview.** *ScientificWorldJournal*. 2013; 2013: 162750.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

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Chinmayi Joshi

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This study is about the antimicrobial potential of *Xylocarpus granatum* leaf extracts against tiger shrimp post-larvae pathogens i.e. *Vibrio harveyi* and *Saprolegnia* sp.

Comments:

1. Authenticity of test organisms should be confirmed.
2. Did the authors perform any additional experiments to decide the concentration of bacteria for infection?
3. In methods, ('Treatment' section) the sentence "The treatments were leaf extract, extracted with ethanol solvent, distilled water or seawater solvent" is confusing.
4. Tables 2-5 contain various values, which should be presented with standard deviation.
5. The authors concluded that "the ethanol and distilled water leaf extract at concentrations of 800 and 1,000 ppm exhibited higher activity in inhibiting and reducing the infection of *V. harveyi* and *Saprolegnia* sp. than antibiotics." Effective concentration of antibiotic should also be mentioned here.
6. If possible, provide the picture showing the difference between infected and extract-treated shrimp.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Antiinfectives from plants

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 13 June 2019

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Ramalingam Ananda Raja

ICAR-Central Institute of Brackishwater Aquaculture, Chennai, Tamil Nadu, India

I give the following suggestions on this manuscript:

1. The Results and Conclusion in the starting of this manuscript itself are confusing and not so clear.
2. *Vibrio spp.* and *Saprolegnia spp.* need NCBI accession number for authentication.
3. How did the authors determine the initial doses for these bacteria and fungi?
4. The Introduction is not elaborate and objectives are not specifically mentioned.
5. The English language needs to be improved, especially tenses.
6. The RPS formula itself is confusing.
7. "Mayor" or "Major"; "damaged" or "damage"?

8. Values should be with S.E or S.D.

9. Please refer to Ananda Raja *et al.* (2017¹).

References

1. Ananda Raja R, Sridhar R, Balachandran C, Palanisammi A, et al.: Pathogenicity profile of *Vibrio parahaemolyticus* in farmed Pacific white shrimp, *Penaeus vannamei*. *Fish Shellfish Immunol.* 2017; **67**: 368-381 [PubMed Abstract](#) | [Publisher Full Text](#)

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Vibriosis in shrimp

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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