Available online on 15.07.2021 at <http://jddtonline.info>

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Copyright © 2021 The Author(s): This is an open-access article distributed under the terms of the CC BY-NC 4.0 which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited

Open Access Full Text Article

Research Article

Immunostimulant Effect of Seaweeds in *Channa punctatus* Challenged by *Aeromonas hydrophila*

Muthukrishnan S^{1*}, Raja P².

¹ Research scholar (Reg. No. 18111282191004), Department of Zoology, St. Xavier's College (Autonomous), Palayamkottai, Tirunelveli -627002, Affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli - 627012, Tamil Nadu, India.

² Assistant Professor, Department of Zoology, St. Xavier's College (Autonomous), Palayamkottai, Tirunelveli -627002, Affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli - 627012, Tamil Nadu, India.

Article Info:

Article History:

Received 11 May 2021
Review Completed 18 June 2021
Accepted 29 June 2021
Available online 15 July 2021

Cite this article as:

Muthu Krishnan S, Raja P, Immunostimulant Effect of Seaweeds in *Channa punctatus* Challenged by *Aeromonas hydrophila*, Journal of Drug Delivery and Therapeutics. 2021; 11(4):20-23

DOI: <http://dx.doi.org/10.22270/jddt.v11i4.4948>

*Address for Correspondence:

Muthu Krishnan. S, Department of Zoology, St. Xavier's College (Autonomous), Palayamkottai, Tirunelveli -627002, Affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli - 627012, Tamil Nadu, India.

Abstract

The current study focused on to evaluate the bioactive potential and immunostimulant effect of seaweeds in striped murrel *Channa punctatus* against the fish pathogen *Aeromonas hydrophila*. The aqueous extracts of seaweeds, *Glacilaria foliifera*, *Acanthophora spicifera* and *Ulva lactuca* were obtained in four different concentrations (25 µL, 50 µL, 75 µL and 100 µL) and evaluated for their immunostimulant potential. Aqueous extract of *G. foliifera* and *A. spicifera* has shown promising activity against fish pathogen. *G. foliifera* exhibited higher bacterial inhibitory zone of 8mm at 100 µL concentration followed by *A. spicifera* with 6mm at same concentration. The disease challenge study was used to ascertain the immunostimulant efficacy of active seaweed extracts. *C. punctatus* was administrated with the *A. hydrophila* and then fed with artificial feed incorporated with seaweeds extract. The survival rate seems to be higher in *G. foliifera* (80%) when compared with *A. spicifera* (60%). Observed result shows that both seaweeds possess active metabolites which may pave the way for the discovery of novel compounds with immunostimulant potential, which can be used in the field of aqua industry to augment disease management.

Keywords: Seaweed, Bioactive compounds, *Aeromonas hydrophila*, *Gracilaria polifera*

INTRODUCTION

Fish is one of the prominent sources of animal protein in developing countries. Indian aquaculture has become a fast growing sector and providing an important supplement to and substitute for stagnating yields from wild fish stocks. World food summit, which agreed to promote the development of environmentally sound and sustainable aquaculture well integrated in to rural, agricultural and coastal development ^{1,2}. As aquaculture production becomes more intensive, the incidence of various infectious diseases has been increased resulting in significant economic losses that ultimately inhibiting the expansion of aquaculture farming. Large scale mortality occurs especially among the freshwater fishes often due to environmental stress followed by pathogenic attacks and parasitic afflictions. However, bacterial infections are the main cause for fish mortality in aquaculture industry ³. Various antibiotics, vaccines and chemotherapeutants have been used for prevention or treatment of diseases. Unfortunately continuous use of antibiotics and vaccines induces the development of new resistant bacteria in the aquatic environment ⁴. These resistant bacterial strains could have a negative impact on the therapy of fish disease and the environment of the fish

farms ⁵. *Aeromonas hydrophila*, gram- negative motile bacilli plays a dominant role as etiological agent of several diseases such as emaciation, asymptomatic septicaemia, haemorrhagic septicaemia, tail rot, fin rot and ulcerative infection in several species including Gouramies and Cat fishes and cause mortality in some severe infection in carps and murels ⁶. This issue creates need to search for new antibacterial agents from available natural sources. The biodiversity of marine environment provides chemical diversity as a source of bioactive compounds which promising to have potential and therapeutic applications ⁷. Seaweeds, marine macroalgae are one of them having a vast potential for screening of antibacterial agents to develop new antibiotics. It is already documented that seaweeds from southeast cost of India has antibacterial property ⁸.

Numerous extracts of seaweeds have been examined for antibacterial activity against pathogen in human, cultivable fish and agriculture crops. Extracts of seaweeds *Codium decorticatum*, *Caulerpa scalpelliformis*, *Gracilariacrasa*, *Acanthophora spicifera*, *Sargassum wightii* and *Turbinaria conoides* from Gulf of Mannar exhibited antibacterial activity against *Vibrio parahaemolyticus*, *Salmonella* sp, *Shewanella* sp, *Escherichia coli*, *Streptococcus pyogenes*, *Klebsiella*

pneumoniae, *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus mirabilis* ⁹. This present study has been attempted to access the immune stimulant efficiency of seaweed organic extracts in snake head murrel, *Channa punctatus*.

MATERIALS AND METHODS

The experimental fish, *C. punctatus* was collected from kumar fish farm, Vannarapettai and transported to CARE aqua laboratory. St. Xavier's College, Palayamkottai, Tirunelveli and acclimatized.

Feed preparation

Preparation of fish feed: Experimental diets were prepared separately using known quantities of ingredients (Table 1). Selected ingredients were powdered and sieved to get fine particles of uniform size. Ingredients were then weighed according to the formulation and hand kneaded by adding sufficient quantity of distilled water and finally made into dough. The dough was then cooked in a closed aluminium container for about 15 min and then cooled. When compared with live feeds, semi moist feeds were widely used in energetic experiments of *C. punctatus* because of their high feed conversion efficiency, easy preparation, less consumption and easy digestion ¹⁰. The following ingredients were used to prepare semi solid feed. Fish meal: Jawala, prawn and anchovy fish were purchased from local fish market and washed in tap water and boiled at 60°C, then dried under sun for 30 days and after drying, powdered using mixer and sieved to required size. The protein content of this fish meal was 60%. Flours: Wheat flour, soybean flour, rice flour and tapioca flour were used. Rice flour constitutes 8g, wheat flour 9g and soy flour 24g and 10.48g of tapioca flour which was used as a binder to improves the stability of the feed. Sun flower oil of 1ml and Vitamin C were also used in fish feed formulation.

Extraction of seaweeds:

Healthy seaweeds such as *Glacilaria foliifera*, *Acanthophora spicifera* and *Ulva lactuca* were collected from the intertidal areas of Tuticorin coast (08° 46' 2.15"N lat; 78°11' 16.05" E long) by handpicking method and washed with seawater, followed with freshwater to remove salt, epiphytes and other suspended materials. The clean algae were dried under the shade and stored at refrigerator. The aqueous extract of seaweeds was prepared following the method ¹¹. 50 g of each dried seaweed powder added with 150 ml of distilled water placed in water bath for 30 minutes at 60°C and then filtered and concentrated.

Antibacterial assay

Filter paper (Whatman No. 1) discs of 6 mm were prepared and sterilized. By using micropipette, discs were loaded with 25µl, 50µl, 75µl and 100µl concentrations of aqueous extract of seaweed extract separately. The antibacterial activity of seaweed extracts was carried out against *Aeromonas hydrophila*. The bacterial culture of 0.1ml was spread in agar surface on petriplate at aseptic condition. The discs were placed at equidistance from each other on the plate and incubated at room temperature for about 48 hour. The inhibitory zone lengths were measured from edge of the disc to end of the clear zone in millimeter.

Experimental condition:

C. punctatus with an average weight of 6.010 ± 2.015g were selected and reared in cement tank of 120 litre capacity. The cement tanks were filled with water (45L). Ten fish were introduced into each tank. Twelve tanks were taken and triplicates were maintained for each sample. The

experimental setup was divided in to 4 groups namely control, *G. foliifera*, *A. spicifera* and *U. lactuca*. The test fish were fed with the control feed without seaweed extract for 20 days. Before the introduction of fish into the tank the initial length and weight of the fish were measured. The tanks were cleaned and refilled with freshwater once in 10days. After 20 days, seaweeds extract mixed feed were provided for 45 days. Water quality parameters like temperature, pH, salinity and dissolved oxygen were measured and recorded for every 10 days.

Disease Challenge Study

Disease challenge study was done on 45th day by inducing *A. hydrophila* at the load of 1 X 10⁴ CFU/ml. Ten fish were kept in 1 litre tray at room temperature and aerated for 1 hr. Bacterial culture were grown in 50ml nutrient broth medium and centrifuged at 12,000 rpm for 3minutes, where after pellets were resuspended in 10 ml of sterile distilled water. Bacterial suspension was inoculated into the water of fish tray ¹².

RESULT AND DISCUSSION

The protein, carbohydrate and lipid composition of ingredient used in the basal diet is reported in table-1. Among the ingredients used, anchovy (60%) has the highest protein followed by jawala (50%) and soy flour (49.65%). The lowest value was noticed in tapioca flour (2.22%). The carbohydrate level was found to be highest in tapioca flour (43.7%) and lowest in anchovy (2.1%). The lipid content was maximum in anchovy (13.9%) and minimum in wheat flour (0.40%).

The percentage composition of ingredient used in the basal diet of *C. punctatus* is given in table-2. The basal diet has anchovy (2.1%), jawala (22.5%), wheat flour (9%), soy flour (24%), rice flour (8%), tapioca (10.48%), vitamin C (0.02%) and 1ml oil. Biochemical compositions were as follows: protein 44.7%, carbohydrate 17.2%, lipid 7.2%, and moisture 30%. The results are represented in table-3.

In the present study antimicrobial activity of seaweeds showed increasing zone of inhibition at increasing concentration (Table 4). Minimum and maximum inhibitory concentration (MIC) of *U. lactuca* and *G. foliifera* with a zone of 2mm (20µl) and 8mm (100 µl) respectively. Likewise in *G. foliifera* the mean value of zone of inhibition was 3mm (50 µl) to 8mm (100 µl). Microbial activity is classified into resistant if the zone of inhibition in millimetre is less than 2mm, if it is 3-5mm intermediate, if the inhibition is 6 or more it is sensitive ¹³.

Water quality parameters in experimental tanks are given in table-5. The pH value of the treatment ranges from 7.5-8.0 and the mean pH was 7.9±0.2. The mean total population of bacteria incorporated in the gut of fishes is shown in table 7. The mean bacterial population densities of *G. foliifera*, *A. spicifera* and *U. lactuca* on 65th day were found to be considerably increased; they were 4.9X10⁵, 6.8X10⁶ and 3.5X10⁵CFU/gm respectively and in control 12.0x 10⁴ CFU/gm in different treatments. In our recorded data the water quality parameters like pH, temperature, salinity and dissolved oxygen content were within the range as suggested ^{14, 15, 16}.

Anon noticed that the application of seaweeds *G. foliifera* and *A. spicifera* promoted the survival, growth of fish; and also prevent the disease of Epizootic ulcerative syndrome (EUS) in murels ¹⁷, similarly our work reveals that the *G. foliifera* and *A. spicifera* had the higher immunostimulant effect in *C. punctatus* while challenging with pathogen *Aeromonas hydrophila* than the *Ulva lactuca*. In which the *G. foliifera*

showed the maximum survival rate of 80% followed by *A. spicifera* (60%) and the *U. lactuca* showed the lowest survival rate of 30%. Number of the dead fishes in disease

challenge experiment is showed in table 7. Higher mortality was observed in the control (without seaweed extract) whereas mortality was lower in *G. foliifera* and *A. spicifera*.

Table 1: Proximate composition of selected ingredients

INGREDIENT	PROTEIN%	CARBOHYDRATE%	LIPID%
Anchovy	60.00	2.10	13.90
Jawala	50.00	3.20	12.70
Tapioca flour	2.22	43.70	0.56
Soybean flour	49.65	6.70	1.45
Rice flour	16.80	25.40	13.80
Wheat flour	12.50	30.40	0.40

Table- 2: Percentage composition of formulated diets

Ingredients	Percentage (%)
Anchovy	25.00
Jawala	22.50
Wheatflour	9.00
Soybeanflour	24.00
Riceflour	8.00
Oil	1.00
TapiocafLOUR	10.48
Vitamin C	0.02

Table 3: Proximate composition of basal diet (semi moist)

BIOCHEMICAL COMPOSITION	COMPOSITION %
Protein	44.7
Carbohydrate	17.2
Lipid	7.2
Moisture	3.0

Table 4: Antimicrobial activity of the seaweed extract on bacterial concentration

SEAWEED	ZONE OF INHIBITION (mm)			
	25µL	50 µL	75 µL	100 µL
<i>Glacilaria foliifera</i>	2	3	4	8
<i>Acanthophora spicifera</i>	-	2	4	6
<i>Ulva lactuca</i>	-	-	-	2

Table 5: Water quality parameters in different seaweeds treated water samples

TREATMENT	pH	TEMPERATURE (°C)	SALINITY (ppm)	DO(mLo2/ML)
CONTROL	(7.5-9.0) 7.9±0.663	(26-28) 26.6±0.8	(1.54-3.67) 2.256±0.764	(3.661-9.515) 3.8±0.473
<i>Glacilaria foliifera</i>	(7.5-9.0) 7.9±0.663	(26-28) 26.6±0.8	(1.44-2.15) 1.674±0.0353	(3.40-19.85) 5.602±1.676
<i>Acanthophora spicifera</i>	(7.5-9.0) 7.9±0.663	(26-28) 26.6±0.8	(1.442-2.15) 1.652±0.243	(4.238-8.35) 12.032±6.99
<i>Ulva lactuca</i>	(7.5-9.0) 7.9±0.663	(26-28) 26.6±0.8	(1.54-2.13) 1.751±0.248	(3.108-4.238) 7.724±7.003

Table 6: Mean population of cells incorporated in the gut of fish

SEAWEEDES	MEAN POPULATION OF CELLS (CFU/g)
	65 TH DAY
CONTROL	12.0X10 ⁴
<i>Glacilaria folifera</i>	4.9X10 ⁵
<i>Acanthophora spicifera</i>	6.8X10 ⁶
<i>Ulva lactuca</i>	3.5X10 ⁵

Table 7: Number of dead fishes in seaweeds treatment

TRIAL	TOTAL NUMBER OF FISH	MORTALITY	SURVIVAL RATE	
			Total number of fish survived	%
CONTROL	10	10	NIL	-
<i>Glacilaria folifera</i>	10	2	8	80
<i>Acanthophora spicifera</i>	10	4	6	60
<i>Ulva lactuca</i>	10	7	3	30

CONCLUSION

Fishes not only a major source of protein for humans but also used as major model organisms for various biological and biomedical researches to screen several drugs. In our study we observed that the seaweeds had effective bioactive compounds to protect them from pathogenic bacteria and increase the survival rate of fish by developing immune power in the fish body. The *G. foliifera* has the higher inhibitory zone of 8mm in 100 µL and 4mm in 50 µL followed by *A. spicifera* the zone of inhibition was 6mm in 100 µL and 4mm in 50 µL. The survival rate of fish seemed to be higher in the *G. foliifera* as 80% followed by *A. spicifera* 60%. From our investigation we conclude that the seaweeds possess immunostimulant potential against the fish pathogen *Aeromonas hydrophila* and it would help in the disease management in aquaculture sector like small scale and also in the large scale fish culture.

ACKNOWLEDGEMENT

We sincerely thank Rev. Fr. Principal and Secretary for having provided necessary facilities for carrying out this work in this great institution.

CONFLICT OF INTEREST

The author declares that he has no conflict of interest.

REFERENCES

1. Susan Panickar. Studies on ecological aspects and the microbial activity of Rice-fish rotational farming system in the wetlands of the Kuttanadu, Kerala. A project report of KRPLLD, TVM (2002).
2. Aloysius M. Sebastian, Production efficiency and sustainability of a Rice- Fish Rotational farming Model in Kuttanad Low lands of Kerala Ph.D Thesis submitted to M. G. University, Kottayam, 2005.
3. Naylor, Rosamond, and Marshall Burke. "Aquaculture and ocean resources: raising tigers of the sea." Annu. Rev. Environ. Resour. 2005; 30:185-218.
<https://doi.org/10.1146/annurev.energy.30.081804.121034>
4. Muniruzzaman M and Chowdhury MBR. "Sensitivity of fish pathogenic bacteria to various medicinal herbs." Bangladesh Journal of Veterinary Medicine, (2004); 2 (1):75-82.
<https://doi.org/10.3329/bjvm.v2i1.1941>
5. Smith P, Hiney M and Samuelsen OB. Bacterial resistance to antimicrobial agents used in fish farming: A critical evaluation of method and meaning. Annual. Review of Fish Diseases, 1994; 4: 273-313. [https://doi.org/10.1016/0959-8030\(94\)90032-9](https://doi.org/10.1016/0959-8030(94)90032-9)
6. Chowdhury, MBR and Baqui MA Bacterial flora in farmed carp (*Labeo rohita*) in Bangladesh. In: Disease in Asian Aquaculture III (eds. T. Flegel and I. H. MacRae), Fish Health Section, Asian Fisheries Society, Manila. 1997; 101-105.
7. Capon, Robert J. "Marine natural products chemistry: Past, present, and future." Australian Journal of Chemistry, 2010; 63(6):851-854. <https://doi.org/10.1071/CH10204>
8. Ravikumar S. "Screening of seaweed extracts against antibiotic resistant post operative infectious pathogens." Seaweed Research and Utilisation, 2002; 24(1):95-99.
9. Lavanya R and Veerappan N. "Antibacterial potential of six seaweeds collected from Gulf of Mannar of southeast coast of India." Advances in Biological Research, 2011, 5(1):38-44.
10. Haniffa MA, Arockiaraj AJ, Sethuramalingam, TA and Sridhar S. "Digestibility of lipid in different feeds by striped murrell *Channa striatus*." Journal of Aquaculture in the Tropics, 2002; 17(3):185.
11. Pise M and Sabale AB "Effect of seaweed concentrates on the growth and biochemical constituents of *Trigonella Foenum-Graecum* L.," Journal of Phytology, 2010; 2 (4):50-56. View at Google Scholar
12. Saleema M, Amalina, NZ and Ina-Salwany MY. The Effects of Inactivated Recombinant Cells Vaccine Encoding Outer Membrane Proteins (OMPs) of *Aeromonas Hydrophila* in African Catfish, *Clarias Gariepinus* (Burchell, 1822). Progress in Aqua Farming and Marine Biology, 2018; 1(1):1-15.
13. Baur, A. W, "Antibiotic susceptibility testing by a standardized single disk method." Am J clin pathol, 1966; 45(4):493- 496.
<https://doi.org/10.1093/ajcp/45.4.ts.493>
14. Boyd, Claude E., and Pillai V. K., "Water quality management in aquaculture." CMFRI special Publication, 1985; 22:1-44.
15. Rowland, Stuart J. "Site selection, design and operation of aquaculture farms." Freshwater Aquaculture in Australia. 1st ed. Brisbane: Rural Press Queensland, 1986; 11-22.
16. Boyd, Claude E., and Craig S. Tucker, Pond aquaculture water quality management. Springer Science & Business Media, 2012.
17. Anon, Fish Farming International 1993; 21(8):6-7