

Dietary Effect of *Padina boergesenii* on Growth, Immune Response and Disease Resistance against *Pseudomonas aeruginosa* in *Cirrhinus mrigala*.

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Research Article

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Abstract

Seaweeds are potential renewable resources in the aquatic environment. The present study was made to evaluate the influence of seaweed *Padina boergesenii* incorporated with basal diet in different concentrations. The Phyto-constituents of the seaweeds were characterized by Gas Chromatography Mass Spectrometry. Diets were formulated to include seaweed meal at elevated levels from 0.5%, 2.5%, 4.5% and 6.5%. The *C. mrigala* fed with *P.boergesenii* incorporated with basal diet for a period of 45 days. The Growth parameters (weight gain, Specific growth rate) and biochemical constituents were observed significantly. The fraction of extracts showed effective inhibitory activity against *Pseudomonas aeruginosa*. Hence, this study suggests that extracts of seaweed *P. boergesenii*, contain potential bioactive compounds with considerable antibiotic activity.

Introduction

Aquaculture is the antiquated form of husbandry. Aquaculture is the cultivating aquatic organisms in marine, brackish water and freshwater zones and they involving interventions in the rearing process to augment the production (NOAA 2018). Aquaculture fish production has enlarged especially over the past few epochs, which has led to intensive fish culture performs. In recent years, aquaculture has become a progressively important part of the world economy. **Aquaculture now occupies half of the world's** aqua food productions, capture fish 170.9 million tons/year in 2016 (FAO 2018). Aquaculture and fisheries are the sources of nourishment and protein. Worldwide nearly 3 billion people receive 20 % of their daily animal protein intake from aqua food and fish and their byproducts (Deivasigamani *et al.*, 2016).

The aquaculture of fish or other aquatic organisms have any illness or any pathogen infection treat to commercially available antibiotics has been applied for many years. The manifestation of antibiotic - antagonistic bacteria and other organisms accompanying by way of fish diseases is a worldwide threat in aquaculture, which has received significant attention in the last few years and continues to increase antibiotic-resistant organisms. Infectious diseases are major problems in the field they cause direct losses of biomass and productivity as well as indirectly affect trade restrictions. The aquaculture animals are incapacitated by various pathogenic-microorganisms and hosts. (Sharifuzzaman *et al.*, 2014). Hence aquatic organisms as a potential and promising source of pharmaceutical agents have increased during the last years (Mayer *et al.*, 1999).

Since ancient times, a macroscopic marine alga has closely associated with human life and has been exhaustively used in numerous ways a source of food feed, fertilizer and medicine. Seaweeds are aborigine non-blossoming plant lacking root, stalk and leaves. Seaweed grows extensively in shallow marine water and estuaries. The marine Macroalage are usually attached to the bottom or other solid structures with a help of "holdfasts". Algae yield a range of valuable natural metabolites that used in the arena of pharmaceuticals and food industry. Currently, the various bioactive complexes such as linoleic acid, Stearic acid, oleic acid, fucoxanthin, fucophlorethol, loliolide, and deschloroelatol are identified using

seaweed. Seaweeds are significant sources of marine environment they having various biological activities (Bouhlal *et al.*, 2011).

Seaweeds contain more than 60 trace elements, compared to terrestrial plants (Soad *et al.*, 2016). Many bioactive compounds isolated from marine macro algae, they are sulphated polysaccharides, peptides, sterols, brominates, aromatics, nitrogen-heterocyclic, nitrosulphuric heterocyclic, dibutanoids, proteins, Alkaloids, flavonoids, pigments terpenoids, starch, and oil fatty acid these compounds having the ability to promote growth, body biochemical constituents in fish and they also help to improve the immunity, antibacterial activity, and anti-stress (Citrasu 2010). Seaweeds contain several immunologically active substances, mainly polysaccharides can modify the activity of some components of the immune system and increase protection against certain diseases. Carrageenan and sodium alginate a polysaccharide abundant in certain red seaweeds induced an increase in macrophage phagocytic activity and in the resistance against bacterial infections.

Plant based proteins have comparatively low price and easily available resources. Anti-nourishment factors (e.g. phytoestrogens protease inhibitors, lectins and phytic acid) and scantling or asymmetry in their vital amino acid melange may compromise growth and health of farmed aquatic species (Mansour Zeynali *et al.*, 2020). Recent experiments pointed out that seaweeds are showing stimulatory effects on the nonspecific immune system of human and animals thus they have attracted the attention of many researchers to use seaweeds as an immunostimulants to improve the health and prevent disease outbreak in animals thereby reduce the use of antibiotics and chemotherapeutics. Using this immunostimulants boost the immune systems and effective against a number of opportunistic and secondary pathogens (Felix *et al.*, 2004). Though, they are the potential source of many commercial and therapeutic sectors, the immense impacts seaweeds are still unaware. In respect of the above factors, the present study was subjected to assess the Phyto - compounds and biological activity of selected seaweed.

Materials And Methods

Experimental animals

The freshwater fish *irrhinus mrigala* (Hamilton 1982) were obtained from Tamil Nadu Fisheries Development Corporation, Bhavani Sagar Dam- Erode Tamil Nadu. About 500 fishes were maintained in the laboratory conditions in a cement tank (1000 L) filled with water (temperature $26.5 \pm 1.43^{\circ}\text{C}$; pH 7.8 ± 1.20 ; R. Chloride 0.1 ± 0.25 mg/l; DO_2 6.5 ± 0.13 mg/L; BOD 13 ± 1.53 mg/l; COD 65 ± 5.30 mg/l) for 15days before commencement of the experiments. During the acclimatization, fishes were fed with rice bran. 80% of water was renewed every day to maintain healthy environment.

Collection and identification of seaweeds

The fresh, well-grown species of algae collected from the coastal area of along the Mandapam, Rameshwaram ($079^{\circ} 20\text{E}$ Long: 09° N Lat) regions, South-east coast of India during June 2018.

Immediately after collection it was thoroughly washed and cleaned with seawater and then freshwater followed by distilled water to remove all holdfasts epiphytes, sand calcareous and other adhering detritus material. The water drained off from thallus and they were spread on blotting paper to remove the excess water. Seaweeds were identified as *Padina boergesenii* with the help of Botanical Survey of India, southern regional center, Coimbatore. The collected samples were air-dried and ground into fine powder according to the method of Gonzalez *et al.*, 2001 and Cho *et al.*, (1999).

Preparation of extract and GC-MS analysis

The powdered material subjected to the soxhlet apparatus were successively extracted using methanol. The Phytochemical constituents of the seaweed sample were analyzed using a Perkin Elmer Clarus Series gas chromatographic system with a capillary column. Identification of products was made by comparison of retention time and fragmentation pattern with known reference complexes as well as with mass spectra in the library search results kept in the software (Turbo. Mass. ver 5.42). The GC-MS results were compared with the standard compounds in the library.

Preliminary Phyto-Chemical tests.

The extracted sample were analyzed to determine the presence of different Phyto-compounds (Batool *et al.*, 2019).

Collection of bacterial strain

The *P. aeruginosa* - bacterial strain was obtained from PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu. Subculture of this bacterium was made on Pseudomonas broth and maintained at 28°C for 24hrs.

Antibacterial susceptibility testing by Kirby-Bauer method.

Antimicrobial susceptibility testing was performed by the disc diffusion method using Muller Hinton agar plate (HI Media Laboratories, Mumbai, India, MV1084), according to the Clinical and Laboratory Standards Institute guidelines. The commercially available antibiotic disc (Rifampicin (2 µg/disc), Vanomycin 30µg/disc, Norfloxacin (10 µg/disc), Ceftriaxone (10 µg/disc), Levofloxacin (2 µg/disc), Cefoxitin (30µg/disc), Gatifloxacin (30µg/disc), Oxacillin (30 µg/disc), Methicillin (10 µg/disc), Nalidixic acid (10 µg/disc) and Neomycin (30 µg/disc) were used to check the multi-drug resistant (MDR) pattern against the *Paeruginosa*. The zone of inhibition measured using HI media antibiotic scale and compared with zone interpretative Chart Bauer *et al.*, 1996.

Antibacterial assays – well diffusion method

Antibacterial activity was evaluated by well diffusion method. Using Muller Hinton agar (pH 7.4). The sterilized media was poured into sterile Petri dishes each plate contains 15 to 20ml of media and then allowed solidify. Prepared sterile cotton swab was used to spread the (*P. aeruginosa*) pathogens and left

for a few minutes. In these plates made 8 mm diameter wells using cork borer. 30 µl of the algal extract was poured into the each well and Milliq water was used as Control. The loaded plates were incubated at 37⁰C for 24hr. After incubation clear inhibition zone was formed around the wells this indicated presence of antimicrobial activity and the zone was measured in mm (Cholaraj *et al.*, 2020).

Feed preparation

Diets were prepared with commercially available feed ingredients, such as Fishmeal and soybean meal were used as protein sources. Rice bran, groundnut oil cake, wheat flour, Tapioca flour was used as carbohydrate sources. Cod liver oil was used as lipid source and egg albumin was used as binding agents were also added vitamins and mineral mixture.

The dough mixer was prepared and cooked in a closed aluminum container at 105⁰C for 20 min followed by cooling at room temperature. Subsequently, the 0.5%, 2.5%, 4.5% and 6.5% seaweed powder was added individually, Cod liver oil, vitamins and egg albumin were added and thoroughly mixed until the stiff dough was obtained. The dough was pelletized by an indigenous hand pelletizer with a mesh size of 0.1 mm diameter (pigeon manufactures, Kolkata, India) and was cut into 3.0 ± 0.97 mm-sized pieces. The pellets were dried at room temperature (27⁰C) until constant weight was reached. The prepared feed was stored individually in airtight plastic containers at -20⁰C until used for the feeding trials Table 6.

Experiment trial

After the acclimatization the fish were divided into triplicates of five groups, each group contains 20 fish. T₁ to T₄ experimental diets were fed with *Pboergesenii* extract mixture at (0.5%, 2.5%, 4.5% and 6.5%), with basal diet and the Control group (T) was devoid of the seaweed extract (Rama Nisha *et al.*, 2014, Sebahattin *et al.*, 2008 and Maria Joao *et al.*, 2016).

Growth parameters and Survival rate

The following growth parameters were evaluated Specific growth rate (SGR), initial weight (IW), Final weight (FW), weight gain (WG), initial length (IL), Final length (FL), Survival rate, feed conversion ratio (FCR), protein efficiency ratio (PER) calculated by using following formula Linga Prabu *et al.*, 2016 and Ramasubramanian *et al.*, 2014.

Survival rate = No of fish treated (Initial) - No of fish remaining (final) × 100

Feed conversion ratio (FCR) = Total feed given (g) / Weight gain (g)

Protein efficiency ratio (PER) = Weight gain by fish (g) / Protein intake (g) × 100

Weight gain (WG) = Final body weight of fish (g) – Initial body weight of fish (g)

Specific Growth Rate (SGR) = Final mean body weight (g) – Initial mean body weight (g) / Time interval (days) × 100

Analysis of muscle biochemical constituents

At the end of 45 days, the fish analyzed for total proteins was determined by the method of Lowry *et al.*, (1951). Total carbohydrate was determined by using the method of Roe (1955). Total lipid content was determined by Folch *et al.*, (1973) method.

Immunological assays

The blood samples were collected from each group of experimental fish. The blood was collected from caudal vein using syringe rinsed with an anticoagulant solution to prevent clotting. The collected blood samples mixed with EDTA solution for further analysis of RBC, WBC, Hb, Packed cell volume, MCV, MCH, MCHC and lysozyme activity were determined by a method of Sahoo *et al.*, 2004 and Vijayakumar 2016.

Statistical analysis

Triplicate samples were used in all experimental groups. Results for each parameters were measured and expressed as the mean ± (SD). Data were analyzed using one-way ANOVA and the comparison of the mean values were done by using Duncan multiple range test using software program SPSS version 16 for windows. Differences were considered statistically significant when $p < 0.05$.

Results And Discussion

Phyto - Chemical analysis.

In this study, we observed positive results of Flavonoids, Alkaloids, Saponins, phenolic compounds, terpenoids, amino acid and carbohydrates (**Table 1**). Glycosides and phlobatannins absence in the *C. racemosa*. Alkaloids, phenolic compounds and amino acid & Protein moderately present in the extract of *C. racemosa*. Higher amount of Flavonoids, Saponins, terpenoids, and carbohydrates are present in *C. racemosa*.

These Compounds have the property of cytostatic, antiviral, anthelmintic, antifungal and antibacterial (Shahidi 1991). Deivasigamani *et al.*, 2016 reported that seaweeds are the source of bioactive compounds them able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities.

Gas Chromatography - Mass Spectroscopy analysis

The bioactive Compounds from methanolic extract of seaweed *P.boergesenii* were analyzed for the presence of secondary metabolites and chemical compounds with respective peak area percentage. Gas Chromatography - Mass Spectroscopy analysis of the methanol extract of *P.boergesenii* showed fourteen peaks which indicating presence of phytochemical constituents (Graph. 1). These Compounds were

identified through Mass spectra compared with NIST library. Extracted seaweed samples contain fourteen phytochemical constituents were characterized and identified that are Chloro-xylenol, Hexadecanoic acid, Arachidonic acid, Stearic acid, Eicosatetraenoic acid, Cis-vaccenic acid, Pyrano, Octadecenoic acid, Phytol, Butanoic acid, 13-Heptadecyn-1-ol, Glycidyl palmitate, Glycidyl oleate and Glucobrassicin and mass spectra chromatogram was presented. Other chemical compounds and their functions based on reports were tabulated (**Table 2**).

Antibacterial susceptibility testing

In this study we have used ten different antibiotic discs (Rifampicin, Vanomycin, Norfloxacin, Ceftriaxone, Levofloxacin, Cefoxitin, Gatifloxacin, Oxacillin, Methicillin Nalidixic acid and Neomycin) to confirm the *P. aeruginosa* is Multi-Drug Resistant (MDR), the observed results pointed out *P. aeruginosa* is resistant to antibiotic discs (**Table 3**).

Antibacterial assays – well diffusion method

In the present study methanol extract of *P.boergesenii* shows significant inhibition activity against *P. aeruginosa*. The minimum inhibitory concentration of *P.boergesenii* showed 23mm

GROWTH PARAMETERS

In the present study, significant elevations in survival, growth rate, weight gain, specific growth rate, protein efficiency ratio, feed conversion ratio, and survival rate of *C. mrigala* fed with different concentration of seaweed extract mixed were observed. The growth parameters of the experimental groups were significantly ($p<0.05$) different from control over the entire experimental period. Among the various treatment groups no significant exists between T_3 and T_4 group. Seaweed-incorporated feeds indicated that the utilization of seaweeds in feed has an effect on better survival and growth of *C. mrigala*.

The maximum **weight gain** was observed in T_3 (4.5% of brown algae – *P. boergesenii*) compared to control. Feed conversion ratio (**FCR**) indicates that the Seaweed-incorporated feed effectively utilized for growth in the dietary treatments, with the best value that was obtained in T_3 (4.5% of brown algae – *P. boergesenii*) compared to other groups and control. Feed intake was improved due to the presence of essential amino acids such as glutamic acid, aspartic acid which can enhance the flavor and taste of the feed. Dietary supplementation of *P. boergesenii* significantly enhances the SGR of *C. mrigala* when compared control diet. where reduced growth was observed with increase the seaweed content in fish feed T_4 experiment 6.5%, therefore we conclude optimum level is T_3 (4.5% of brown algae) **Table 4**.

Biochemical constituents

In the present study, Biochemical constituents of the *C. mrigala* were significantly different ($P<0.05$) in all experimental setup when compared to the control group. Among the various treatment groups no

significant exists between T₃ and T₄. Highest protein level in T₃ (4.5% of brown algae) compared with control and other experiments, followed by Carbohydrate content was high in all treated groups when compared with control. The high level was detected in experiment T₃ (4.5% of *P. boergesenii*). Total lipid content in all treated groups was not significant compared to control (**Table 4**). Over all Experiment T₃ with 4.5 % of *P. boergesenii* shows good activity.

HAEMATOLOGICAL PARAMETERS

The WBC, RBC, Hb, PCV, MCV MCH and MCHC count was significantly different from control during the experimental period. Among the various treatment groups no significant exists between T₃ and T₄. **RBC** count were high in group T₃ (4.5 % brown algae), whereas lower in control. High **WBC** count was observed in T₄ (6.5% brown algae), among other experimental groups while lower WBC count was observed in the control group. Other parameters like Hb, PCV, MCV, MCH and MCHC were compared with control, the T₃ (4.5% brown algae) experiment shows higher activity compare to control. **PCV** level high in T₃ (4.5 % brown algae) whereas low level were recorded in control group. **MCHC** level was high in T₃ (4.5 % *P. boergesenii*) compared to control **Table 5**.

The experimental fish were challenged with *P. aeruginosa* pathogen, 94 % survival was observed in experiment T₃ fed with 4.5% of brown algae compared to control 20% (Fig. 1). Post challenge study WBC count (Fig. 2) and lysozyme activity (Fig. 3) was increased significant level except for the control group. The reduction of Haematological parameters in fish feed having lowest supplementation of *P. boergesenii*.

Discussion

In this study we investigated the Phyto - compounds and biological activity of *P.boergesenii* and *C. mrigala* fed with dietary supplementation with extracts of *P.boergesenii* led to increase the growth performance, chemical body composition and different immunological parameters.

Based on the in vitro examination of antimicrobial, Phyto-chemical and bio active compounds, *P.boergesenii* was selected as an immunostimulant to incorporate in the diet of *C. mrigala*. Several studies have shown that seaweeds or its extracts have different biological activities, including, antitumor (Xu *et al.*, 2004), antiprotozoal, antiviral antioxidant (Cox *et al.*, 2010) and cytotoxic activity against the human cancer cell lines. Active compounds from seaweeds were found to be active against human and animal particularly fish bacterial pathogens. Seaweeds contain many fatty acids and secondary metabolites they having higher antibacterial activity against gram-positive bacteria and gram-negative bacteria. Antimicrobial susceptibility test of *P. aeruginosa* showed that resistant to different antibiotics. The current results similar to findings were observed Yuji Morita *et al.*, 2014 reported that *P. aeruginosa* is having multifactorial mechanisms and Besides *P. aeruginosa* has a natural protection from numerous antimicrobials due to the bacterium's external film obstruction, the presence of multidrug efflux carriers, and endogenous antimicrobial inactivation.

In the present study *P.boergesenii* showed positive results of Flavonoids, Saponins, terpenoids and those compounds have the property of cytostatic, antiviral, anthelmintic, antifungal and antibacterial (Shahidi 1991). Deivasigamani *et al.*, 2016 reported that seaweeds are the source of bioactive compounds them able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. The bioactive Compounds from methanolic extract of seaweed *P.boergesenii* were analysed for the presence of secondary metabolites and chemical compounds with respective peak area percentage. Seaweeds are source of polysaccharides, Fatty acids (omega 3), different vitamins and minerals. Those compounds having the ability to promote growth, biochemical constituents in fish and they also help to improve the non-specific immunity, antibacterial activity, and anti-stress. Similar findings were also reported in the fish, fed with *A. paniculata* they fed for fish that responsible for increase the feed intake and give resistant to invading pathogens (Citrasu 2010).

In the present study, *. mrigala* fed with dietary seaweed *P.boergesenii* showed improved growth parameters in dose dependent increase the Survival rate, Weight gain, Specific growth rate in three experimental group compared to control group. This is in agreement with the results of Prabhu *et al.*, 2018. The decrease of fish growth and feed utilization at levels of *P.boergesenii* incorporation with 6.5 % due to Seaweeds having higher amount of anti-nutritional factors (Saponins, tannins phytic acid) they may affect fish growth. Correspondingly Natify *et al.*, 2015.

Biochemical constituents are important quality trait of aquatic animals for marketing (Brinker *et al.*, 2011 and Jayakumar *et al.*, 2016). In the present study, increased protein carbohydrate and lipid content in experiment T₃ comparted to control and other experimental groups.It indicates that supplement feed additives have the ability to promote the absorption and storage of the nutrients in muscles of *C. mrigala*, similar findings reported by Ergun *et al.*, 2009 and Naser *et al.*, 2015. Whereas reduced biochemical constituents increasing inclusion of *P.boergesenii* meal, this is in agreement with the results of Shapawi *et al.*, 2015.

The results indicated that *irrhinus mrigala* fed with had higher level of RBC, WBC, HCT, this might be due to the enhancement of the non-specific immune system of the *Cirrhinus mrigala*.

The findings from this study are supported by previews studies showed that dietary supplementation of seaweeds can improve the immunity of fish (Balasubramanian *et al.*, 2008 and Hari Krishnan *et al.*, 2003). Uthayakumar *et al.*, 2012 reported enhancement of non-specific immunity through the applications of Natural immunostimulants. In post challenging study the administration of methanol extract of 4.5% of brown algae has increase SR in *Cirrhinus mrigala*, compared to control. *P. boergesenii* meal has improved the nonspecific immune parameters and disease resistance against *P. aeruginosa*, this might due to the enhancement of the non-specific immune system of the fish by seaweed *P. boergesenii* meal. There is strong experimental evidence of *P. boergesenii* that feeding can modify the activity of fish immune system by stimulating the non-specific immune response and increase the disease resistance in several fishes reported by Rao *et al.*, 2006, radhika *et al.*, 2017 and Lobo *et al.*, 2018.

Hematological parameters are useful indicators of health status in fish (Mobakane EM and Moya NAG 2019). In our study fishes were fed with seaweed mixed diet and the experiment was carried out 45 days, the blood parameters have increased from the first day to last day. It improved the fish health. From this study, we have concluded that seaweed as a good food ingredient and also it enhances the non-specific immunity and increases disease resistance of *C. mrigala*.

Conclusion

The presence of active antibacterial compounds alkaloids, Flavonoids, Saponins, terpenoids and fatty acids such as Hexadecanoic acid, Arachidonic acid, Octadecenoic acid, Phytol in the experimental feed containing *Padina boergesenii* extract enhanced the growth, biochemical content, immune response and protection against *P. aeruginosa*. The experiment T₃ with 4.5% concentration seaweed incorporated diet is optimum to stimulate the immune response. The base line information will be of important to the fish farming. Hence seaweeds could be effectively beneficial to aqua-farmers, pharmaceutical and therapeutics application in future.

Declarations

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Conflicts of interest

The authors declare that there are no conflicts of interest.

Code availability: Not applicable

Author contribution

V. Ramasubramanian and C. Ragunath conceptualized and designed the study, C. Ragunath, Experimented, collected needed data and preparing the text, figures, and table. All authors reviewed the manuscript and approved it for submission.

Declarations

This is following international, national, and institutional guidelines as it relates to methods concerning the care and use of animals.

Consent to participate: Not applicable

Consent for publication: Not applicable

Data availability statement

The authors confirm that all relevant data are available within the manuscript.

Animal Ethics Declarations

No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated invertebrate species.

References

1. Balasubramanian G, Sarathi M, Venkatesan C, John TD, Sahul Hameed AS, 2008. Oral administration of antiviral plant extract of *Cynodon dactylon* on a large scale production against white spot syndrome virus (WSSV) in *Penaeus monodon*. *Aquaculture*, 279, pp.2-5.
2. Bauer AW, Kirby WM, Sherris JC, Turk M 1966. Antibiotic susceptibility by a standardized single disk method. *Am J Clin. Pathol*, 45pp. 493–496, <https://europepmc.org/abstract/med/5325707>.
3. Bhakuni, D.S. and Rawat, D.S., 2006. Bioactive marine natural products. *Journal of the American Chemical Society*, 128 (13), pp.4494-4494.
4. Bouhlal R, Haslin C-Chermann, Collicec, Jouault S, Siquin C, Simon G., Cerantola S, Riadi, HBourgougnon N, 2011. Antiviral activities of sulphated Polysaccharides isolated from *Sphaerococcus coronopifolius* (and *Boergesenii ellathuyoides* (Rhodophyta, Ceramiales). In *Marine Drugs*, vol. 9: pp. 1187-1209, DOI:[10.3390/md9071187](https://doi.org/10.3390/md9071187)
5. Brinker A, 2011. Fish meal replacement by plant protein substitution and guar gum addition in trout feed, Part I: Effects on feed utilization and fish quality *Aquaculture*, pp. 350-360, DOI: [10.1016/j.aquaculture.2010.09.041](https://doi.org/10.1016/j.aquaculture.2010.09.041)
6. Chandrasekaran, M., Senthilkumar, A. and Venkatesalu, V., 2011. Antibacterial and antifungal efficacy of fatty acid methyl esters from leaves of *Sesuvium portulacastrum* L. *European Review for Medical and Pharmacological Sciences*, 15, pp.775-780.
7. Cho J.Y., Jin H.J., Lim H.J., Whyte J.N.C, Hong Y.K. 1999. Growth activation of the microalga

Isochrysisgalbana by the aqueous extract of the seaweed Monostromanitidum. Journal of Applied Phycology, 10, pp.561–567, DOI: 10.1007/s10811-006-9123-x.

8. Cholaraj Ragunath, Yohannan Aron Santhosh Kumar, Iwar Kanivalan and Sruthi Radhakrishnan, 2020.

Phytochemical Screening and GC-MS Analysis of Bioactive Constituents in the Methanolic Extract of *Caulerpa racemosa* and *Padina boergesenii*, Current Applied Science and Technology, Vol. 20 No.3, Pp 380-393, DOI: 10.14456/cast.2020.24

9. Citarasu, Thavasimuthu, 2010. Herbal biomedicines, A new opportunity to aqua- culture industry. Aquaculture. Int., 18, pp.403-414, DOI: 10.1007/s10499-009-9253-7.

10. Deivasigamani B and Vasukisubramaniyan 2016. Applications of immunostimulants in aquaculture: a review: International Journal Of Current Microbiology And Applied Sciences ISSN: 2319-7706 vol5 pp. 447-453, DOI: <http://dx.doi.org/10.20546/ijcmas.2016.509.048>.

11. Dhanasekaran Linga Prabu, Narottam P Sahu, Asim K Pal, Subrata Dasgupta Ashalaxmi Narendra 2016. Immunomodulation and interferon gamma gene expression in sutchi cat fish, *Pangasianodonhypophthalmus*: effect of dietary fucoidan rich seaweed extract (FRSE) on pre and post challenge period, Aquaculture Research, 47, pp.199–218, DOI: 10.1111/are.12482

12. Ergun S, Soyuturk M, Guroy B, Guroy D, Merrifield D, 2009. Influence of Ulva meal on growth, feed utilization, and body composition of juvenile Nile tilapia (*Oreochromis niloticus*) at two levels of dietary lipid, Aquaculture. Int., 17, pp. 355-361.

13. FAO, 2004. The State of World Fisheries and Aquaculture. Rome, DOI: <http://www.fao.org/3/a-i3720e.pdf>
accessed 16.08.19.

14. FAO, 2014. The State of World Fisheries and Aquaculture. Rom, DOI: <http://www.fao.org/3/a-i3720e.pdf>
accessed 16.08.19.

15. FAO, 2016. Probioticss in animal nutrition – Production, impact and regulation by Yadav S. Bajagai, Athol

16. Klieve, Peter J. Dart and Wayne L. Bryden. In: Makkar, H.P.S. (Ed.), FAO Animal Production and Health Paper No. 179, DOI: <http://www.fao.org/3/a-i5933e>.
17. FAO, 2018. The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals.

Rome, DOI: <http://www.fao.org/3/i9540en/I9540EN>.

17. Felix S., Herald Robins P, Rajeev A., 2004. Immune enhancement assessment of dietary incorporated marine

algae *Sargassum wightii* (Phaeophyceae/Punctariales) in tiger shrimp *Penaeus monodon* (Crustacea / Penaeidae) through prophenoloxidase (proPO) systems, Indian J Mar Sci 33(4) pp.361-364, DOI: http://hdl.handle.net/1234_56789/1691.

18. Folch, J., Lees, M, Bloane-Stanley, G.H. 1957. A simple method for the isolation and purification of total

lipids from animal tissue, *J. Biol. Chem.*, 266, pp.497–509, DOI: <http://www.jbc.org/content/226/1/497.long>.

19. Gerwick, William H, Ballantine, David L, Velez, Sylvia M, Alexander, Evelina, Guevara, Pablo 1985, Antibiotic activity of lipid-soluble extracts from Caribbean marine algae, *Hydrobiologia* 151, pp.463–469, DOI: 10.1007/BF00046168

20. Gonzalez Del Val A, Platas G, Basilio A, 2001. Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). *Int. Microbiol.* 4 pp.35-40, DOI: [10.1007/s101230100006](https://doi.org/10.1007/s101230100006).

21. Gopalakrishnan CN, Binumol T, 2016. Preliminary studies on the effect of bioactive substances of *Hypnea*

musciformis (Wulf) Lamour on the growth of seedlings in green gram, *Vigna radiata* L, *J Phytol* 8, pp.1–6.

22. Harikrishnan R, Rani M, Balasundaram C. 2003. Hematological and biochemical parameters in common

Carp, *Cyprinus carpio*, following herbal treatment for *Aeromonas hydrophila* infection, *Aquaculture*, 221, pp.41-50, DOI: 10.1016/S0044-8486(03)00023-1.

23. Jayakumar, N., Francis, T., Jawahar, P., Rajagopalsamy, C. B. T., Santhakumar, R. and Subburaj, A, 2016.

Acute cadmium toxicity induced impairments in the liver and kidney of freshwater catfish, *Heteropneustes fossilis* (Bloch). Indian journal of science and technology, 9, pp.1-6.

24. Batool, R., Khan, M.R., Sajid, M. et al. Estimation of phytochemical constituents and in vitro antioxidant

potencies of *Brachycton populneus* (Schott & Endl.) R.Br.. BMC Chemistry 13, 32 (2019).
<https://doi.org/10.1186/s13065-019-0549-z>

25. Lee, K.L., Lee, S.H. and Park K.Y., 1999. Anticancer activity of phytol and Eicosatrienoic acid identified from *Perilla* leaves. Journal of the Korean Society of Food Science and Nutrition, 28, pp.1107-1112.

26. Lobo, G., Pereira, L.F., Gonçalves, J.F.M, 2018. Effect of dietary seaweed supplementation on growth performance, antioxidant and immune responses in European sea bass (*Dicentrarchus labrax*) subjected to rearing temperature and salinity oscillations. Int Aqua Res **10**, pp.321–331, DOI: 10.1007/s40071-018-0208-3.

27. Lowry, O.H., Rosenbrough, W.J., Fair, A.L., Randall, R.J. 1951. Protein measurement with the Folin phenol

reagent, J. Biol. Chem., 193, pp.265–275, DOI: <http://www.jbc.org/content/193/1/265.long>.

28. Maqsood, S., Kittiphattanabawon, P., Benjakul, S., Sumpavapol, P., Abushelaibi, A. 2015. Antioxidant activity of date (*Phoenix dactylifera* var. Khalas) seed and its preventive effect on lipid oxidation in model systems, International Food Research Journal, 22(3), pp.1180-1188,
[http://www.ifrj.upm.edu.my/22%20\(03\)%202015/\(42\)](http://www.ifrj.upm.edu.my/22%20(03)%202015/(42)).

29. Maria Joao Peixoto, Emilio Salas- Leitona, Luis Ferreira Pereira, Augusto Queiroza, Fernando Magalhaesa,

RuiPereirad, Helena Abreud, Pedro AlexandreReisa, Jose, Fernando MagalhaesGonc, alvesa, Rodrigo Otavio de Almeida Ozorio 2016. Role of dietary seaweed supplementation on growth performance, digestive capacity and immune and stress responsiveness in European sea bass (*Dicentrarchuslabrax*), Aquaculture Reports 3, pp.189–197, DOI: 10.1016/j.aqrep.2016.03.005.

30. Matanjun, Patricia, Mohamed, Suhaila, Mustapha, Noordin M, Muhammad, Kharidah, 2009. Nutrient content of tropical edible seaweeds, *Eucheuma cottonii*, *Caulerpa lentillifera* and *Sargassum polycystum*, Journal of Applied Phycology, volume 21, pp. 75–80, DOI: 10.1007/s10811-008-9326-4.

31. Mayer C, Moritz R, Kirschner C, Borchard W, Maibaum R, Wingender J, Flemming HC, 1999. The role of intermolecular interactions: studies on model systems for bacterial biofilms, *Int J BiolMacromol* 26 pp.3-16, DOI: 10.1016/S0141-8130(99)00057-4.
32. Mcdonell, G.E. and Ruseell, A.D., 1999. Antiseptics and disinfectants activity, action and resistance. *Clinical Microbiology Reviews*, 12(1), pp.147-179.
33. Nasser A. Al-Asgah, El-Sayed M. Younis, Abdel-Wahab A. Abdel-Warith , Faozi S. Shamlol, 2016. Evaluation of red seaweed *Gracilaria arcuata* as dietary ingredient in African catfish, *Clarias gariepinus*, *Saudi Journal of Biological Sciences*, Volume 23(2), Pp.205-210, DOI: 10.1016/j.sjbs.2015.11.006
34. National oceanic and atmospheric administration (NOAA) 2018, <https://www.noaa.gov>
35. Pongprayoon, U., Baeckstream, P., Jacobsson, U., Lindstream, M. and Bohlin, L., 1992. Antispasmodic activity of beta-damascenone and e-phytol isolated from *Ipomoea pes-caprae*. *Planta Medica*, 58(1), 19-21.
36. Shapawi, I. Ebi, A.S.K. Yong, W.K. Ng, 2014. Optimizing the growth performance of brown-marbled grouper, *Epinephelus fuscoguttatus* (Forsk.) by varying the proportion of dietary protein and lipid levels, *Animal Feed Sci. Technol.*, 191, pp. 98-105, DOI: 10.1016/j.anifeedsci.2014.01.020.
37. D, Mohaideen, 2017. A Study on Immunostimulatory Property of Few Seaweeds Injected Intraperitoneally, *Int J Curr Pharm Res*, 9(4) pp.135-139.
38. Ragunath, C., Komathi, S. and Ragunathan, R., 2018. Molecular Characterization of *Leptospira interorgans*, isolated from animal samples. *International Journal of Pure and Applied Bioscience*, 6(2), pp.995-1004, DOI: 10.18782/2320-7051.6400.
39. Rama Nisha P, Elezabeth Mary A, Uthayasiva M, and Arularasan 2014. Seaweed *Ulva reticulata* a Potential Feed Supplement for Growth, Colouration and Disease Resistance in Fresh Water Ornamental Gold Fish, *Carassius auratus* *Aquac Res Development* 2014, 5:5, DOI: 10.4172/2155-9546.1000254.
40. Rao YV, Das BK, Jyotirmayee P, Chakrabarti R, 2006. Effect of *Achyranthes aspera* on the immunity and

survival of *Labeo rohita* infected with *Aeromonas hydrophila*, Fish Shellfish Immunology, 20 pp. 263–273.

41. Roe, J.H. 1955. The determination of sugar and blood and spinal fluid with anthrone reagent. J. Biol. Chem.,

212, 335–343, DOI: f4ffab9cac51dcace5ffd20c fe80b9f75967

42. P, 2002. Mineral content of edible marine seaweeds, *Journal of Food Chemistry*, Volume 79(1), Pp.23-26, DOI: 10.1016/S0308-8146(02)00171-1.

43. Sahoo, Meher, Mohapatra, Saha, Jana, Reddy 2004. Immune responses in different fullsib families of Indian

major carp, *Labeo rohita*, exhibiting differential resistance to *Aeromonashydrophila* infection. *Aquaculture* 238, pp.115–125, DOI: 10.1016/j.aquaculture.2004.06.008.

44. Sebahattin Ergun, Murat Soyuturk, Betul Guroy, Derya Guroy, Daniel Merrifield 2008. Influence of Ulva

meal on growth, feed utilization, and body composition of juvenile Nile tilapia (*Oreochromis niloticus*) at two levels of dietary lipid, *Aquacult Int*, DOI 10.1007/s10499-008-9207-5.

45. Shahidi and Wanasundara, 1992. Phenolic antioxidants, *Critical Reviews in Food Sciences and Nutrition*, 32,

46. 67.

47. Shanmughapriya S, Manilal A, Sujith S, Selvin J, Kiran GS, Seenivasan KN, 2008. Antimicrobial activity of

seaweeds extracts against multiresistant pathogens, *Ann Microbiol*, 58 (3), pp.535-541.

47. Sharifuzzaman, S., Austin, Brian. 2014. Probiotics for Disease Control in Aquaculture. 189-222, Diagnosis

and Control of Diseases of Fish and Shellfish, First Edition, *John Wiley & Sons Ltd*, DOI:10.1002/9781119152125.ch8.

48. Soad M., .Mohy El-Din., Amani M.D. El-Ahwany, 2016. Bioactivity and phytochemical constituents of marine red seaweeds (*Janiarubens*, *Corallinamediterranea* and *Pterocladia capillacea*). *Journal of Taibah University for Science*, pp.471-478, DOI: 10.1016/j.jtusci.2015.06.004.

49. Uthayakumar V, Ramasubramanian V, Velayudham, Senthilkumar, Dhanabalan, Venkatesan, Brindha,

Harikrishnan, Ramasamy, 2014. Biochemical characterization, antimicrobial and hemolytic studies on skin mucus of fresh water spiny eel *Mastacembelus armatus*, Asian Pacific Journal of Tropical Biomedicine 2. Pp.863–869, DOI: 10.1016/S2221-1691(12)60325-6.

50. Vijaya Kumar 2016. Haematological indices of Indian major carps cultured in West Godavari region of

Andhra Pradesh. International Journal of Applied Research 2016; 2(9): pp.674-677, <http://www.allresearchjournal.com/archives/2016/vol2issue9/PartJ/2-9-130-730>.

51. Ramasubramanian V, Munirasu S, Uthayakumar V, Kiruba A, 2014. Effect of live feed *Mesocyclops aspericornis* survival, growth, biochemical constituents and energy utilization of the freshwater fish *catla catla*, Journal of Aquaculture Feed Science and Nutrition, pp 23-31.

Tables

Table 1: Qualitative phytochemical analysis + + + highly Presence of the compound, ++ moderately presence – absence of the compound.

S. No	Phytochemicals	Inference
1	Alkaloids	+ +
2	Flavonoids	+ + +
3	Saponins	+ + +
4	Phenolic compounds and tannins	+ +
5	Glycosides	-
6	Terpenoids	+ + +
7	Phlobatannins	-
8	Amino acid & protein	+ +
9	Carbohydrate	+ + +

Table 2: GC-MS analysis of *Padina boergesenii*, and their biological activity, molecular formula and molecular weight are showed.

PEAK	R. TIME	Name of the compound	Molecular Weight	Molecular formula	Biological activity
10.07	9.731	Chloroxyletol	156.609	C ₈ H ₉ C ₁₀	Anti-bacterial Mcdonell <i>et al.</i> , 1999
3.878	20.570	Hexadecanoic acid, methyl ester	270.457	C ₁₇ H ₃₂ O ₂	Anti- fungal, antibacterial, Antioxidant, antimicrobial and Anticancer activity Chandrasekaran <i>et al.</i> , 2011
10.617	21.111	n-Hexadecanoic acid	256.430	C ₁₇ H ₃₄ O ₂	
0.370	21.776	Pyrano [4,3-b] benzopyran-1,9-dione, 5a-methoxy-9a-methyl-3-	308.374	C ₁₇ H ₂₄ O ₅	
0.592	22.651	Arachidonic acid	304.474	C ₂₀ H ₃₂ O ₂	
1.416	23.722	11,14-Octadecadienoic acid, methyl ester	294.479	C ₁₉ H ₃₄ O ₂	
3.204	23.862	9-Octadecenoic acid (Z)-, methyl ester	296.500	C ₁₉ H ₃₆ O ₂	Anticancer and antimicrobial Bhakuni <i>et al.</i> , 2006
0.905	24.017	Phytol	296.539	C ₂₀ H ₄₀ O	anticancer anti-inflammatory antimicrobial Antispasmodic activity and vitamins E, K1 Lee <i>et al.</i> , 1999
12.631	24.507	cis-Vaccenic acid	282.468	C ₁₈ H ₃₄ O ₂	Anticancer Pongprayoon <i>et al.</i> , 1992
0.448	28.969	Stearic acid, 3-(octadecyloxy)propyl	595.050	C ₃₉ H ₇₈ O ₃	Antifungal ragunath <i>et al.</i> , 2020

Table 3: Antibacterial susceptibility testing, Interpretative criteria are up to 14 mm resistant, 15 – 16 intermediate, above 17 sensitive.

S. No	Antibiotic disc	Zone of inhibition (mm)
1	Gentamicin	3mm
2	Amikacin	Nil
3	Ciprofloxacin	2.3mm
4	Neomycin	Nil
5	Tobramycin	Nil
6	Ceftazidime	1mm
7	Oxacillin	Nil
8	Meropenem	1.5
9	Imipenem	3.4mm
10	Vanomycin	2mm

Table 4: Growth and biochemical parameters of experimental fish *Cirrhinus mrigala*, Mean \pm SD (n=3) mean values within the same row sharing the same superscript are statistical significant level (P<0.05).

Diets	SGR	FCR	PER	WG	LG	Carbohydrate	Protein	Lipid
T	0.98 \pm 0.26^{bc}	1.92 \pm 0.23^{bc}	2.18 \pm 0.18^h	0.59 \pm 0.24^f	0.77 \pm 0.45^b	16.43 \pm 0.22 ^e	11.72 \pm 0.57 ^h	3.51 \pm 0.40 ^e
T₁	1.28 \pm 0.56 ^{bc}	1.40 \pm 0.16 ^c	3.05 \pm 0.31 ^f	0.92 \pm 0.21 ^{df}	1.74 \pm 0.36 ^a	19.32 \pm 0.35 ^d	14.79 \pm 0.31 ^g	3.93\pm 0.22^e
T₂	2.04 \pm 0.47 ^b	1.22 \pm 0.21 ^b	6.17 \pm 0.10 ^e	0.57 \pm 0.25 ^f	2.04 \pm 0.36 ^a	21.46 \pm 0.29 ^{bc}	16.92 \pm 0.10 ^e	3.58 \pm 0.39 ^c
T₃	3.86\pm 0.51^a	1.05 \pm 0.14^b	9.29\pm 0.32^a	1.74\pm 0.23^{ab}	2.30\pm 0.15^a	24.16\pm 0.59^a	18.04\pm 0.32^a	2.80 \pm 0.61 ^{bc}
T₄	3.20 \pm 0.43 ^{ab}	1.42 \pm 0.21 ^a	7.85 \pm 0.34 ^c	1.44 \pm 0.19 ^{bcd}	2.37 \pm 0.30 ^a	22.83 \pm 0.47 ^b	17.60 \pm 0.34 ^c	2.42 \pm 0.34 ^{abc}

Table 5: Hematological parameters of experimental fish *Cirrhinus mrigala*, Mean \pm SD (n=3) mean values within the same row sharing the same superscript are statistical significant level (P<0.05).

Diets	RBC	WBC	HB	PCV	MCV	MCH	MCHC
T	1.08 ± 0.10 ^c	13.30 ± 0.33 ^d	7.76 ± 0.60 ^c	15.11 ± 0.11 ^c	95.93 ± 3.5 ^c	42.42 ± 2.43 ^c	38.92 ± 1.27 ^c
T ₁	1.23 ± 0.06 ^c	14.89 ± 0.17 ^c	8.30 ± 0.29 ^b	16.28 ± 0.58 ^{bc}	98.98 ± 2.62 ^c	51.49 ± 0.96 ^{bc}	49.22 ± 1.16 ^b
T ₂	1.50 ± 0.26 ^b	15.17 ± 0.28 ^{bc}	8.83 ± 0.15 ^{aaa}	16.41 ± 0.05 ^b	112.86 ± 5.06 ^b	53.67 ± 3.13 ^b	51.67 ± 1.42 ^b
T ₃	1.86 ± 0.06^a	16.20 ± 0.18^a	9.27 ± 0.39^a	18.21 ± 0.27^a	136.62 ± 4.08^a	62.91 ± 2.36^a	55.78 ± 2.92^a
T ₄	1.53 ± 0.10 ^b	15.63 ± 0.64 ^{ab}	9.16 ± 0.32 ^{aa}	16.28 ± 1.81 ^{bc}	114.87 ± 5.41 ^{bc}	59.32 ± 3.08 ^a	51.72 ± 0.87 ^b

Table 6: Nutrient Composition of experimental diets (g%)

Ingredients	(T)	Exp1	Exp2	Exp3	Exp4
Fish meal	30	30	30	30	30
Vitamin & mineral	4	4	4	4	4
Soybean	21.5	21.5	21.5	21.5	21.5
Tapioca starch	15	14.5	12.5	10.5	8.5
Groundnut oil cake	10	10	10	10	10
Binder	1	1	1	1	1
Cod liver oil	6.5	6.5	6.5	6.5	6.5
Wheat bran	12	12	12	12	12
Seaweed powder	0	0.5	2.5	4.5	6.5
Proximate compositions					
Moisture %	7.77 ± 0.13	7.81 ± 0.05	8.34 ± 0.08	8.86 ± 0.08	9.55 ± 0.13
Crude Protein	34.93 ± 0.17	35.04 ± 0.3	35.92 ± 0.14	38.32 ± 0.14	39.72 ± 0.45
Crude Fibre	5.24 ± 0.06	5.83 ± 0.09	5.69 ± 0.17	5.12 ± 0.23	6.11 ± 0.58
Ether extract	9.16 ± 0.06	10.04 ± 0.09	11.76 ± 0.10	11.92 ± 0.08	11.30 ± 0.05
Total ash	7.07 ± 0.13	7.48 ± 0.05	7.69 ± 0.08	7.96 ± 0.08	7.25 ± 0.12
Grass energy	3905 kcal/kg	3931 kcal/kg	3905 kcal/kg	3915 kcal/kg	3901 kcal/kg

Figures

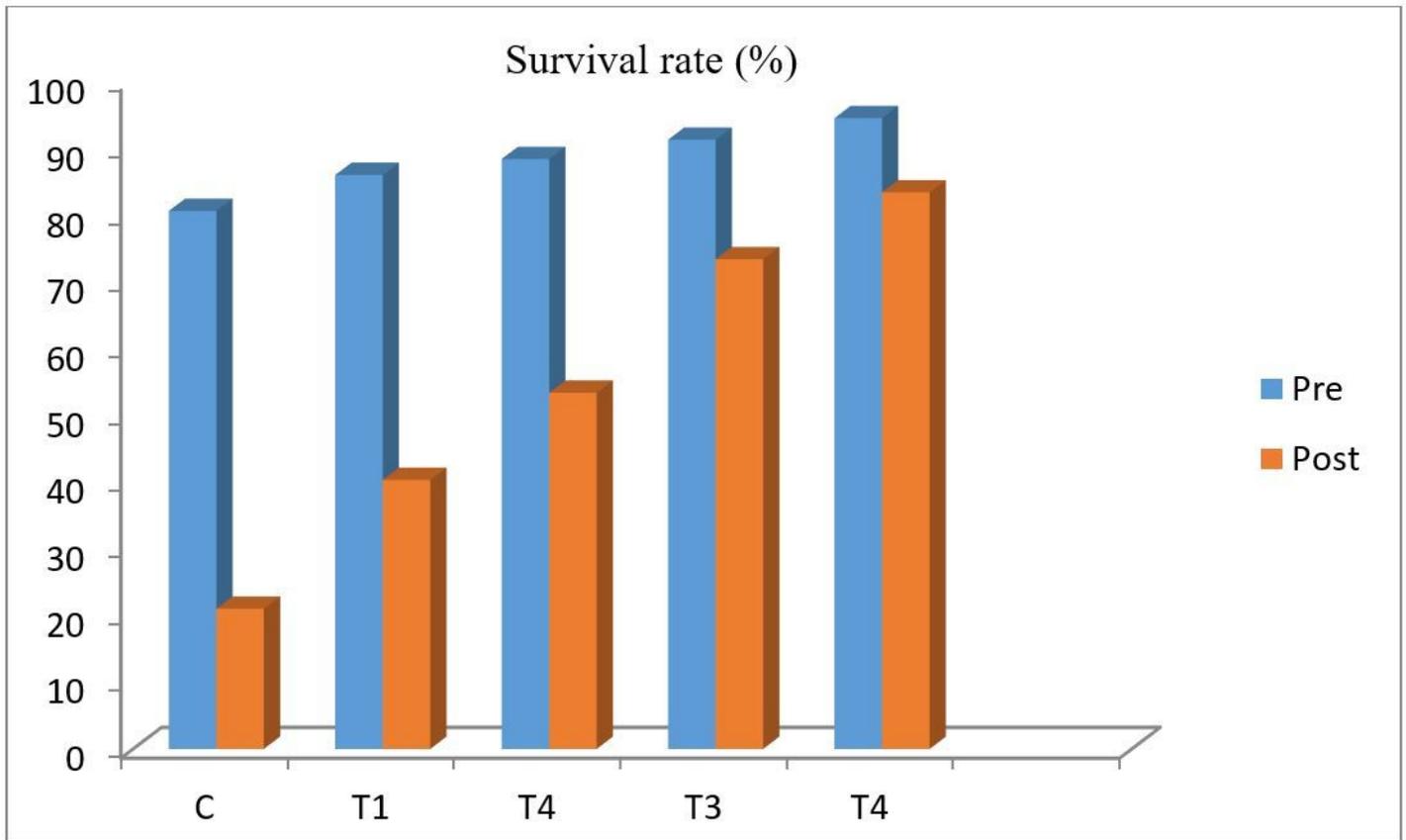


Figure 1

Survival rate of *C. mrigala*, Pre- before challenging study, post- after challenging study

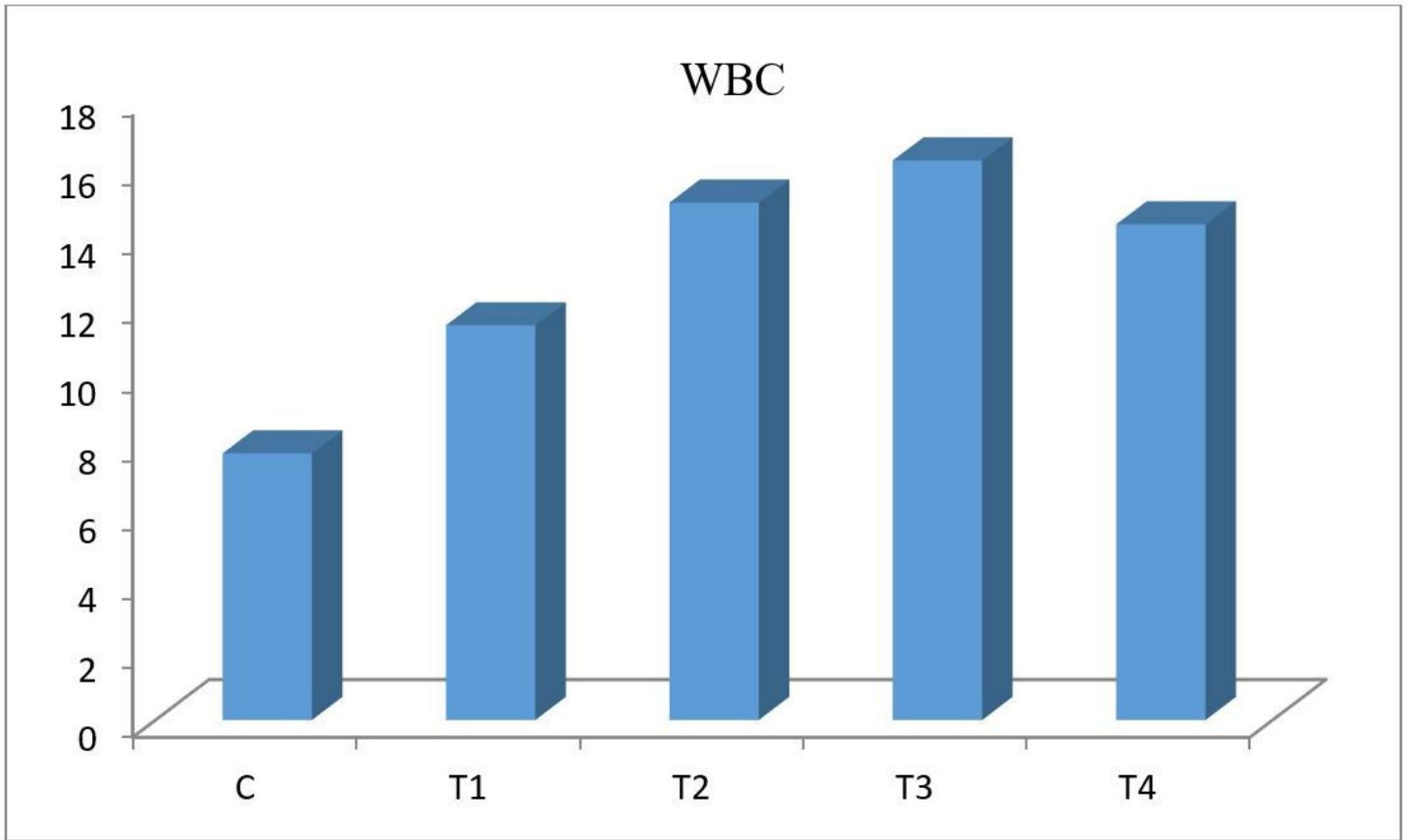


Figure 2

WBC after challenging study of *C. mrigala*

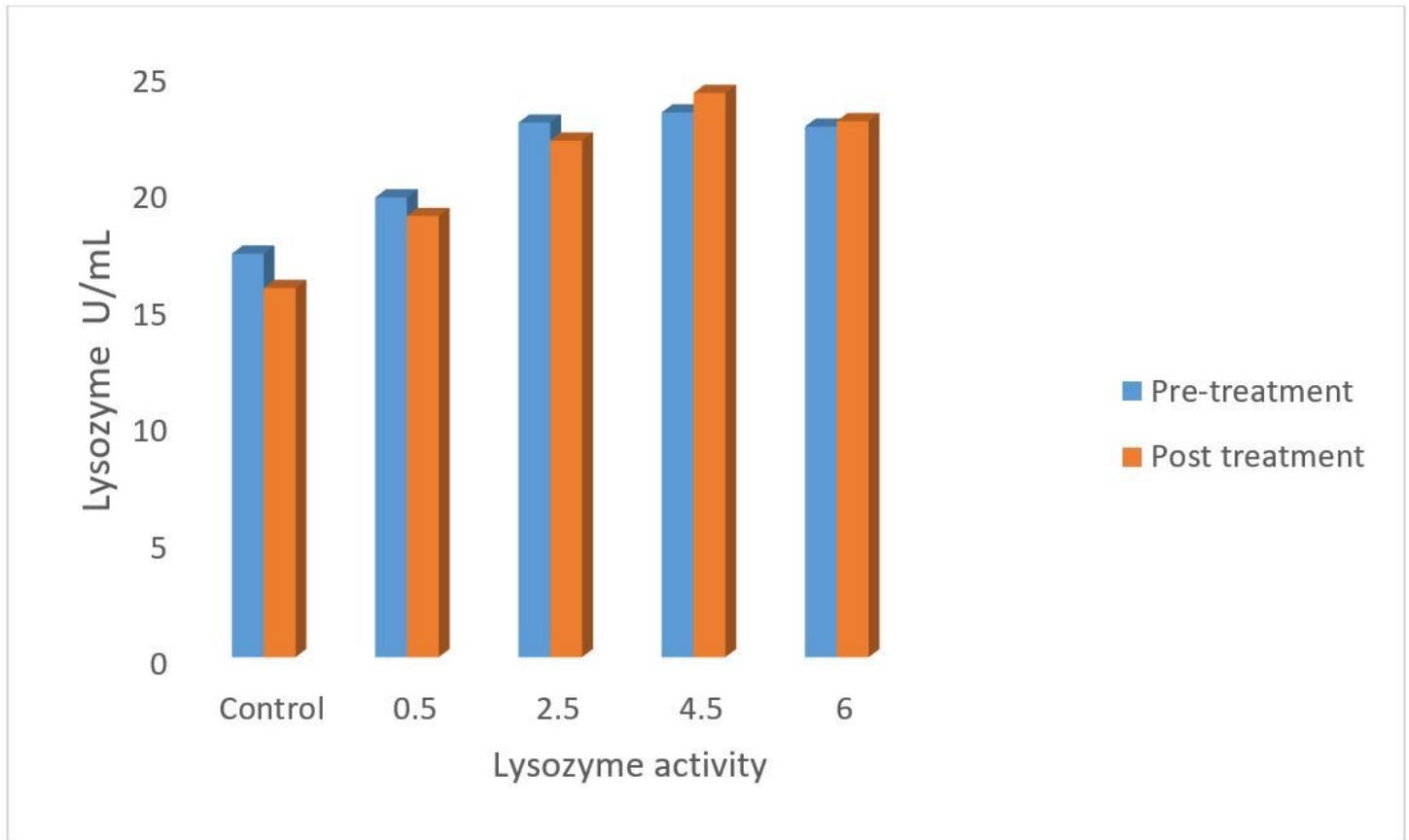


Figure 3

The effect of *P. boergesenii* on Lysozyme activity of *C. mrigala* for pre- challenge and post – challenge in each sampling period. Data expressed as mean \pm SD, n= 3). Different superscript values are statistically significant $P \leq 0.05$

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