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Prophylactic potential of ethanolic leaves extract of Muli bamboo (*Melocanna baccifera*) against *Aeromonas hydrophila* infection in *Labeo rohita*

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Abstract
From the time immemorial, medicinal plants are being used for various purposes starting from as source of nutraceuticals to therapeutic agent due to the presence of phyto-bioactive/ phytochemicals compounds in them. The present study is designed for the evaluation of preventive efficacy of a bamboo (*Melocanna baccifera*), which is abundant in north-eastern part of India. Based on the earlier study bamboo leaves alcoholic extract (BLAL) at 0.1% were fed to *Labeo rohita* fingerling prior to the challenge against *Aeromonas hydrophila*. The study revealed that 0.1% BLAL has the efficiency in preventing bacterial infection in fish and thus it can be concluded as non-toxic phyto-prophylactic which can induce a nonspecific immune response and reduces the probabilities of bacterial infection in *L. rohita* fingerlings.

Keywords: Bamboo, phytochemical, phyto-prophylactic, *Aeromonas hydrophila*, nonspecific immune responses

Introduction
Aquaculture now serves an important sector for food production, carry keynote value in human nutritional security to the food basket of the world. It is not only nutritional security but it also contributes to agricultural export significantly. According to FAO, fish accounts for almost 17% of animal protein supply to the global population and 6.5% of total protein [1]. According to FAO, about one billion people throughout the world rely on fish as their prime source of animal protein [2]. To meet these ever-increasing demands, aquaculture has expanded quickly and is now one of the fastest growing food-producing agro-based industry in the world. As per the assumption of FAO [2] by 2030, over half of the fish consumed by the people throughout the globe will be produced by aquaculture. Nowadays, rapidly increasing and intensifying culture systems lead to poor physiological conditions of the cultured animals and drastically increasing their susceptibility to harmful pathogens. Also, the long-term sustainability of the intensified aquaculture systems is under question due to various concerns such as finite availability of critical inputs, environmental deterioration, the emergence of new diseases, spread of them with pre-existing pathogens, failure in implementation of biosecurity measures *etc.*

In order to increase profitability, the culture practices have lead to the worsening of the aqua-environment through the indiscriminate use of chemotherapeutics, excessive feeding, overstocking *etc.* resulting in increased stress to the animals and consequent incidence of diseases. Among aquatic pathogens, the bacterial disease has now become a primary constraint to aquaculture growth and addressing health questions, therefore it becomes an urgent requirement for sustaining the growth of the aquatic animal.

Aeromonas hydrophila infection in Aquaculture systems
A. hydrophila serves as the most common bacterial disease in aquaculture systems [3] and because of its severity and clinical appearance the disease is known as MAS/ motile aeromonad septicemia or haemorrhagic septicemia or ulcer disease or red-sore disease *etc. A. hydrophila* is a ubiquitous, gram-negative, facultative anaerobe, oxidase-positive, rod-shaped bacterium of the family aeromonadaceae which is commonly isolated from freshwater ecosystems. MAS outbreaks have a major impact on total production of aquaculture systems and at present no vaccine is commercially available to prevent, because of the vast serotypes of *A. hydrophila* [4].

The indiscriminate uses of chemotherapeutics by farmers to control the catastrophic result of *A. hydrophila* infection lead to the circumstances where the major microbial pathogens are making themselves resistant against antimicrobial compounds (AMR-anti microbial resistance and superbug genesis) through the mechanism of transformation, conjugation and transduction, predominantly when the treatment doses are at sub-therapeutic level and applied for extended period of time [5]. To adhere to the sustainability of the aquatic systems and food safety we have to look for alternative strategies to counter the pathogens [6].

The herbal alternative as immunoprophylactic agent

From the ancient time many herbal preparations and their derivatives were used for mitigating disease and stressed condition in all living beings. The significance of herbal medicines is because of their cost efficacy, biodegradability, efficacy with minimal deleterious effect either on target organisms or the aquatic environment *etc*. Phytochemicals or botanicals that are commonly found in plants, referred as a diverse group of phytogenic active compounds which biosynthetically originated from primary metabolites of plants [7], which includes flavonoids, alkaloids, phenolics, ascorbic acid, saponin, rotenin, terpenoids, steroids, essential oils *etc*. These metabolites are having enormous potential to be used as either as therapeutic or prophylactic entities and they can be an excellent eco-friendly alternative for chemotherapy in aquaculture systems without or very minimal toxic effect [8].

Among several medicinal plants available in north-eastern part of India, bamboo plant is bestowed with several nutraceutical properties. Bamboo plants are highly abundant in this part of the country and its leaves, shoots, roots, fruits and flowers are having lots of unique and miraculous property in them. India is the second in terms of bamboo species diversity globally and contributes nearly 13% of the total area of the country with the diversity of 136 species in about 18 genera [9]. And in India, it is North-East which contributes about 66% of total bamboos resources of India [10]. In the most part of the world, shoot is only part in bamboo which considered as an important part with ancestral medicinal value in it, but actually, the remaining parts like leaves, stem, fruits etc. are also having immense potential because of their unique phytoconstituents. In the present study the bamboo leaves which generally considered as waste and let to degrade, is being chosen to evaluate its competence against bacterial infection in fish.

Materials and methods

Sample collection and preparation of extract

For the present study most abundant bamboo variety (80%) in north-eastern India, *i.e.*, *M. baccifera* was chosen [11]. The bamboo sample *M. baccifera*, which is locally available and called as Muli bamboo by the tribes of Tripura, was collected from Lembucherra, Agartala, India (longitude: 91.277847 and latitude: 23.829321) during the month of June to July (monsoon). The validation of the identified species was done with the help of the department of Botany, Tripura University, Agartala. Extraction of active compounds from bamboo plant leaves was done by using a non-polar solvent (90% ethanol). Leaves were dried in the shade and then grounded. Grounded samples were soaked in ethanol and kept in a shaker for 48 h and then centrifuged at 2460 g for about 10 minutes. The supernatant was collected and kept in an amber bottle at 4°C until further use. The supernatant was evaporated in the rotary evaporator at 40° to 45 °C. The collected extracts were then kept at -20 °C until further use. The solvent and solute ratio used for the extraction was 10:1 [11-13].

Collection and maintenance of fish

Healthy *L. rohita* fingerlings (average length 15.7±1.5 cm and weight 20.2±1.5 g) were collected from the farm of College of Fisheries, Tripura and they were acclimatized for about 15 days in fiber reinforced plastic (FRP) tanks of 500 l capacity. Fishes were fed with the pelleted feed collected from College of Fisheries, Lembucherra twice a day at 2% of their body weight. The 15 days acclimatized fish were adapted to control feed for further 7 days and before the onset of the experiment, experimental fish fasted for one day.

Preparation of herbal diets

Two purified diets (control diet -0.0% and BLAL diet -0.1% of Kg-1 feed) were prepared by incorporating the calculated amount of BLAL (bamboo leaves alcoholic) extract in the diets (Table 1) as per treatment doses [11]. The ingredients required for the formulation of the feed as mentioned in Table 1. Before that, a toxicity study was conducted with BLAL extract in rohu fingerlings (average length of 10.3 ± 1.5 cm and weight 15.5 ± 1.5 g) and no mortalities were observed even at the higher dose of 20 mg kg BW−1.

Table 1: Formulation of experimental diets (100 g) for the experiment

Ingredients	Quantity (in g)	
	Control diet	**BLAL Diet (diet T2)**
1. Casein	39.0	39.0
2. Gelatin	8.0	8.0
3. Dextrin	31.0	31.0
4. CMC	2.0	2.0
5. Veg oil	3.0	3.0
6. Cod liver oil	3.0	3.0
7. Vit-min mixture*	6.0	6.0
8. BHT	0.2	0.2
9. Betain	0.2	0.2
10. Cellulose	7.7	7.6
11. Extract (BLAL)	0.0	0.1
Total	100	100
Proximate composition of diets		
Moisture	10.14±0.11	9.98±0.12
Crude Protein	35.85±0.1	36.38±0.1
Total Lipid	2.04±0.5	2.06±0.9
Ash	19.81±0.04	19.41±0.03
Carbohydrate	32.17±0.24	32.16±0.17

*****Composition of vitamin-mineral premix (quantity 2.5 kg-1): Vitamin A, 5500000 IU; Vitamin D3, 1100000 IU; Vitamin B2, 2000 mg; Vitamin E, 750 mg; Vitamin K, 1000 mg; Vitamin B6, 1000 mg; Vitamin B12, 6 mcg; Calcium Pantothenate, 2500 mg; Nicotinamide, 10 g; Choline Chloride, 150 g; Mn, 27,000 mg; I, 1000 mg; Fe, 7500 mg; Zn, 5000 mg; Cu, 2000 mg; Co, 450 mg; L-lysine, 10 g; DL- Methionine, 10 g; Selenium, 50 mg l-1.

Experimental design for *A. hydrophila* infection

For the present study, a pure culture of *A. hydrophila* was recultured first in nutrient agar and later in specific media of *Aeromonas i.e.* RS media (Rimler shott). Further, the bacteria were grown in nutrient broth for 24 h and then centrifuged at 3000 g for 10 min. The supernatant was discarded, and the pellet was resuspended in autoclaved PBS, pH 7.2 and OD (optical density) of the solution was adjusted to 0.885 at 456 nm which correspondents to 1x107 cells ml-1. During the challenge, study fish were peritoneally injected with 100 µl and kept under observation for about 7 days. The confirmation of the infection was accomplished after re-isolating the bacteria from the dead fish [15, 16].

From our earlier study [11] it was found that feeding with 0.1% BLAL for 7 days is sufficient to elicit the maximum immune response in rohu. Based on the findings, the present study was designed to evaluate the efficacy of BLAL in preventing systemic bacterial infection in fish. Two groups of acclimatized and apparently healthy rohu fish (average length 15.7±1.5 cm and weight 20.2±1.5 g) were kept in FRP tanks and fed with control and BLAL diet (diet T2 with - 0.1% of extract), respectively for 7 days. Two times feeding up to satiation level and 30% water exchange was done at regular intervals [11]. After 7 days of feeding, fish were exposed to bacterial infection in the aquariums as described in table 2. Three treatments with two replicates were used for the study in 6 aquariums (2'x1'x1'). Total of 60 numbers of fish were used for the study.

Table 2: Different treatment groups for the challenge study

S. No.	Treatments
1.	Positive control - Fish fed with control diet for 7 days and exposed to *A. hydrophila* infection
2.	Treatment - Fish fed with BLAL diet for 7 days and exposed to *A. hydrophila* infection
3.	Negative control - Fish fed with control diet for 7 days and not exposed to *A. hydrophila*

Cumulative mortality (%): (total number of fish died / total number of fish infected) / 100

Statistical Analysis

SPSS (SPSS Inc., Chicago IL, USA) was used for the statistical analysis of data. Results were represented as a mean ± standard error. Comparative analysis of mean values was determined using one-way ANOVA and Duncan's test. Probability levels of 0.05 were used to find out the significance in various cases.

Result of Challenge study

Cumulative mortality of diet T2 fed *L. rohita* fingerling group (for the seven days) were significantly (p<0.05) lower than the positive control group (Fig. 1).

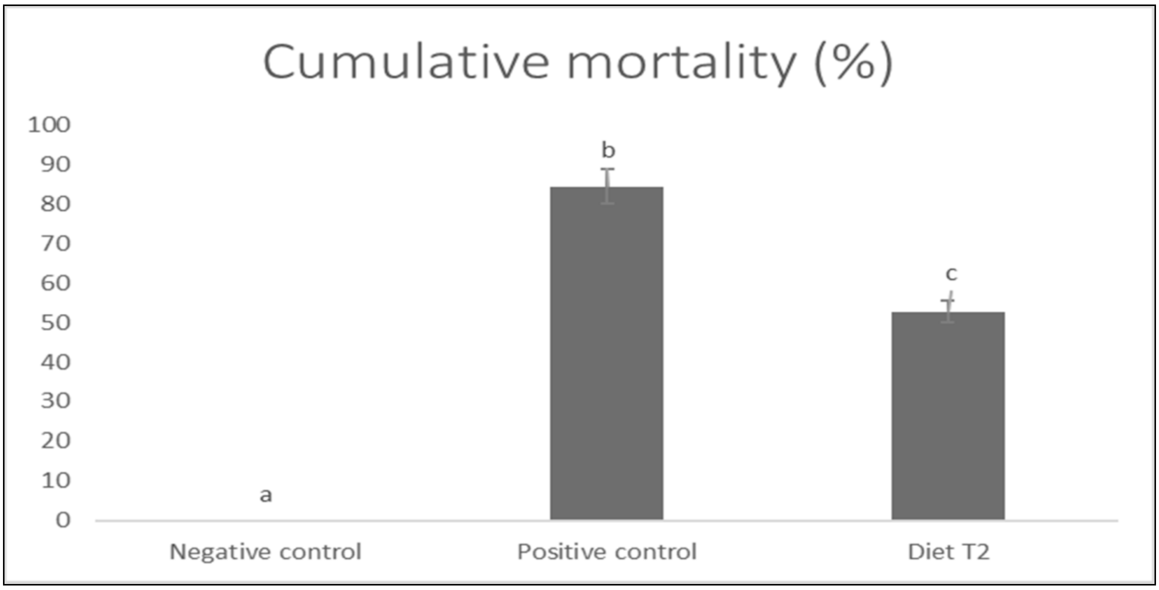

Fig 1: Cumulative mortality of *L. rohita* fingerlings challenged with *A. hydrohila* after 7 days of feeding with BLAL extract based diet (diet T2). Data (mean ± SE) with different letters (a, b and c) significantly differ (p<0.05) among different experiment groups (Negative control, positive control and diet T2 fed fishes).

Discussion

In the present study, cumulative mortality in the BLAL extract fed group at 0.1% was significantly (p<0.05) lower than positive control. The possible cause for the survival of fish under *A. hydrophila* infection may be due to the increase in non-specific immune responses in fish after feeding with BLAL extract incorporated diet [11]. These results corroborate with the finding of Christybapita *et al.* [17] and Logambal *et al.* [18] in *O. mossambicus*, treated with *Eclipta alba* leaf extract and *Ocimum sanctum*, respectively.

Moreover, a similar type of findings was also obtained by an intraperitoneal administration of *Solanum trilobatum* Leaf extracts in Tilapia [19]. Studies by Logambal *et al.* [18] and Das *et al.* [14] revealed that leaf extract of *O. sanctum* is effective against *A. hydrophila*. Sahu *et al.* [20] also reported the immunostimulatory and diseases resistance effect of mango kernel in rohu against *A. hydrophila* infection. Similarly, extracts from *Euphorbia hirta*, *Aegle marmelos* [21] and *Lawsonia inermis* [22] have been utilised for enhancing the resistance of *Cyprinus carpio* against *A. hydrophila* infection.

The rise in the non-specific immunity and capability to survive against the stressed condition in the fish may be the immuno-boosting effect of bioactive compounds (lectins, alkaloids, polysaccharides, phenolic compounds. glycosides, flavonoids, tannins, anthocyanins, terpenoids, sterols, saponin *etc*.) present in the bamboo extract [23]. These phytochemicals also allow the fish to counter the environmental stressful situation like low pH, oxidative stress [11] *etc.* which eventually lead to an immunoprophylactic potential against opportunistic pathogens.

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References
1. FAO. Food and Agriculture Organization. The State of World Fisheries and Aquaculture, FAO, Rome, 2010.
2. FAO. Food and Agriculture Organization. The State of World Fisheries and Aquaculture, FAO, Rome, 2014.
3. El-Sayed A-FM. Tilapia Culture. Wallingford, Oxon.: CABI Publishing. 2006, 294.
4. Vivas JB, Carracedo J, Riano BE, Razquin P, Lopez-Fierro. Behavior of an *Aeromonas hydrophila aroA* live vaccine in water microcosms. Applied Environ. Microbiol. 2004; 70(249):2702-2708.
5. Cabello FC. Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. Environ Microbiol. 2006; 8:1137-1144.
6. Kelly AR. Medicated Feed for Food Fish. United States Department of Agriculture: Southern Regional Aquaculture Centre. 2013, 6.
7. Arts IC, Hollman PC. Polyphenols and disease risk in epidemiologic studies. The American Journal of Clinical Nutrition. 2005; 81:317-325.
8. Madhuri S, Mandloi AK, Govind P, Sahni YP. Antimicrobial activity of some medicinal plants against fish pathogens. Int. Res. J. Pharm. 2012; 3:28-30.
9. Nirmala C, Sharma ML, David E. A comparative study of nutrient components of freshly emerged, fermented and canned bamboo shoots of *Dendrocalamus giganteus* Munro. J Am. Bamboo. Soc. 2008; 2:33-39.
10. Sarmah AS, Thomas M, Goswami K, Haridashan, Borthakur SK. “Rattan and bamboo fora of North-East India in a conservation perspective,” in Sustainable Management of Forests, A. Arunachalan and M. L. Khan, Eds., International Book Distributors, Dehradun, India, 2010, 37-45.
11. Khan MIR, Saha RK, Saha H. Muli bamboo (*Melocanna baccifera*) leaves ethanolic extract a non-toxic phyto-prophylactic against low pH stress and saprolegniasis in *Labeo rohita* fingerlings. Fish and Shellfish Immunology, 2018, 10.1016/j.fsi.2017.11.047.
12. Cheung LM, Cheung PCK, Ooi VEC. Antioxidant activity and total polyphenolics of edible mushroom Exract, Food Chem. 2003, 81:249-255.
13. Singh HB, Singh RS, Sandhu JS. Herbal medicines of Manipur. Daya Publishing House, 241 Delhi, 2003; 6, 11, 16, 25, 31, 36.
14. Das R, Raman RK, Saha S, Singh R. Effect of *Ocimum sanctum* Linn. (Tulsi) extract on the immunity and survival of *Labeo rohita* (Hamilton) infected with *Aeromonas hydrophila* Aquaculture Research. 2015; 46:1111-1121.
15. Das BK, Pradhan J. Effects of Supplementation Diet Containing Microcystis Aeruginosa on Haematological and Biochemical Changes in Labeo Rohita Infected with Aeromonas Hydrophila. J Aquac Res Development. 2015; 6:315. doi:10.4172/2155-9546.1000315
16. Anusha M. Effect of water born iron on pathophysiological responses of *Cirrhinus mrigala* fingerlings. M.F.Sc. Thesis submitted to Central Agricultural University, Imphal, 2015,
17. Christybapita D, Divyagnaneswari M, Michael RD. Oral administration of *Eclipta alba* leaf aqueous extract enhances the non-specific immune responses and disease resistance of *Oreochromis mossambicus*. Fish Shellfish Immunol. 2007; 23:840-852.
18. Logambal SM, Venkatalakshmi S, Michael RD. Immunostimulatory effect of leaf extract of *Ocimum sanctum* Linn. in *Oreochromis mossambicus* (Peters). Hydrobiologia. 2000; 224 430:113-120.
19. Divyagnaneswari M, Christybapita D, Dinakaran RM. Immunomodulatory activity of *Solanum trilobatum* leaf extracts in *Oreochromis niloticus*, In: M.G. Bondad-Reantaso, C.V. Mohan, M. Crumlish and R.P. Subasinghe (eds.). Diseases in Asian Aquaculture VI. Fish Health Section, Asian Fisheries Society, Manila, 2008, 221-234.
20. Sahu S, Das BK, Pradhan J, Mohnpatra BC, Mishra BK, Sarangi N. Effect of *Magnifera indica* kernel as a feed additive on immunity and resistance to *Aeromonas hydrophila* in *Labeo rohita* fingerlings. Fish and Shellfish Immunology. 2007; 23(1):109-118.
21. Pratheepa V, Ramesh S, Sukumara N. Immunomodulatory effect of *Aegle marmelos* leaf extract on freshwater fish *Cyprinus carpio* infected by bacterial pathogen *Aeromonas hydrophila.* Pharmaceutical Biology. 2010; 48(11):1224-1239.
22. Fereidouni MS, Soltanian S. Effect of Henna (*Lawsonia inermis*) extract on the immunity and survival of common carp, *Cyprinus carpio* infected with *Aeromonas hydrophila.* Int. 244 Aquat. Res. 2016; 8:247-261.
23. Venkatalakshmi P, Vadivel V, Brindha P. Role of phytochemicals as immunomodulatory agents: A review. International Journal of Green Pharmacy. 2016; 10(1):1.