



## Isolation of bacterial fish pathogen *Aeromonas hydrophila* and therapeutic effects of medicinal plants on its invasion

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### Abstract

*Aeromonas hydrophila*, a bacterial pathogen, was isolated from *Pangasius hypophthalmus*. For pathogenicity test, different doses were injected intramuscularly in *Barbonymus gonionotus*. Crude extracts were prepared from various parts *Azadirachta indica*, *Curcuma longa*, *C. zedoaria*, and *Callotropis gigentia* and applied to *B. gonionotus* for 7 days. Bath treatment was done up to their tolerance level and well ventilation was confirmed for aeration and 50% water was exchanged daily. Minimum inhibitory dose was detected as 7 mg/ml. High inhibitory effect was observed in case of *A. indica* and mixed extract of *A. indica* and *C. gigentia*. Both *A. indica* and *C. gigentia* showed the best result with 90-95% recovery of infected fish at a dose of 7 mg/l. *C. zedoaria* showed moderate to weak effect with 50-60% recovery at the same dose. The present study showed that medicinal plants would be an effective control measure against *A. hydrophila*.

**Keywords:** Fish pathogen, fish disease, therapeutic effect, medicinal plant, herbal plant, *Aeromonas hydrophila*

### INTRODUCTION

Bacterial fish diseases especially bacterial hemorrhagic septicemia and motile aeromonas septicemia in freshwater fish cause great losses (Roberts 1989, Lio Po *et al.* 1992). Motile aeromonas septicemia is probably the most common bacterial disease of freshwater fish. This disease has been associated with several members of the genus *Aeromonas*, including *A. hydrophila*, *A. sobria*, *A. schuberti* and *A. veronii* (Suthi 1991). Chowdhury *et al.* (2003) studied ulcer type of disease in the small-scale rural farmer's pond and recovered a number of susceptible bacterial pathogens from the lesions and kidney of the ulcer affected fishes during mostly January and February. Among them, *A. hydrophila* was detected as more pathogenic than others. In Bangladesh, *Aeromonas* are frequently observed in farmed fishes as well as in the water (Iqbal *et al.* 1996, Dipu 2012). *Aeromonas hydrophila* was isolated from the suspected EUS affected *Heteropneustes fossilis* (Rashid *et al.* 2008).

Among all other bacteria, *Aeromonas* is the major bacterial fish pathogens which are widely distributed in aquatic organisms in nature (Chowdhury 1988). In coastal regions, fish have also been suffered from *Aeromonas* (Rahman 2005). Involvement of bacteria is very important in producing diseases in the farmed fishes in Bangladesh (Chowdhury 1998). There is strong evidence that many EUS affected fish die as a result of septicemia caused by opportunistic bacterial pathogen, *Aeromonas* sp., notable *A. hydrophila* (Khan *et al.* 2011).

There were problems in preserving virulent bacterial isolates for long time. For this reason new virulent bacterial isolates was collected. Bacterial isolates was recovered from pangus cultured in Valuka fish farm project. Medicinal plants have been used for the treatment of infectious diseases. Medicinal plants as the alternative agents are effective to treat the infectious diseases. Some of local herbs and desert plants were reported to inhibit the pathogenic bacteria. For

traditional control measures using synthetic drugs are often harmful to ecosystem, fish and consumer.

To avoid harmful effects of diseases, medicinal plants could be an alternative measures to control. There are many medicinal plants are available in Bangladesh such as margosa tree or neem (*Azadirachta indica*), Indian barberry or turmeric (*Curcuma longa*), zedoary root or shoti (*C. zedoaria*), bowstring hemp or akand (*Callotropis gigantea*). All of these medicinal plants have high medicinal value for various diseases control. Proper use of these medicinal plants could bring a lot of success in controlling fish diseases which is also suitable from ecological and economical perspective. The present work will contribute a lot to develop treatment techniques with low cost therapy using medicinal plants, available locally.

Thus the present study was undertaken to experimentally infected pangus fish, *Pangasius hypophthalmus*, with *A. hydrophila* and its control trial with a view to attain the collection and identification of virulent bacterial isolate, challenge test for pathogenicity evidence, artificial infection and therapeutic measures with medicinal plants and To examine therapeutic effect of medicinal plants on the experimental infection of fish under laboratory condition.

## METHODOLOGY

For isolation and experimental infection laboratory stock pathogens were used but due to their very low virulent characters, fresh isolates were collected from diseased wild and farmed *P. hypophthalmus* from “Valuka Fish Farm Project” for using in the study (Figure 1). Methods of bacterial isolation, their characterization and pathogenicity test were followed according to the method described by Barrow and Feltham (1993) and Chowdhury and Muniruzzaman (2002).

At first glassware (Petri dishes, test tubes, L-sticks, mortar, conical flasks, vials, measuring cylinder etc.) were washed, dried and sterilized at 170°C for 1 hour by a dry sterilizer. The plastic materials were autoclaved at 121°C for 15 minutes.

0.85 g NaCl was poured into distilled water to make the volume 100 ml. This was called physiological saline (PS=0.85% NaCl). The preparation was mixed nicely by vortex mixer. All the PS were autoclaved at 121°C for 15 min and kept at 4°C for future use.

Body cavities of the collected fish were opened by the help of sterilized scissors. After that sterile inoculating loop were used and samples collected. Sample were culture in TSA petridishes by sterile inoculating loops.

Plate dilution technique was applied. TSA plates incubated at 25°C for 48 hours for colony appearances.



Figure 1: Sampling site, “Valuka Fish Farm Project” [Source: Banglapedia (2014)]

After growing bacteria in an agar plate, carefully a single colony was taken by sterile inoculating loop and cultured in a agar plate and incubated at 25°C for 48 hours for colony appearances. *Aeromonas* bacteria was identified based on morphological, physiological and biochemical characters of the isolates.

For bacterial suspension preparation 10 mg bacterial sample was added with 1 ml sterile PS in a sterile test-tube and mixed nicely with rotator mixer. Bacterial suspension was prepared when the fish conditioning was confirmed and the fish were ready for injection.

Apparently healthy Java barb (*Barbonymus gonionotus*), weighing 8-10 g, were collected. There were acclimatized in aquarium with aeration for two days and checked for any disease before exposing them to pathogenicity test. Three healthy fish against each bacterial pathogen were injected intramuscularly with 0.1 ml of bacterial suspension at the base of dorsal fin. Control fishes (three fish) received only sterile 0.85% PS and kept in an another aquarium.

For experimental infection, three types of bacterial samples were used. Different doses like 10-1, 10-2, 10-3 and 10-4 were applied. All fishes died due injection with certain sign and symptoms. Re-isolation was done from dead fish.

Medicinal plants were collected from the adjacent area of Bangladesh Agricultural University, Mymensingh. They

were brought to the laboratory immediately after collection and preserved for using in the preparation of herbal extracts.

The desired parts of plants were cut into small pieces which grinded by hammering. The large particulate from the extracts were screened out by passing through fine meshed cloth. The crude extracts were then preserved in the refrigerator at low temperature for future use in the efficacy test.

Collected medicinal plants were applied against the bacterial fish pathogen, the effective plants were investigated to determine their minimum inhibitory dose (MID) that can inhibit the infectivity of bacterial pathogen. Two different doses, 8 mg/ml and 7 mg/ml of the plant extracts were applied.

## RESULTS

**Bacterial isolates and pathogenicity:** The identified bacterial isolates were investigated to detect their infectivity response to the experimentally challenged fish. Out of the three recovered bacterial isolates one isolate was found to be pathogenic (Table 1).

**Table 1:** Determination of pathogenicity of the three recovered bacterial isolates, *Aeromonas hydrophila*

Laboratory code	Pathological changes (%)	Infectivity to fish
PK <sub>2</sub> VS <sub>1</sub>	80-90	+++
PFAF <sub>1</sub>	40-50	+
PFAF <sub>2</sub>	0	-

+++ , high pathogenicity; ++ , medium pathogenicity; + , low pathogenicity; - , no pathogenicity

**Medicinal plants against fish pathogenic bacteria:** Out of the total number of effective herbal plants, Neem and Akand were detected as highly effective against the growth of the bacterial culture. In the case of *A. hydrophila*, Turmeric, Shoti were found to be medium effective. On the other hand, combination of Akand and Neem were found to strongly inhibit the infectivity of *A. hydrophila* bacteria (Table 2).

**Table 2:** Efficacy test for native medicinal plants on pathogenic bacterial growth

Plants name	Growth inhibitory
<i>Azadirachta indica</i>	+++
<i>Callotropis gigentia</i>	++
<i>Curcuma longa</i>	++
<i>Curcuma zedoaria</i>	+
<i>A. indica</i> and <i>C. gigentia</i>	+++

+++ , high; ++ , medium; + , low; - , not effective against pathogen

**Determination of minimum inhibitory dose (MID):** MID of *A. indica* was determined as 7 mg/ml against *A. hydrophila*. *C. gigentia* was found to performed strong inhibitory response at 8 mg/ml. Combination of *A. indica* and *C. gigentia* were found to be strongly inhibit the growth of bacteria pathogen at the MID of 7 mg/ml. *C. longa* showed medium inhibitory response at MID of 6 mg/ml on the bacterial isolates. *Curcuma zedoaria* also showed lower inhibitory response at MID of 7 mg/ml on the bacterial isolates (Table 3).

**Table 3:** Determination of minimum inhibitory dose against bacterial pathogen

Name of plant	Dose (mg/ml)	Inhibitory effect after exposure		
		1 <sup>st</sup> day	3 <sup>rd</sup> day	7 <sup>th</sup> day
<i>A. indica</i>	7	++	++	+++
	8	+	++	+++
<i>C. gigentia</i>	7	+	++	++
	8	+	++	+++
<i>C. longa</i>	6	+	++	++
	7	+	++	+++
<i>C. zedoaria</i>	6	+	+	++
	7	+	++	++
<i>A. indica</i> and <i>C. gigentia</i>	6	+	+	+++
	7	++	++	+++

+++ , high; ++ , medium; + , low; - , not effective

**Therapeutic effect of medicinal plants:** Among the four treatments the highest recovery was obtained in *A. indica*. It recovered up to 90% of the experimentally infected fish with in the 7 days of experimental period. The lowest recovery was found in *C. zedoaria* which recovers up to 60% of the infected fish. Combined treatment of *A. indica* and *C. gigentia* recovered up to 95% of the experimentally infected fish. *C. longa*, recovered up to 70 % of pathological changes (Table 4).

**Table 4:** Therapeutic effects of medicinal plants on *Barbonymus gonionotus*

Medicinal plants	Dose (mg/ml)	Recovery of fish (%)
<i>A. indica</i>	7	90
	8	80
<i>C. gigentia</i>	7	80
	8	75
<i>C. longa</i>	6	50
	7	60
<i>C. zedoaria</i>	6	70
	7	80
<i>A. indica</i> and <i>C. gigentia</i>	7	95
	8	90
Control	No doses	0

## DISCUSSIONS

The present study was designed to recover fresh isolate of *Aeromonas* pathogen and to examine the therapeutic effect of selected medicinal plants against common bacterial fish pathogen responsible for causing disease in cultured fishes. A total of three new bacterial isolates were recovered from naturally infected fish. Among them one isolate was detected as pathogenic, the rest two were non pathogenic. Selected new isolates showed high infectivity causing lesions on the body of the experimental fish. Basic results of this study regarding medicinal plants correspond with the works done by Muniruzzaman (2004) and Rahman (2005). The present study revealed that some medicinal plants have an important role to inhibit the growth of bacteria producing disease in fish. But the efficacy of the collected medicinal plants were found to be varied from species to species. All the medicinal plants in present study were found effective against the bacterial pathogen. Mixture of the *A. indica* and *C. gigentia* showed the highest effect against the bacterial pathogens tested. Individually *C. gigentia* and *C. longa* showed medium inhibitory response. Traditionally *C. gigentia* is used alone or with other medicinal plants to treat common diseases such as fevers, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting, diarrhoea (Caius 1986). However, bacterial diseases by *A. hydrophila* could be reduced by the application of the medicinal plants extracts, used in present study.

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