

## Research Article

### **A study on the control of *Aeromonas hydrophila* infection in the cat fish by medicinal plants.**

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**Abstract:** *Aeromonas hydrophila* was obtained from various sources and identified on the basis of growth on selective media, phenotypic and biochemical characters. The isolates (Ah1-Ah9) were subjected to antibiotic susceptibility and resistance pattern tests. The isolates showing maximum resistance to most of the commercial antibiotic discs used were selected. The selected isolate (Ah7) was further subjected to check its susceptibility towards six medicinal plants (*Mentha piperita*, *Azadirachta indica*, *Andrographis paniculata*, *Phyllanthus niruri*, *Solanum nigrum*, *Ruta chalepensis*) by using their solvent extracts through agar gel diffusion method. Two plant extracts ( *Andrographis paniculata* and *Azadirachta indica* ) were prominently showing maximum zone of inhibition (29mm and 27mm). This isolate (Ah7) was used to infect fresh water channel cat fishes. A significant association was found in control of *Aeromonas hydrophila* infection in the fish treated with medicinal herbs. While in untreated fish, it resulted in death with severe infected lesions.

**Keywords:** *Aeromonas hydrophila*, antibiotics, channel cat fish (*Ictalurus punctatus*), medicinal herbs, solvent extraction.

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#### **INTRODUCTION**

*Aeromonas* of the *Hydrophila* group occur ubiquitously in aquatic environment, fresh food including fish meat, poultry, raw milk and salad vegetables. *Aeromonas hydrophila* are gram negative, rod shaped, facultative intracellular bacteria with aquatic distribution and is the causative agent of ulcerative disease syndrome (UDS) in fish [12]. They are often referred to as complex disease organisms that are associated with bacterial hemorrhagic septicemias and other ulcerative condition or red sore disease in fishes.

Motile *Aeromonas* primarily cause disease in cultured warm water fishes: carp (*Cyprinus carpio*), channel cat fish (*Ictalurus punctatus*), striped bass (*Morone saxatilis*), an large mouth bass (*Micropterus salmoides*). The pathogens may also affect a variety of cool water species, but is not necessarily restricted to fresh water environment [2].

*Aeromonas hydrophila* is recognized as a scourge of freshwater fish farming world wide and is considered to be a major problem, in being the causative agent of haemorrhagic septicemia and Epizootic ulcerative syndrome (EUS) [11]. Pathologic conditions attributed to members of the *Aeromonas*

complex may include dermal ulceration, tail or fin rot, ocular ulcerations, erythrodermatitis, hemorrhagic septicemia, red sore disease, red rot disease, and scale protrusion disease. In the acute form of disease, a fatal septicemia may occur so rapidly that fish die before they have time to develop a few gross signs of the disease. When clinical signs of infection are present, affected fish may show exophthalmia, reddening of the skin, and an accumulation of fluid in the scale pockets [8].

*Aeromonas hydrophila* is the causative agent of the disease known as “haemorrhagic septicaemia”, “ulcer disease” or “red-sore disease” [10, 25]. Catfish with septicemic or latent infections had enlarged nuclei in the bronchial epithelium and that there was a significant correlation between the presence of these gill lesions and the severity of hepatic and pancreatic lesions and increased bacterial acetyl-cholinesterase activity in the brain tissue of moribund fish [24]. *Aeromonas hydrophila* has received particular attention because of its association with human diseases.

The prevention and treatment of both human and fish diseases by the extensive use of antimicrobial agents have undoubtedly contributed to an increase in the frequency of resistant strains [22]. Additionally,

since antimicrobial agents are released into the surrounding water during treatment of bacterial fish diseases, there is a direct negative impact on the aquaculture environment [3, 26]. The use of antibiotics and other chemotherapeutics for controlling disease has been criticized for their negative impacts. Application of medicinal herbs in diseases management is gaining success, because herbal treatment is cost effective, eco-friendly and has minimal side effects. Traditional herbal medicines seem to have the potential immune-stimulation. The use of medicinal plant is an alternative to antibiotics in fish health management [5]. These herbs are not only safe for consumers but also widely available throughout Asia and they also have a significant role in aquaculture [6]. Many studies have been proved that herbal additives enhanced the growth of fishes and also protected from the diseases. And also plant medicine have minimal side effects, are easily biodegradable, inexpensive, and locally available, and extracts are easily prepared. Medicinal herbs have played a major role in the development of modern medicine and continue to be widely used in their original form [17].

*Aeromonas hydrophilia* outbreaks has a major impact in aquaculture and at present, no vaccine is commercially available to prevent such incident [29]. Under these circumstances, studies on the prevention and treatment of diseases in fishes using medicinal herbs have assumed significance especially with the advent of multidrug resistant strains. Enormous economic loss is created because of *Aeromonas* infection in fishes. In the present study an attempt has been made to understand the curative potential of medicinal plant extracts on the fishes infected by *Aeromonas hydrophilia*.

## MATERIALS AND METHODS

### Sampling and microbiological examination

The samples from soil, cow dung, prawn, well water, slaughter house waste, fruits washed water, mucous membrane of fish, fish intestine, meat washed water were collected for the source of *Aeromonas hydrophilia* and designated as Ah1-Ah9 respectively. These samples were plated on Muller Hinton Agar and incubated at 37 °C under aerobic condition. After incubation the honey yellow colored colonies were isolated and further pure cultured on Phenol Red Ampicillin Starch Agar and were identified as *Aeromonas hydrophilia*. On the basis of colony morphology, gram staining, motility and biochemical characteristics.

### Antibiotic susceptibility tests

The susceptibility of the isolates to the following antibiotics Ampicillin (30µg), Chloramphenicol (30µg), Gentamycin(30µg) Kanamycin(30µg), Tetracycline (30µg), Streptomycin (30µg), Ofloxacin (30µg), Amoxicillin(30µg),

Erythromycin (30µg), Norfloxin (30µg) were performed using disc diffusion method. The isolate showing maximum resistance towards the number of antibiotics used, was selected and opted for further study.

### Solvent extract preparation

Leaves of *Mentha piperita*, *Ruta chalepensis* and *Azadirachta indica*, *Andrographis paniculata*, *Phyllanthus niruri*, *Solanum nigrum*, were collected from Nilgiris district and Salem district respectively. The leaves were washed thoroughly in tap water, and dried at room temperature for 15 days separately. The dried plant materials were powdered. The powder (1gm/10ml) was soaked in different solvents like ethyl acetate, ethanol, chloroform and petroleum ether for 3 days in a shaker at room temperature separately. Then the extracts were filtered through cheese cloth and the extracts were used for antimicrobial activity test.

### Culture preparation for the test

The isolate showing resistance towards most of the antibiotic discs was selected as previously mentioned and inoculated into peptone broth (peptone-0.4gm, pH-7.2) and incubated at 37°C for 24 hours in individual tubes. The bacterial cells were harvested by centrifuging at 5000g for 15mins. The bacterial pellet was washed thrice with PBS (Phosphate Buffered Saline) and the cells were counted by haemocytometer. The bacterial cells were diluted to approximately 10<sup>5</sup>CFU/ml before use [21].

### Assessment of antimicrobial activity

The agar well diffusion method [4] was used for screening the antimicrobial activity of *Mentha piperita*, *Ruta chalepensis* *Azadirachta indica*, *Andrographis paniculata*, *Phyllanthus niruri* and *Solanum nigrum*, against *Aeromonas hydrophila* isolate. Muller Hinton agar plates were inoculated with the organisms, by evenly spreading 0.1ml of the bacterial inoculum on the media with the help of a sterile 'L' glass rod. The inoculated plates were allowed to dry in the incubator at 37°C for 10 minutes, then four wells (6mm in diameter) were punched in, using a sterile cork borer and the wells were filled with the plant extract (50µL). Control wells are loaded with the solvents without plant extracts and the standard drug (Chloramphenicol) for comparison and assessment, which were run parallel in separate plates. The plates were incubated at 37°C for 24hours. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition for the extracts and then comparing it with the standard drug and control. The plants extract showing maximum zone of inhibition towards *Aeromonas hydrophila* (Ah7) were selected as superior for the treatment of infected fish.

### Field trial using fish

The fresh water fishes, Cat fish (*Ictalurus punctatus*) were procured from fish market (Erode) and it was grown in fish tank for one week to accustom to the normal environmental conditions as well as healthy fish feed was given. In this experimental three (F1, F2, F3), fresh water fishes (*Ictalurus punctatus*) were used for infection. The infection was made by creating a wound which was done by gently abrading the skin on the trunk region with the help of sterile blade.

And the three fishes were left separately in individual tanks. The two fishes (F1 and F2) were infected with the organism but treated with powdered leaves of plants through fodder following infection, which induced maximum zone of susceptibility in the organism *Aeromonas hydrophila* (Ah7) used in the test to study their antimicrobial activity. This included two plants (*Andrographis paniculata* and *Azadirachta indica*), 0.5mg of the plant powder was mixed with the feed. This feed mixed with powdered leaf was fed twice a day following the second day of infection for curing of the disease. The fish designated F3 was used as control by infecting with the *Aeromonas hydrophila* (Ah7) culture but without being subjected to treatment following infection and were left for observation.

## RESULT AND DISCUSSION

### Microbial examination and biochemical characteristics

On PRSA (Phenol Red Starch Ampicillin Agar), pink colored colonies indicate the growth of *Aeromonas hydrophila* on its selective media. A microscopic examination of gram staining and motility indicate gram negative bacilli most of the isolates actively motile, excepting a few. The biochemical characteristics of *Aeromonas hydrophila* isolates are given in Table-1.

### Antibiotic sensitivity tests

*Aeromonas hydrophila* isolates showed varied sensitivity, resistance patterns against the ten antibiotic discs used. Among the nine isolates studied, Ah7 showed the maximum resistance towards seven antibiotic out of the ten used. And this isolate (Ah7) was used as a source to infect the cat fishes for study (Table-2).

### Assessments of solvent extract activity

Out of the solvent extracts of six medicinal plants used to test their antibacterial activity against Ah7, the ethanol extract of two plants (*Azadirachta indica* and *Andrographis paniculata*) induced the maximum sensitivity with a zone diameter of 27mm and 29mm respectively (Table-3) and Graph-1.

**Table-1: Biochemical characteristics of *Aeromonas hydrophila* isolates (Ah1- Ah9) isolates.**

BIOCHEMICAL CHARACTERISTICS	REACTIONS
<b>Carbohydrate fermentation</b>	
Lactose	+
Glucose	+
Mannitol	+
<b>Hydrolysis test</b>	
Starch hydrolysis	+
Cesain hydrolysis	+
Gelatin hydrolysis	+
Esculin hydrolysis	+
Oxidase and Catalase test	+
Gas from glucose	+
Hemolysis	+
Ampicillin susceptibility	S
<b>IMVIC test</b>	
Indole	+
Methyl red	+
Voges prosekaur	+
Citrate utilization	+

+ = positive. - = negative, S = sensitive

Table-2 Antibiotic sensitivity tests

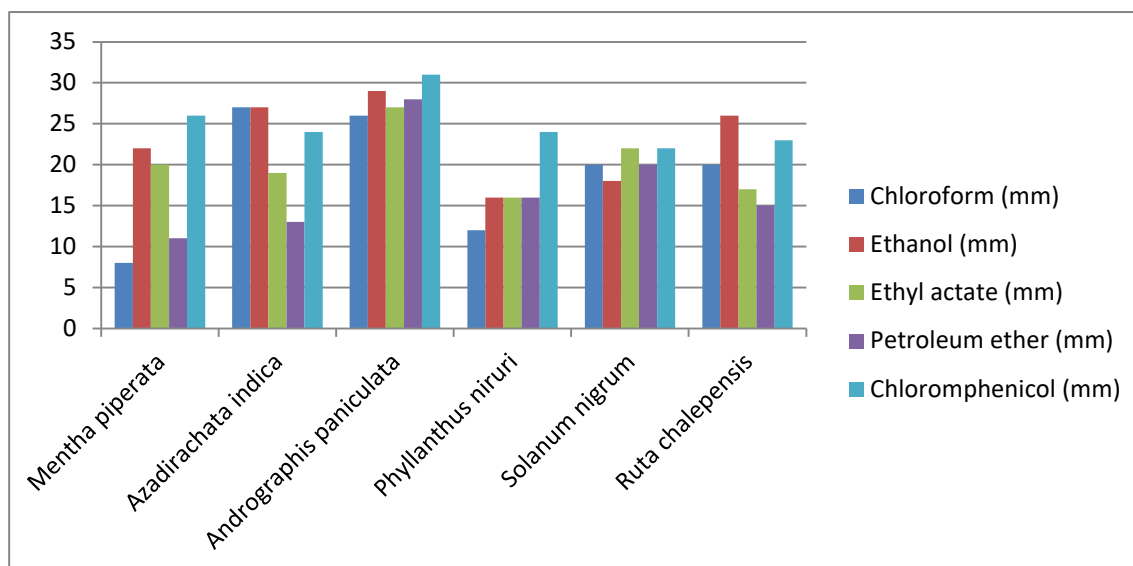
Antibiotics (30µg Himedia)	Ah1	Ah2	Ah3	Ah4	Ah5	Ah6	Ah7	Ah8	Ah9
Streptomycin	S	S	I	S	S	S	R	R	I
Penicillin	R	R	I	R	I	I	R	R	R
Ampicillin	I	S	R	R	S	R	R	S	R
Amoxicillin	R	R	R	R	I	R	R	R	R
Ofloxacin	S	S	S	S	S	S	S	I	I
Chloromphenicol	S	I	S	S	S	I	S	S	S
Tetracycline	R	S	R	R	S	I	R	R	I
kanamycin	S	I	I	I	I	I	R	R	I
Erythromycin	I	I	I	I	I	I	R	I	I
Norfloxin	I	S	S	I	I	S	S	S	I

S= Sensitive ( $\leq 14$ mm), R= Resistance ( $\geq 16$ mm), I= Intermediate (14-16mm).

Table-3 Antimicrobial activity of medicinal plants against *Aeromonas hydrophila* (Ah7) isolates

Plant used	Chloroform		Ethanol		Ethyl Acetate		Petroleum Ether		Chloromphenicol C
	C	T	C	T	C	T	C	T	
<i>Mentha piperata</i>	0	8	0	22	0	20	0	11	26
<i>Azadirachta indica</i>	0	27	0	27	0	19	0	13	24
<i>Andrographis paniculata</i>	0	26	0	29	0	27	0	28	31
<i>Phyllanthus niruri</i>	0	12	0	16	0	16	0	16	24
<i>Solanum nigrum</i>	0	20	0	18	0	22	0	20	22
<i>Ruta chalepensis</i>	0	20	0	26	0	17	0	15	23

C= Control (mm), T= Test (mm)



Graph-1: Antimicrobial activity of medicinal plants against *Aeromonas hydrophila* (Ah7) isolates

### Results of field trials

The wound infection began as a dermal lesion with hemorrhage and inflammation. This skin ulcer was surrounded by a bright rim of red tissue. All the three fishes (F1, F2, F3) were subjected to infection. F1 and

F2 infected fishes were treated with the medicinal plants following the 2<sup>nd</sup> day of infection. There was gradual reduction in the rate of infection. Out of two fishes were recovered from the lesions within four days of application of the medicinal plants in feed and water

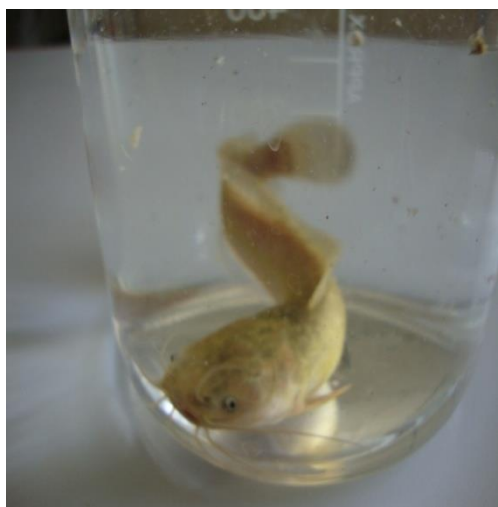
and were healthy. The untreated fish (F3) was infected severely; the lesion spread through out the trunk with total reddening and the finally caused death of the fish (Figure1 and 2).



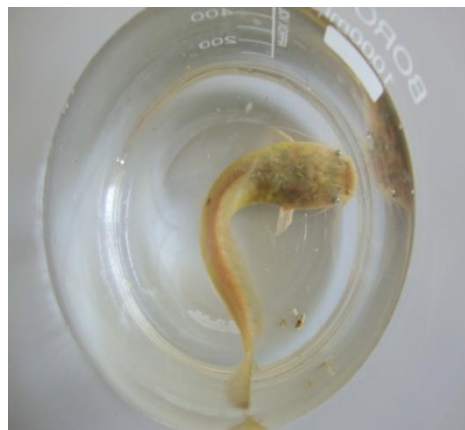
**Fig-1 (a): Cat fish (F1) after infection reddish injury visible**



**Fig-1 (b): Cat fish (F2) after infection reddish injury visible**



**Fig-1(c): Cat fish (F3) as a control**



**Fig-2(a): Cat fish F1 after treatment**



**Fig-2(b): Cat fish F2 after treatment**



**Fig-2 (c) :Cat fish F3 – untreated resulted in death of the fish**

As it was mentioned earlier in the introduction, that *Aeromonas* are the bacteria associated with bacterial hemorrhagic septicemia and ulcerative condition or red sore disease in fish. And the *Aeromonas hydrophila* and other motile *Aeromonas* are among the most common bacteria in fresh habitats throughout the world, and these bacteria frequently cause disease among cultured and fresh fishes. [20]. As reported by [14, 9] the *Aeromonas* is also found in fresh

water environment and food samples where they survive and multiply in low temperature.

When clinical signs of infection are present, affected fish show exophthalmia, reddening of the skin, and an accumulation of fluid in the scale pockets, death of these fish occur due to fatal septicemia [8]. Similarly during infection the abdomen of fish become distended as a result of an edema and scales may bristle out from the skin to give a “washboard” appearance. The gills with hemorrhage and ulcers may develop on the dermis [19]. And the cat fish with septicaemic or latent infections had enlarged nuclei in the bronchial epithelium and a significant correlation between the presence of gills lesions and the severity of hepatic and pancreatic lesions were identified. These results were identical with the results obtained by [24].

In this study medicinal plant as an important therapeutic aid for various ailments and the application of medicinal herb in disease management especially as an alternative to antibiotic in fish health was experimented. Out of the six , two medicinal plants were chosen and used as ailment for treatment of fish infected with *Aeromonas hydrophila* the organism isolated from various sources.

The *Aeromonas* are also found in prawn and cultured fish, the prawn isolates were resistant to Bacitracin the prawns were also used for the isolation of *Aeromonas hydrophila* [15, 27]. Systemic infections were characterized by diffuse necrosis in several internal organs and the presence of melanin-containing macrophages in the blood [28], and internally the liver and kidneys are target organs of an acute septicemia. Followed by the infection, the treatments with the selected medicinal plants were given and it was observed that the lesion and inflammation gradually subsided. Recently, research has been initiated to evaluate the feasibility of using herbal medicines in fish diseases management. [1]. In India, 500 medicinal plant spices are used to treat diseases in humans. Plants have been used as traditional medicine since time immemorial to control bacterial, fungal and viral diseases [6]. Many infectious microorganisms are resistant to synthetic drugs, hence an alternative therapy is very much needed, Phytomedicines usually have multiple effects, their actions often going beyond the symptomatic treatment of diseases [18]. Chemotherapeutic agents, which are isolated from plants, have been proven effective against drug resistant bacteria [23]. Compound such as volatile oils, phenolic compound including tannins, saponins, alkaloids, polysaccharides and polypeptides were shown to be effective alternatives to antibiotics [6], hence herbal extract is used for the antagonistic effects against grouped pathogens.

Use of medicinal plant is an alternative to antibiotics in fish health management [6] said that they have significant role in aquaculture. And many studies proved that herbal additives enhanced the growth of fish and also protected from the diseases [7].

The increased use of antibiotics has resulted in the development of resistant bacteria [13]. In recent years, misuse of antibiotics resulting in multi-drug resistance among bacteria has accelerated the search for drugs and dietary supplements effective against such multi drug resistant bacteria. It has been reported that in sales of botanical medicines increased by over 37% in 1995 [16].

The two effective medicinal plants used in the study were *Andrographis paniculata* and *Azardirachta indica*, which showed a remarkable effect in the treatment of infection in fish.

An attempt was made to prove the use of medicinal herbs in the treatment of infection in fish. According to this study and over derived results it has been indicated that herbal treatment can be used as an alternative to antibiotic treatment against drug resistant bacteria.

## CONCLUSION

The confirmatory reports of our studies indicate herbal medicine in fish health management and curing of disease proves to be prompt alternate. Further studies can be carried out on valuable herbal resources and standardization of dosage. It is conclude from the results obtained that herbal treatment for *Aeromonas hydrophila* infection in fish by both *Andrographis paniculata* and *Azardirachta indica* are equally effective. Improvement in herbal drugs can be tried in different plants and combination. This can also be tried in other fishes so that it will be a big boom to aqua culturists.

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