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***In vitro* and *in vivo* Antibacterial Studies of Medicinal  
Plant on Motile Aeromonad Septicemia in Fish  
Caused by *Aeromonas hydrophila***

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**Abstracts:** *Aeromonas hydrophila*, gram negative facultative anaerobic short bacillus, causes red fin disease, haemorrhagic septicemia, motile aeromonad septicemia and other infections in *Carassius auratus*. Multiple drug resistance of some strains of *A. hydrophila* presents a major threat in the control these diseases. Recently much attention has been paid to extracts and biologically active compounds isolated from plant species. In this studies ten plants leaves were assayed against *Aeromonas hydrophila* both *in vitro* and *in vivo*. The effective plant was found to be *Phyllanthus emblica* which shows the 24 mm zone diameter which was the nearest to oxytetracyclin and further choose for the *in vivo* study. *Tagetes erect*, *Azadirachta indica*, *Calotropis procera* also showed nearer activity nearer to the control antibiotic. There were no effect of *Momordica charantia*, *Achyranthes aspera*, *Centella asiatica* on aeromonads disease as the solvent alcohol solely gave the zone diameter of 14 mm. When incorporated *P. emblica* leaf extract in infected gold fish at a dose of 25 mg g<sup>-1</sup> of body weight, the *A. hydrophila* infection was cured within 12-15 days under laboratory condition.

**Key words:** *Aeromonas hydrophila*, *Carassius auratus*, medicinal plants, antibacterial activity, agar well diffusion method

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## INTRODUCTION

*Aeromonas hydrophila* causes red fin disease, haemorrhagic septicemia, motile aeromonad septicemia and other infections by in gold fish, *Carassius auratus*. The organism *Aeromonas hydrophila* is a gram negative facultative anaerobic short bacillus. It is motile and grows wide range of temperature 11.2-40.5°C. It produces exotoxin which exhibit toxicity to the carp. *A. hydrophila* infection is a zoonotic disease. It causes bacteremia, cellulites, myonecrosis, eethyma gangrenosum in human. The Gold Fish, *Carassius auratus* is an ornamental fish contain the golden color pigment due to the presence of erythrofore pigments. This fish has high susceptibility to motile aeromonads and are commonly valuable for experimental animals.

For this studies 10 plant species were selected, which was based on their use by traditional Indian healers or their reported antimicrobial activities in an attempt to find bioactive medicines for use in the treatment haemorrhagic septicemia, skin ulcers and wounds. The plants are *Phyllanthus emblica*, *Tagetes erecta*, *Azadirachta indica*, *Calotropis procera*, *Aloe vera*, *Citrus limon*, *Carum copticum*, *Momordica charantia*, *Achyranthes aspera*, *Centella asiatica*. The plant part chosen was leaves. The leaf of this plant contains tennins, Polyphenols, corilagin, alkaloids, phyllantidine, flavonoids etc as antimicrobials.

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To control bacterial diseases in fishes usually the chemotherapeutic agents like chloramphenicol, tetracycline, streptomycin etc are used. There are wide ranges of current antibiotics that are used for the treatment of bacterial infections but are still some challenges to be met in microbial chemotherapy. One of the problems in the development of resistance of chemotherapeutic agent is due to abuse of these drugs. Some strains of *A. hydrophila* show multiple drug resistances. Some time the chemotherapeutic agents also accumulate inside various organs of fish and become toxic to them. Because of the side effects and the resistance that pathogens build against antibiotics, recently much attention has been paid to extracts and biologically active compounds isolated from plant species. The use of plant extracts, as well as other alternative forms of medicinal treatments, is enjoying great popularity in late 1990s (Cowan, 1999). The acetone extracts of the roots of *Rumex crispus* and *Acinos rotundifolius* demonstrated significant inhibitory effects against *A. hydrophila* (Uluhanli *et al.*, 2005). Studied the efficacy of plant extracts in inhibiting *Aeromonas hydrophila* and *Listeria monocytogenes* in refrigerated cooked poultry (Hao *et al.*, 1998).

The main objective of this research was to determine the phytochemicals effectiveness of the plants extracts against the microorganism which cause diseases in the gold fish by both *in vitro* and *in vivo*. The antibiotic sensitivity test was determined only by agar well diffusion method and it gave clear zone of inhibition. These results were compared with the potent antibiotics that are effective against these diseases. *In vivo* studies of the infected fish were carried out with the most effective plant extract with the feed.

## MATERIALS AND METHODS

### Collection of Samples

Infected live fishes were anaesthetized by using chloroform. Inoculum from the infected part of the fish was taken out under aseptic conditions and the infected part was inoculated into nutrient broth and incubated at 27°C for 24 h.

### Isolation, Screening, Selection and Identification of *Aeromonas hydrophila*

The isolation of *Aeromonas hydrophila* was carried out with Rimler Shotts agar plate. One milliliter of the preinoculum was plated and incubated at 27°C for 24 h in an inverted position. The colonies were screened and biochemically identified as *Aeromonas hydrophila* (Bergey's Manual) (Holt *et al.*, 2000).

### Plant Material and Preparation of Extracts

The plant material used in this study consisted of *Phyllanthus emblica*, *Tagetes erecta*, *Azadirachta indica*, *Calotropis procera*, *Aloe vera*, *Citrus limon*, *Carum copticum*, *Momordica charantia*, *Achyranthes aspera* and *Centella asiatica* which were collected from different parts of Bangalore and Dharwad, Karnataka, India during October 2005. For 8 plant species fresh leaves were washed under running tap water, air dried and shade dried for three weeks. *Calotropis procera* and *Carum copticum* leaves were thick so sun dried carried out. Then the leaves were grounded using blender to get the powdery form.

### Solvent Extraction

Twenty five gram of dried plant was extracted with 100 mL of 70% alcohol in conical flasks sealed with foil and allowed to stand for 72 h. They were filtered with Whatman Filter paper No. 1 to obtained crude ethanolic extracts and stored at 4°C when not in use.

### Antibacterial Assay

The antimicrobial properties were evaluated by the agar well diffusion method using Mueller Hinton agar (Hi-media) for the assay. The microorganisms were activated by inoculating a loopful of the strain in the nutrient broth (25 mL) and incubated at 27°C for 24 h.

One milliter of inoculum was inoculated into the 45-50°C cooled agar and plated. Using the cork borer wells was made and different alcohol extracts having 25 mg mL<sup>-1</sup> concentration were transferred using a micropipette. Then the plates were kept in the refrigerator for 5 min for diffusion and incubated at 27°C for 24 h.

The control experiment was carried out with oxytetracyclin of Ranbaxy Company, India i.e., the drug of choice for the aeromonad infection and solvent alcohol.

### Animal Testing

Ten gold fish (GF1-GF10) were obtained local fish aquarium centers and maintained in the lab condition. Isolated and identified *Aeromonas hydrophila* were cultured on Rimler Shotts agar medium and further diluted from 10<sup>-1</sup> to 10<sup>-15</sup> dilutions in saline water were carried out. Ten fishes were injected at the caudal peduncle region with 0.5 mL the serially diluted solution 10<sup>-6</sup> to 10<sup>-15</sup>, respectively with 1 mL disposable insulin syringe. The fishes after being inoculated with the inoculum showed several symptoms like lack of appetite, swimming abnormalities, pale gills, blotted appearance and skin alliterations. The leaf extract was mixed with the fish feed at dosages of 25 mg g<sup>-1</sup> of body weight of fish and feed daily for a period of 12-15 days.

## RESULTS AND DISCUSSION

The organism isolated from the fish was confirmed as *Aeromonas hydrophila* shown (Table 1).

The antibacterial activities of the extracts obtained from the plants under study and the standard antibiotic was determined by the agar well diffusion method (well diameter 6 mm) was shown in the Table 2.

The effective plant was found to be *Phyllanthus emblica* which shows the 24 mm zone diameter which was the nearest to oxytetracyclin and further choose for the animal study. *T. erect*, *A. indica*, *C. procera* also showed nearer activity nearer to the control antibiotic. There were no effect of *M. charanti*, *A. aspera*, *C. asiatica* on aeromonads disease as the solvent alcohol solely gave the zone diameter of 14 mm.

The *in vivo* studies showed the days of recovery of the fish from the disease and shown in Table 3.

*P. emblica* leaf extract has the healing capacity for the *A. hydrophila* infection of the gold fish at a dose of 25 mg g<sup>-1</sup> of body weight of the fish for a period of 12-15 days under laboratory condition.

Table 1: The biochemical characteristics of *Aeromonas hydrophila*

Reactions	Results
Indole	+ve
Methyl red	+ve
Voges-proskauer	+ve
Citrate utilization	-ve
H <sub>2</sub> S production	+ve
Urea hydrolysis	-ve
Gelatin hydrolysis	+ve
D-glucose	+ve (A and G)
Cellobios	-ve
Growth temperature	27°C

Table 2: Inhibitory properties (inhibition zone diameter in mm) of different plant extract on *Aeromonas hydrophila*

S. No.	Plant extracts	Zone of inhibition (mm)
1	<i>Phyllanthus emblica</i>	24
2	<i>Tagetes erect</i>	22
3	<i>Azadirachta indica</i>	20
4	<i>Calotropis procera</i>	20
5	<i>Aloe vera</i>	15
6	<i>Citrus limon</i>	16
7	<i>Carum copticum</i>	16
8	<i>Momordica charanti</i> ,	14
9	<i>Achyranthes aspera</i>	8
10	<i>Centella asiatica</i>	6
<b>Standards</b>		
	Oxytetracyclin	26
	Alcohol	14

Table 3: Effect of oxytetracyclin and *P. emblica* leaf extract (25 mg g<sup>-1</sup> b.wt.) *in vivo*

Gold fishes	Days of recovery from infection	
	Oxytetracyclin	<i>P. emblica</i>
GF.1	10	15
GF.2	10	15
GF.3	10	14
GF.4	10	15
GF.5	11	13
GF.6	9	14
GF.7	9	12
GF.8	9	12
GF.9	10	12
GF.10	9	12

Multiple drug resistance of some strains of *A. hydrophila* presents a major threat in the control of the motile aeromonad septicemia in fish and much attention has been paid to extracts and biologically active compounds isolated from plant species. In this study the plant species *Phyllanthus emblica* leaf extract showed the same antimicrobial activity as compare to the drug of the choice, oxytetracyclin. The days of recovery from the disease also only 1.2-1.5 times less compared to the control. The *Phyllanthus emblica* leaves contain tannins, polyphenols, terchebin, terchebin corilagin, alkaloids like phyllantidin and phyllantine which acts as antimicrobial to kill or inhibit the bacteria.

Result of this kind herald an interesting promise of designing a potentially active anti bacterial agent of plant origin. Many plants have become sources of important drugs and the pharmaceutical industries have come to consider traditional medicine as a source of bio-active agents that can be used in the preparation of medicine (Aboaba *et al.*, 2006).

The present study confirmed the antibacterial effect of the *Phyllanthus emblica* leaf extract which can be treated apart from the synthetic drug to overcome the toxicity and the resistance of the bacteria. But this study was confined only for laboratory conditions hence it can also be further considered for studying the inhibitory action in natural ecosystem for the commercial usages.

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