International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

Successive Extraction from Leaves of *A.Barbadensis* and its Antibacterial Activity Against *Aeromonas Hydrophila* Infected Common Carp

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Abstract: ***The Aim of the present study was to determine the efficacy of different crude extracts obtained from the leaves of A.barbedensis against the pathogenic organism A.hydrophila, whichvery harmful and major problem are creating to the aquaculture environment. The organism was collected from the organs of experimentally infected common carp and the antibacterial activity was done for all extracts derived from the successive extraction. The zone of inhibition was measured for each extracts.***

Keywords: Successive extraction, *A.barbadensis*, Antibacterialactivity, common carp

1. Introduction

A. hydrophila is the causative agent of MAS (motile *Aeromonas* septicaemia). Both farmed and wild fishes have been found to be affected by this disease. Fishes becomesusceptible to the disease condition in their intensive culture system by *Aeromonas hydrophila.* Thedisease was characterized by swollen abdomen, red mouth, haemorrhage in external surface andsurrounding the anus [1]. Hemorrhagic septicemia is a common bacterial disease caused by *Aeromonas species*[2].Aeromoniasis in Indian major carps poses one of the major threats in aquaculture. Occurrence of skin lesions with haemorrhages due to A. hydrophila and the effective antibiotic treatment was reported in a carp (Cyprinus carpio) hatchery farm in Turkey[3]. *A. hydrophila* was frequently observed in various species of diseased farmed and wild freshwater fishes in different locations of Bangladesh[4]. It was recognized as a causative agent of ulcer type disease occurred in farmed fishes[5].

During the last few years, fish health problembecame a major concern to aquaculturist in all overthe world. In south-east Asian countries, fishproduction was badly affected by the outbreak offish disease, such as Epizootic UlcerativeSyndrome (EUS) in 1980. Important bacterial fishpathogens, including*Aeromonas* spp.,*Pseudomonas* spp., and *Flexibacter columnaris*, areregularly isolated from fish and become primarypathogenic agents frequently reducing theproduction of cultured freshwater fish. In late1980, a total of 125 tons of carp were lost in Java(Indonesia) due to bacterial disease infection .The disease was causedby bacterium *Aeromonas hydrophila.* This bacterium was not only causing mortality oncommon carp but also on catfishes and snakeheadfish[6].Bacterial hemorrhagicsepticemia due to strain of *Aeromonas hydrophila* may be transmitted through the water, diseased and healthy fish, other affected vertebrates, and favored by external as well as internal parasites[7].

Aloe vera Linne or *Aloe barbadensis* Miller is a succulentfrom the Aloe family (400 different species) with its origin in African continent. Its thick leaves contain the water supply for the plant to survive long periods of drought[8].The recent researches on Aloe Vera are appreciable. In the previous study, A. Vera aqueous and alcoholic extracts were prepared by decoction and hot percolation process. Alcoholic extracts displayed higher antibacterial and anti-fungal activity than aqueous extract[9].

Biological activities of Aloe vera include promotion of wound healing, antifungal activity, hypoglycaemic or antidiabetic affects, and anti-inflammatory. Anticancer, immunostimulatory and gastro protective properties[10].The rising incidence in multidrug resistance amongst pathogenic microbes has further necessitated the need to search for newer antibiotic sources. Because of its wide usage and availability, this study was set out to investigate the antimicrobial activity of the Aloe vera[11]. The present work has been made an attempt to determine the antibacterial activity of A.barbadensis against Aeromonas hydrophila infected common carps.

2. Materials and Methods

2.1 Collection of Fish

25 healthy Common carps (*Cyprinus carpio*) with same sized were collected from Tamilnadu fish farm,Thiruporur,Chennai.Fish were purchased and transported to the fish wet lab with aseptically and well aerated conditionto carry out the study. Fish were fed with routine fish feed collected from the same fish farm. Before starting the experiment fish were acclimatized for 15 days.

2.2 Experimental test

Experiment was performed at the wet disease laboratory of the Department of fish Immunology. Experimental fishes

with the average bodyweight of 20.5gm were used for the experimental test against the test organism.

2.3 Collection of Test Organism

Pure culture of *A.hydrophila* was collected from Fish immunology Lab, VELS University, Pallavaram, Chennai. This organisms was swabbed on selective media for the confirmation of the organism andthis was maintained on nutrient agar slants for future work.

2.4 Collection and Extraction of Aloe vera plant

Leaves of *A.barbadensis* were collected from in and around college campus of VELS University. The voucher specimen was submitted to Prof.P.Jayaraman, Ph.D. Institute of Herbal Botany, Plant Anatomy Research Centre, Tambaram, and Chennai for taxonomic identification of the plant.

The entire gel portion of the plant was removed and the leaf portion was dried and ground for the successive extraction using Soxhlet apparatus. Differentsolvents like Petroleum ether, Chloroform, Ethanol, Methanol and Water were used for theextraction. The extracts were further transferred to vacuumevaporator for getting the crude extracts. These crude extracts were used for the antibacterial activity against the *A.hydrophila*.

2.5 Antibacterial Sensitivity test

The Muller-Hinton Agar was used for the antibacterial activity. The medium was sterilized and poured into the sterile petri plates. After solidification plates were loaded with *A.hydrophila*from the infected fish by streaking eventually on the surface of the medium using sterile cotton swab. The wells were prepared by using sterile steel borer. The wells were loaded with 25µl of the crude extract and the commercial drug tetracycline.The antibacterial activity was performed for each extract. After incubation the zone of inhibition were measured.

3. Result and Discussion

3.1 Extraction of *Aloe barbadensis* leaf

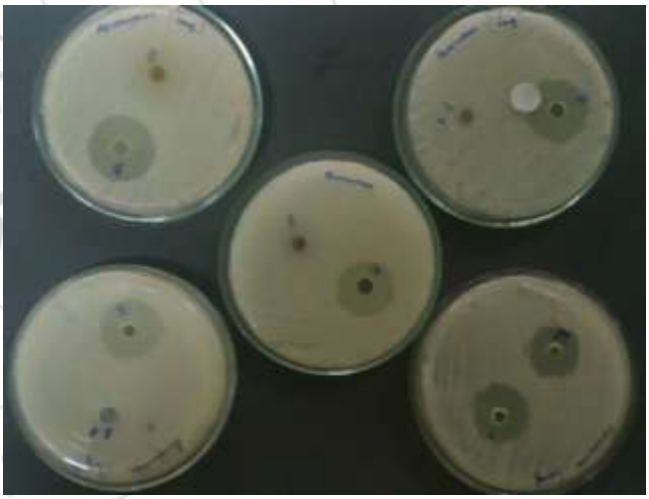
20 gms dried powder was packed in Sohxlet apparatus for the successive extraction by using low polar solvent to high polar solvent (Petroleum ether, chloroform, ethanol, methanol and water). Extraction was made for each solvents. After each extraction the extracts were filtered through What Mann filter paper and subjected to dryness for getting the crude extract. This crude extract was stored at Refrigerated condition for future use.

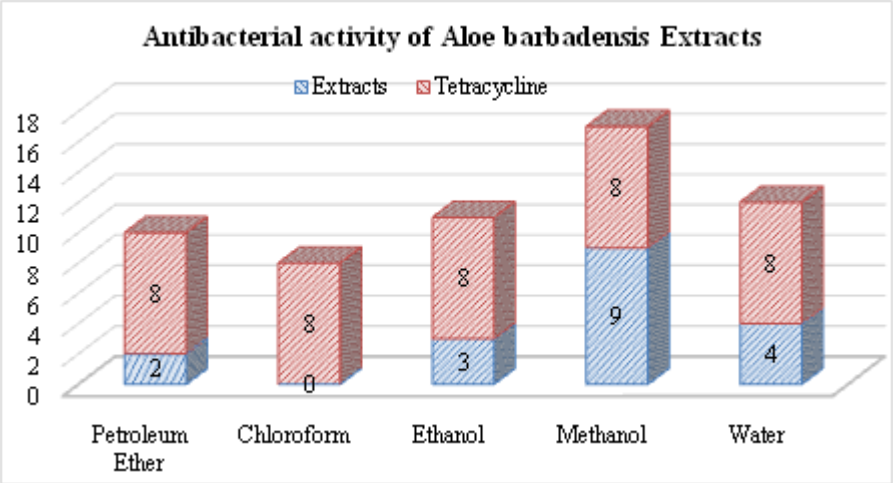
3.2 Experimental Test

The virulent strain was obtained by repeated injection of viable cells in to the fish by intramuscularly till the lesions were formed. After the lesion formed from the fish the swab was made from the fish and swabbed on the Aeromonas selective media for getting the pure culture. Antibacterial activity was performed for the different extracts against the pathogenic organism collected from the infected fish.

3.3 Antibacterial Activity

In this study, the antibacterial activity was performed for *A.hydrophila* against all extracts. In petroleum ether extract showed very little activity. There was no antibacterial activity was observed in chloroform extract and minimum activity was seen in ethanol and water extracts .Methanol extract showed more activity when compared to all other extracts. Tetracycline was used as positive control.





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