Antibacterial Activities of three medicinal plant extract against Fish Pathogens

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Abstract
The antimicrobial activities of ethanol, chloroform and acetone extracts of three plant species were studied. The extracts of Asparagus racemosus, Withania somnifera and Macuna pruriens were tested in vitro against four fish pathogens by well diffusion method. Aeromonas hydrophila, Pseudomonas fluorescens, Vibrio cholera and Klebsiella pneumoniae were used in the present investigation. Acetone extracts of A. racemosus, W. somnifera and M. pruriens were active against A. hydrophila, P. fluorescens and K. pneumonia. The ethanol extract M. pruriens showed various antibacterial activities (1.3-1.6 cm/200μl) against the entire tested microorganism. Acetone and chloroform extract of A. racemosus and M. pruriens showed minimum inhibitory zone (0.6 cm/200μl). The maximum inhibitory zone was exhibited in the ethanol extract of M. pruriens (1.7 cm/200μl) against A. hydrophila, and chloroform extract of A. racemosus showed higher inhibition (1.9 cm/200μl) against K. pneumonia.

Keywords: Asparagus racemosus, Withania somnifera, Macuna pruriens, Extracts, Fish pathogen, Antibacterial activity.

Introduction
India has a rich heritage of knowledge on plant based drugs both for use in preventive and curative medicines. A country like India is very much suited for development of drugs from medicinal plant as it is rich in biodiversity. The medicinal value of plants has assumed a more important dimension in the past few decades. This is due largely to the discovery that extracts from plants contain not only minerals and primary metabolites but also a diverse variety of secondary metabolites. The search for biologically active compounds has been vigorous in recent years due to the growing cases of microbial resistance to the time honored antibiotics (Dimayuga and Garcia, 1991). In recent years numbers of studies have been reported dealing with antimicrobial screening of extracts of medicinal plants (Gundidza and Gazu, 1993; Perumalsamy and Ignacimuthu, 2000). A variety of compounds are accumulated in plants accounting for their constitutive antimicrobial activities (Callow, 1983).

Aquaculture has a growing activity for the last twenty years worldwide and impressive development has been attended by some practices potentially damaging human and animal health (Naylor, 2005). Various medicinal plants have been used for years in daily life to treat diseases all over the world. Bacterial diseases are responsible for heavy mortality in wild and cultured fish. Disease outbreaks have been causing severe losses and hence farmers are frequently using chemotherapy to control and prevent diseases, which has several drawbacks such as development of resistant pathogens and accumulation (Chakraborti, 2006). The characteristics of the plants that inhibit microorganisms are important for fish health. The recent expansions of aquaculture has lead to a growing interest in understanding fish disease, so that they can be treated or prevented and have atleast partial success (Stokopf, 1993).

Asparagus racemosus is an herb belonging to the family Asparagaceae, commonly called as Shatavari. The plant grows throughout the tropical and sub tropical parts of India up to an altitude of 1500m. The roots are the part that finds use in various medicinal preparations. The major active constituents of A. racemosus are saponins (/-Shatavariins I-IV) that are present in the roots. It has been used in ayurveda as a galactagogue, diuretic, antispasmodic and nerve tonic (Sharma et al., 2000; Purihar and Hemmni, 2004). Extracts and formulations prepared from A. racemosus exhibited various immuno pharmacological actions such as increases in white cell counts, haemagglutinating and haemolytic antibody titres in cyclophosphamide treated mouse ascetic sarcoma (Diwanay et al., 2004).
Withania somnifera belongs to the family Solanaceae, commonly known as swagandha. It is a shrub reaching about 150 cm in height. This plant grows widely in all drier parts of sub tropical India. It has general animating and generative qualities and is used among others for the treatment of nervous exhaustion, memory related conditions and insomnia, it helps countered, chronic fatigue, weakness, dehydration, bone weakness. It involves in the activation of macrophages, Phagocytosis and increased activity of the lysosomal enzymes.

Mucuna pruriens is a climbing shrub, which is belonging to the family Fabaceae and the use of Mucuna pruriens is documented in siddha medicine for a number of uses, including as a potent aphrodisic. It has been used for remedying tuberculosis, diabetes and cancer. It is used for producing fertilizer and improving the soil quality. Mucuna pruriens is also used to prepare various formulations, which are used as medicines or remedies against different diseases (Nadakoshi, 1954).

Among the fish pathogens A. hydrophila is the most common bacterial pathogen in freshwater fish causes tail or fin rot and haemorrhagic septicemia. Vibriosp also cause haemorrhagic septicemia in the skin of tail and fins. Pseudomonas sp causes haemorrhage in the tail and fins and ulceration of the skin. Considering the aforesaid, the objective of the present study was to evaluate the effect of A. racemosus (root) Withania somnifera (root) and M. pruriens (seed) on some bacterial strains viz. A. hydrophila, P. fluorescens, V. cholerae and K. pneumoniae.

Materials and Methods
Plant material and preparation of extract
Asparagus racemosus (root), Withania somnifera (root) and Mucuna pruriens (seeds) were purchased from the local market and were dried and made into powder. 20gram of each plant parts were extracted separately with 150 ml of ethanol, chloroform and acetone solvent for 8 hours by using soxhlet equipment. The solvent was made to evaporate so as to make the final volume, one fourth of the original volume. It was then stored in air tight bottles for further microbiological assays.

Test organisms
The standard bacterial cultures used in this investigation are Aeromonas hydrophila strain no: 646 (MTCC), Pseudomonas fluorescens, Vibrio cholerae and Klebsiella pneumoniae were obtained from the Department of Microbiology, Sadakathullah Appa college, Tirunelveli-11, India.

Antimicrobial assay
The antimicrobial assays were done by Kirby-baeyer method (Casida,1986). Midnight pathogenic bacteria cultures were used for our study. Nutrient agar plates were prepared and the extracts thus obtained were injected into a sterilized well of 100 μm diameter in amounts of 200 μl. Well injected with 200 μl of pure ethanol, chloroform and acetone served as controls. The plates were then incubated at 37±℃ for 24 hours. The inhibitory activity of the compounds in various solvents was determined by measuring the size of inhibition zones of different solvents.

Results and Discussion
The antibacterial activity of plant A. racemosus, W. somnifera and M. pruriens extracted in different solvents against the bacterial pathogens are shown in Table 1. The compounds extracted in different solvents exhibited different antibacterial activity. The antibacterial activity of A. racemosus against different fish pathogens were shown in Table- 1. Acetone and chloroform extract of root of A. racemosus showed inhibition against A. hydrophila, P. fluorescens and K. pneumoniae. Chloroform extract of A. racemosus showed higher inhibition (1.9cm/200 μl) against K. pneumoniae. Methanol extract of A. racemosus showed considerable in vitro antibacterial efficacy against E.coli, V. cholerae, B.subtilis and S.aureus, and it increases phagocytic activity of macrophages (Mandal, 2000).

The antibacterial activities of ethanol, acetone and chloroform extract of W. somnifera showed marked inhibition towards all the bacterial strains tested. The antibacterial activity of V. cholerae was much reduced as compared to that of other bacterial strains. Ethanol and acetone extract of W. somnifera showed maximum inhibitory zone of 1.1 and 1.2 cm/ μl respectively against V. cholerae (Onkarappa, 2005) reported that W. somnifera, Glycyrrhiza glabra and Leucus aspera showed variable antimicrobial activity against bacteria. Earlier studies of Witherin A are believed to cause anti inflammatory effect on granuloma tissue in rats (Davis, 2000). They were found to be effective against B.cereus, Streptomyces sp. and P. fluorescens. It showed complete inhibitory action on fungus Aspergillus fumigatus, aspergillus terreus, Pencillus funiculosus (Abou-Dough, 2002).
Table 1. Antibacterial activity of three plants against fish pathogens (Disc/200μl)

<table>
<thead>
<tr>
<th>Micronorganisms</th>
<th>Zone of Inhibition (cm)</th>
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<tr>
<td></td>
<td>A. racemosus</td>
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<tr>
<td></td>
<td>Ethanol</td>
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<tr>
<td>A. hydrophila</td>
<td>0.9</td>
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<tr>
<td>P. fluorescens</td>
<td>0.9</td>
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<tr>
<td>V. cholerae</td>
<td>0.8</td>
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<tr>
<td>K. pneumonia</td>
<td>1.2</td>
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The antibacterial activities of *M. prureins* were shown in Table 1. Ethanol extract of *M. prureins* were found inhibitory to all the bacterial strains tested. Earlier studies reported that *M. prureins* act as a potent curative agent for endosulfan induced testicular toxicity and hepatotoxicity. (Balamurugan, 2005). Methanol extract of *M. prureins* inhibit the growth of *B. pumilillus, E. coli, V. choleraca* (Yerra Rajeshwar, 2005). *M. prureins* seeds showed antibacterial activity against *E. coli, S. dysenteriae, S. typhi* and *B. subtilis* (Ashok Kumar, 2009). (Damodaran, 1937) reported that seeds of *Mucuna prureins* contain L-dopa, a dopamine precursor utilized in Parkinson’s disease.

The herbal plants may be used as a potential and promising source of pharmaceutical agents against fish pathogens (Bahaduria,2004) reported the activity of *Solanum dulcamara* against human pathogens. Medicinal herbs as immunomodulants increase resistance to disease by enhancing non specific and specific defense mechanisms (Venkatalakshmi, 2001). The results suggest the presence of high concentration of an active principle in all the extracts of the tested three plants which showed potential antibacterial activity.

References


Damodaran and Ramasamy,1937. Isolation of 1, 3,4 dihydroxy phenylalanine from the seeds of *Mucuna prureins*. *Biochemical Journal,* 31:21-49.


Aquaculture is growing rapidly all over the world since fish forms the primary source of animal protein. However, it also faces many problems which affect its development. Disease management is a major requirement for the successful development of aquaculture. Various factors have been related to the apparent increased incidence of disease. Environmental factors and poor water quality sometimes resulting from increased self-pollution due to effluent discharge and pathogen transfer via movement of aquatic organisms appear to be an important underlying cause of epizootic ulcerative syndrome (EUS). Over the past two decades, EUS has had a serious effect on tropical fisheries resulting in heavy economic losses. A large number of reports are available on a possible association between EUS changes and seasonal factors such as rainfall, flooding and water quality parameters such as low temperature, low pH and low dissolved oxygen levels. A diverse group of biotic agents viz. viruses, bacteria and cutaneous ectoparasites may cause skin lesions which are subsequently colonised by Aphanomyces invaders and ultimately lead to EUS.

*Coleus aromaticus* Benth belongs to the family *Lamiaceae* (local name *Oosavali* in Tamil and *Navarpachi* in Malayalam) and is well known for its medicinal properties like antihypertensive, chemopreventive, anti-oxidant and antiepileptic potential. Other folk uses include treatment of ulcers, boils, swellings, urogenital and wound healing properties. It is used by local Indian people for its therapeutic efficacy against common cold, cough, fever, headache.
Comparative study of two herbal treatments in EUS-affected murrels

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and indigestion. The leaves are said to have specific action on the bladder and to be very useful in urinary disease and vaginal discharges.

The henna plant, Lawsonia inermis L., belongs to the family Lythraceae (local name Maruthan). Henna leaves, flowers, seeds, stem bark and roots are used in traditional medicine to treat a variety of ailments such as rheumatoid arthritis, headache, ulcers, diarrhoea, leprosy, fever, leucorrhoea, diabetes, cardiac disease and wound healing. Chloroform and aqueous extracts of leaves of the plant were capable of inhibiting the growth of microorganisms that are involved in causing burn wound infections.

The snakeheads (family Channidae), commonly called murrels, have a good market value due to their excellent taste, low fat, fewer intramuscular spines and medicinal qualities. Diseased specimens of two types of murrels were selected for a comparative study on the effectiveness of the two herbs Coleus aromaticus and Lawsonia inermis in treating wounds caused by EUS in the fish.

Methodology

Diseased giant murrels (Channa marulius) of length 28 cm and weight 250 g were collected from the Bahavani sagar reservoir and diseased spotted murrels (Channa punctatus) length 20-24 cm and weight 350-400 g were purchased from the local market in Truvanoveli district. The infected individuals were separated and were transported to the Centre for Aquaculture Research and Extension. Most of them showed lesions and deformed fins on the upper side and a few showed reddish spots on the bottom.

These diseased murrels were kept in cement tanks (3 m × 1 m × 1 m) and were fed with semi-moist feeds. A number of wound healing techniques, viz spraying chemicals into ponds or adding antibiotics in the feed, have been previously described. A study carried out in 1993 noticed that the application of neem and turmeric leaves prevented the spread of infection and promoted the survival and growth of fish.

In order to treat the diseased murrels, two types of herbal pastes, viz Coleus aromaticus and Lawsonia inermis paste, were prepared.
Results and conclusion

When compared to Lawsonia inermis paste, Coleus aromaticus paste was more effective in wound treatment. Coleus-treated individuals showed complete wound healing on the fifth day of treatment whereas Lawsonia-treated murrels showed slower recovery and all the external wounds disappeared on the eighth day of treatment. The antimicrobial activity of Coleus showed maximum inhibition zone against Aeromonas hydrophilia compared to the Lawsonia herb. Based on the results of the present investigation, Coleus aromaticus is more effective against EUS on murrels.

Application of Coleus aromaticus paste to EUS-affected C marulius (top) and Lawsonia inermis paste to C punctatus (bottom).

Every day the fishes were taken from the rearing tanks. Wound healing was observed and treatment was repeated for 10 days. Meanwhile the rearing tanks were cleaned and supplied with well-oxygenated water from a nearby borewell. Water quality parameters were as follows: pH (7.63 ± 0.28), salinity (2.07 ± 0.02 ppt), dissolved oxygen (9.4 ± 0.30 ml/litre) and ammonia (0.26 ± 0.02 ppm).

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