Antibacterial Activity of some Macrophytes against Fish Pathogens

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Abstract- This paper embodies the antibacterial activity of aqueous extracts of three macrophytes *Polygonium amphibium*(leaves, rhizome), *Potamageton pectinatus*(leaves) and *Ipomea aquatica*(leaves, rhizome) against fish pathogen *Pseudomonas aeruginosa* isolated from freshwater fishes. During the study it was found that the strongest antibacterial activity among the above mentioned macrophyte species were obtained by the aqueous extract of *Polygonium amphibium*(leaves) with inhibition zone of 12.9mm followed by *Polygonium amphibium*(rhizome) with inhibition zone of 12mm. The aqueous extract of *Ipomea aquatica*(rhizome) marked 9.7mm inhibition zone followed by *Potamageton pectinatus*(leaves) with inhibition zone of 8mm while *Ipomea aquatica*(leaves) marked 7mm inhibition zone against *Pseudomonas aeruginosa*

I. Introduction

Fishes are cold blooded aquatic vertebrate animals which breathe by means of pharyngeal gills, propelling and balancing themselves by means of fins (Jhingaran, 1982). Fishes are a treasured resource in terms of food, sports and aquarium and for other scientific studies. The fish population of aquatic systems plays a significant role in the human economy (Talwar and Jhingaran, 1991). They offer job opportunities to millions of skilled and unskilled rural people, uplifting their economic levels in general and of the fisherman community in particular. Fish is the cheapest source of protein supply to rural community where potentialities for production exist (Forese and Pauly, 1998).

Fishes are susceptible to several bacterial infections (like Infectious dropsy, Furunclosis, Cotton mouth disease, Tail Rot or Fin Rot, Tuberculosis, Columnaris, Vibriosis), mainly when reared in high density conditions. Fishes living in tropical waters are more susceptible and prone to diseases than fishes living in temperate waters, because the former provides favourable conditions for introduction and spread of diseases (Hatha and Joice, 2005).

Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids which have been found *in vitro* to have antimicrobial properties. Compounds that inhibit pathogens but have little toxicity to host cells are candidates for developing new antimicrobial drugs (Agatemor, 2009).

II. Material and Methods

Macrophytes were collected from the Upper Lake (Bhopal, M.P.) Epiphytic and extraneous matter was removed by washing first by lake water and then with distilled water and transported to the laboratory in polyethylene bags at ice temperature. The fresh weight of these macrophytes was recorded. The macrophytes were identified by using special identification keys and by experts in the respective fields (Adoni, 1985) and identified as *Polygonium amphibium, Potamageton pectinatus* and *Ipomea aquatica.* The samples were shade dried for about 90 hours and weighed again. Then the individual parts of samples were separated from each other and their individual weight was also recorded. Also the rhizome was cut into pieces so that more of moisture will be released and it was dried properly. After that all the individual parts of these samples were separately powdered in a mixture grinder.

Plant material was extracted by using a Soxlet extractor with distilled water. Extraction of each part of plant powder (20 g) was done at room temperature in 150 ml of distilled water for about 8 to 10 hours.

The diseased fishes were brought from Shahpura Lake and Itwara Fish Market (Bhopal) and were ice preserved till carried to the laboratory and preserved in refrigerator. The diseased fishes were identified by common symptoms such as dizziness, red spots on the body and whitish patches on and near the gills etc. Nutrient agar media was prepared on five Petri plates. About 20 ml of nutrient agar was poured in each Petri plate and it was allowed to stand for few minutes to solidify.

The diseased fishes were scratched using sterilized needle at different body spots such as mouth, gills, tail and streaked in five agar plates which were put in an incubator for about 48 hours at 37^{o}C. The growth of certain bacterial colonies where found in the form of slightly yellow coloured and white. The growth of some fungal colonies where also noticed. The new plates containing cultural agar media were prepared. The bacterial colonies that had grown on the previous plates were separated and enoculated aseptically on new plates to make cultural plates of pure bacterial colonies to avoid destruction by fungal colonies. The bacteria were identified by IMVIC test (Cappuccino and Sherman, 1995) which includes a series of four tests: Indole test, Methyl red test, Voges-proskauer test and Simmon's citrate test. The antibacterial activity of distilled water extract was evaluated against one Gram-negative bacterium by paper disc method as described by Dulger (2005). The discs with macrophyte extracts were placed on nutrient agar plates. The plates were incubated overnight at 37^{o}C. The diameters of the inhibition zones were measured in millimetres.

III. Results

A 20g weight from each part of macrophyte were taken and extracted in 150ml of distilled water for about 8-10 hours. The total extract collected from extractor was found to be around

100ml which was further heated to concentrate the extract solution and stored at 15°C.

Table 1: Showing fresh and dry weight of different macrophytes.

S. No.	Macrophytes	Fresh weight (g)	Dry weight (g)
1	*Polygonium amphibium* (rhizome)	180	45
2	*Polygonium amphibium* (leaves)	100	25
3	*Ipomea aquatica* (rhizome)	150	35
4	*Ipomea aquatica* (leaves)	200	50
5	*Potamageton pectinatus* (leaves)	175	35

Table 2: The weight of different parts of macrophytes, the distilled water used for extraction and the total extract received

Plant name	Dry Weight	Distilled water used	Total extract received
Polygonium amphibium (rhizome)	20g	150ml	100ml
Polygonium amphibium (leaves)	20g	150ml	100ml
Ipomea aquatica (rhizome)	20g	150ml	90ml
Ipomea aquatica (leaves)	20g	150ml	90ml
Potamageton pectinatus (leaves)	20g	150ml	100ml

The bacteria which were cultured on the agar plates were identified by IMVIC test as:

Indole Test:- 5 ml portions of medium from a pure culture were inoculated and incubated at 35±0.5 °C for 24 hours. 0.2-0.3 ml of test reagent was added and it was gently shaken. The culture was allowed to stand for about 10 minutes and results were recorded. The absence of dark red colour in the amyl alcohol surface constituted a negative test.

Methyl Red Test:-10 ml portion of medium from a pure culture were inoculated and incubated at 35±0.5°C for five days. 5 drops of methyl red indicator were added to 5 ml of culture and it was again incubated for 48 hours. The absence of red colour constituted a negative test.

Voges-Proskauer Test:- 5 ml portion of medium were incubated for 48 hours at 35±0.5°C. 0.6 ml of napthol solution and 0.2 ml of KOH solution were added to 1 ml of culture. The absence of colour change from pink to crimson at the surface within 5 minutes constituted a negative test.

Simmon's Citrate Test:- Agar medium was inoculated by the streaking technique using a light inoculums and it was incubated for 48 hours at 35±0.5°C. The colour change of medium from green to blue constituted a positive test.

The bacteria were identified as *Pseudomonas aeruginosa*. It is a rod shaped bacteria which is characterized by abundant thin, white growth with the medium turning slightly green. In IMVIC test, it shows its confirmation only when Citrate test is positive while with the remaining tests are found negative as shown in Table 3.

Table 3: showing identification of different bacteria by IMVIC Test.

Organism	Indole test	Methyl Red test	Voges-Proskauer test	Citrate test
E. coli	+	+	-	-
Enterobacter aerogenes	_	_	+	+
Klebsiella pneumonia	_	±	±	+
Shigella dysenteriae	±	+	-	-
Salomonella typhimurium	-	+	-	+
Proteus vulgaris	+	+	-	±

Psedumonas aeruginosa	_	_	_	+
Alcaligenes faecalis	_	_	_	±
Styphylococcus aureus	_	+	±	_
Streptococcus lactis	-	+	-	-
Micrococcus luteus	_	_	_	_
Corynebacterium xerosis	_	_	_	_
Bacillus cereus	_	_	±	_

The extracts of different plant parts of macrophytes showed various inhibition zones. Among these plant distilled water extracts, *Polygonium amphibium* (leaves) showed the inhibition zone of 12.9 mm followed by *Polygonium amphibium* (rhizome) with inhibition zone of 12 mm which was followed by *Ipomea aquatica* (rhizome) and *Potamageton pectinatus* (leaves) with inhibition zones of 9.7 mm and 8 mm respectively while as, *Ipomea aquatica* (leaves) showed the inhibition zone of 7 mm.

Table 4: Showing antibacterial activity of aqueous extracts against *Pseudomonas aeruginosa.*

S. No.	Macrophytes	Inhibition zone (mm)
1	*Polygonium amphibium* (rhizome)	12.0±0
2	*Polygonium amphibium* (leaves)	12.9±0
3	*Ipomea aquatica* (rhizome)	9.7±0
4	*Ipomea aquatica* (leaves)	7.0±0
5	*Potamageton pectinatus* (leaves)	8.0±0

IV. Conclusion

From the present study it can be concluded that there are several macrophytes that have the potential to treat several types of diseases without producing any side effects by inhibiting the growth (activity) of pathogenic microbes that are responsible for several fish fatal and other bacterial diseases. The leaves of *Polygonium amphibium* was found to have the maximum antibacterial activity against fish pathogens. So, the need of moment is to explore more number of plant species and natural products residing in such type of plants that could be potential sources for the reduction of bacterial diseases and others likely.

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