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Research Article

PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL SCREENING OF MEDICINAL PLANTS AGAINST AEROMONAS HYDROPHILA

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ABSTRACT

The objective of the present study is to screen antimicrobial activity of methanolic extracts of eight medicinal plants (*Ocimum sanctum, Psidium guajava, Punica granatum, Tinospora cardifolia ,Withinia sominifera, Allium sativum, Terminalia arjuna and Mentha arvensis)* against the common fish pathogen *Aeromonas hydrophila* . Phytochemical analysis of the extracts revealed the presence of alkaloids, carbohydrates, flavonoids, saponins, terpenes and phenolic compounds. Among the eight medicinal plant extracts, *T.cardifolia and W.sominifera* showed a wider spectrum of antibacterial activity and expand least MIC and MBC values against *A.hydrophila*. All the other herbal extracts showed a moderate antibacterial activity against the test pathogen. The significant antibacterial activity of active extracts were compared with standard antibiotics Kanamycin, Neomycin and Penicillin. The results obtained in the present study suggested that *T. cardifolia* and *W.sominifera* can be used for treating diseases caused by *A.hydrophila* in aquaculture practices.

Keywords: Medicinal herbs, *Aeromonas hydrophila*, Antibacterial activity, Phytochemical analysis.

INTRODUCTION

Aeromonas hydrophila, the most common bacterial pathogen has been recognized as the causative agent for tail/fin rot and haemorrhagic septicemia especially in freshwater fishes[2]. *Aeromonas hydrophila* is a persistent problem in murrel fish farms and outbreak of diseases such as Motile Aeromonad Septicemia (MAS) or Epizootic Ulcerative Syndrome (EUS), results in mass mortality. Currently, bacterial infections in aquaculture, including Motile Aeromonad Septicemia, are mainly controlled by antibiotics. However, recently, the use of antibiotics in aquaculture has been discouraged since their use will lead to development of drug resistance thereby reducing drug efficacy. Moreover, the accumulation of antibiotics both in the environment and in the fish is a potentially risky to consumers as well as non-target species [1]. To overcome such problems, use of environment friendly natural products has been promoted as an alternative to control bacterial infection in aquaculture. In this regard use of medicinal herbs having antimicrobial activity are recommended against several pathogenic bacteria as traditional medicines for treatment of diseases [5, 6, 12 & 13]. Since the herbal medicines have minimal side effects and are easily biodegradable, inexpensive and locally available, they have been recommended as an alternative tool for bacterial disease management in aquaculture [3]. The present study was attempted to evaluate the antibacterial activity of extracts of from eight medicinal plants against the dreadful pathogen *Aeromonas hydrophila.*

MATERIALS & METHODS

Leaves of *Ocimum sanctum, Psidium guajava, Punica granatum,Tinospora cardifolia,* and *Mentha arvensis* and root, peel and bark of *Withinia sominifera, Allium sativum* and *Terminalia arjuna* were collected from in and around Tirunelveli (8.44^{o}N, 77.44^{o}E). All the plant samples were shade dried and powdered and extracted successively with methanol and distilled water using a Soxhlet extractor. The pathogen *A.hydrophila* was freshly cultured (18-24 hr) from the mother culture preserved at CARE Microbiology Laboratory. Phytochemical analysis of methanol extracts of selected plant samples were made following the procedure of Brinda et al 1981[4]. The disc diffusion assay (Kirby-Bauer Method) was used to screen the herbal extracts for their antibiotic activity (Prescott *et al.,* 1990)[10]. Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentration (MBC) were determined according to Vollekova *et al* (2001)[14].

RESULTS AND DISCUSSION

The results of the phytochemical screening revealed the presence of alkaloids, carbohydrates, flavonoids, saponins, terpenes and phenolic compounds (Table 1). associated with antimicrobial activities and curative properties against pathogens [9].The invitro antibacterial studies showed a significant inhibitory activity against the tested bacterium *A.hydrophila* (Fig.:1). The inhibition zones produced were significantly higher for the methanol extract of *T. cardifolia, W. sominifera* and *A. satium* when compared to the positive control (Neomycin, Kanamycin) (Fig: 2). Among the above three plants *T. cordifolia* showed the maximum zone of inhibition (23.0mm) against invitro growth of *A.hydrophila* when compared to the positive control *Kanamycin* (22mm). The other two plants *W. sominifera and A. satium* also showed significant inhibitory effect (21mm, 20mm) when compared to the control Neomycin (18mm) (Fig:2). In a recent survey by Samy and Ignacimuthu (2000)[12], *Aerva lanata* (L.) Juss. Ex Schult. (Amaranthaceae), *Abutilon indicum* Sweet (Malvaceae), *Tephrosia pupurea* Pers.(Fabaceae), *Sida acuta* Burm (Malvaceae), *Syzygium cumini* and *Toddalia asiatica* were found not to exhibit any antimicrobial properties. Similarly, our study failed to show any activity for, *M. arvensis*. The solvents, methanol and distilled water are used as negative control which did not show inhibition zones. Penicillin used as positive control exhibited no other inhibitory effects. The MIC values for T. cardifolia and W. sominifera are estimated as 0.780mg/ml and 1.560mg/ml respectively (Table 2). The highest MIC value was observed in *M.arvensis* (50mg/ml). The MBC value was determined from the viable counting method (Table 3). The MBC values of *T. cardifolia* and *W. sominifera* are similar to their MIC values (Table 3). The traditional healers use primarily water as solvent but in this study, the extracts prepared using methanol provided more consistent antimicrobial activity due to more solubility of active principles. Penicillin did not show inhibition against *A. hydrophila.*. Among the eight medicinal plants methanolic extracts of *T.cardifolia and W.sominifera* showed a broad spectrum of antibacterial activity and least MIC & MBC values against *A. hydrophila*. Ruban *et al* (2008)[11] too reported that methanolic extract of *Croton zambesicus* had lowest MIC & MBC values against *E.coli* and *S.aureus.* There are many published reports on the effectiveness of traditional herbs against gram-positive and gram-negative microorganisms, and as a result, plants are still recognized as the bedrock for modern medicine to treat infectious diseases [7, 8].

Hence the results of the present investigation revealed that the methanol extracts of above two plants will be used as good bactericidal agents against *Aeromonas hydrophila.* Further studies are also being carried out to test the efficacy of the herbal extracts in fish models in vivo.

Table 1: Preliminary Phytochemical Analysis of Methanolic Extracts of Medicinal Plants.

Medicinal Plants	Steroids	Terpeniods	Alkaloids	Phenol	Saponin	Tannin	Flavonoids	Sugar
Ocimum sanctum	_	_	_	_	_	+	+	_
Psidium guajava	_	_	+	_	+	_	_	+
Punica granatum	_	+	_	+	_	+	_	_
Tinospora Cardifolia	+	+	+	+	+	+	+	_
Withinia sominifera	+	+	+	+	+	+	+	+
Allium sativum	_	_	_	_	+	+	+	+
Terminalia arjuna	_	+	_	+	+	+	+	_
Mentha arvensis	+	_	_	_	-	+	+	_

(+) present; (-) absent

Table 3: Antibacterial activity of Medicinal plants against fish pathogen *Aeromonas hydrophila*. Values are given as mean±standard deviation

Medicinal Plants	Zone of inhibition (mm)	
	Methanol Extract	Water Extract
Ocimum sanctum	15.3±1.24	-
Psidium guajava	12.6±2.49	-
Punica granatum	7.66±2.05	-
Tinospora cardifolia	23.0±3.74	6.7±1.69
Withinia sominifera	21.3±2.86	5.0±0.81
Allium sativum	17.6±2.41	-
Terminalia arjuna	20.3±2.86	-
Mentha arvensis	3.66±1.20	-

Table 4: Antagonistic activity of commercial antibiotics against fish pathogen *Aeromonas hydrophila*. Values are given as mean±standard deviation.

Antibiotics	Zone of inhibition (mm)
Neomycin	18±2.51
Kanamycin	22±3..65
Penicillin	_

Table 2: Minimum Inhibitory Concentration (MIC) values of Methanolic Extracts of Medicinal Plants against *A.hydrophila*

Medicinal Plants	Extracts concentratiom mg/ml								
	0.780	1.560	3.125	6.25	12.5	25	50	100	200
Ocimum sanctum	-	-	-	-	α	+	+	+	+
Psidium guajava	-	-	-	α	+	+	+	+	+
Punica granatum	-	-	-	-	-	α	+	+	+
Tinospora cardifolia	α	+	+	+	+	+	+	+	+
Withinia sominifera	-	α	+	+	+	+	+	+	+
Allium sativum	-	-	α	+	+	+	+	+	+
Terminalia arjuna	-	-	α	+	+	+	+	+	+
Mentha arvensis	-	-	-	-	-	-	-	α	+

- Resistance (growth of bacteria)

+ Concentrations show no turbidity (inhibition of baterial growth)
α Least concentration showing no turbidity (MIC)

Table 3: Minimum Bacterial Concentration (MBC) values of Methanolic Extracts of Medicinal Plants against *A. hydrop*

Medicinal Plants	Extracts concentration mg/ml								
	0.780	1.560	3.125	6.25	12.5	25	50	100	200
Ocimum sanctum	-	-	-	α	+	+	+	+	+
Psidium guajava	-	-	-	α	+	+	+	+	+
Punica granatum	-	-	-	-	α	+	+	+	+
Tinospora cardifolia	α	+	+	+	+	+	+	+	+
Withinia sominifera	-	α	+	+	+	+	+	+	+
Allium sativum	-	-	α	+	+	+	+	+	+
Terminalia arjuna	-	-	α	+	+	+	+	+	+
Mentha arvensis	-	-	-	-	-	-	-	α	+

- Resistance (growth of bacteria)

+ Bactericidal Concentrations
α Minimum Bactericidal Concentrations (MBC)

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