

Screening of antimicrobial activity of mangrove plant *Acanthus ilicifolius* on shrimp and fish pathogens

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ABSTRACT

The anti-microbial activity of ethyl acetate, ethanol and methanol extract of leaf, flower, seed and root of the plant *Acanthus ilicifolius* collected from Kakinada mangrove forest was studied. The crude plant extracts of ethyl acetate, ethanol and methanol were tested against pathogenic bacteria such as *Aeromonas hydrophila*, *Vibrio harveyi* and *Escherichia coli*. The ethyl acetate, ethanol and methanol extracts of the different parts of the *A. ilicifolius* exhibited strong to moderate activity against test microorganisms. The inhibition zone exhibited by ethyl acetate extract was significantly higher than that produced by ethanol and methanol extracts.

Keywords: Antimicrobial activity, Crude extract, *Acanthus ilicifolius*, agar well diffusion method.

INTRODUCTION

Acanthus ilicifolius (acanthaceae), is locally known as “Alchi” and commonly called as holy leaved *Acanthus* [1]. It is a gregarious, sparingly branched, evergreen shrub, 0.6-1.5 meters in height. It is a plant of marshy habitat distributed widely throughout the mangroves of India and different parts of the Asian countries. The plant grows luxuriously by the side of the Ganges in Sunderbans, the shrub is also planted as a sand-binder along the banks of tidal rivers and lakes [2]. *A. ilicifolius* is a medicinal plant used against rheumatism, paralysis, asthma and snake bite, analgesic, anti inflammatory, smallpox and ulcer [1, 3]. Phytochemical screening indicates the presence of flavonoids and terpenes [4, 5]. The flavonoids present in the plant were found to have hepato-protective and antioxidant activities [6]. The present study deals with the antimicrobial activity of different organic solvent extracts of leaf, flower, seed and root of *A. ilicifolius* collected from Kakinada against shrimp pathogen *Aeromonas hydrophila*, fish pathogen *Aeromonas harveyi* and human pathogen *Escherichia coli*.

MATERIALS AND METHODS

Collection of Plant:

Fresh leaf, seed, flower and root of *Acanthus ilicifolius* were collected from Kakinada mangrove forest, East Godavari District, Andhra Pradesh, in the month of September, 2014. The leaf, seed, flower and root were air-dried, coarsely powdered and were subjected to extraction.

Preparation of extracts:

One gram of each of the coarsely powdered plant material (Leaf, Flower, Seed and Root) was soaked in 10ml of solvents (ethanol, methanol and ethyl acetate) for a period of one week at room temperature. Then the extracts were

filtered and concentrated by rotary vacuum evaporator (Buchi-R 420). The final concentration was adjusted to 100mg/ml for the antimicrobial activity.

Antimicrobial study:

The pure culture of *V. harveyi* (MTCC No: 3438), *A. hydrophila* (MTCC No: 1739) and *E. coli* (MTCC No: 1678) were used as test microorganisms for antibacterial activity. The agar well diffusion method [7] was employed to assess the antibacterial activity. Sterile Muller–Hinton Agar (MHA) plates were taken and inoculated with 25 µl of 18 hour young cultures of respective pathogens with a sterile micropipette and spread with L-shaped glass rod to get the lawn of bacterial culture. In each of these plates, wells of 6mm in diameter were made using a sterile cork borer No.4 [8]. Exactly 25 µl of each extract was taken and filled in respective wells and allowed to diffuse at room temperature for 2 hrs. The respective solvents were used as controls. The plates were incubated for 24 hrs at 37°C. The antimicrobial activity was evaluated by measuring the diameter of the Inhibition Zone (in mm)

RESULTS AND DISCUSSION

The antimicrobial activity of *A. ilicifolius* leaf, stem, flower and root extract of *A. hydrophila*, *V. harveyi* and *E. coli* is shown in Table 1.

Ethyl acetate extracts of seed showed strong inhibition zone with 12mm against *V. harveyi* and 10mm against *A. hydrophila* whereas leaf, flower and root showed moderate and mild inhibition zones against all three bacteria. Ethanol extract of seed showed strong inhibition zone with 11mm and root with 9mm against *V. harveyi* whereas leaf, flower extracts showed moderate to mild inhibition zone against three pathogens. Methanol extract of flower showed inhibition zone against *A. hydrophila* with 8mm and whereas seed showed moderate inhibition zone against *E. coli* with 5mm, leaf and root extracts showed mild inhibition zones against three tested pathogens.

The ethyl acetate and ethanol extract of seed showed strong inhibition zones against both shrimp and fish pathogens than leaf, flower and root extracts of *A. ilicifolius* in the present study. The antifungal and antibacterial activity of methanol extract of *A. ilicifolius* is also reported by Chandrashekhar and Varahala Rao [9]. According to Ganesh and Jannet [2], the methanol and ethanol extract of leaf, stem and root of *A. ilicifolius* showed considerable inhibitory activity against *E. coli* than that of the present study. These variations may be due to method of extract preparation, concentration of extract used for antimicrobial activity, period of incubation etc. The leaf extract and fraction of *A. ilicifolius* effectively inhibit *V. harveyi* growth in tiger prawns, decrease the prevalence of attacks, and increase the tiger prawns survival from *V. harveyi* attack [10]. *A. hydrophila* causes disease in fish known as motile *Aeromonas septicemia* (MAS) and red-sore disease. The seed extract of *A. ilicifolius* can prevent these infectious diseases in fish.

Table 1. Antimicrobial activity of different parts of *A. ilicifolius* against pathogenic bacteria

S. No.	Micro organism	Zone of inhibition in mm											
		Ethyl acetate extraction				Ethanol extraction				Methanol extraction			
		Leaf	Seed	Flower	Root	Leaf	Seed	Flower	Root	Leaf	Seed	Flower	Root
1	<i>Vibrio harveyi</i>	1	12	2	2	2	11	5	9	0	1	0	0
2	<i>Aeromonas hydrophila</i>	1	10	2	2	0	1	1	0	1	0	8	1
3	<i>Escherichia coli</i>	2	1	5	1	0	1	0	0	1	5	2	0

CONCLUSION

According to preliminary studies, it is concluded that *A. ilicifolius* contains biologically active compounds which have ability to inhibit the growth of pathogenic bacteria. The work can further be extended to isolate the pure compounds and evaluate the antimicrobial activity against a wide range of pathogenic bacteria.

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