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Hemolytic and Anti Microbial Effect in the Leaves of *Acanthus ilicifolius*

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ABSTRACT

Acanthus ilicifolius is a very potential plant and it has more phyto chemical compounds. In present study, anti microbial and hemolytic effects of *Acanthus ilicifolius* leaves extracts of chloroform and aqueous in isolated protein. The antimicrobial activity of crude extract against bacterial and fungal pathogens showed the clear inhibition zone against *Vibrio cholerae* and *Aspergillus niger* in chloroform extract and aqueous extracts showed clear inhibition zone for *Pseudomonas* sp. and *Candida albicans*. Both the extracts exhibited hemolytic activity which was estimated as 10.80 ht mg^{-1} for chloroform extract and 9.5 ht mg^{-1} for aqueous extract. The partial purification of protein is done by using DEAE cellulose. On SDS-PAGE the crude protein yielded three well defined bands at 100.5, 52 and 21.4 kDa in both the extracts.

Key words: *A. ilicifolius*, hemolytic, anti microbial, protein, DEAE cellulose, SDS-PAGE

INTRODUCTION

Antibiotics used are no longer desirable because of concerns about bacterial resistance (Wallace, 2004). Increased awareness of the potential problems associated with the use of antibiotics stimulates research efforts to identify alternatives to their use. Novel approaches in the development of new antimicrobial have been carried out, such as compounds to treat diseases or to improve animal growth include dietary use of probiotics, prebiotics (Higgins *et al*., 2007), organic acids (Immerseel *et al*., 2006), medicinal herbs (Arab *et al*., 2006; Du and Hu, 2004)

Hemoglobin S differs from HbA in the substitution of valine for glutamic acid in the sixth position of the **β**-chain amino acid sequence. Hemoglobin HbSS, in whom all the hemoglobin type is HbS, always manifest features of sickle cell disease, which may be fatal in childhood. The hemolytic activity of the extracts was also performed to rule out the possible cytotoxic mechanism and to check the safety of the phytocompound thus making it suitable for the preparation of natural drugs.

Acanthus ilicifolius is a commonly available as back mangrove plant in almost all the coastal states of India. It is a folklore medicinal plant used mainly against rheumatism, paralysis, asthma and snake-bites, skin disease, ulcer. A decoction of the plant with sugar candy and cumin is used in dyspepsia with acid eructations (Kathiresan and Ramanathan, 1997; Ramanathan, 2000) It is also considered to be a diuretic and is used as a cure for dropsy and bilious swellings. In Goa, the leaves are employed as an emollient fomentation in rheumatism and neuralgia (Ananda and

Sridhar, 2002). The leaves are bruised and soaked in water for external application and are also used as an expectorant. The analgesic, anti-inflammatory (Kanchanapoom *et al*., 2001) and leishmanicidal (Kapil *et al*., 1994) properties of *A. ilicifolius* have been documented, whilst have reported the antioxidant and hepatoprotective properties of the plant (Babu *et al*., 2001). Hence, the present study was undertaken to evaluate the protective effects of *Acanthus ilicifolius* extract and its comparison with active principle hemolytic and anti microbial effects.

MATERIALS AND METHODS

Plant material: Chemical reagents were purchased to Sigma Chemicals Co. (St. Louis, MO). The fresh leaves of *Acanthus ilicifolius* was collected during September 2009 in Parangipettai, Chidambaram, Tamil Nadu, India. The leaf was identified and authenticated by Botanist at Annamalai University, Annamalai Nagar, Tamilnadu, India. The specimen was deposited in the departmental herbarium unit.

Extraction of crude toxin: Aqueous extraction: The aqueous extract of sponge was prepared by squeezing the sand-free specimens in triple distilled water. The resultant solution was filtered and dialyzed by using Sigma dialysis membrane-500 (Av Flat width-24.26 mm, Av. Diameter -14.3 mm and capacity approx -1.61 mL cm^{-1}) against D-glucose to remove the excess water. The supernatant so obtained was lyophilized (Labcono Freeze Dry System) and stored at 4°C in a refrigerator for further use as crude aqueous extract.

Chloroform extraction: Crude toxin was extracted following the method of Bakus (1981) with certain modifications. The sponge was dried in air for 2 days and after that 10 g sponge tissue was shocked with 200 mL of chloroform, covered and kept standing for 5 h. The solvent was then removed after squeezing the sponge and filtered through Whatman No. 1 filter paper. The solvent was evaporated at low pressure by using a Buchi Rotavapor R-200 at 45°C in refrigerator for further use as crude chloroform extracts.

Antimicrobial activity: Petri dishes with nutrient agar and PDA agar were inoculated with six different species of bacteria and fungus. Sponge extracts were sterilized by passing each through a 0.22 :m Millipore GV filter (Millipore, U.S.A). Round paper discs w with a radius of 0.8 cm were dipped into each sponge extract and placed in the center on inoculated Petri dishes. The bacterial and fungal colonies were allowed to grow overnight at 37 and 20°C respectively and then the inhibition zone around the disc was measured.

Protein estimation: Protein estimation was done as described by Lowry and Lopez (1946) using Bovine serum Albumin at the rate of 1 mg mL^{-1} as the standard. Different concentrations of the standard ranging from 0.1 to 1 mg mL^{-1} were taken and made up to 1 mg mL^{-1}. Then 5 mL of alkaline copper reagent was added, mixed well and allowed to stand for 10 min at room temperature. Then 0.5 mL of diluted Folins phenol reagent was added and mixed well. The mixture was incubated for 30 min at room temperature. The absorbance at 720 nm was read spectrophotometrically. The protein concentrations of *S. fibulatus* extracts were estimated.

Partial purification of crude protein: Partial purification of the crude extract was carried out using DEAE Cellulose Anion Exchange chromatography according to the procedure of Stempein *et al*. (1970) method.

Gas chromatography: Gas chromatography of the crude extract was done as described. Identification of fatty acid was carried out on the basis of retention times of the standard mixtures of fatty acids.

Hemolytic assay: The micro hemolytic test was performed in 96 well 'V' bottom micro titer plates. Different rows were selected for chick blood. Serial two fold dilutions of the crude toxin were made in 100 mL of normal saline. This process was repeated up to the last well. Then 100:l of RBC was added to all the wells. Appropriate controls were included in the test. To the 1% RBC suspension 100:l was added normal saline, which served as negative control. The plate was gently shaken and then allowed to stand for two hours at room temperature and the results were recorded. Uniform red colour suspension in the wells was considered as positive hemolysis and a button formation in the bottom of these wells was considered as lack of hemolysis. Reciprocal of the highest dilution of the crude toxin showing pattern was taken as 1 Hemolytic Unit (HU).

SDS-PAGE: One dimension sodium dodecyl sulphate (SDS) Polyacrylamide gel electrophoresis (PAGE) was carried out following the modified method of Laemmli (1970) SDS-PAGE was run on vertical slab gel system. Proteins were electrophorised on 12% separating gel (0.75 mm thickness) overlaid with 5% stacking gel. A 10% (w/v) stock solution was prepared in de ionized water and stored in room temperature.

RESULTS AND DISCUSSION

The present study to investigated bioactivities of protein isolated from *A.ilicifolius* leaves extracts. Chloroform extract of mangrove plant yielded a total amount of 5.5 g crude extract from 500 g of *A.ilicifolius*. Similarly aqueous extract pf total amount of 4.85 g of crude extract. Table 1 The crude aqueous and chloroform extract was determine the protein level of in our sample in our result suggested that chloroform extract 1.520 mg mL^{-1} and followed by aqueous extract 1.234 mg mL^{-1} (Table 2). The crude of aqueous and chloroform extracts at different concentration of 5, 10 and 15 mg mL^{-1} were tested against 6 species of bacteria viz *Pseudomonas* sp., *Streptococcus aureus*, *Vibrio parahaemolyticus*, *Vibrio cholerae*, *E.coli* and *Vibrio parahaemlyticus* and 3 species of fungus, *A. flavus*, *A. niger* and *Candida albicans*. The results showed that the crude aqueous extract inhibit the growth of *V. cholerae* whereas in the chloroform extract a clear inhibition zone were observed only against *Pseudomonas* sp. The inhibition zone was measured and it was found to be 2.7 cm for *Pseudomonas* sp. and 1.8 cm for *Vibrio cholerae* (Table 4). The results showed that the crude aqueous and chloroform extracts against fungal stains. In the aqueous extract inhibit the growth of *A.niger* whereas in the chloroform extract a clear

Table 1: Preparation of crude extract

Name of the solvent	Yield (in grams for 500 g of sample)
Chloroform	5.50
Aqueous	4.85

Table 2: Protein estimation

Type of extract	Absorbance at 750 nm	Concentration of protein (mg mL^{-1})
Chloroform	1.9	1.520
Aqueous	1.2	1.234

Table 3: Hemolytic activity of *Acanthus ilicifolius*

Type of extract	Amount of protein (mg)	Total hemolysis	Hemolytic titre	Specific hemolytic activity (HT mg^{-1})
Aqueous	1.520	4	16	9.5
Chloroform	1.234	4	16	10.8

Table 4: Anti microbial activity of *Acanthus ilicifolius*

Microbial sp.	Name of the microbe	Inhibition zone measurement (cm)
Bacteria	*Vibrio cholerae*	1.8
	Pseudomonas sp.	2.7
Fungus	*Aspergillus niger*	3.1
	Candida albicans	2.9

inhibition zone was observed only against *C. albicans*. The recent work was mention that maximum zone inhibition showed in *Trichosporon begelli* and *Candida albicans* and minimum zone inhibition was showed in *Aspergillus niger* and *Penicillium chrysogenum* in *Spinifex littoreus* grass (Thirunavukkarasu *et al*., 2010). The inhibition zone was measured and it was found to be 3.1 cm for *A. niger* and 2.9 cm for *Candida albicans*. Burholder and Bedford (1978) isolated two bromo compounds from *Verongi fistularies* and *V. vauliformis* that inhibited the growth gram positive and gram negative bacteria. The crude chloroform extract induced pronounced hemolysis on chicken blood. The hemolytic titer in case of chloroform extract found to be 16 and its specific hemolytic activity was estimated to be 10.8 HT mg^{-1} of protein (Table 3). The hemolytic titer of aqueous extract of marine *Acanthus ilicifolius* was found to be 16 and its Hemolytic Activity was found to be 9.5 HT mg^{-1} of protein. SDS-PAGE on 12% gel, the crude protein toxins yielded 5 bands in aqueous extract and 8 bands in chloroform extract of *A. ilicifolius*. Ranging from 7.8 to 116 kDa with three well defined bands at 100.5, 52 and 21.4 kDa defined bands at in both extracts. Proteins have recently been found in ginseng. Pananotin, from the roots of *Panax notoginseng* was found to be toxic to *Coprinus comatus, Physalospora piricola* and the phytopathogens, *Botrytis cinerea* and *Fusarium oxysporum* Other proteins isolated from the *Panax* family (panaxagin and quinqueginsin) also exhibit antifungal activity (Lam and Ng, 2002).

CONCLUSION

The chloroform and aqueous extracts of *A. ilicifolius* have great potential asantimicrobial proteins against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes. And also it has great effect of hemolytic activity in chicken blood. Plant extracts against resistant bacteria leads to new choices for the treatment of infectious diseases.

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