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Antimicrobial activity of Acanthophora spicifera

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ABSTRACT

The anti bacterial activity of ethanolic extract and its bioactivite guided fraction of the seaweed Acanthophora spicifera (ACS) was studied by disc diffusion method using Muller Hinton agar medium and the zone of inhibition was compared that of standard ciprofloxacin (5µg/ disc). All the tested extract and guided fractions showed potential antibacterial activity to that of the standard drug against the tested organisms. The MIC for all the test samples was fixed at 300 µg/ml. Hence the presence study validated the scientific documentation of the anti infective property of the seaweed ACS.

Keywords: Acanthophora spicifera; Antibacterial activity; Disc diffusion method

INTRODUCTION

Marine sources of organisms servers as a new area for exploitation to lead focused discovery with set targets in achieving new drugs. Blue green algae, (Cynobacteria) a potential source known with novel bioactive components including toxins. Macro algae which include seaweed are important sources of protein, iodine, vitamins and minerals. Hence, in the past few decades there has been enormous flow of research unveiled with respect to antioxidant, antitumor and immune-modulatory potentials (Furuswas and furuswa, 1998). Acanthophora spicifera, a Rhodophycean algae belonging to the largest classification of seaweed commonly known as spiny seaweed, Family: Rhodomelaceae. This is wide in distribution through tropics and sub tropics and it was the most abundant red algae found on reef flats. These economically important red algae are located in Rameswaram coast (Umameheswara Roa 1970).

This seaweed was utilised for various pharmacological effects so as to treat Eczema and gallstone. *Acanthophora spicifera* which yield 12% of agroid serves as good source of food for human consumption since decades (Chennubotla, 1981). Many edible seaweeds were reported to posses varied bioactive constituents exhibiting varied pharmacological effects Viz anti cancer, anti-hypertensive, immunomodulatory, antioxidant and antibacterial. (Mohamed et,2012). The algae was documented to posses phenolic components ex-

* Corresponding Author Email: lavanyalavakumar@gmail.com Contact: +91-Received on: 21-03-2012 Revised on: 27-03-2012 Accepted on: 29-03-2012 hibiting antioxidant activity when extracted with methanol. Further, the same was found to be dose dependent with petroleum ether and ethyl acetate fraction. Hepato-protective effect was significant with ethanolic extract and the anti-oxidant status of the extract showed tremendous effect with respect to enzymes superoxide dismutase, catalase and glutathione peroxidise the same was justified with the presence of the vitamins like A, E, C in this algae (Vasanthi). Various phytoconstituents were reported from *Acanthophora spicifera* like conjugated eicosopentanoic acid and conjugated Arachidonic acid (Basker et al.,) steroids cholest-4-ene-3 alpha, 6-beta-diol,lauric acid, o-phthalic acid (wahidulla et al 1998).

Hence, upon careful screening of the literature it was evidenced that the red algae *Acanthophora spicifera* was evidenced to possess lack of documentation with respect to the antimicrobial potential of the algae. In the present study an attempt was made to explore the antimicrobial potential of the algae with respect to activity guided fraction resulting from the ethanolic extract of the seaweed

MATERIALS AND METHOD

Marine algae collection

The fresh red algae *Acanthophora spicifera* (Family: Rhodomelaceae, Ceramiales) was collected from Mandapam, during the month of March 2008 from Ramaeshwaram coast, Tamil Nadu, India. It is identified and authenticated by Dr. Krishnamurthy, Institute of algology, Anna nagar, Chennai. The voucher specimen (VCP/09-345) was deposited in the Department of Pharmacognosy, Vels college of Pharmacy, Chennai 117.

Extraction of Acanthophora spicifera

Dried, pulverized *A. spicifera* (1 kg) was extracted with 5 liters of alcoholic using soxhlet apparatus for 24 hrs. The extract was filtered, and the filtrate was evaporated by rotary vacuum evaporator and sample was freeze dried for further use. The percentage yield of ethanol extract was found to be 20.22% w/w. The ethanolic extract was subjected for preliminary qualita-

tive chemical analysis (Kokate, 1994; Harborne, 1998).

Separation of activity guided fraction of ethanolic fraction

The crude extract was fractionated with various solvents ranging from non polar to polar solvents like hexane, acetone, and ethyl acetate. The ethanolic crude extract was subjected for fractionation with hexane to give a hexane-soluble fraction and hexane in-

| Table 1: Anti-bacterial act | tivity of various | fractions of of | Acanthophora s | picifera |
|-----------------------------|-------------------|-----------------|----------------|----------|
| | livity of various | | Acunthophoru 3 | picijciu |

| Description | Staph | CONS | Candida | E.coli | Klebsiella | Pseudomonas | S.typhii | |
|---------------|--------|--------|----------------|-------------|---------------------------------------|-------------|----------|--|
| Control | + | + | + | + | + | + | + | |
| Ciprofloxarin | 5 | 5 | 5 | 5 | 5 | 5 | 5 | |
| Hexane | > 200 | > 200 | > 200 | > 100 | > 50 | > 50 | > 200 | |
| | < 300 | < 300 | < 300 | < 200 | < 100 | < 100 | < 300 | |
| Acetone | > 200 | > 200 | > 100 | > 200 | > 200 | > 100 | > 100 | |
| | < 300 | < 300 | < 200 | < 300 | < 300 | < 200 | < 200 | |
| Ethyl acetate | > 100 | > 100 | > 100 | > 100 | > 100 | > 50 | > 50 | |
| | < 200 | < 200 | < 200 | < 200 | < 200 | < 100 | < 100 | |
| . | S.para | S.para | Enterna handen | Ch i walla | A - : : : : : : : : : : | Sheritia | Proteus | |
| Description | typnii | typnii | Enterobacter | Snigella | Achenobacior | | vulgaris | |
| | A | В | | | | | 5 | |
| Control | + | + | + | + | + | + | + | |
| Ciprofloxacin | 5 | 5 | 5 | 5 | 5 | 5 | 5 | |
| Hexane | > 50 | > 50 | > 100 | > 100 < 200 | > 50 | > 50 | > 50 | |
| | < 100 | < 100 | < 200 | > 100 < 200 | < 100 | < 100 | < 100 | |
| Acetone | > 50 | > 200 | > 200 | > 100 < 200 | > 50 | > 200 | > 100 | |
| | < 100 | < 300 | < 300 | > 100 < 200 | < 100 | < 300 | < 200 | |
| Ethyl acetate | > 50 | > 50 | > 50 | > 100 < 200 | > 50 | > 50 | > 50 | |
| | < 100 | < 100 | < 100 | > 100 < 200 | < 100 | < 100 | | |

Table 2: Zone of inhibition (mm) of various fraction of Acanthophora spicifera

| Description | Standard | Hexane | Acetone | Ethyl acetate | Ethanolic |
|-------------------------------------|----------------|---------|---------|---------------|-----------|
| Organisms | ciprof-loxacin | extract | extract | extract | Extract |
| Staphylococcus aureus | 18 | 18 | 20 | 20 | 19 |
| coagulase-negative staphylococci | 16 | 20 | 22 | 22 | 18 |
| Candida albicans | 12 | 10 | 12 | 10 | 11 |
| Escherichia coli | 25 | 12 | 14 | 12 | 11 |
| Klebsiella pneumoniae | 19 | 16 | 19 | 18 | 10 |
| Pseudomonas aeruginosa | 26 | 19 | 18 | 14 | 15 |
| Salmonella typhi | 24 | 16 | 14 | 14 | 16 |
| Salmonella paratyphi A | 22 | 12 | 13 | 11 | 12 |
| Salmonella para typhi B | 26 | 15 | 15 | 17 | 13 |
| Enterobacter aerogenes | 20 | 20 | 15 | 12 | 10 |
| Shigella dysenteriae | 25 | 14 | 11 | 17 | 17 |
| Acinetobacter bau-mannii | 22 | 18 | 14 | 18 | 16 |
| Serratia liquefaciens | 24 | 12 | 12 | 16 | 14 |
| Proteus vulgaris | 22 | 12 | 16 | 15 | 13 |

soluble residue. The hexane-insoluble residue was partitioned with acetone and acetone soluble fraction was collected. The acetone insoluble fractions were further partitioned with ethyl acetate. There is no remaining insoluble residue was noted. The ethyl acetate fraction was dried in a rotary evaporator at 40°C to yield a dry extract. Further microbial screening was peformed with hexane, acetone and ethyl acetate residues.

Bacteria

Escherichia coli, Staphylococcus aureus, coagulasenegative staphylococci, Candida albicans, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Salmonella para typhi A, Salmonella para typhi B, Enterobacter aerogenes, Shigella dysenteriae, Actinobacter baumanni, Seratia liquefaciens and Proteus vulgaris

Medium MH agar, Ciprofloxacin discs (5 μ g/ disc) were obtained from Hi- media Laboratories limited, Mumbai-400 086.

Anti-bacterial activity (N.Deepa et al 2004)

The plates were prepared MH agar and the extracts of various dilutions were added and allowed to solidify and dry. A loop full of bacterial cultures was inoculated and incubated at 37°C for 24 hours. Results were read by the presence or absence of growth of organisms (Table 1) and the MIC was determined. The same procedure was followed for the investigation of all the extracts. The zone of inhibition shown by various extract on tested organisms was also recorded based on the MIC concentration. (Table 2)

RESULTS AND DISCUSSION

Both the extract and the bio active guided fractions of *Acanthophora spicifera* demonstrated anti-bacterial activity as shown in Table 1, against the tested bacteria. The results of all the extracts were as comparable with that of the standard Ciprofloxacin (5 micrograms / disc) (Table 2).

The results of the present study indicated the antimicrobial properties of ethanolic extract and the bioactivity guided fraction viz., hexane, acetone and ethyl acetate. The same was comparable with that of the standard Ciprofloxacin (5 micrograms / disc) against the tested organisms. The presence of flavonoids, tannins, glycosides are found to be present as eveidenced by the qualitative chemical analysis. These phytoconstituents could be the key players in exhibiting the antimicrobial property which is evidenced in the study. However the role of these phyto-constituents in the anti-bacterial property has to be explored in detail in the near feature. Among the tested organisms all samples showed better activity. In particular the ethyl acetate fraction demonstrated efficient activity among the tested bacteria (Table 2).

CONCLUSION

These findings of the present study support beneficial effects of the extract as well as the bioactivity guided fraction against the pathogenic organisms. Further investigations with respect to validation of bioactive component which may be responsible for the expressed pharmacological property and may lead to identification of the novel template with potent biological activity. This may throw light on the minds of the researchers for future development of new entity in phytomedicine with potent anti-infective property.

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