

Screening of antibacterial and antifungal activities of red marine algae *Acanthaphora spicifera* (Rhodophyceae)

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Abstract

Infectious diseases are one of the main causes of high morbidity and mortality in human beings around the world. Currently the marine is an important source of medicinal products since the marine ecosystem, particularly seaweeds are directly exposed and are susceptible to ambient micro organisms such as bacteria fungi and viruses. In the present study, we assessed the antibacterial and antifungal activity of various extracts of *Acanthaphora spicifera* such as petroleum-ether, chloroform and methanol against *Escherichia coli*, *Bacillus subtilis*, *Bacillus palmitus*, *Pseudomonas aeruginosa*, and against *Candida albicans*, *Microsporum gypseum*, *Aspergillus niger* respectively by disc diffusion techniques. The methanolic extract of *Acanthaphora spicifera* was showed higher antibacterial and antifungal activity compare to other two extracts. The minimum inhibitory concentration of methanolic extract of *Acanthaphora spicifera* was found to the range of 100 μ g.ml⁻¹ to 50mg.ml⁻¹ on test organisms. The antibacterial and antifungal activity of methanolic extracts showed similar activity to that of ciprofloxacin and amphotericin respectively.

Keywords: *Acanthaphora spicifera*, antibacterial activity, antifungal activity

Introduction:

Infectious diseases are one of the main causes of high morbidity and mortality in human beings around the world, especially in developing countries [1]. The severity of the diseases have increased in recent years and the emergence of multidrug resistant strains[2].since the failure of the treatment the marine environment covers a wide thermal range, pressure range, and nutrient range and it has extensive photic and non-photic zones. This extensive variability has facilitated an extensive biodiversity that for exceeds that of the terrestrial environment. Despite this fact, research into the use of marine natural products as pharmaceutical agents is still in its infancy in many countries around the world, mainly in developing countries [3]. Seaweeds serve as an important source of bioactive natural substances. Red algae are mainly used as human food, feed and medicine [4,5].Seaweed have been used as medicine and food stuff in the Asian diet for centuries as it contains carotenoids, low calorie food, vitamins, minerals and dietary fibers [6] . In addition to these seaweeds are potentially good sources of protein, polysaccharides and fibers [7,8]. Many metabolites isolated from

marine algae have been shown to possess bioactive effects and they show antibacterial activity [9,10,11,12]. Compounds with cytotoxic, antiviral, antihelminthic, antifungal and antibacterial activities have been detected in red algae [13, 14]. The degree of antibacterial activity of various family of sea algae such rhodophyceae, chlorophyceae, thephaeophyceae are 80, 62.5 and 61.9% respectively. The maximal antifungal activity of shown by sea algae such red, brown and green algae are 37. 33.3, and 8.3% respectively [15]. In the present study we assessed the antimicrobial activity of *acanthaphora spicifera* It is a red Algae grows up 8cm in height and is widely distributed in the Mandapam coastal area of Tamilnadu India.

Materials and Method:

Collection of marine algae:

Fresh algae of *Acanthaphora spicifera* (Rhodophyceae) were collected from different locations of Mandapam area, Tamilnadu, South East Coast of India. In the early morning 5 am to 11.30am during which the tidal height was from 0.77 meter to 0.08 meter (lat 9° 15' N; long 79° E). Then the algae were washed thoroughly with sea water to remove

extraneous materials and brought to the laboratory in plastic bag containing water to prevent evaporation. Samples were then shade dried until constant weight obtained and ground in an electric mixer. The powdered samples subsequently stored in refrigerator [16].

Preparation of Extracts:

The shade dried material was extracted with analytical grade petroleum ether, chloroform and methanol for 8 hours by continuous hot percolation in Soxhlet apparatus [17]. The extracts were dried under vacuum. The dry drug extract were dissolved in dimethyl formamide (DMF) and tested for antibacterial and antifungal activities.

Test Micro-Organism:

The bacterial strains such as *Escherichia coli*, *Klebsilla pneumoniae*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus palmitus*, *Pseudomonas aeruginosa* and the fungal strains such as *Candida albicans*, *Aspergillus niger* and *Microsporum gypseum* are used for the present study were received from the Department of medical Microbiology, Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalainagar, Tamilnadu, India.

Antimicrobial Testing of Extracts:

The test extracts at a concentration of 5% w/v were prepared using sterile dimethyl formamide as solvent. Ciprofloxacin were taken as the standard for antibacterial activity at a concentration of 0.5 μ g. The antibacterial activity was tested by using the filter paper disc diffusion method [18] employing 24 hours cultures of the above mentioned organisms. The test organism were seeded into sterile nutrient agar medium by uniformly mixing one ml of inoculums with 20ml sterile melted nutrient agar cooled to 48-50°C in a sterile Petri dish. The medium was allowed to solidify. The different extracts of test and standard drugs as well as blank were impregnated in what Mann filter paper disc

placed and the placed on solidified medium in the Petri dishes were left undisturbed for two hours at room temperature. The Petri dishes were incubated at 37°C for 24 hours and the zone of inhibition was measured.

The amphotericin were taken as the standard for antifungal activity. The sterile yeast nitrogen base (Hi-Media) with 2% agar was inoculated by a rotating swab (soaked in standard inoculums suspension) over the surface of the media. Extract impregnated discs were placed on the agar and incubated at 37°C for 18 hours. The solvent DMF was used as control. The clear zone of inhibition was measured. The antibacterial and antifungal activity of extracts which exhibits maximum activity is to measure for minimum inhibitory concentration. The extracts were further tested against the bacterial and fungal strains for its antibacterial, antifungal efficiency at different concentration (100mcg/ml, 500mcg/ml, 1mg/ml, 10mg/ml, and 50mg/ml) by using the filter paper disc diffusion method. The zone of inhibition was calculated by measuring the minimum dimensions of zone of no bacterial and fungal growth around the filter paper disc.

Result and Discussion:

The antibacterial activity and antifungal efficiency of various solvent extracts of algae *Acanthaphora spicifera* are shown in table 1 and 2. The antibacterial and antifungal activity of various extract of *Acanthaphora spicifera* were tested against various micro organism. The methanol extracts showed maximum antibacterial and antifungal activity than petroleum ether and chloroform extracts the zone of inhibition were ranged between 15.2 to 6.2 mm against bacterial strains and 15.1 to 2.8mm against fungal strains. From the study the methanol extract shows promising antibacterial activity and antifungal activity against all bacterial strains and fungal strains and its showed maximum activity against *Escherichia coli* (15.2mm) similarly the same

Table 1: Antibacterial activity of various extract of *Acanthaphora spicifera* against various bacterial strains. Ciprofloxacin is used as reference standard.

Microorganism	Zone of Inhibition (mm)*				
	Pet. Ether Extract	Chloroform extract	Methanol Extract	Ciprofloxacin standard (100 mcg/ml)	Blank DMF
<i>Bacillus subtilis</i>	8.38 ± 0.37	6.5 ± 0.32	14.31 ± 0.27	19.16 ± 0.32	0
<i>Bacillus palmitus</i>	7.05 ± 0.26	-	15.16 ± 0.27	19.90 ± 0.16	0
<i>Staphylococcus aureus</i>	7.16 ± 0.18	-	12.21 ± 0.24	17.85 ± 0.26	0
<i>Pseudomonas aeruginosa</i>	8.21 ± 0.24	-	14.25 ± 0.22	19.06 ± 0.23	0
<i>Escherichia coli</i>	9.23 ± 0.25	-	15.26 ± 0.16	20.18 ± 0.29	0
<i>Klebsiella pneumoniae</i>	8.93 ± 0.20	6.21 ± 0.24	10.95 ± 0.12	17.83 ± 0.25	0

* Zone are Mean ± SD for n=6; - No zone of inhibition

Table 2: Antifungal activity of various extract of *Acanthaphora spicifera* against various fungal strains Amphotericin is used as reference standard.

Microorganisms (Yeast / Fungi)	Zone of Inhibition (mm)*				
	Pet. Ether Extract	Chloroform extract	Methanol Extract	Standard (100 mcg/ml)	Blank DMF
<i>Aspergilus niger</i>	3.28±0.52	-	13.42±0.56	16.26±0.42	0
<i>Microsporum gypseum</i>	6.42±0.48	2.8±0.4	15.12±1.06	24.18±0.52	0
<i>Candida albicans</i>	8.62±0.56	-	11.24±0.48	20.32±0.94	0

* Zone are Mean ± SD for n=6; - No zone of inhibition

Table 3: Minimum Inhibitory Concentration of Methanolic extract

Test Microorganisms	Zone of Inhibition (mm)					
	100 mcg/ml	500 mcg/ml	1 mg/ml	10 mg/ml	50 mg/ml	Standard*
<i>Bacillus subtilis</i>	10	11	11	15	16	19
<i>Bacillus Palmitus</i>	8	11	11	6	14	20
<i>Staphylococcus aureus</i>	7	5	7	11	12	18
<i>Pseudomonas aeruginosa</i>	7	6	10	11	15	19
<i>Escherichia coli</i>	9	11	10	11	15	17
<i>Klebsiella pneumoniae</i>	7	8	10	11	13	18
<i>Aspergilus niger</i>	7	-	9	-	11	17
<i>Microsporum gypseum</i>	6	-	8	11	12	18

* Standard for Antibacterial – Ciprofloxacin (100 mcg/ml). For antifungal – Amphotericin (100 mcg/ml).

- No zone of inhibition

extract showed higher activity against *Microsporum gypseum* (15.1mm)

The further study carried out only with methanolic extract. The minimum inhibitory concentration of methanol extract was tested against bacterial and fungal strains. The 100µg/ml concentration showed inhibitory effect against bacterial and fungal strains as shown in the table-3. As the concentration increases from 100µg/ml, 500µg/ml, 1mg/ml,

10mg/ml, 50mg/ml, the inhibitory effect also increases proportionality the maximum inhibitory effect absorbed with 50mg/ml of methanol extract as compare to standard drug. From the study its absorbed that if the crude extract is purified it could show similar activity when compare to standard antibacterial ciprofloxacin and antifungal agent amphotericin.

The active principles of algae are responsible for antibacterial and antifungal activity. However some algae extracts were unable to exhibit antibacterial and antifungal activity against tested bacterial and fungi strains. As suggested by these strains may have some kind of resistance mechanism e.g. enzymatic inactivation, target sites modification and decrease intracellular drug accumulation as the concentration of the compound used may not be sufficient [19]. As for the effectiveness of the extraction method some studies have shown that methanol extraction yield higher antimicrobial activity than n-hexane and ethyl acetate [20]. It is clear that the use of organic solvents always provides a higher efficiency in extracting antimicrobial active compound [21]. Our studies showed that the active compounds were present in all of the organic solvents used in the present study. It can be concluded that the methanol extract of algae possess a significant antibacterial and antifungal activities.

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