

Antibacterial activity of ethanol extracts of seaweeds against fish bacterial pathogens

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Abstract. – Crude extracts were prepared using the solvent ethanol from the seaweeds viz., *Gracilaria edulis*, *Calorpha peltada* and *Hydroclathres* sp. and screening for their antibacterial activity against six bacterial pathogens were also carried out. The test bacterial strains were *Escherichia coli*, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus faecalis* and *Bacillus cereus*. Ethanol extract of *Gracilaria edulis* inhibited growth of all the test organisms except *Bacillus cereus* and *Enterobacter aerogenes*. Seaweed extract of *Calorpha peltada* was found effective against a number of Gram negative and Gram positive bacteria such as *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus faecalis*. *Hydroclathres* sp. extract inhibited the growth of *Pseudomonas aeruginosa* only out of the six tested pathogens.

Key Words:

Marine algae, Fish bacterial pathogens, Antibacterial activity.

Introduction

Selective utilization of marine algae as potential source of pharmaceutical agents has been increasing in recent years. Many of the seaweeds possess bio-active components which inhibit the growth of some of the Gram positive and Gram negative bacterial pathogens. The algal extracts were used as a curative and preventive agent for various diseases such as antibiotics, anti-helminthics, cough remedies, antihypertensive, antitumour and antidiarrhoea. Recently we have embarked on the chemical investigation of marine algae with a special accent on their bioactive properties¹.

The antifouling activity of extracts of nine macro algae against bacteria, fungi, diatom, has

been investigated in relation to season in bi-monthly samples. Of the extracts tested 48.2% were active against at least one of the fouling organisms and of these extracts 31.2% were seasonally active with a peak of activity².

Most of the bioactive substances isolated from marine algae are chemically classified as brominated, aromatics, nitrogen-heterocyclic, nitrosulphuric-heterocyclic, sterols, dibutanoids, proteins, peptides and sulphated polysaccharides. The crude extract thus obtained is subjected to broad based biological screening for antifungal, antiviral, antibacterial, antimalarial, antifilarial, hypoglycaemic and antifertility activity³. On other hand, the algae are also used as food stuff, animal fodder, fertilizer, industrial material such as agar and minor medicines⁴. The green algae *Calorpha peltada* contains 1-4 diacetoxy butadiene and fatty esters which possess antibacterial, anti-ichthyotoxic and anti-hypertensive properties.

Antibacterial activity has been detected in a number of seaweeds collected from the coast of Mandapam to Kanyakumari. The predominantly found fish bacterial pathogens are *Streptococcus* sp., *Pseudomonas* sp., *Bacillus* sp., *Staphylococcus* sp., *Enterobacter* sp. and *Escherichia coli*. The slime layer that covers the outer surface of fish has been found to contain bacteriae viz., *Pseudomonas* sp., *Acinetobacter* sp., *Moraxella* sp., *Alkaligenes* sp., *Micrococcus* sp., *Serratia* sp., *Vibrio* sp. and *Bacillus* sp.⁵. The antimicrobial activity of the selected species of marine algae were not uniform. The highest antibacterial activity was found in the class Rhodophyceae (80%) followed by the Chlorophyceae (62.5%) and the Phaeophyceae (61.9%). The maximum antifungal activity was observed in the red algae 37%, brown algae 33.3% and green algae 8.3% activity. *Staphylococcus aureus* was the most susceptible bacterial pathogen followed by *Vibrio* sp.⁶.

Eleven species of seaweeds were tested for their activity against *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*. Acetone extracts of *Caulerjia cupressoides* showed maximum inhibitory activity against *Escherichia coli* and propanol extracts of *Gracilaria edulis* showed maximum inhibitory effect against *Klebsiella pneumonia*⁷ were observed.

Forty four species of seaweeds were screened for antibacterial activity. All the species with antibacterial activity were active against Gram positive bacteria whereas only two species *Asparagopsis taxiformis* and *Osmundea hybryda* were active against mycobacteria. The production of secondary metabolites with antimicrobial activities by the macro algae was also studied under different conditions, although no common trend for bioactivity was absorbed⁸.

Materials and Methods

Collection of Seaweeds

The algal samples viz., *Gracilaria edulis*, *Calorpha peltada* and *Hydroclothres* sp. were collected from the coast of Mandapam to Kanyakumari. The collected samples were cleaned with sea water and fresh water to remove all epiphytes and dried in room temperature and powdered as per the method⁹.

Preparation of Algal Discs

The crude extracts from the seaweeds were prepared by solvent extraction method using soxhlet apparatus (Oberoi Scientific Corporation, India). Ethanol was used as solvent. The crude extracts were kept for evaporation to remove the solvent by intermittent shaking. The crude extracts were transferred to air tight containers and stored in a refrigerator for further usage. 6 mm diameter of Whatman No. 1 sterile filter paper discs were impregnated. Control discs were prepared by just loading the sterile discs with the solvent ethanol alone. The positive control discs were prepared by the impregnation of the sterile discs with 0.1% concentration of the ampicillin antibiotics. Triplicates were maintained for all the tests, including controls, their mean and standard error were calculated and tabulated.

Isolation and Identification of Fish Pathogens

Samples were collected from the infected fish using sterile swabs. After the collection of patho-

genic bacteria from fish they were lawn cultured on the blood agar, eosine methylene blue (EMB), *Pseudomonas* isolation agar and *Bacillus* selective agar.

After incubation the morphology and biochemical activities of the colonies were observed and identified as *Escherichia coli*, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus faecalis* and *Bacillus cereus*¹⁰.

Antibacterial Assay

The sterilized Petri plates were poured with Muller Hinton agar medium and labelled. The 0.1 ml of identified test organisms viz., *Escherichia coli*, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus faecalis* and *Bacillus cereus* were inoculated and spreaded on the agar medium using sterilized "L" rod so as to make lawn. The agar surface was allowed to dry for five minutes. Then the prepared algal extract impregnated discs were placed over the agar using sterilized forceps. After 24 hours of incubation at 37°C the zone of inhibition was measured and tabulated.

Results

The ethanol extract at 1% concentration of *Gracilaria edulis* produced a maximum zone of 13.7 mm against *Staphylococcus aureus* and a minimum zone of 3.1 mm against *Enterobacter aerogenes*. The 1% concentration of ethanol extract from *Calorpha peltada* produced a maximum zone of 14.9 mm against *Streptococcus faecalis* and minimum zone of 1.4 mm against *Enterobacter aerogenes*. The extract of 1% concentration from *Hydroclothres* sp produced maximum zone of 14.9 mm against *Pseudomonas aeruginosa* and minimum zone of 1.3 mm against *Enterobacter aerogenes*. However, no antibacterial activity was found against *Bacillus cereus*.

In 0.3% concentration of *Gracilaria edulis* and *Calorpha peltada* no antibacterial activity against *Bacillus cereus* was observed. The ethanol extracts of *Calorpha peltada* at 0.3% and 0.6% concentration also not showed an inhibitory activity against *Enterobacter aerogenes*.

The ethanolic extract of *Hydroclothres* sp. of 0.3%, 0.6% and 1% concentrations didn't provoke a zone of inhibition against *Bacillus*

Table 1. Showing zone of inhibition of various crude algal extracts against test pathogens.

Test algae	Concentration (%)	Bacterial pathogens showing zone of inhibition (mm)						
		<i>Escherichia coli</i>	<i>Bacillus cereus</i>	<i>Enterobacter aerogenes</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus faecalis</i>	
Gracilaria edulis	0.3	10.5 ± 0.5	-	1.5 ± 0.2	9.9 ± 0.5	12.5 ± 0.6	9.1 ± 0.4	
	0.6	11.1 ± 0.3	1.9 ± 0.1	1.9 ± 0.2	10.5 ± 0.7	13.0 ± 0.6	10.8 ± 0.5	
	1.0	11.9 ± 0.2	4.2 ± 0.3	3.1 ± 0.3	11.2 ± 0.9	13.7 ± 0.7	12.1 ± 0.5	
Calorpha peltada	0.3	12.0 ± 0.4	-	-	6.6 ± 0.3	12.2 ± 0.4	9.8 ± 0.3	
	0.6	12.5 ± 0.6	1.1 ± 0.2	-	7.1 ± 0.3	12.6 ± 0.5	11.2 ± 0.4	
	1.0	13.2 ± 0.7	2.7 ± 0.2	1.4 ± 0.2	7.8 ± 0.4	13.1 ± 0.3	14.9 ± 0.6	
Hydroclathres sp.	0.3	5.9 ± 0.2	-	-	13.4 ± 0.8	7.9 ± 0.2	1.2 ± 0.5	
	0.6	6.3 ± 0.3	-	-	14.1 ± 0.8	8.5 ± 0.2	8.5 ± 0.2	
	1.0	6.8 ± 0.4	-	1.3 ± 0.2	14.9 ± 0.9	9.2 ± 0.3	5.2 ± 0.2	
Positive control	0.1% Ampicillin impregnated discs	14.1 ± 0.3	12.5 ± 0.4	13.3 ± 0.2	14.6 ± 0.3	14.2 ± 0.5	13.6 ± 0.2	
Control (Ethanol saturated sterile discs)	-	-	-	-	-	-	-	

-, No activity; Mean ± S.E (standard error).

cereus and *Enterobacter aerogenes*. The *Bacillus cereus* and *Enterobacter aerogenes* were found resistant against all the tested algal extracts (Table I).

The ethanol impregnated negative control discs showed no zone of inhibition against all the test bacterial pathogens and the 0.1% concentration of ampicillin impregnated positive control discs showed 14.1, 12.5, 13.3, 14.6, 12.8 and 13.6 mm for *Escherichia coli*, *Bacillus cereus*, *Enterobacter aerogenes*, *Staphylococcus aureus* and *Streptococcus faecalis* respectively.

Discussion

Antibacterial activity of red, brown and green algae against both Gram positive and Gram negative bacteria has been established by several scientists. The best antibacterial activity was recorded with *Gracilaria edulis*¹¹ extract. *Calorpha peltada* was showed inhibitory activity against *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus faecalis*¹².

Methanolic extracts of six marine algae belongs to Rhodophyceae (*Corallina officinalis*), Phaeophyceae (*Cystoseria barbata*, *Dictyota dichotoma*, *Halopteris filicina*, *Cladostephus spongiosus*, *F. verticillatus*) and Chlorophyceae (*Vulva rigida*) from North Aegean sea (Turkey) were studied for the antibacterial activity against pathogenic microbes, three Gram positive bacteria viz. *Staphylococcus aureus*, *Micrococcus luteus*, *Enterococcus faecalis* and three Gram negative bacteria viz. *Escherichia coli*, *Enterobacter aerogenes*, *Escherichia coli* (O157:H7) *in vitro*. Extracts of the test marine algae except *Clostridium officinalis* showed inhibition against *Staphylococcus aureus* and highest inhibition activity among all the extracts was shown to *Enterobacter aerogenes*¹³.

In the present work ethanolic extract of *Gracilaria edulis* inhibited most of the tested bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus faecalis*. The antibacterial activity of *Hydroclathrus* sp. extracts against the same test organisms was proven to be effective against *Pseudomonas* sp. alone⁷. The test algae showed more or less equal zone of inhibition or slightly greater against some pathogens when compared with positive control ampicillin impregnated positive control discs.

Crude extracts prepared using the solvent acetone from the seaweeds of the family Chlorophyceae, Rhodophyceae and Phaeophyceae were already screened for the antibacterial activity against following pathogens: *Staphylococcus aureus*, *Proteus vulgaris*, *Salmonella typhi*, *Vibrio cholerae*, *Escherichia coli* and *Citrobacter* sp.¹⁴. Similar results regarding the activity against *Staphylococcus aureus* and *Escherichia coli* were observed in the present study.

The antibacterial and antifungal activity of 82 marine algae were analyzed from crude extracts of fresh and lyophilized samples against three Gram positive, two Gram negative bacteria and one yeast using agar diffusion technique¹⁵. The highest percentage of active taxa was found in Phaeophyceae (84%) followed by Rhodophyceae (67%) and Chlorophyceae (44%). *Bacillus cereus* was the most sensitive test microorganisms and *Pseudomonas aeruginosa* was the most resistant, but in the present study *Pseudomonas aeruginosa* along with *Staphylococcus aureus*, *Streptococcus faecalis* and *Escherichia coli* showed high sensitivity.

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