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Antibacterial and Antioxidant activity of Marine Seaweeds.

N. Hemashenpagam[1]*, S. Aswathy[1], T. Selvaraj[2] and A. Panneerselvam[3]

[1]Department of Microbiology, Hindustan College of Arts and Science, Coimbatore - 641028, Tamil Nadu, India.
[2]Department of Plant Life Sciences, Faculty of Agricultural Science, AMBO University, Ethiopia.
[3]Department of Botany and Microbiology, A.V.V. M. Sri Pushpam College (Aut), Poondi - 613503, Thanjavur, Tamil Nadu, India.

Abstract

The potentials of antimicrobial activity and antioxidant property of eight seaweeds were studied. The seaweeds weres tested for inhibition against human pathogens (*Staphylococcus aureus, Pseudomonas aeruginosa*) from clinical samples. The solvent extract of *Turbinaria ornata* showed maximum zone of inhibition against *Staphylococcus aureus.* Methonal extracts showed larger zones of inhibition than hexane *Ulva fenestrata and Turbinaria ornate* showed higher radical scavenging activity than other algae.

Keywords: antibacterial activity, antioxidant, hydrogen peroxide, MIC, seaweed

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years. An impressive number of modern drugs have been isolated from medicinal plants mainly based on their use in traditional medicine. Seaweeds or benthic marine algae are the oldest members of the plant kingdom, extending back many hundreds of millions of years. They have little tissue differentiation, no true vascular tissue, no roots, stems, or leaves, and no flowers. Algae range in size from microscopic individual cells to huge plants more than 100 feet long. Seaweed lives either in marine or brackish water environment. Seaweed is high in potassium which is a plus for cancer patients. They are rich in Calcium which has been shown to help fight colon cancer. Seaweed and vegetables are natural detoxifiers. Seaweed and algae can serve as excellent sources of minerals such as iron, iodine, potassium, and trace minerals and they are used as food, medicinal source etc.

Nowadays, the use of antibiotics has increased significantly due to heavy infections and the pathogenic bacteria becoming resistant to drugs is common due to indiscriminate use of antibiotics. It becomes a greater problem of giving treatment against resistant pathogenic bacteria. Moreover the cost of the drugs is high and also they cause adverse effect on the host, which include hypersensitivity and depletion of beneficial microbes in the gut (Idose *et al.*, 1968). Decreased efficiency and resistance of pathogen to antibiotics has necessitated the development of new alternatives. Many bioactive and pharmacologically important compounds such as alginate, carrageen and agar asphycocolloids have been obtained from seaweeds and used in medicine and pharmacy (Siddhanta *et al.*, 1997).Fatty acids are isolated from micro algae that exhibited antibacterial activity. Many workers revealed that the crude extracts of Indian seaweeds to the reactive against Gram-positive bacteria (Kandaswamy, 2008). Methanol extracts of fifty-six seaweeds collected from South African coast, belonging to Chlorophyceae, Phaeophyceae and Rhodophyceae showed antibacterial activity with members of Phaeophyceae showing the highest antibacterial activity (Vlachos *et al.*, 1997).

Minimum inhibitory concentration (MIC, is the lowest concentration of antimicrobial that will inhibit the visible growth of microorganisms after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agent (Andrews 2001).

A free radical is a molecule with one or more unpaired electrons in the outer orbital. Many of these free radicals, in the form of reactive oxygen and nitrogen species, are an integral part of normal physiology. Free-radical scavengers are antioxidants which can provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species and subsequent lipid peroxidation, protein damage and DNA strand breaking . The most commonly used synthetic antioxidants at present are butylated hydoxy anisole (BHA), butylated hydoxy toluene (BHT) Propylgallate (PG) and test butylatedhydroquinone. However, these synthetic antioxidants have side effects such as liver damage and carcinogenesis (Sherwin *et*

*Corresponding Author
email: *nhema10@yahoo.co.in*

al., 1990). Therefore, there is a need for isolation and characterization of natural antioxidant having less or no side effects, for use in foods or medicinal materials to replace synthetic antioxidants. Marine algae have received special attention as a source of natural antioxidants recently. Seaweeds are known source of pharmacological and food additives with potential health effects like antioxidative and anticarcinogenic (Jayanta *et al.*, 2008).

In this investigation extracts of eight types of marine algae such as Chlorphyceae (*Ulva fenestrata)*, Rhodophyceae (*Gracillaria, Kappaphycus alvarezii, Kappaphycus weighii*) and Phaeophyceae (*Sargassum wightii, Turbinaria conoides, T.ornata*) were studied against pathogenic microbes (*Pseudomonas aeruginosa Staphylococcus aureus)* and minimum inhibitory concentration was determined. and *in vitro* antioxidant activity was determined for two genera *Ulva fenestrata* and *Turbinaria ornata* using standard procedures.

MATERIALS AND METHODS

Collection of seaweed

Seaweeds were collected from Rameswaram coastal area, Tamilnadu and Orissa coastal area, India. The seaweeds were transported to the laboratory in sterile polythene bags at 0°C temperature. Algal samples were cleaned of epiphytes and extraneous matter, and necrotic parts were removed. Plants were washed with seawater and then in fresh water.

Solvent extraction

Preparation of methanol and hexane extracts of seaweed

In the laboratory, samples were rinsed with sterile distilled water and were shade dried. The samples were dried at room temperature, powdered, and sieved. They were then cut into small pieces and powdered in a mixer grinder. The powder was dissolved with methanol (1:10w/v) and hexane and kept at room temperature for overnight than the extract was filtered through a filter paper. The filtered extract was concentrated by steam distillation .The crude extracts were tested for their antibacterial activity against the pathogens. The powder extract was then dried at room temperature. The dried sample was dissolved in double distilled water and stored in -20 °C for further use.

Isolation and identification of human pathogens from clinical specimen:

Staphylococcus aureus and *Pseudomonas aeruginosa* were isolated from infectious pus, throat swab and blood sample from the clinical specimens from a diagnostic laboratory, Bangalore and preserved at low temperature. The bacterial isolates were identified by gram staining and biochemical characterization.

Antimicrobial assay:

Muller Hinton agar plates were prepared and inoculated with one ml culture per 20 ml in molten media and poured into a Petridish, Wells were created using 6mm cork borer. 50µl sample was added to the wells and with solvent as the control. The plates were kept for incubation at 37°C for 24 hours. Methonal and Hexane were used as the negative control for the antimicrobial assay. Chloramphenical antibiotics disc were used as positive control.

Minimum Inhibitory concentration:

Muller Hinton agar plates were prepared and inoculated with one ml of culture per 20 ml in molten media and poured on Petri dish, wells were created using 6mm cork borer and 10, 20, 30, 40 and 50µl extracts were added into the wells and kept for incubation. The minimum inhibitory concentrations were obtained.

Antioxidant activity of seaweeds by measurig H_2O_2 scavenging activity:

Ability of the seaweed extracts to scavenge hydrogen peroxide was determined as described by Gulcin *et al.* (2004). One ml of the extract was rapidly mixed with 2 ml of 40 mM phosphate buffered (pH 7.4) hydrogen peroxide solution. The absorbance was measured at 230 nm in the UV spectrophotometer after 10 minutes of incubation at 37ºC against a blank (without hydrogen peroxide).The positive control (ascorbic acid 5mg/ml) was also maintained and checked for OD at 230nm.

The percentage of scavenging of hydrogen peroxide was calculated using the following formula.

$$\text{Percentage} = A1/A0 \times 100$$

(A0 – Absorbance of control; A1 – Absorbance of sample)

RESULT AND DISCUSSION

Isolation of *Staphylococcus aureus* and *Pseudomonas aeruginosa* from clinical samples:

Staphylococci aureus and Pseudomonas aeruginosa were isolated from the infectious pus, blood, throat swab of the samples (Table1).

Antimicrobial activity of seaweeds against *Staphylococcus and Pseudomonas*:

Marine Seaweeds were checked for the antibacterial activity against the human pathogens and the results showed that they are effective against only Gram positive bacteria and not for the Gram negative bacteria. Antibacterial test result indicated that among the eight algal sample, only two sample *Turbinaria ornata* and *Ulva fenestrata* showed antibacterial activity against *Staphylococcus aureus* in the preliminary assay

(50µl) and only methanolic extracts were very effective (Table 2). The methanolic and hexane extracts of *Turbanaria ornata* showed zones of inhibitions against *Staphylococcus aureus* .The zone diameter was15mm for methanolic extract and 9mm for hexane extract. The methanol extract of *Ulva fenestrata* showed zones of inhibitions of 12mm diameter. *Sargassum wightii* showed an inhibitions zone of 9mm and hexane extracts of *Turbinaria ornata, Sargassum wightii* showed low activity.

Antimicrobial activity with standard antibiotics:

Staphylococcus aureus and *Pseudomonas aeruginosa* were checked with the standard antibiotic chloramphenicol which showed zone of inhibitions of 16mm, 20 mm diameter respectively.

Table 1. Results of the biochemical test on clinical samples

S. No.	Biochemical test	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
1	Indole	-	-
2	Methyl red	+	-
3	Voges Proskauer	+	-
4	Citrate	-	+
5	Oxidase	+	+
6	Catalase	+	+
7	TSI		K/K
8	Nitrate Reduction Test	+	–
9	Gelatin	+	–

Minimum inhibitory concentration:

The results suggest that 50µl is the minimum inhibitory concentration (MIC) for the three algae which showed antibacterial activity.

Antioxidant activity by measuring H_2O_2 scavenging activity:

Antioxidant activity was measured by adding H_2O_2 to the 1ml test sample and positive control. The results showed that the antioxidant activity was higher in *Ulva fenestrata* and *Tubanaria ornata*.

Table 3. Radical Scavenging Activity of Marine seaweeds with Ascorbic acid as the control (1 gm in 10ml)

S. No.	Concentration(µg)	Volume of sample (ml)	Volume of a H_2O_2 added (ml)	OD at 230nm
1	20 (CONTROL)	0.2	0.8	0.433
2	40 (CONTROL)	0.4	0.6	0.514
3	60 (CONTROL)	0.6	0.4	0.548
4	80 (CONTROL)	0.8	0.2	0.640
5	100(CONTROL)	1.0	-	0.670
6	*Ulva fenestrata*	0.5	0.5	3.0
7	*Turbinaria ornata*	0.5	0.5	3.0
8	*Sargassum wightii*	0.5	0.5	1.67

From the results given in table 3 it is inferred that the antioxidant activity was higher in the seaweeds when compared to ascorbic acid .

Table 2. Antimicrobial activity of methonal and hexane extracts of seaweeds against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

S. No.	Name of the seaweeds	Zone of inhibition in diameter (mm)			
		Methanol extract		Hexane extract	
		Staphylococcus aureus	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
1	*Turbinaria conoides*	No zone	No zone	No zone	No zone
2	*Turbinaria ornata*	15mm	No zone	9mm	No zone
3	*Sargassum wightii*	9mm	No zone	No zone	No zone
4	*Gracillaria folifera*	No zone	No zone	No zone	No zone
5	*Gracillaria verucosa*	No zone	No zone	No zone	No zone
6	*Kappaphycus strian1*	No zone	No zone	No zone	No zone
7	*Kappaphycus strain2*	No zone	No zone	No zone	No zone
8	*Ulva fenestrata*	12mm	No zone	8mm	No zone

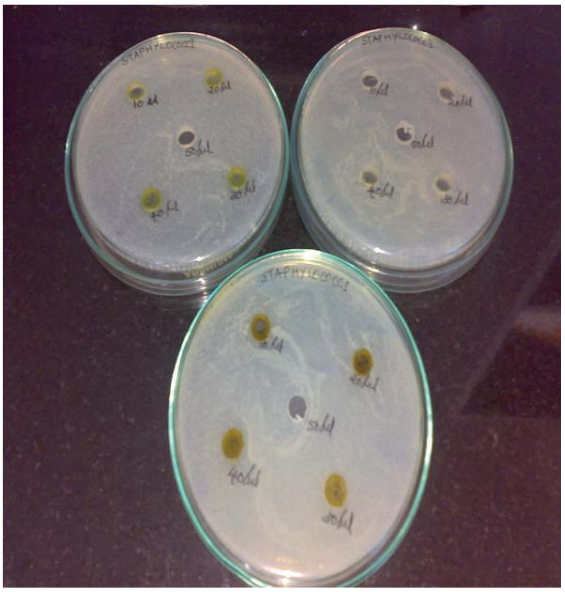
Table 4. Hydrogen peroxide scavenging activity of seaweeds based on time intervals

S. No.	Time intervals	Positive control OD	*Ulva fenestrata* OD	*Turbanaria ornata* OD	*Sargassum wightii* OD
1	1hr	2.514	3	3	1.67
2	2hr	2.599	3.1	3.5	1.88
3	3hr	2.768	3.24	3.8	1.9
4	4hr	2.89	3.33	3.90	1.98
5	5hr	2.90	3.56	4.2	2.00
6	6hr	2.99	3.66	4.6	2.11

Higher scavenging activity was observed in *Turbinaria ornata* after 6hr of incubation. (Table 4)

DISCUSSION

The pathogenic organisms tested were more susceptible to the crude extracts of algae used. In the present investigation, extracts of a marine algal species were tested against the bacterial pathogens by agar diffusion method and antioxidant property of H_2O_2 radical scavenging were determined. The results of preliminary screening tests were summarized which revealed that the three algal species possess antibacterial activity. The results of the present study were similar to that of Tuney *et al.*, (2006). The gram-positive bacteria were more effectively controlled by the extracts of algae when compared to the Gram-negative bacteria. Taskin *et al.*, (2001), also made similar observations, indicating that the more susceptibility of Gram-positive bacteria to the algal extracts might be due to the differences in their cell wall structure and their composition, acting as barriers to many environmental substances including antibiotics (Tortora *et al.*, 2001) is the presence of thick murine layer in the cell wall also prevents the entry of the inhibitors.



The ability of seaweeds to scavenge H_2O_2 was determined according to the method of Ruch and Gulcin *et al.*, which indicated that *Turbinaria ornata* (.o5µg/ml) has a maximum H_2O_2scavenging activity. Rao and Parekh (1981) tested the extracts of *Enteromorpha intestinalis* and *G. corticata* for antibacterial activity. They found that the algae were active throughout the year with a peak during the winter season. In the investigation *Turbinaria ornata* showed high antibacterial activity and high H_2O_2 radical scavenging activity. From the present investigation, it was observed that *Turbinaria ornata* could be used as antibacterial agent for the effective control of pathogens and as antioxidant to preserve the food products. Differences between the results of the present investigation and results of other studies might be due to the production of bioactive compounds related to the seasons, extractions, methods and organic solvents used for extraction..Finally it can be concluded from the study that extracts of algal species used in the present investigation showed better antibacterial activity against the pathogens studied. So they might be potential sources of bioactive compounds and should be investigated further to identify and purify these antibacterial substances.

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