

Full Length Research Paper

Evaluation of *in vitro* antibacterial property of seaweeds of southeast coast of India

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The *in vitro* antibacterial activities of seaweeds belong to Chlorophyceae (*Caulerpa racemosa* and *Ulva lactuca*), Rhodophyceae (*Gracillaria folifera* and *Hypneme muciformis*) and Phaeophyceae (*Sargassum myricocystum*, *Sargassum tenneerimum* and *Padina tetrastomatica*) were studied against both Gram-negative and Gram-positive pathogenic bacteria. Methanolic extracts of all seaweed extracts tested in the present study exhibited broad spectrum of antibacterial activity. Chlorophyceae members showed high antibacterial activity than other members of the algae tested in the present investigation. *Escherichia coli* alone resistant to all the seaweed extracts except *S. tenneerimum*. Results of the present study confirmed the potential use of seaweed extracts as a source of antibacterial compounds.

Key words: Seaweeds, antibacterial activity, methanolic extracts, pathogenic bacteria.

INTRODUCTION

Bacterial infection causes high rate of mortality in human population and aquaculture organisms. For an example, *Bacillus subtilis* is responsible for causing food borne gastroenteritis. *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* cause diseases like mastitis, abortion and upper respiratory complications, while *Salmonella* sp. causes diarrhea and typhoid fever (Leven, 1987; Jawetz et al., 1985). *P. aeruginosa* is an important and prevalent pathogen among burned patients capable of causing life-threatening illness (Boyd, 1955). Preventing disease outbreaks or treating the disease with drugs or chemicals tackles these problems. Nowadays, the use of antibiotics 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 (Sieradzki et al., 1999). 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 (Smith et al., 1994). Many bioactive and pharmacologically important compounds such as alginate, carrageen and agar as phycocolloids have been obtained from sea-weeds and used in medicine and pharmacy (Siddhanta et al., 1997). Fatty acids are isolated from micro algae that exhibited antibacterial activity (Kellam et al., 1988). Many workers revealed that the crude extracts of Indian seaweeds are active against Gram-positive bacteria (Rao and Parekh, 1981). Methanolic extracts of fifty-six sea-weeds collected from South African coast, belonging to Chlorophyceae, Phaeophyceae and Rhodophyceae showed antibacterial activity. Among them, Phaeophyceae members showed highest antibacterial activity (Vlachos et al., 1997).

In this investigation, antibacterial activity of seven marine algae belonging to families such as Chlorophyceae (*Caulerpa racemosa* and *Ulva lactuca*), Rhodophyceae (*Gracillaria folifera* and *Hypneme muciformis*) and Phaeophyceae (*Sargassum myricocystum* and *Sargassum tenneerimum* and *Padina tetrastomatica*) was studied against pathogenic microbes (*Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Escherichia coli*, *Pseudomonas*

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Table 1. Antibacterial of different algal extracts (10 µl/disc) against pathogenic bacteria.

Bacteria	Inhibiting zone diameter in mm (mean ± SD)							Positive control- Chloramphenicol
	<i>C. racemosa</i>	<i>U. lactua</i>	<i>G. folifera</i>	<i>H. musciformis</i>	<i>S. teneeriumum</i>	<i>S. myriocysterm</i>	<i>P. tetrastomatica</i>	
<i>K. pneumonia</i>	15 ± 0.41	12 ± 0.51	14 ± 0.26	13 ± 0.59	8 ± 0.45	13 ± 0.58	12 ± 0.54	9 ± 0
<i>E. aerogens</i>	15 ± 0.32	15 ± 0.56	12 ± 0.54	12 ± 0.31	8 ± 0.65	13 ± 0.59	15 ± 0.52	10 ± 0
<i>E. coli</i>	ND*	ND*	ND*	ND*	12 ± 0.87	ND*	ND*	14 ± 0
<i>P. aeruginosa</i>	14 ± 0.26	13 ± 0.78	13 ± 0.65	12 ± 0.95	8 ± 0.31	12 ± 0.95	13 ± 0.54	11 ± 0
<i>M. luteus</i>	14 ± 0.35	14 ± 0.65	13 ± 0.35	13 ± 0.54	14 ± 0.69	12 ± 0.31	14 ± 0.26	10 ± 0
<i>E. faecalis</i>	15 ± 0.51	12 ± 0.83	14 ± 0.51	12 ± 0.75	12 ± 0.43	14 ± 0.35	13 ± 0.59	12 ± 0
<i>S. aerus</i>	16 ± 0.36	17 ± 0.38	14 ± 0.32	12 ± 0.69	12 ± 0.54	14 ± 0.26	13 ± 0.35	10 ± 0
<i>S. faecalis</i>	12 ± 0.61	14 ± 0.65	10 ± 0.32	14 ± 0.83	11 ± 0.23	14 ± 0.35	ND*	11 ± 0
<i>B. subtilis</i>	15 ± 0.89	14 ± 0.51	12 ± 0.31	12 ± 0.65	15 ± 0.65	13 ± 0.78	12 ± 0.54	12 ± 0

ND*: Antibacterial activity was not detected.

aeruginosa, *Micrococcus luteus*, *Enterobacter faecalis*, *Streptococcus faecalis*, *Staphylococcus aureus* and *Bacillus subtilis*).

MATERIALS AND METHODS

Marine algae were collected by hand picking from the submerged marine rocks at Koodankullam village, Tirunelveli District of Tamil Nadu, India during low tide. Seaweeds were collected during May 2007 and were identified by Dr. J. Ramalingam, senior scientist, Centre for Marine Fisheries Research Institute, Mandapam, Tamil Nadu, India.

Extract preparation

Algal samples were cleaned of epiphytes and extraneous matter, and necrotic parts were removed. Plants were washed with seawater and then in fresh water. The seaweeds were transported to the laboratory in sterile polythene bags at 0°C temperature. In the laboratory, samples were rinsed with sterile distilled water and were shade dried, cut into small pieces and powdered in a mixer grinder. The algal powdered samples were extracted in methanol thrice by soaking for overnight at room tempera-

ture. The extracts from three consecutive soaking were pooled and evaporated under reduced pressure. The residues (crude extracts) obtained were finally dried under vacuum pressure. The crude extracts were tested for their antibacterial activity against the pathogens used in the present investigation.

Test organisms

Test microorganisms were clinical isolates and maintained in the laboratory of School of Biological Sciences, Madurai Kamaraj University, Madurai Tamil Nadu, India. The pathogenic bacteria were cultured individually on Muller-Hinton broth at 37°C for 18 h, before inoculation for assay. 100 µl of broth culture, which contain $10^7 - 10^8$ number of bacteria per ml was added to Tryptic soy agar medium (Hi-media) and poured to sterile petri dishes and allowed for solidification.

Antibacterial assay

Antibacterial activity was evaluated by agar diffusion method (Bauer et al., 1966). 20 mg of crude extract was dissolved in 1 ml of 20% of DMSO. From this stock solution 10 µl of each extract was loaded on sterile antibiotic discs

(6 mm diameter) (Hi-media company) and air-dried. After drying, discs were placed on the Tryptic soy agar. Chloramphenicol antibiotic disc and disc loaded with 10 µl of respective solvent were used as positive and negative control respectively.

RESULTS

In the present investigation, extracts of a marine algal species were tested against the bacterial pathogens by agar diffusion method. The results of preliminary screening tests were summarized in Table 1, which revealed that all algal species possess antibacterial activity.

Crude extracts of all tested algae exhibited high antibacterial activity against the pathogens tested (Table 1). All the crude extracts of algae inhibited the growth of all the pathogens except one or two bacterial pathogens. Crude extract of *U. lactua* showed high inhibiting activity against *S. aureus* (17 mm), Bacterial pathogen *E. coli* was resistant to all extracts tested in the present study except that of *S. teneenimum* which showed inhibiting

zone diameter of 12 mm (Table 1). *G. foliferae* and *S. teneerimum* showed lowest inhibiting activity on *E. faecalis*, *K. pneumoniae* and *E. aerogens* and diameter of the inhibition zone was 8 mm (Table 1).

Gram-positive bacteria were more sensitive than Gram-negative bacteria. Among Gram-positive bacteria *S. aureus* was more susceptible to all the algal extracts. *S. aureus* growth was highly inhibited by the extract of the *U. lactuca* and *C. racemosa*: the diameter of the inhibition zone of respective algal extracts was 17 and 16 mm, respectively. Next to *S. aureus*, *B. subtilis* was susceptible to all the extract of algae used in the present study (Table 1).

DISCUSSION

Hornsey and Hide (1974) reported that 151 species of marine algal crude extracts showed inhibitory activity against pathogenic bacteria. But variation in antibacterial activity may be due to the method of extraction, solvent used in extraction and season at which samples were collected. They also reported that *G. corticata* did not show antibacterial activity. In contrast to our investigation, the results showed that methanolic extract of *G. foliferae* inhibiting the growth of all pathogenic bacteria except *E. coli*. 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.

Perez et al. (1990) observed that the extract of *Ulva lactuca* had no antibacterial activity. In contrast, results of our study showed that *U. lactuca* inhibited all the test organisms except *E. coli*. This difference in results may be due to time and place sample collection. In 2001, Gonzalez del val et al. (2001) reported that the methanolic extract of *Padina pavonica* showed antibacterial activity only against *B. subtilis*. However, in our study the methanol extract of *P. tetrastromatica* inhibited the growth of *K. pneumoniae*, *E. aerogens*, *M. lectues*, *E. faecalis*, *S. aureus*, *P. aeruginosa* and *B. subtilis*. It was unable to inhibit the growth of *E. coli* and *S. faecalis*. In contrast, Tuney et al. (2006), reported that acetone, methanol, and diethyl ether extracts of *P. pavonica* had no antibacterial activity, but ethanol extract of *P. pavonica* showed weak activity against *E. faecalis*, *P. aeruginosa* and *E. coli*.

Different species of marine algae were collected and analyzed for their antibacterial activity from different parts of the world. Reichelt and Borowitzka (1984) and Salvador et al. (2007), screened many species of algae for their antibacterial activity. They reported that the members of the red algae exhibited high antibacterial activity. In contrast, green algae (chlorophyceae) were the most active. In this study *Caulerpa racemosa* and *Ulva lactuca* members of green algae were more active compared to other groups of algae screened for their antibacterial activity. *Ulva lactuca* inhibited the growth of *K.*

pneumoniae actively and produced 17 mm diameter inhibition zone.

In the present study, it was observed that *E. coli* was resistant to methanol extract of all algae except *S. teneerimum*. Ozdemir et al. (2006). Salvador et al. (2007) also reported that *E. coli* was resistant to the extracts of *Dictyota dichotoma*, *Malopteris filicine*, *Cladostephus spongiosus*, *F. verticillatus* and *Ulva rigida*. The results of Tuney et al. (2006) also coincided with our study. They reported the antibacterial activity of the methanolic extracts of *U. rigida*, *E. Linza*, *P. pavonica*, *C. sniosa*, *D. Linearis*, *D. membranacea*, *C. mediterranea*, *E. siliculosus*, *C. rubrum*, *G. gracilis* and *A. nojadiformis*. They also reported that diethyl ether extract of the above mentioned algae inhibited the growth *E. coli*. It may be due to the diethyl ether used as an organic solvent used for extraction. Diethyl ether provides a higher efficiency in extracting compounds for antimicrobial activities (Febles et al., 1995; Lima-Filho et al., 2002).

The results of the present study revealed that Gram-positive organisms were more susceptible to the crude extracts of algae used. Tuney et al. (2006) also reported that Gram-positive bacteria were more effectively controlled by the extracts of algae used in their study compared to Gram-negative bacteria. Taskin et al. (2001), also made similar observations, indicating that the more susceptibility of Gram-positive bacteria to the algal extract was due to the differences in their cell wall structure and their composition (Paz et al., 1995). In Gram-negative bacteria, the outer membrane acts as a barrier to many environmental substances including antibiotics (Tortora et al., 2001). The presence of thick murine layer in the cell wall also prevents the entry of the inhibitors (Martin, 1995).

Differences between the results of the present investigation and results of other studies may be due to the production of bioactive compounds related to the seasons, method, organic solvents used for extraction of bioactive compounds and differences in assay methods. Finally it can be concluded from the study that extracts of algal species used in the present investigation showed better antibacterial activity against pathogens used. They are potential sources of bioactive compounds and should be investigated for natural antibiotics. But further research should be made to identify and purify these antibacterial substances.

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