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Research Article

ANTI-PATHOGENIC ACTIVITY OF GREEN SEAWEEDS (CHLOROPHYCEAE) FROM THE COAST OF RAMANATHAPURAM, TAMILNADU

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Abstract

The present study mainly focused on green seaweeds were collected from Keelakarai, Ramanathapuram district, Tamil Nadu. The antibacterial activity of ethanol extract of four green seaweeds Caulerpa sertularioides, Enteromorpha intestinalis,Ulva lactuca, Ulva reticulata were tested against nine human bacterial pathogens viz., Bacillus cereus, Escherichia coli, Enterococcus faecalis, Klebsiella pneumonia, Micrococcus luteus, Pseudomonas aeruginosa, Pseudomonas aureus, Salmonella typhi and Staphylococcus aureus were analysed. When compared positive control, ethanol extracts of four green seaweeds activity were considered as good.The results revealed,the present study confirmed the potential use of green seaweeds as a good source of antibacterial agent.

Key Words: Antipathogenic, Anti bacterial activity, Seaweed, Chlorophyceae and Human pathogen

INTRODUCTION

The marine ecosystem is the treasure place for many natural resources (Anandhanand Sornakumari, 2011). Marine seaweeds aresimplest group of marine algae where they are present nearby Seashore and in rock regions of breaches. They inhabitingthe intertidal zones of estuaries, lagoonsand inthe seaacross theworldplayanimportantrolein the marine ecosystems.Seaweeds are among the important marine living resources withtremendous commercial application. Seaweeds have been traditionally used in human and animal nutrition. Seaweeds are rich source of bioactive compounds such as carotenoids, dietary fibres, protein, essential fatty acids, vitamins and minerals. Important polysaccharides such asagar, alginates and carrageenans obtained from seaweeds are used in pharmaceutical as well asin the food industries (Bocanegra*et* al., 2009).Most of the secondary metabolites produced byseaweeds have bacteriocidal or the antimicrobial compounds derived from seaweeds consist ofdiverse groups of bacteriostatic properties brominates phenols, oxygen heterocyclic, terphenols,sterols, polysaccharides, dibutenolides peptides and proteins. Although most of the antibioticsfound from terrestrial sources are used as therapeutic agents to treat various diseases, the oceanshave enormous biodiversity and potential to provide novel compounds with commercial value(Hay, 1996; Smit, 2004).

The antimicrobial activity was regarded as an indicator to detect the potent pharmaceutical capacity of macro algae for its synthesis of bioactive secondary metabolites (Gonzolez*et* al., 2001; Smit, 2004). Seaweeds contain several bioactive compounds like antiviral, antibacterial, antifungal, antioxidant and hypertensive properties. Since the finding of antibacterial and antifungal activities in many species of marine algae from different part of the world and the isolation of some bioactive compounds from them (Hornsey and Hide, 1974; Reichelt and Borowitzka, 1984). There are number of reports regarding the medicinal importance of seaweeds belongingto Chlorophyceae, Phaeophyceae and Rhodophyceae from the corners of the world(Kandhasamyand Arunachalam, 2008; Val *et al.*, 2001; Srivastava*et al.*, 2010; Rao*et al.*, 1982; Reichelt and Borowitzka, 1984; Zheng*et al.*, 2001; Tuney*et al.*, 2006; Selvaraj*et al.*, 2006; Taskin*et al.*, 2007; Salvador *et al.*, 2007; Margret *et al.*, 2008). The present study is also intended to evaluate antibacterial activity of four green seaweeds (Chlorophyceae) collected from coastal area of Ramanathapuram, Tamil Nadu against nine human bacterial pathogens.

MATERIALS AND METHODS

Collection of Specimen

The following four green seaweeds viz.,

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Caulerpasertularioides (S.Gmelin) Howe, *Enteromorphaintestinalis*(Linnaeus)Nees, *Ulvalactuca* (Linnaeus) and *Ulvareticulata*(Forsskal) (Fig – 1) were collected from coastal area of Ramanathapuram, Tamil Nadu.

Fig. 1. Collected Green Seaweeds

Study area

The above said green seaweeds were collected from Keelakkarai, Ramanathapuram coast, Tamil Nadu. The sample were cleaned with fresh seawater and their in distilled water to remove all the unwanted impurities, epiphytes and adhering sand particles morphologically distinct thallus of algae were placed in new polythene bag (every seaweeds in separate bags) and kept in an ice box containing slush ice and transported to the laboratory. Further they were washed thoroughly using tap water to remove the salt on the surface of the seaweed then dried the water off and samples were spread on the blotting paper to remove excess of water. They were then dried samples were in the room temperature. The dried samples were chopped into fine fragment with the help of mixer. The powder samples were stored in refrigerator for further use.

Preparation of Seaweed Extracts

The seaweed powders (10 g) were extracting successfully with 50 ml of ethanol for 3 days at room temperature and extracts were collected and concentrated. The extracts were filtered through Whatman No.1 filter paper. This ethanol seaweed extracts used for antibacterial studies.

Collection of Pathogens (Bacteria)

The bacterial species *Bacillus cereus*, *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Pseudomonas aureus*, *Salmonella typhi* and *Staphylococcus aureus*were obtained from Centre for Advance Studies in Botany, University of Madras, Chennai, Tamil Nadu.

Antibacterial Assay

Preparation of media

The growth media employed in the present study included Nutrient agar and Nutrient broth.The medium was adjusted to pH 7.4 and sterilized by autoclaving at 15 lbs pressure (121^{0}C) for 15 minutes.

Sub culturing of microorganisms

The pure culture of microorganism was maintained on nutrient agar slants by frequent sub culturing. The culture was stored at 4^{0}C.

Preparation of inoculum

Each organism was recovered for testing by sub culturing on fresh media. A loopful inoculum of each bacterium was suspended in 5ml of nutrient broth and incubated overnight at 37^{0}C. These overnight cultures were used as inoculum.

Antimicrobial activity

Antimicrobial activity was demonstrated by modification of the method described by Barry and Thornsberry, (1985). 0.1 ml of the diluted microbial culture was spread on sterile nutrient agar plate. The pre-soaked and dried discs of 6mm diameter of What man No.1 filter paper were then placed on the seeded plates and gently pressed down to ensure contact. At the same time standard antibiotic of Tetracycline (30µg/ disc) was used as reference or positive control. Respective solvents without plant extracts served as negative control. The plates were incubated at 37°C for 24 hours. After the incubation period, the diameter of the inhibition zone around the plant extract saturated discs were measured and also compared with the diameter of inhibition zone of commercial standard antibiotic discs. The inhibition zone around the discs were measured and recorded as the difference in diameter between the disc (6mm) and growth free zone.

RESULTS

The antibacterial activity of ethanol extract of four green seaweeds *Caulerpasertularioides*, *Enteromorphaintestinalis*, *Ulvalactuca* and *Ulvareticulata* were tested against 9 human bacterial

Table 1 Antibacterial activity Showing Zone of Inhibition of Ethanol Extracts of Green Seaweeds against Human Pathogenic Bacteria

Sl.No	Seaweed Extracts (Ethanol)	Human Pathogenic Microorganisms (Bacteria) Zone of Inhibition (mm) (mean ±SD)								
		Bc	*Ec*	*Ef*	*Kp*	*Ml*	*Pa*	*Pau*	*St*	*Sa*
1	*Caulerpasertuloides*	5.5±0.23	4.5±0.33	6.5±0.65	4.0±0.54	3.5±0.78	4.5±0.76	6.0±0.26	5.0±0.83	4.5±0.21
2	*Enteromorphaintestinalis*	6.5±0.10	5.5±0.13	8.0±0.32	5.0±0.46	8.5±0.32	9.5±0.23	8.0±0.72	9.0±0.22	5.5±0.21
3	*Ulvalactuca*	7.0±0.59	---	6.0±0.24	6.0±0.35	6.0±0.68	3.0±0.82	4.5±0.13	9.5±0.72	7.0±0.54
4	*Ulvareticulata*	7.0±0.03	4.5±0.22	6.0±0.83	8.0±0.81	8.5±0.22	8.5±0.31	7.5±0.79	9.5±0.66	6.5±0.38
5	Positive Control (Tetracyline)	10.0±0.32	11.2±0.42	10.5±0.33	10.0±0.43	10.0±0.42	11.0±0.30	10.0±0.81	11.5±0.82	9.0±0.71

Bc - Bacillus cereus: Ec - Escherichia coli: Ef - Enterococcus faecalis: Kp - Klebsiella pneumonia: Ml - Micrococcus luteus: Pa - Pseudomonas aeruginosa: Pau - Pseudomonas aureus:St - Salmonella typhi: Sa - Staphylococcus aureus

pathogens viz., *Bacillus cereus*, *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Pseudomonas aureus*, *Salmonella typhi* and *Staphylococcus aureus*were presented in Table 1. The inhibition zones observed for each algal extract were compared with that of the standard Tetracyclin.

Caulerpasertularioides

The maximum inhibition zone was recorded in *Enterococcus faecalis*(6.5 mm) followed by *Pseudomonas aureus*(6.0 mm) , *Bacillus cereus* (5.5 mm) and *Salmonella typhi*(5.0 mm). The minimum inhibition zone showed in *Micrococcus luteus*(3.5 mm).

Enteromorpha intestinalis

The highest inhibition zone was showed in *Pseudomonas aureus*(9.5 mm) followed by *Salmonella typhi*(9.0 mm) and minimum inhibition was recorded in *Staphylococcus aureus* and *Enterococcus faecalis*(5.5 mm).

Ulva lactuca

The maximum inhibition zone was observed in *Salmonella typhi*(9.5 mm) followed by *Bacillus cereus* and *Staphylococcus aureus*(7.0 mm) and minimum inhibition zone showed in *Pseudomonas aeruginosa*(3.0 mm). *Ulvalactuca*extract was inactive in *Enterococcus faecalis*.

Ulva reticulata

The maximum activity was obtained in *Salmonella typhi* (9.5 mm) followed by *Micrococcus luteus* and *Pseudomonas aeruginosa*(8.5 mm). The low inhibition zone was exhibited in *Enterococcus faecalis* (4.5 mm). The four green seaweeds have antibacterial activity against tested human pathogens except in *Enterococcus faecalis*.

DISCUSSION

The antibacterial activity of ethanol extract of four green seaweeds was tested against 9 human bacterial pathogens. When compared positive control out of four green seaweeds *Enteromorpha intestinalis, Ulva lactuca* and *Ulva reticulate* activity were considered as good.In *Enteromorpha intestinalis* the highest inhibition zone was showed in *Pseudomonas aureus*(9.5 mm) followed by *Salmonella typhi* (9.0 mm) and minimum inhibition was recorded in *Staphylococcus aureus* and *Enterococcus faecalis*(5.5 mm). In *Ulva lactuca* the maximum inhibition zone was observed in *Salmonella typhi*(9.5 mm) followed by *Bacillus cereus* and *Staphylococcus aureus*(7.0 mm) and minimum inhibition zone showed in *Pseudomonas aeruginosa*(3.0 mm). *Ulval actuca* extract was inactive in *Enterococcus faecalis*.In *Ulva reticulate* the maximum activity was obtained in *Salmonella typhi* (9.5 mm) followed by *Micrococcus luteus* and *Pseudomonas aeruginosa* (8.5 mm). The low inhibition zone was exhibited in *Enterococcus faecalis*(4.5 mm).Seaweeds act as potential bioactive compounds of interest for pharmaceutical applications (Solomon and Santhi, 2008). There are numerous reports of macroalgae derived compounds that have a broad range of biological activities like antibiotic, antiviral, antineoplastic, antifouling, anti-inflammatory, cytotoxic and antimitotic (Naqvi*et al.*, 1980; Caccamese*et al.*, 1981; Fenical and Paul, 1984; Hodgson, 1984; Ballesteros *et al.*, 1992; Bhosale*et al.*, 2002).Vallinayagame*t al.*, (2009) screened the antibacterial activities of four important seaweeds namely *Ulva lactuca*, *Padina gymnospora*, *Sargassum wightii* and *Gracilaria edulis* against human bacterial pathogens *Staphylococcus aureus, Vibrio cholera, Shigella dysentriae, Shigella boydii, Salmonella paratyphii, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The maximum activity was recorded from the extract of *Gracilaria edulis* against *Pseudomonas aeruginosa*.

Chileblbtissame*t al.*, (2009) evaluated the antibacterial activity of methanolic extracts from 32 macroalgae for the production of antibacterial compounds against *Escherichia coli, Staphylococcus*

aureus, Enterococcus faecalis and *Klebsiellapneumoniae*. Their results indicated that these species of seaweed have the antibacterial activities, which makes them interesting for screening for natural products. MelikaNazemi*et al.*, (2010) conducted *in vitro* antibacterial screening tests against selected clinical isolates of bacteria. Methanolic and diethylether extracts demonstrated activity against *Esherichia coli* and *Bacillus subtilis* microbes tested. Minimum inhibitory concentration and minimum bactericidal concentration were determined using bacterial broth dilution methods. The extracts showing good antimicrobial activity are undergoing further analysis to identify the active constituents. Prakash*et al.*, (2005) studied the antibacterial activity of 45 extracts of nine algae namely *Sargassum wightii*, *Chaetomorpha antenna*, *Ulva fasciata, Amphiroa fragillisima, Gracillaria edulis*and *Enteromorpha* sp. against the pathogens. The Otitis media infected bacterial pathogens were isolated from 25 infected patients. The isolated bacterial species were Gram positive and Gram negative such as *Haemophilis influenza, Streptococcus pneumoniae, Streptococcus pyogenes, Staphylococcus aureus*and *Maraxella catarrhalis*. Bioassay was carried out with 45 extracts *Halmyedia floresia* crude was found to produce maximum growth inhibition against the bacterial pathogens. Five solvents were used for the extraction of antimicrobial of which butanol showed maximum extraction of antimicrobials.

Unci Ney *et al.*, (2006) studied the antimicrobial activity of methanol, acetone, diethyl ether and ethanol extracts of 11 seaweed species against *Candida* sp., *Enterococcus faecalis, Staphylococcus aureus, Streptococcus epidermidis, Pseudomonas aeruginosa* and *Escherichia coli* by disc diffusion method. Diethyl ether extracts of fresh *Cystoseira mediterranea, Enteromorpha linza, Ulva rigida, Gracilaria gracilis* and *Extocarpus siliculosus* showed effective results against all tested organisms. However, diethyl ether extracts of some species, such as *Padina pavonica, Colpomenia sniosa, Dictyota linearis, Dictyopteris membranaceaea, Ceramiumrubrum*and *Acanthophoranojadiformis*gave different results. A significant difference in antimicrobial activity was not observed between the acetone and methanol extracts of each algae.

The maximum antibacterial activity shown by three green seaweeds viz., *Enteromorpha intestinalis, Ulva lactuca* and *Ulva reticulate* in the present work confirms to the earlier work. Disc diffusion methods are extensively used to investigate the antimicrobial activity of natural substances and plant extracts.The screening and scientific evaluation of seaweed extracts against microbes may provide new antimicrobial substances.

CONCLUSION

The above said results reveal that, Seaweeds are the potential source for bioactive compounds and it should be thoroughly being investigated for natural antibiotic properties. But, further studies may be made to identify and evaluate the actual substances which are responsible for the antibacterial property.

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