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Research Article

Medicinal Properties of Gren Seaweeds, *Ulva fasciata* (Delile, 1813) and *U. reeticulate* (Forsskal, 1777*)*

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ABSTRACT

The increasing resistance of pathogens and the side effects of antibiotics have made it vital to find new alternative compounds from different sources. In the present study, the antibacterial potential of two green seaweeds was screened against 10 human pathogenic bacteria. The seaweeds were extracted with Acetone, Petroleum ether and Methanol and screened. The crude acetone and methanol extracts of the *Ulva fasciata* showed significant antibacterial activity with the inhibition zone range of 2-8 mm. The petroleum ether extract of *U. reticulata* also exhibited wide spectral activity with the zone of inhibition level of 1 – 5 mm. In partial purification of active extract of the *U. fasciata* by silica gel column chromatography, the fraction 50%A:50%M showed prominent antibacterial activity against all the human pathogens and the zone of inhibition was ranged between 4 -11 mm at 100 µg/disc concentration which indicated the presence of non-polar to intermediate polar active compounds. This semi- purified fraction showed prominent activity against *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Bacillus subtitles*. Thus, the seaweeds are gaining more and more importance in the pharmaceutical industry along with their nutritional properties all over the world.

Keywords: *Ulva fasciata; Ulva reticulata*; Antibacterial activity

INTRODUCTION

The World Health Organization (WHO, Geneva) has estimated that around 1500 people die each hour from infectious diseases globally and half of these are children under five years of age (Meylears et al., 2002). Increased prevalence of diseases, resistance of bacterial strains to drugs, high cost of industrialized medicines and side effects caused by synthetic drugs are some of the factors contributing to the central role of natural products in health care (Johann et al., 2007 and Prem Anand, 2013). Worldwide, there has been a renewed interest in marine natural products and nearly all forms of life in marine environments (e.g., algae, sponges, corals, tunicates, nudibranches) have been investigated (Chellaram, et al., 2009, 2013, Prem Anand, et al 2011, 2012, Faulkner, 2000). In this regard, seaweeds have been getting much attention. Seaweeds are extraordinarily sustainable resource in the marine ecosystem and have been used as food stuff in Asia for centuries as they contain carotenoids, dietary fibres, proteins, essential fatty acids, vitamins and minerals. They also have been reported to have some of the valuable medicinal components such as antibiotics, laxatives, anticoagulants, anti-ulcer products and suspending agents in radiological preparations (Fayaz et al., 2005). Marine algae have been the source of about 35% of the newly discovered metabolites followed by sponges (29%) and cnidarians (22%) (Ireland et al., 1993). The present study was done with two seaweeds, *Ulva fasciata* and *U. reticulata* (green algae) belonging to the family Ulvaceae with the objectives (i) to prepare the crude extracts using various solvents of different polarity like Acetone, Petroleum ether and Methanol (ii) to test the antibacterial activity of the crude extract of marine algae against a range of gram positive and gram negative bacterial strains (iii) to localize the polarity of active compound through partitioning of the active crude extracts (iv) to partially purify the active extract through bio-assay guided silica gel column chromatography.

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MATERIALS AND METHODS

Collection of Seaweeds

The healthy seaweeds, *Ulva fasciata and U. reticulata* were collected from the inter-tidal areas of Vizhinjam and Tuticorin coasts respectively along the south coast

of India. After identification using standard keys, seaweeds were rinsed with seawater followed by fresh water to remove epiphytes and adherents then air dried in shade under room temperature and then immediately subjected to extraction.

Preparation of Extracts

The seaweeds after drying were weighed and finely powdered; about 25 gram of each sample was soaked in 250 ml of various solvents such as Acetone, Petroleum ether and Methanol separately and kept for 48 hours at -18^0 C. After 48 hours the samples were filtered and the filtrate allowed for evaporation (Becerro et al., 1994). The concentrated residues were weighed and stored in refrigerator for antibacterial sensitivity assay.

Test Organisms

The Acetone, Petroleum ether and Methanol crude extracts of seaweeds were screened against 10 human pathogenic bacteria such as *Escherichia coli* (MTCC 50), *Shigella sonnei* (MTCC 2957), *Bacillus subtilis* (MTCC 1133), *Salmonella typhimurium* (MTCC 1357), *Staphylococcus aureus* (MTCC 3160), *Vibrio cholerae* (MTCC 3906), *Enterobactor faecalis* (MTCC 2729), *Klebsiella pneumoniae* (MTCC 3384), *Strptococcus pyogenes* (MTCC 1923) and *Micrococcus luteus* (MTCC 4821) obtained from Microbial Type Culture Collection and Gene Bank (MTCC) Chandigarh, India.

Antibacterial Sensitivity Assay

The antibacterial activity was evaluated by following the standard agar disc diffusion method using Muller Hinton agar (Becerro et al., 1994). The 10 human pathogenic bacteria were inoculated into nutrient broth and incubated for 24 hrs prior to their use in the antibacterial assay. The sterile Whatman no.1 filter paper discs of 6 mm diameter were loaded with 250 µg of the crude extracts and air dried; then placed aseptically over plates of Muller Hinton agar swabbed with test pathogens (10^6cells/ml) and incubated at room temperature for 24 hrs. Control plates were maintained separately. The zone of inhibition around the discs after the incubation was measured from the edge of the disc to the end of clear zone in millimeter.

Partitioning of Crude Extract

The partitioning of the active crude extracts was done by following the method of Riguera (1997) and Wright (1998) to asses the polarity and localize the active component. The Acetone crude extract of *U. fasciata* and Petroleum ether extracts of *U. reticulata* were partitioned separately between ethyl acetate and water and then this water phase was subsequently partitioned against n-butanol (Wright, 1998; Slattery et al., 1995). The partitioned phases (Ethyl acetate, butanol and water) were collected separately; evaporated; and subjected to antibacterial assay at the concentration of 250 µg per disc.

Bioassay Guided Column Chromatography

The active crude acetone extracts of *Ulva fasciata* were admixed with silica gel grade 200-400 mesh (LOBA chemical) (Wright, 1998) at the proportion of 200 mg of crude extract for 400 mg of silica gel (230-400 µm). The admixture was loaded into the column (30 x 1.7 cm size) filled with silica gel (230-400 µm) and pre calibrated. The TLC was used to asses the separation of compounds and to select the solvent system of increasing polarity from non-polar to polar. The solvent system Hexane, Acetone and Methanol were used and the eluted step gradient series were 100% Hexane, 75% Hexane: 25% Acetone, 50% Hexane: 50% Acetone, 25% Hexane: 75% Acetone, 100% Acetone, 75% Acetone: 25% Methanol, 50% Acetone: 50% Methanol, 25% Acetone: 75% Methanol, 100% Methanol. The resultant 9 fractions were collected separately and subjected to antibacterial sensitivity assay at 100 µg/disc concentration.

RESULTS

Antibacterial Activity

Invariably, all the solvent extracts of *U. fasciata* and *U. reticulata* exhibited antibacterial activity (Table 1). The acetone extracts of *U. fasciata* showed remarkably higher activity against all the human bacterial pathogens with the zone range of 2- 8 mm. The maximum zone of inhibition was recorded against the *Bacillus subtilis* and mild activity observed in *Staphylococcus aureus, Streptococcus pyogenes* and *Micrococcus luteus*. The methanol extract also showed good activity but the zone of inhibition was comparatively lower than the acetone extract while minimum level activity observed in the petroleum ether extract. Interestingly, antibacterial activity was more common against gram positive bacteria than gram negative bacteria. The Petroleum ether extracts of *U. reticualta* showed wide spectral activity. The inhibition zone was ranged from 1- 5 mm, the maximum inhibition was observed in *Bacillus subtilis*; minimum in *Vibrio cholerae* and *Micrococcus luteus* and other strains inhibited moderately. The methanol and acetone extracts were also found to have broad spectral activity against seven and eight pathogens respectively. The acetone and petroleum ether extracts of *U. fasciata* and *U. reticulata* were selected for further screening.

Partitioning

The ethyl acetate phase of both seaweeds exhibited higher activity; modest in butanol phase and no activity in water phase (Table 2). The ethyl acetate phase of *U. fasciata* exhibited wide spectral activity with the zone range of 3 - 8 mm, with maximum inhibition observed in *Streptococcus epidermis* and minimum against *Escherichia coli, Bacillus subtilis, Vibrio cholerae, Enterobacter faecalis* and *Micrococcus luteus*. In butanol phase, the zone of inhibition was ranging between 1 and 6

Table 1: Antibacterial activity of crude extracts at various solvents from *Ulva fasciata* and *U. reticulata*

S.No	Human pathogens	*Ulva fasciata*			*Ulva reticulata*		
		A	PE	M	A	PE	M
		Zone of inhibition level (mm) at 250µg/disc					
1	*Escherichia coli*	3	1	3	2	3	1
2	*Shigella sonnei*	3	2	1	1	2	1
3	*Bacillus subtilis*	8	3	1	1	5	2
4	*Salmonella typhimurium*	3	1	1	1	2	-
5	*Staphylococcus aureus*	3	1	1	-	2	-
6	*Vibrio cholerae*	2	1	T	-	1	1
7	*Enterobacter faecalis*	4	3	2	1	5	2
8	*Klebsiella pneumoniae*	3	1	1	1	2	1
9	*Streptococcus pyogenes*	2	1	1	1	4	1
10	*Micrococcus luteus*	2	-	T	2	1	-

Key: A-Acetone; PE – Petroleum ether; M- Methanol; - No activity; Low (Trace to < 1mm); **Moderate (1mm to 4mm); High (above 4mm)**

Table 2: Antibacterial activity of partitioned fraction of *Ulva fasciata* and *U. reticulata*

S. No	Human pathogens	*Ulva fasciata*			*Ulva reticulata*		
		Zone of inhibition level (mm) at 250µg/disc					
		W	EA	B	W	EA	B
1	*Escherichia coli*	-	3	1	-	4	1
2	*Shigella sonnei*	-	4	2	-	4	1
3	*Bacillus subtilis*	-	3	1	-	5	1
4	*Salmonella typhimurium*	-	4	1	-	3	1
5	*Staphylococcus aureus*	-	5	6	-	7	5
6	*Vibrio cholerae*	-	3	-	-	2	-
7	*Enterobacter faecalis*	-	3	-	-	2	T
8	*Klebsiella pneumoniae*	-	4	2	-	3	2
9	*Streptococcus pyogenes*	-	8	-	-	5	4
10	*Micrococcus luteus*	-	3	-	-	2	1

Key: W-Water; EA-Ethylacetate; B- Butanol; - No activity; Low (Trace to < 1mm); Moderate (1mm to 4mm); High (above 4mm)

mm and showed activity against 60% of pathogenic bacteria.

The partitioned ethyl acetate phase of *U. reticulata* was observed to have wide spectral activity. The zone of inhibition level was ranged from 2-7 mm. The remarkable inhibition was against *Staphylococcus aureus* and moderate against *Vibrio cholerae, Enterobacter faecalis* and *Micrococcus luteus* and mild activity in remaining pathogens. In the butanol phase, zone of inhibition was varied between trace to 5 mm and showed activity against 90% of the human bacterial pathogens.

Bioassay Guided Column Chromatography

The results of semi-purified column fractions of *Ulva fasciata* are presented in Table 3. All the fractions showed activity at least against two of the pathogens. Out of 9 fractions, the fraction 7 (50% Acetone: 50% methanol) showed significant activity and inhibition zone ranged from 4 – 11 mm, *Klebsiella pneumoniae* was found to be very sensitive and *Micrococcus luteus* was inhibited slightly and moderate level activity exhibited against all the other pathogens. The fractions 2 (75% Hexane: 25% Acetone), 8 (25% Acetone: 75% Methanol) and 9 (100% Methanol) showed broad spectral activity followed by the fraction 3 (50% Hexane: 50% Acetone), fraction 4 (25% Hexane: 75% Acetone) and fraction 5 (100% Acetone). The fraction 1 (100% Hexane) showed antibacterial activity only against two human pathogens.

DISCUSSION

Seaweeds provide a rich source of bioactive compounds as they are able to produce a great variety of secondary metabolites which are mainly reported to show defense against herbivores, fouling organisms and pathogens; they also play a role in reproduction, protection from UV radiation and as allelopathic agents (Hay, 1996; Watson and Cruz-Rivera, 2003). These secondary metabolites with varying ecological role could be used to cure human diseases.

In the present study, the antibacterial potential of *U. fasciata* and *U. reticulata* was evaluated and the results revealed that both seaweeds posses wide range

Table 3: Antibacterial activity of column fractions of *Ulva fasciata*

S.No	Human pathogens	Column fraction								
		100% H	75% H:25%A	50%H:50% A	25%H:75%A	100% A	75% A: 25%	50% A: 50% M	25% A: 75% M	100% M
		Zone of inhibition level (mm) at 100µg/disc								
1	*Escherichia coli*	-	3	1	2	2	-	8	4	3
2	*Shigella sonnei*	-	4	1	2	2	-	6	3	2
3	*Bacillus subtilis*	-	-	-	-	-	-	10	5	2
4	*Salmonella typhimurium*	-	3	1	2	2	-	8	2	3
5	*Staphylococcus aureus*	-	5	1	2	2	-	7	-	1
6	*Vibrio cholerae*	-	3	1	2	2	4	7	4	-
7	*Enterobacter faecalis*	-	-	-	-	-	-	5	-	-
8	*Klebsiella pneumoniae*	1	5	3	3	3	8	11	9	5
9	*Streptococcus pyogenes*	-	3	1	2	2	5	6	4	3
10	*Micrococcus luteus*	1	2	1	2	4	5	4	4	2

Key: H -Hexane; A – Acetone; M – Methanol; - No activity; Low (Trace to < 1mm); Moderate (1mm to 4mm); High (above 4mm)

of antibacterial compounds. The disc diffusion assay was selected as it was primarily considered as preliminary tool to assess the antibacterial potential of seaweeds (Hun et al., 1994). The advantage of such a screening is that it is simple, less time-consuming and requires only a small quantity of the materials to be screened. In this investigation, all the solvent extracts showed higher antibacterial activity in gram positive bacteria than gram negative bacteria. These differences in susceptibility towards bioactive secondary metabolites can be accredited to differences in cell wall structure of Gram-positive bacteria compared to Gram-negative bacteria (Hawak et al., 1998).

In *Ulva fasciata,* though all the solvent extracts showed broad spectral activity, acetone extracts showed remarkable antibacterial activity than that of methanol and petroleum ether extract. It was similar to the observation of Osman et al. (2010) who also noted that the acetone extracts of *U. fasciata* had shown higher antibacterial activity against human pathogens than methanol and ethanol extracts. This result indicates that the acetone was the best solvent for extracting the bioactive compounds from seaweeds; followed by methanol and petroleum ether extracts. This could probably due to the difference in the solubility of bioactive metabolites in the corresponding solvents (Osman et al., 2010). The observation of higher activity in acetone and ethyl acetate extracts was further supported by the report of Chellaram et al. (2009). Secondary metabolites from green algae were found to exhibit substantial biological activity against human pathogens and many studies have documented the bioactivities of crude extracts from seaweeds against human pathogenic bacteria (Seenivasan et al., 2010; Kolanjinathan and Stella, 2009; Cox et al., 2010; Srinivasa Rao and Parek, 1981; Selvin and Lipton, 2004). The observation of wide activity in different solvents could be attributed to the production of biogenic compounds with different polarity such as indoles, terpenes, acetogenins, phenols, fatty acids and volatile halogenated hydrocarbons, which were prominently isolated from marine algae (Watson and Cruz-Rivera, 2003).

U. reticulata showed higher activity in petroleum ether extracts followed by methanol and acetone. It is found to be contrast to the report of Karthikaidevi et al. (2009), who observed lower antibacterial activity in petroleum ether extracts of *U. reticulata* from Vedalai coast of Gulf of Mannar, southeastern India and observed comparatively higher activity in methanol and acetone extracts. This can be attributed to the variation in the production of secondary metabolites which is influenced by geographical location, different seasons and pressure faced by the seaweeds from herbivores and fouling organisms (Manivannan et al., 2011).

During partitioning of crude extracts, the compounds are reported to be distributed according to their polarity (Chellaram, et al., 2011, Riguera, 1997). This gradient partitioning helps to the next step of chromatographic separation as the separation systems, which are used for the purification, depends to a large extent on the polarity of the compounds (Wright, 1998). The gradient partitioning of *U. fasciata* and *U. reticulata* revealed higher activity in the ethyl acetate phase, indicating the intermediate polar nature of active compounds. The activity observed in ethyl acetate fractions could be attributed to the presence of lipophylic compounds such as terpenoids, acetogenins and compounds of mixed biosynthesis (Hay and Steinberg, 1992; Ragan and Glombitza, 1986; Pereira and Valentine, 1999). The observed lower activity in the butanol phase could be attributed to the presence of different compounds in seaweeds.

In the attempt of bioassay guided partial purification of crude extracts using silica gel column chromatography, totally 9 fractions were collected and observed higher activity in 7th fractions (50% Acetone: 50% Methanol). The present observation was corroborated with Jebasingh et al. (2011) who also reported higher activity in 100% acetone fraction of green seaweed *Caulerpa scalpelliformis*. In a similar study, Manilal et al. (2009) recorded wide spectrum activity of red seaweed *Falkenbergia hillebrandii* in the fraction comprised petroleum ether and ethylacetate.

CONCLUSION

This study clearly indicates the bio-potential of *U. fasciata and U.reticulata* against human pathogenic bacteria with significant activity. The observed wide spectral of activity revealed the presence of different compounds and these compounds can be isolated and studied for their various properties. This investigation further increases our interest to utilize these wonderful renewable marine resources to combat the prevalence of diseases and subsequent mortality.

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