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Antibacterial Activity and Biochemical Constituents of Seaweed *Ulva lactuca*

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Abstract: The antibacterial activity of seaweed extracts of 9 different solvents were checked against 11 human pathogens and 5 fish pathogens. The acetone extract of *Ulva lactuca* showed broad spectrum of antibacterial activity when compared to other solvent extracts. The *Ulva lactuca* had high content of protein (20.8%) followed by 13.27% carbohydrates and 4.4%. of lipid content.

Key words: Marine Macro Algae · Solvent Extraction · Antimicrobial Activity · Human Pathogens · Fish Pathogens · Nutrients

INTRODUCTION

Marine environment is an exceptional reservoir of biologically active natural products, many of which exhibit structural features that has not been found in terrestrial natural products [1]. Marine natural products such as secondary or non-primary metabolites produced by living organisms have been exploited by people for a variety of purposes including food, fragrance, pigments, insecticides and medicines [2]. Approximately 2500 new metabolites were reported from a variety of marine organisms from 1977 to 1987 [3]. Seaweeds refer to any large marine benthic algae that are multicellular, macrothallic and thus differentiated from most algae that are of microscopic size. Seaweeds are one of the most important marine resources of the world.

Marine algae are an inexhaustible source of chemical compounds that produce wide variety of biologically active secondary metabolites. Marine algae have become an important target for the biotechnology industry because of the large number of bioactive compounds recently discovered from them. Seaweeds are the renewable living resources which are also used as food, feed and fertilizer in many parts of the world. Seaweeds are of nutritional interest as they contain low calorie food but rich in vitamins, minerals and dietary fibres [4]. In addition to vitamins and minerals seaweeds are also potentially good sources of protein, polysaccharides and fibres [5, 6]. The lipids which are present in very small amount are unsaturated and afford protection against cardiovascular diseases.

Seaweeds have been recognized as potential sources of antibiotic substances. The production of antimicrobial activities was considered to be an indicator of the seaweeds to synthesize bioactive secondary metabolites [7, 8]. There are numerous reports of compounds derived from macro algae with a broad range of biological activities, such as antibacterial activities [9-19]. Like other plants, seaweeds contain various inorganic and organic substances which can benefit human health [20]. Many bioactive compounds are extracted from seaweeds [21-23]. Seaweeds have been screened extensively to isolate life saving drugs or biologically active substances all over the world [24- 26]. Many workers revealed that the crude extracts of Indian seaweeds are active against Gram-positive bacteria [27].

Many of the seaweeds possess bio-active components which inhibit the growth of some of the Gram positive and negative bacterial pathogens [28]. Bacterial infection causes high rate of mortality in human population and aquaculture organisms [29]. Nowadays there is an increasing demand for biodiversity in the screening programmes for selecting therapeutic drugs from natural products, the marine organisms especially seaweeds are of with immense interest, since they are having a broad range of biological activities such as antibacterial, antifungal, antiviral, antitumor, anti-inflammatory and antioxidants [30].

Marine seaweed *Ulva lactuca* was selected for the present study because it was available throughout the year in the Gulf of Mannar Coast of Tuticorin. Present study was aimed to investigate the biochemical content

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and to study the antibacterial properties of the seaweed extracts of 9 different solvents against 11 human and 5 fish pathogens.

MATERIALS AND METHODS

Collection of Seaweed Samples: Fresh seaweed *Ulva lactuca* was collected from intertidal regions (1-4m depth) of Tuticorin Coast of Gulf of Mannar Marine National Park. The taxonomic identification of species was done using standard literature and taxonomic keys. The collected samples were cleaned well with the seawater until unwanted impurities, adhering sand particles and extraneous matter like epiphytes, pebbles, shells were removed and it was immediately brought to the laboratory in sterile plastic bags containing sea water in order to prevent evaporation. It was then washed thoroughly with tap water and distilled water to remove the surface salty materials. It was air dried for 1 week and later ground in an electric mixer. The powdered samples were subsequently stored in the refrigerator for future use.

Extraction of Seaweeds: The powdered seaweed sample was soaked in different solvents such as Methanol, Ethanol, Butanol, Acetone, Chloroform, Ethyl acetate, Xylene, Toluene and water and extracted. The samples in the ratio of 1:4 w/v were kept for 24 hours at room temperature and were then filtered with Whatman No.1 filter paper. The filtrate obtained was evaporated, concentrated and stored at 4ºC. The concentrates were reconstituted with their respective extracts [31, 32].

Test Organisms: Extract were tested against 10 human pathogens viz., *Enterobacter feacalis-MTCC-2729, Shigella sonnei- MTCC-2957, Bacillus subtilis-MTCC- 1133, Vibrio cholera-MTCC-3906, Salmonella typhimurium-MTCC- 1357, Escherichia coli-MTCC-50, Staphylococcus aureus- MTCC-3160, Streptococcus pyogenes- MTCC- 1923, Klebsiella pneumonia-MTCC-3384, Micrococcus luteus- MTCC- 4821, Staphylococcus epidermis-MTCC- 2639* and 5 fish pathogens such as *Vibrio harveyi, Vibrio sclintis, Vibrio parahaemolyticus, Vibrio alginolyticus, Vibrio anguillarum*. The cultures were received from Fisheries College, Tuticorin. The bacterial cultures were grown in nutrient agar and stored at 4°C.

Antibacterial Assay: The seaweed extracts were screened against ten human pathogens and five fish pathogens. The antimicrobial activity was evaluated using well-cut diffusion technique [33]. Wells were punched out using a sterile 0.7 cm cork borer in nutrient agar plates inoculated with the test microorganism. About 50 µl of the different algal extract were transferred into each well. For each microorganism, negative controls were maintained where pure solvents were used instead of the seaweed extract. All plates were incubated at 4°C for 2 hours. To prevent drying the plates were covered with sterile plastic bags. The plates were later incubated at 37°C for 24 hours [34].The result obtained by measuring the inhibition zone diameter for each well and expressed in millimetre [35].

Biochemical Assay: The amount of protein present in the sample was estimated by mixing the sample with analyticaland folin phenol reagent then measured the absorption of the colour was read at 660nm in the spectrophotometer following Lowry's [36] method. The lipid was estimated by the method of Folch*et al*., [37]. In brief for the estimation of lipid content, the dried samples were finely ground and the fat was extracted with chloroform and methanol mixture. After extraction the solvent was evaporated and the extracted materials were weighed and the percentage of the fat content was calculated. The amount of carbohydrate present in the sample was estimated by following the method of Hedge and Hofreiter [38].

RESULTS

The antibacterial activity of the seaweed *Ulvalactuca*extracts of 9 different solvents were tested against 11 human pathogens viz., *Enterobacter faecalis, Shigella sonnei, Bacillus subtilis, Vibrio cholera, Salmonella typhimurium, Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumonia, Micrococcus luteus, Staphylococcus epidermis*were are presented in Table 1 and 5 fish pathogens such as *Vibrio harveyi, Vibrio sclintis, Vibrio parahaemolyticus, Vibrio alginolyticus, Vibrio anguillarum* (Table 2). The antibacterial activity of the solvents were in the order of Acetone>Methanol>Butanol>Ethylacetate>Chloroform >Ethanol>Toluene> Xylene > Water aqueous. The extract using acetone showed very high activity against *E. coli* (26 mm). Moderate activity of this extract was seen against *B. subtilis* (20mm), *S.typhi* (20mm), *S.epidermis* (19mm) and *V. cholera* (18mm). Less activity was observed for *S. sonnei* (17mm), *E. faecalis* (16mm*), S. pyogens* (16mm), *K. pneumonia* (16mm) *S. aureus* (15mm) and *M. luteus* (15mm).

Table 1: Antibacterial activity of green seaweed *Ulva lactuca* against human pathogens

		Zone of inhibition (mm) in diameter								
S.No	Pathogens	A	M	B	EA	C	E	T	X	W
1	*Enterobacter faccalis*	16	11	11	11	12	10	11	10	-
2	*Shigella sonnei*	17	14	12	12	12	9	10	11	-
3	*Bacillus subtilis*	20	13	16	14	17	12	12	14	-
4	*Vibrio cholera*	18	14	17	17	14	13	10	17	16
5	*Salmonella typhimurium*	20	14	17	17	12	18	12	-	19
6	*Escherichia coli*	26	15	11	11	13	13	10	-	-
7	*Staphylococcus aureus*	15	13	15	15	10	12	14	10	-
8	*Streptococcus pyogenes*	16	19	12	12	13	10	13	11	-
9	*Klebsiella pneumonia*	16	14	14	14	13	11	9	9	-
10	*Micrococcus luteus*	15	26	14	14	18	11	9	11	-
11	*Staphylococcus epidermis*	19	11	16	14	11	14	14	-	-

C- Chloroform, M- Methanol, B- Butanol, T-Toluene, EA-Ethyl Acetate, W-Water Aqueous, X-Xylene, A-Acetone, E-Ethanol.

Table 2: Antibacterial activity of green seaweed *Ulva lactuca* against fish pathogens

		Zone of inhibition(mm) in diameter								
S.No	Pathogens	A	B	EA	M	E	C	T	X	W
1	*Vibrio harveyi*	18	19	18	18	11	-	12	12	16
2	*Vibrio sclintis*	21	16	13	13	11	11	11	9	-
3	*Vibrio parahaemolyticus*	18	19	16	12	10	6	-	-	-
4	*Vibrio aigino*	20	16	17	12	15	11	12	10	-
5	*Vibrio anguillarum*	21	11	12	13	8	11	-	-	-

C- Chloroform, M- Methanol, B- Butanol, T-Toluene, EA-Ethyl Acetate, W-Water Aqueous, X-Xylene, A-Acetone, E-Ethanol.

The methanolic extract exhibited highest zone of inhibition against *Micrococcus luteus*was (26mm) followed by *S.pyogens* (19mm). Moderate activity was seen in *E. coli* (15mm), *S.sonnei*, *V. cholera S. typhi*, *K pneumonia* (14mm)], *B. Subtilis* and *S .aureus* (13mm). Low activity was seen in *E. faecalis* (11mm).Similar antimicrobial activities were exhibited by both butanolic and ethyl acetate extracts in 9 pathogens. The highest zone of inhibition was seen in *V. Cholera* and *S. typhi* was 17 mm in both these extracts. Lowest activity was seen in *E. faecalis* and *E. coli* (11mm) in both theseextracts. In the chloroform extract the highest zone of inhibition was seen in *M. luteus* (18mm) and the lowest was seen in *S.aureus* (10mm). In the ethanolic extract the highest zone of inhibition was seen against *S. typhi* (18mm) and the lowest was seen in *S.sonnei* (9mm). In the toluene extract the high zone was seen against *S. epidermis* (14mm) and *S. aureus* (14mm). Low activity was seen in *K. pneumonia* and *M. luteus*. In the xylene extract *S. typhi, E. coli* and *S. epidermis* did not show any antibacterial activity. In the water extract the highest activity was seen in *S.typhi* (19mm) and the moderate activity in *V. cholera* (16mm).

Regarding the antibacterial activity against fish pathogens again the acetone extracts showed broad spectrum of activity against all the tested fish pathogens. This was followed by butanol, ethyl acetate, methanol, ethanol, chloroform, toluene, xylene and water extracts. Acetone extracts showed highest activity against *Vibrio sclintis* and *Vibrio anguillarum* (21mm). Moderate activity was seen against *V.sclintis* (21mm) and *V. alginolyticus* (20mm). Low activity was seen against *V. harveyi* and *V. parahaemolyticus* (18mm). In the butanolic extract the highest activity was shown against *V. harveyi* and *V. paraheamolyticus* (19mm). Moderate activity was seen against *V. sclintis* and *V. alginolyticus* (16mm). Very low activity was seen against *V. anguillarum* (11mm). In both ethyl acetate and methanolic extract similar zone of inhibition was seen by *V. harveyi* (18mm) and *V. sclintis* (13mm). Moderate activity was exhibited against *V. paraheamolyticus* (16mm) and *V.alginolyticus* (17mm). Low activity was seen against *V. anguillarum* (12mm). In the methanolic extract low activities were seen against *V. paraheamolyticus* and *V. alginolyticus* (12mm). In the ethanol extract high activity was seen against

Table 3: Nutritional status of green seaweed *Ulva lactuca*

S.No	Parameters	Amount mg/ g
1	Protein	20.8mg/ g
2	Corbohydrate	13.27mg/ g
3	Lipid	4.4mg/ g

V. alginolyticus (15mm). Moderate activity was seen by *V. harveyi* and *V. sclintis* (11mm) and *V.paraheamolyticus* (10mm).Very poor activity was seen against *V.anguillarum* (8mm). Regarding the chloroform extracts moderate activity was seen against *V. sclintis, V. alginolyticus* and *V. anguillarum* (11mm). Poor activity was exhibited against *V. paraheamolyticus*. In the toluene extract moderate activity was seen against *V.harveyi*, *V.alginolyticus*(12mm) and *V.sclintis* (11mm). No antibacterial activity was seen in *V. paraheamolyticus* and *V. anguillarum* in both toluene and xylene extract. In the xylene extract moderate activity was seen against *V.harveyi* (12mm) and *V.alginolyticus* (10mm). Low activity was seen in *V.sclintis* (9mm). Water extract showed moderate activity against *V.harveyi*(16mm). None of the other pathogens showed antibacterial activity in the water extract. Regarding the biochemical content of the seaweed *Ulva lactuca* it had 20.8% of protein content followed by 13.27% of carbohydrates and 4.4 % of lipid content and it was shown in Table 3.

DISCUSSION

Seaweeds contain various inorganic and organic substances which can benefit for human health [20]. Compounds with cytostatic, antiviral, anthelmintic, antifungal and antibacterial activities have been detected in green, brown and red algae [39, 40]. Seaweeds have effective antibacterial activity against human pathogenic bacteria. It was reported that 151 species of marine algal crude extracts showed inhibitory activity against pathogenic bacteria [41]. Several different organic solvents have been used to screen algae for antibacterial activity [42]. In this study 9 different solvents were used for extraction. Among them the acetone extract had high broad spectrum of activity against all the pathogens. Our present findings are consistent with some earlier reports of Kolanjinathan and Stella [43] who indicated that acetone was the best solution for extracting the effective antimicrobial materials from *Sargassummyricystum*, *Turbinariaconoides*, *Hypneamusiformis*, *Gracilariaedulis* and *Halimediagracilis*. Hornsey and Hide [41]; Kandhasamy and Arunachalam [29]; Moreau *et al*., [44] also reported variation in antibacterial activity may be due to the method of extraction, solvent used in extraction and season at which samples were collected. Perez *et al*., [45] observed that the extract of *Ulva lactuca* had no antibacterial activity. In contrast, results of our study showed that *U. lactuca* inhibited mostly all the organisms in all the solvents tested except for the water extract which inhibited only two organisms. This difference in results may be due to time and place of sample collection. Our results correlated with the results of Chiheb *et al*., [46] who reported that methanol extracts of *Enteromorphacompressa* (Linnaeus) and *Cystoseiratamariscifolisa* (Hudson) Papen fuss showed activity against every bacterial strain tested. They also reported that the extract of *Ulva lactuca* (Linnaeus) has a larger inhibition diameter against the pathogenic bacteria. Reichelt and Borowitzka [47] and Salvadov *et al*., [48] screened many species of algae for their antibacterial activity. They reported that the members of the red algae exhibited high antibacterial activity. In contrast the green algae (chlorophyceae) were the most active species. Present results are in accordance with those of Kandhasamy and Arunachalam [29] who reported that green algae (chlorophyceae) were the most active division than others and also agreed with that of Fareed and Khairy [49] who showed that *U.lactua* (Chlorophyceae) were more active than *J. Rubens* (Rhodophyceae). Toluene extracts also inhibited both the Gram positive and negative organisms. Our result is in accord with the results of Padmakumar and Ayyakkannu [50] they used a mixture of toluene and methanol to extract antibacterial substances from *Enteromorphaintestinalis*. It is clear that using organic solvents always provides a higher efficiency in extracting compounds for antimicrobial activities compared to water-based methods [51, 52]. Our results coincided with the results of Parekh *et al.,* [53] who reported that the extracts obtained with acetone,ethyl alcohol and ether showed higher antimicrobialactivity than chloroform extracts. In our study even xylene extracts proved to a good solvent for extraction which in turn exhibited good antibacterial activity. Water extract was not very suitable in extraction of compounds since it did not show any antibacterial activity. In the antibacterial activity against fish pathogens acetone extract was rated first. This was followed by butanol, ethyl acetate, methanol, ethanol, chloroform, toulene, xylene and water. This shows that the seaweed extract can be useful for aquaculture remedies after further purification.

In the present study protein, carbohydrate and lipid content of the seaweed *Ulva lactuca was* analysed. The sea weed has high protein content (20.8%) and this results coincided with the results of Manivannan *et al.,* [54] who described that protein concentration of *C. glomerata* was about (20.38 ± 0.73%). This result also agreed with the results of Sobha *et al*., [55] who reported *Ulva*sp. have the rich sources of protein and vitamins. Carbohydrate concentration (13.27%) and the lipid content (4.4%) were low when compared to the protein content. Kaliaperumal *et al*., [56] reported the fresh *Ulva lactuca* contain 25.8% of protein, 16.27% of carbohydrate and 7.4% of lipid. In the present study nutrient content slightly decreased and this may be due to the lipid oxidation and protein, carbohydrates denatured during air drying. Lipid-soluble extracts from marine macroalgaehave been investigated as a source of substances with pharmacological properties.

CONCLUSION

The seaweed *Ulva lactuca*is having very good antibacterial activity. Further purification of active compounds can be used as a source of antibiotics for the treatment of disease causing pathogens. High protein content shows that it can be used as food/feed for human and animal consumption if proper methods of cooking are used to remove the phenol content.

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