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Full Length Research Paper

Antibacterial and antihemolytic activities of *Enteromorpha intestinalis* in Caspian Sea Coast, Iran

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Enteromorpha **is one of the filamentous green-algal genus and has a widespread distribution in Caspian Sea Coast. This study aimed at assaying the antimicrobial activities of** ***Enteromorpha intestinalis*** **in South of Caspian Sea. Antimicrobial activities of hydroalcoholic extracts of five different gram negative and positive bacteria including** ***Staphylococcus aureus*****,** ***Bacillus subtilis*****,** ***Pseudomonas aeruginosa*****,** ***Salmonella typhimurium*** **and** ***Proteus mirabilis*** **were investigated. The extract was primarily screened for their possible antimicrobial effects using disc diffusion methods. The potential antibacterial activities at different concentrations of the extract were elucidated. The extract displayed variable degrees of antimicrobial activities on different bacteria. Among gram positive bacteria, the** ***B. subtilis*** **(with wider zones of inhibition) was found to be more sensitive than** ***S. aureus*****. Among gram negative** ***P. aeruginosa*** **was found to be more resistant than** ***P. mirabilis*** **and** ***S. typhimurium*****. The extract did not show any harmful effects on erythrocytes and, in fact, exhibited potent antihaemolytic activity with IC_{50} of 323±11.7 µg ml^{1} compared with 235±9 µg ml^{-1} for vitamin C which served as positive control. Extract show antihemolytic activity against hydrogen peroxide (H_2O_2) induced hemolysis. Our findings suggest the possibility of using the** ***E. intestinalis*** **as a novel source of natural antimicrobial and antihemolytic agent for pharmaceutical industries.**

Key words: *Enteromorpha intestinalis*, antibacterial activity, antihemolytic activity.

INTRODUCTION

Novel marine natural products isolated from marine bacteria, fungi, sponges, worms, fish and plant (Mayer et al., 2011). These products are widely distributed in the plant kingdom and classified in six major chemical classes, namely, polyketides, terpenes, peptides, alkaloids, shikimates and sugars. Marine natural product showed antibacterial, anticoagulant, antimalarial, anti-inflammatory, antiprotozoal, antituberculosis and antiviral effects (Soltani et al., 2011; Jung et al., 2007; Abad et al., 2008; Carballeira, 2008; Mayer et al., 2007). Algae have been a very important source of bioactive natural products and many species of red, green and brown macro and micro algae have been screened to see if they contain substances with antibacterial activity (Sasidharan et al., 2010). There are number of reports concerning the inhibiting activities from macroalgae against human bacterial and fungal pathogens. Those products have been applied for bacterial diseases in other organisms such as fish and plant for many years (Bansemir et al., 2006). *Enteromorpha* sp. Is a tubular alga that is known

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Abbreviations: DMSO, Dimethyl solphoxamide; **PBS,** phosphate buffered saline.

to be a dominant species in saline coastal sea. In the shallow coastal zone of the southern Caspian Sea especially algae *Enteromorpha* sp. dominant benthic plants. This alga predominantly is found attached to rocky and stony shores or mixed with other genera (such as *Cladophora* sp. and *Ulva* sp.). In this study we determined antibacterial activity on the several grams positive and negative bacteria (*Salmonella typhimurium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Proteus mirabilis*) in *Enteromorpha intestinalis* extract in order to understand the usefulness of this alga as a foodstuff as well as a medicine.

MATERIALS AND METHODS

Chemicals

Muller Hinton Agar, Nutrient broth and Dimethyl solphoxamide (DMSO) were purchased from Merck (Germany). All other chemicals were of analytical grade or purer.

Collection and preparation of sample

Samplings were carried out in the southern coast of the Caspian Sea in Sari, Mazandaran Province, Iran, in summer 2010. Samples of *Enteromorpha* were collected manually from the rocks. Harvested macroalgae were stored in plastic bags for transport to the laboratory. Voucher specimen of species were pressed and stored in 5% formalin for identification according to Burrows (1991), Voucher (No. 122) are deposited in herbarium (Qaemshahr branch, Islamic Azad University, Qaemshahr, Iran). Biomass was rinsed with fresh water to eliminate other materials such as sand, shells, etc. The macroalgae were stored in the laboratories.

Collection and preparation of algal extracts

Dried materials were coarsely ground before extraction. 5 g of dried materials was extracted by maceration with 70% ethanol (1 h sonication, filtered; repeated 2 times). The extract was then separated from the sample residue by filtration through Whatman No. 1 filter paper. The resultant extracts were concentrated in a rotary evaporator under reduced pressure until a crude solid extract was obtained which were then freeze-dried for complete solvent removal (0.9 g).

Microorganisms used and determination of antibacterial activity

Five bacterial strains (gram positive and negative) were selected for the study. Gram positive species were *B. subtilis* and *S. aureus* while the gram negative species were *P. mirabilis*, *P. aeruginosa* and *S. typhimurium*. Each bacterial strain was incubated in nutrient broth at 37°C overnight (14 h), and test bacterial solutions were prepared with the same broth to give a concentration of 1.5×10^8 CFU ml^{-1}. Suspensions of microorganisms were transferred onto the surface of Muller Hinton Agar media and spread evenly over the entire surface of the plates. Blank discs (6.4 mm, Padtan Teb, Iran) impregnated with 20 µl of a serial 20-fold dilution of extract compounds (100, 50, 25, 12.5, 6.25, 3.125 and 1.565 mg ml^{-1}) were prepared using 50% DMSO. The plates spread with bacteria were incubated at 37ºC for 24 h. After incubation, the inhibition zones formed around the disks were measured (Androw, 2001). Gentamycin disc (10 µg), Cefalexin disc (30 µg) and tetracycline disc (30 µg) were used as positive control.

Antihemolytic activity

Preparation of rat erythrocytes

Male Wistar rats were sacrificed under anesthesia and blood was collected by heart puncture in heparinized tubes. Erythrocytes were isolated and stored according to the method described by Yuan et al. (2005). Briefly, blood samples collected were centrifuged (1500×g, 10 min) at 4°C; erythrocytes were separated from the plasma and buffy coat and were washed three times by centrifugation (1500×g, 5 min) in 10 volumes of 10 mM phosphate buffered saline (pH 7.4 phosphate buffered saline; PBS). The supernatant and buffy coats of white cells were carefully removed with each wash. Washed erythrocytes were stored at 4°C and used within 6 h for further studies.

Antihemolytic activity of extract against H2O2 induced hemolysis

Antihemolytic activity of the extract was assessed as described by Naim et al. (1976) with slight modifications. The erythrocytes from male Wistar rat blood were separated by centrifugation and washed with phosphate buffer (pH 7.4). The erythrocytes were then diluted with phosphate buffered saline to give 4% suspension. 1 g of extract ml-1 of saline buffer was added to 2 ml of the erythrocyte suspension and the volume was made up to 5 ml with saline buffer. The mixture was incubated for 5 min at room temperature and then 0.5 ml of H_2O_2 solution in saline buffer was added to induce the oxidative degradation of the membrane lipids. The concentration of H_2O_2 in the reaction mixture was adjusted to about 90% hemolysis of blood cells after 240 min. After incubation, the reaction mixture was centrifuged at 1500 rpm for 10 min and the extent of hemolysis was determined by measuring the absorbance at 540 nm corresponding to hemoglobin liberation.

Statistical analysis

Experimental results are expressed as means±SD. All measurements were replicated three times. The data were analyzed by analysis of variance ($p<0.05$) and the means separated by Duncan's multiple range test.

RESULTS AND DISCUSSION

Determination of antibacterial activity

The inhibitory effects of the concentrations of *E. intestinalis* extract on the growth of various gram positive and negative bacteria of disc diffusion method is shown in Table 1. The extract showed activity against gram positive as well as gram negative bacteria and inhibitory effects were augmented with increase in extract concentrations. The *E. intestinalis* extract displayed variable degrees of antimicrobial activity on different bacteria. The *B. subtilis* was found to be more sensitive among gram positive bacteria with being the most sensitive (widest zones of inhibition) than *S. aureus*. Among the negative gram *P. aeruginosa* was found to be

Table 1. Antibacterial activity of *E. intestinalis* extract.

Bacteria name	Concentration of extract (mg/ml)							Tetracyclin	Cephalexin
	100	50	25	12.5	7.25	3.125	1.565		
	Inhibition zone (mm)								
Salmonella typhimurium	12.06	10.33	9.3	-	-	-	-		
Staphylococcus aureus	10.1	0.85	0.75	-	-	-	-		
Pseudomonas aeruginosa	-	-	-	-	-	-	-		
Bacillus subtilis	10.33	7.1	-	-	-	-	-	3.05	0.92
Proteus mirabilis	12	11	10.66	10.23	9.96	7.46	-	1.67	-

- no activity.

most resistant than *P. mirabilis* and *S. typhimurium*. In general, gram negative bacteria were more resistant than gram positive bacteria. Studies by other researchers reveal same type of results (Soltani et al., 2011). The *P. aeruginosa* was found to be the most resistant among all bacteria (without zones of inhibition).

Antihemolytic activity

Hemolysis has a long history in use of measuring free radical damage and its inhibition by antioxidants but only few studies have been performed with erythrocytes in whole blood. In this study, we used a biological test based on free radical-induced erythrocytes lyses in rat blood. This assay is useful either for screening studies on various molecules and their metabolites, especially those having an oxidizing or antioxidizing activity and molecules having a long-term action (Djeridane et al., 2006). Lipid oxidation of rat red blood cell (RBC) membrane mediated by H_2O_2 induces membrane damage and subsequently hemolysis. The effect of extract was tested and found that they did not show any harmful effects on erythrocytes in fact, exhibited strong antihaemolytic activity with IC_{50} = 323±11.7 µg ml^{-1} compared with 235 ± 9 µg ml^{-1} for vitamin C which served as positive control.

Conclusions

Natural products are in great demand owing to their various biological properties and bioactive components which have proved to be useful against large number of effects like antibacterial, anticoagulant, antimalarial, anti-inflammatory, antiprotozoal, antituberculosis and antiviral effects. The studies for bioactive molecules in marine organisms have been growing in the last decade (Mayer et al., 2011). It has been proved that extracts of *E. intestinalis* showed antibacterial and antihaemolytic activities. The present work represents the screening of antibacterial activities in *E. intestinalis* extract. This species collected from the coast of Caspian Sea showed more potent antibacterial activity against of *S. aureus* and *P. mirabilis* than *S. typhimurium* and *B. subtilis. P. aeruginosa,* extract showed any antibacterial activity. Identification of the antioxidant compounds of this extract will lead to their evaluation in considerable commercial potential in medicine, food production and the cosmetic industry.

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