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Evaluation of antibacterial activities in *Cladophora glomerata* and *Enteromorpha intestinalis*

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ABSTRACT

Cladophora and *Enteromorpha* are the filamentous green-algal genuses that have a widespread distribution in Caspian Sea Coast. The aim of this study was to assess the antimicrobial activities of *Cladophora* and *Enteromorpha* in South of Caspian Sea. Antibacterial activities of hydroalcoholic extracts of five different gram negative and positive bacteria including *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Proteus mirabilis* were investigated. The extract was primarily screened for their possible antibacterial effects using disc diffusion methods. The potential antibacterial activities at different concentrations of the extract were elucidated. The extracts of *Cladophora* and *Enteromorpha* displayed variable degrees of antimicrobial activities on different bacteria. Among gram positive bacteria the *Staphylococcus aureus* (with wider zones of inhibition) was found to be more sensitive than *Bacillus subtilis* and among the gram negative *Salmonella enteritidis* was found to be more resistant than *Pseudomonas aeruginosa* in the extract of *Cladophora glomerata*. On the other hand, in the extract of *Cladophora glomerata*, the *Staphylococcus aureus* was found to be more sensitive among gram positive bacteria. In the extract of *Enteromorpha intestinalis*, the *Bacillus subtilis* was found to be more sensitive among gram positive bacteria. In general, gram negative bacteria were more resistant than gram positive bacteria. Studies by other researchers revealed the same type of results. The *Pseudomonas aeruginosa* was found to be the most resistant among all bacteria (without zones of inhibition). Our findings suggest that the possibility of using the *Cladophora glomerata* and *Enteromorpha intestinalis* as the novel sources of natural antimicrobial and antioxidant agents for pharmaceutical industries.

1. Introduction

Enteromorpha and *Cladophora* are marine seaweeds present almost year round and attached to the submerged rocks, logs or other hard surfaces by a holdfast along the coastal region. Wind and wave action cause the algae to break free from the holdfast and wash up on shore. *Cladophora* is a genus of reticulated filamentous green algae, while the *Enteromorpha* first begins growing; it forms a single row of cells and after more cell division create to form a tube shape. Bioactive natural products can invigorate immunity as well as anti-bacterial (Stirk et al., 2007), anti-viral, antifungal (Volka et al., 2006), antioxidant (Le Tutour et al., 1998), anti-tumor (Jiao et al., 2009) and anti-inflammatory activities. There is an increasing need for new antibiotics in medicine for human and other organism because

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of the evolving resistance of microorganisms to existing antibiotics. Competition for life led to the product of antimicrobial defense strategies in algae, offer a particularly rich source of potential new antibacterial substance (Bansemir et al., 2006). Antibacterial activity of extracts studied in some of red, brown and green algae demonstrated that vary in different seaweeds and is proportional with the content of active compounds (Bansemir et al., 2006). Antibacterial activity has been widely investigated for new classes of antibiotics with novel structures that are effective against human pathogens (Vlachos et al., 1999, Abourriche et al., 1999 and Kajiwara et al., 2006). In the shallow coastal zone of the southern Caspian Sea the filamentous algae *Cladophora* sp. and *Enteromorpha* sp. are dominant benthic plants. They predominantly are found to be attached to rocky and stony shores.

This work determines the antibacterial activity of the several gram positive and negative bacteria (*Salmonella typhimurium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Proteus mirabilis*) in response to *Cladophora glomerata* and *Enteromorpha intestinalis* extracts. The present paper reports the results of a research aimed to verify the antibacterial activity in the extract of the green algae *Cladophora* and *Enteromorpha* from the floating benthic populations of south coast of the Caspian Sea in Sari, Iran, in order to understand the efficacy of this algae as a foodstuff as well as in medicine and recommends its use in human diets and commercial purpose.

2. Materials and Methods

2.1. Chemicals

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

2.2. Collection and preparation of sample

Samplings were carried out in the southern coast of the Caspian Sea in Sari, Mazandaran Province, Iran, in summer 2011. Samples of *Enteromorpha intestinalis* and *Cladophora glomerata* were collected manually from the rocks (Figure 1). Harvested macro-algae were stored in plastic bags for transport to the laboratory. Voucher specimen of species were pressed and stored in 5% formalin according to Burrows (1991), Voucher (No. 122 for *Enteromorpha intestinalis* and No. 121 for *Cladophora glomerata*) are deposited in herbarium (Qaemshahr branch, Islamic azad university, Qaemshahr, Iran). Biomass was rinsed with fresh water to eliminate other materials such as sand and shells. The macroalgae were stored in the laboratories for further analysis.

2.3. Collection and preparation of algal extracts

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

2.4. Microorganisms used and determination of antibacterial activity

Five bacterial strains (gram positive and negative) were selected for this study. Gram positive species were *Bacillus subtilis* and *Staphylococcus aureus* while the gram negative species were *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*. Each bacterial strain was incubated in nutrient broth at 37ºC overnight (14 h), and the 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, 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 (30µg) and Tetracycline disc (30µg) were used as positive control (Figure 2).

Figure 1. Sampling location (1.Satellite image 2. Place image 3. Algae attached to rock)

2.5. 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. The IC_{50} values were calculated from linear regression analysis.

3. Results

3.1. Determination of antibacterial activity of Cladophora glomerata

The inhibitory effects of the concentrations of *Cladophora glomerata* extract on the growth of various gram positive and negative bacteria by disc diffusion method is shown in Table 1. The extract showed activity against gram positive as well as gram negative bacteria and the inhibitory effects were augmented with increase in extract concentrations. The *Cladophora* extract displayed variable degrees of antimicrobial activity on different bacteria. The *Staphylococcus aureus* was found to be more sensitive among gram positive bacteria. Among gram negative *S. typhimurium* was found to be more resistant than *P. mirabilis*. In general, gram negative bacteria were more resistant than gram positive bacteria. The *Pseudomonas aeruginosa* was found to be the most resistant among all bacteria (without zones of inhibition).

3.2. Determination of antibacterial activity of Enteromorpha intestinalis

The inhibitory effects of the concentrations of *Enteromorpha intestinalis* extract on the growth of various gram positive and negative bacteria of disc diffusion method is shown in Table 2. The extract showed activity against gram positive as well as gram negative bacteria and inhibitory effects were augmented with increase in extract concentrations the same as *Cladophora* extract. The *Enteromorpha* extract displayed variable degrees of antimicrobial activity on different bacteria. The *Staphylococcus aureus* was found to the most sensitive among gram positive bacteria. In contrast to *Cladophora* extract, among gram negative *P.mirabilis* was found to be most resistant than *S. typhimurium*. In general, gram negative bacteria were more resistant than gram positive bacteria. The *Pseudomonas aeruginosa* was found to be the most resistant among all bacteria (without zones of inhibition).

4. Discussion

Natural products are in great demand owing to their various biological properties and bioactive components which have been shown to have antibacterial, anticoagulant, anti malarial, anti-inflammatory, anti protozoal, anti tuberculosis and anti viral effects. The studies

for bioactive molecules in marine organisms have been growing in the last decade (Mayer et al., 2011). The purpose of this study was to evaluate the capability of different genus of seaweed from the coastal of the Caspian Sea on the growth of different bacteria. This study revealed different levels of bioactivity of the extracts from the two seaweeds analysed as well as different susceptibilities of the different gram positive and gram negative species of bacteria. Seaweeds extracts have the broadest antibacterial spectrum on different bacteria. Other studies also demonstrated that the extract from seaweeds including green, brown and red algae such as *Laminaria japonica*, *Kjellmaniella crassifolia*, *Gracilaria verrucosa* and *Ulva pertusa* showed strong antimicrobial activities against *Escherichia coli* and *Erwinia carotovora* (Kajiwara et al., 2006). In green algae such as *Cladophora* and *Enteromopha*, there are several studies about antibacterial activities on different bacteria species. *Cladophora alba, Cladophora rupestris* and *Cladophora glomerata* showed various inhibition activity against several species of *Vibrio* spp. *Enteromorpha compressa* showed weak antimicrobial activity on *Pseudomonas anguilliseptica* and no antimicrobial activity on *Vibrio anguillarum*. This work demonstrated the screening of antibacterial activities of *Cladophora glomerata* and *Enteromorpha intestinalis* extracts. Those species collected from the coast of Caspian Sea showed different levels of antibacterial activities. The *Cladophora glomerata* extract showed more potent antibacterial activity against of *Staphylococcus aureus* and *Proteus mirabilis* than *Salmonella typhimurium* and *Bacillus subtilis*. Also, the *Cladophora glomerata* extract showed more potent antibacterial activity against of *Staphylococcus aureus* and *Proteus mirabilis* than *Salmonella typhimurium* and *Bacillus subtilis*. On the other hand in *Enteromorpha intestinalis* extract showed more potent antibacterial activity against *Salmonella typhimurium* and *Proteus mirabilis* than *Bacillus subtilis* and *Staphylococcus aureus*. In *Pseudomonas aeruginosa*, extract showed no antibacterial activity in both of them.

In conclusion, our results showed that the investigated seaweeds may be a potential source of antibacterial compounds which could be used for the prevention and treatment of some bacteria. Differences between the results of the present investigation and the results of other studies may be due to the production of bioactive compounds related to the solvents used for the extraction or place of sampling. Moreover, all the investigated seaweeds could be a potential source of biologically active compounds. Identification of the antibacterial compounds of those extract will lead to their evaluation in considerable commercial potential in medicine, food production and the cosmetic industry.

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Table 1.Antibacterial activity of *Cladophora glomerata* extract.

	Concentration of extract (mg/ml)						
Bacteria name	100	50	25	12.5	7.25	3.125	1.565
	Inhibition zone(mm)						
Salmonella typhimurium	7.7	6.3	-	-	-	-	-
Staphylococcus aureus	22.5	21.8	18	13.8	8.7	-	-
Pseudomonasa aeruginosa	-	-	-	-	-	-	-
Bacillus subtilis	11.5	9.2	8.2	-	-	-	-
Proteus mirabilis	15	13.8	12	-	-	-	-

- no activity

Table 2. Antibacterial activity of *Enteromorpha intestinalis* extract.

Bacteria name	Concentration of extract (mg/ml)						
	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	-	-	-	-
Pseudomonasa aeruginosa	-	-	-	-	-	-	-
Bacillus subtilis	10.33	7.1	-	-	-	-	-
Proteus mirabilis	12	11	10.66	10.23	9.96	7.46	-

- no activity

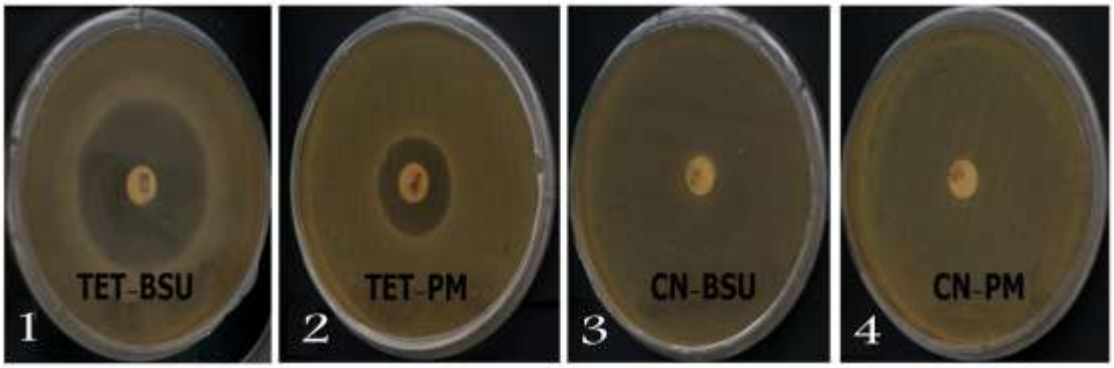

Figure 2. Disc diffusion assay (A =Disc impregnated with antibiotics; B = Negative control; 1, 2= Bacillus subtilis and Proteus mirabilis with Tetracycline disc respectively; 3, 4= Bacillus subtilis and Proteus mirabilis with Cefalexin disc respectively).

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