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RESEARCH ARTICLE

Antibacterial and Antifungal Effects of Some Marine Algae

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Summary

Ethanolic crude extracts from seven marine algal species belong to Clorophyceae *(Cladophora glomerata, Enteromorpha linza, Ulva rigida),* Phaeophyceae *(Cystoseira barbata, Padina pavonica)* and Rhodophyceae *(Corallina officinalis, Ceramium ciliatum)* from the coast of Vona, Turkey were evaluated in vitro for the antibacterial and antifungal activity of six bacteria and two fungus with the paper disc agar diffusion methods. The marine algae analysed of *Cladophora glomerata* and *Padina pavonica* were the species with the strongest activities against the broadest spectrum of test organisms. In particular, *Enteromorpha linza* and *Padina pavonica* showed the highest antifungal activity against *Aspergillus niger,* while *Cladophora glomerata* showed the highest antibacterial activity against *Staphylococcus aureus.* On the other hand, it was found that extracts of all marine algae were significant antimicrobial activity against *Staphylococcus aureus, Salmonella typhimurium* and *Aspergillus niger.* The minimal inhibition concentrations (MIC) of the crude extract obtained from *Enteromorpha linza* and *Padina pavonica* ranged from 2.5 to 10 mg/ml and 1.25 to 10 mg/ml, respectively.

Keywords: *Antimicrobial effect, Marine algae*

Bazı Deniz Alglerinin Antibakteriyel ve Antifungal Etkileri

Özet

Türkiye'de Vona kıyısından toplanan Clorophyceae *(Cladophora glomerata, Enteromorpha linza, Ulva rigida),* Phaeophyceae *(Cystoseira barbata, Padina pavonica)* ve Rhodophyceae *(Corallina officinalis, Ceramium ciliatum)* familyalarına ait yedi deniz alg türünden elde edilen etanol ektraktlarının altı bakteri ve iki fungusa karşı *in vitro* koşullarda antibakteriyel ve antifungal aktiviteleri kağıt disk difüzyon metodu ile değerlendirilmiştir. Deniz alglerinin analizi sonucunda *Cladophora glomerata* ve *Padina pavonica* türleri test edilen organizmalara karşı en yüksek aktiviteye sahip olduğu görülmüştür. Özellikle, *Enteromorpha linza* ve *Padina pavonica, Aspergillus niger* türüne karşı en yüksek fungal aktiviteyi gösterirken *Cladophora glomerata* ise *Staphylococcus aureus* türüne karşı en yüksek antibakteriyel aktiviteyi göstermiştir. Bunun yanısıra tüm alg türlerinin ekstraktları *Staphylococcus aureus, Salmonella typhimurium* ve *Aspergillus niger* türlerine karşı yüksek derecede antimikrobiyal aktivite gözlenmiştir. *Enteromorpha linza* ve *Padina pavonica*'dan elde edilen kuru ektraktların minimal inhibisyon konsantrasyonu (MİK) sırasıyla 2.5-10 mg/ml ve 1.25-10 mg/ml değişen aralıktadır.

Anahtar sözcükler: *Antimikrobiyal etki, Deniz algleri*

INTRODUCTION

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [1]. Marine algae are found to be important sources of useful bioactive substances since two decades [2]. More than 150.000 macroalga or seaweed species are found in the oceans of the globe but only a few of them were identified [3]. Secondary or primary metabolites obtained from these organisms may be potential bioactive compounds of interest for the pharmacological industry [4]. Many substances obtained from marine algae such as alginate, carragenean and agar as phycocolliods have been used for decades in medicine and pharmacy [5]. Marine secondary metabolites are organic compounds produced by microbes, sponges, seaweeds, and other marine organisms [6]. There are numerous reports of macroalgae derived compounds that have a broad range of biological activities, such as antibiotic, antiviral, anti-neoplastic, antifouling, anti-inflammatory, cytotoxic and antimitotic [7-12].

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In this investigation, antibacterial and antifungal activity of seven marine algae belonging to Chlorophyta (*Cladophora glomerata*, *Enteromorpha linza*, *Ulva rigida*), Phaeophyta (*Cystoseira barbata*, *Padina pavonica*) and Rhodophyta (*Ceramium ciliatum*, *Corallina officinalis*) were studied against pathogenic organisms (*Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenesis*, *Pseudomonas aeruginosa*, *Bacillus cereus*) and two fungus (*Candida albicans*, *Aspergillus niger*) *in vitro*.

MATERIAL and METHODS

Plant Material and Preparation of Plants Extracts

The marine algae of Chlorophyta (*Cladophora glomerata*, *Enteromorpha linza*, *Ulva rigida*), Phaeophyta (*Cystoseira barbata*, *Padina pavonica*) and Rhodophyta (*Ceramium ciliatum*, *Corallina officinalis*) were collected at a depth of 1-2 m from the coast of Vona Bay Perşembe, Ordu in May 2008 and were identified by used the study of Aysel et al.[13] Samples were washed with tap water to remove epiphytes and other marine organisms and then washed with distilled water. Fresh marine algae were dried at 23-25°C for 1-2 weeks and powdered. The extract of the marine algae were prepared according to the methods described by Ertürk et al.[14] and Holopainen et al.[15], with slight modification. Dried leaves and twigs of the Seaweeds were extracted with 95% ethanol (20 g 1/5 ethanol) at room temperature. The extracts were kept at 4°C for a day, and they were filtered through 45 µm membrane filter, and then the solution was dried with an evaporator. The crude extracts were stored at -20°C until used.

Microorganisms Tested and Culture Media

Strains of bacteria and fungus were obtained from ATCC (American Type Culture Collection, Rockville, Maryland). Antimicrobial activities of seven crude extract samples obtained from the seven marine algae species were assayed against *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 10876), *Aspergillus niger* (ATCC 9642), *Salmonella typhimurium* (ATCC 14028), *Listeria monocytogenes* (NCTC 11994), *Escherichia coli* (ATCC 25922), *Candida albicans* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853).

The species of bacteria were grown in Mueller Hinton Agar (Merck) and Mueller Hinton Brot (Merck). *C. albicans* and *A. niger* were grown in Sabouraud Dextrose Broth (Difco) and Sabouraud Dextrose Agar (Oxoid). The concentrations of bacterial suspensions were adjusted to 10^8 cells/ml, while those of fungal suspensions to 10^7 cells/ml.

Antifungal Assay and Antibacterial Assay

Antibacterial and antifungal activities were measured using methods of diffusion disc plates on agar [16]. In order to test antibacterial and antifungal activity, the fractions of seven marine algae samples were dissolved in 70% ethanol. Mueller Hinton Agar medium (Merck) (20 ml) for bacteria and Sabouraud Dextrose Agar (Oxoid 20 ml) for fungus were poured into each 15 cm petri dish. All bacterial strains were grown in Mueller Hinton Broth medium (Merck) for 24 h, at 37°C, and *C. albicans* and *A. niger* were grown in Sabouraud Dextrose Broth (Difco) at 37°C for 48 h. Growth was adjusted to OD (600 nm) of 0.1 by dilution with Mueller Hinton Broth medium (Merck) and Sabouraud Dextrose Broth medium (Difco) for bacteria and fungus, respectively. Suspension (100 µl) with approximately 10^8 bacteria and fungus per milliliter was placed in petri dishes, over agar and dispersed. Then, sterile paper discs (Oxoid, CT09988, 6 mm diameter) were placed on agar to load 15 µl of each plant (20 mg/ml). One hundred units of nystatin for fungus and Ampicillin and Cephazolin for bacteria, all obtained from a local pharmacy, were used as positive controls and alcohol as a negative control. Inhibition zones were determined after incubation at 37°C for 48 h. All tests were made in triplicate.

Minimum Inhibition Concentration

The agar dilution method, described by Vanden Berghe and Vietinck was used for the antibacterial screening with slight modifications [17]. Instead of 96 well microtitre plates 24 well tissue culture (Corning) plates were used. The crude extracts of seven marine algae sample were dissolved in 70% ethanol and physiological Tris buffer (Amresco 0826-500G) 1:4 and mixed with an equal amount of 3% agar solution at 45°C to a final concentration of 10, 5, 2.5 and 1.25 mg of (1 g/ml) extract/ml. From the solutions 400 µl was transferred into each well of the tissue culture (Corning) plate. After solidification each well was inoculated with 10 µl of freshly prepared bacterial suspension of 10^8 bacteria/ml and incubated at 37°C for 24 h. Ampicillin and Cefazolin, obtained from a local pharmacy, were used at 10, 5, 2.5 and 1.25 mg/ml (1 g/ml stock) as positive control for bacteria, nystain was used as positive control for fungus, and 70% alcohol was used as negative control. The bacterial growth was assessed by a stereo microscope after the incubation period. All tests were made in triplicate.

RESULTS

Crude extracts of seven marine algae from coast of Vona Bay were tested against bacteria (*S. aureus* (ATCC 25923), *B. cereus* (ATCC 10876), *S. typhimurium* (ATCC 14028), *L. monocytogenes* (NCTC 11994), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) and fungus *C. albicans* (ATCC 25922, *A. niger* (ATCC 9642). Antibacterial and antifungal activities were determined by using paper disc agar diffusion method and the results are summarized in *Table 1*.

Cladophora glomerata and *Padina pavonica* were the species with the strongest activities against the broadest spectrum of test organisms. In particular, *Enteromorpha linza* and *Padina pavonica* showed the highest antifungal activity against *Aspergillus niger* (17 mm and 17 mm/15 µl inhibition zone, respectively), while *Cladophora glomerata* showed the highest antibacterial activity against *Staphylococcus aureus* (17 mm/15 µl inhibition zone). On the other hand, *Cystoseria barbata* has the lowest inhibitor activity against the tested organisms. Besides; it was found that extracts of all marine algae were significant antimicrobial and antifungal activity against *Staphylococcus aureus, Salmonella typhimurium* and *Aspergillus niger*.

Evaluation of MICs of the crude extracts obtained from some marine algae by means of agar dilution experiment method is reported in *Table 1*. All fractions at concentrations higher than 1.25 mg/ml showed antibacteral and antifungal activity.

The crude extract sample from *Cladophora glomerata* required an MIC of >1.25 mg/ml for *E. coli, S. aureus, S. typhi, L. monocytogenes* and *P. aureginosa* and of >10 mg/ml for *B. cereus*. The crude extract sample from *Enteromorpha linza* required an MIC of 10 mg/ml for *B. cereus* and a lower MIC value (>1.25 mg/ml) against *A. niger*. In addition; *Padina pavonica* required an MIC of >1.25 mg/ml for *E. coli, S. aureus, P. aeruginosa* and *A. niger*. *S. aureus* was less activity to ampicillin and highly resistant to cefazolin with 10-30 µg, respectively whereas *Cladophora glomerata* showed activity against *S. aureus*. Moreover, the antifungal activity of *Enteromorpha linza* and *Padina pavonica* was stronger than the standard antifungal (100 units of nystatin) against *Aspergillus niger* and *Candida albicans*.

DISCUSSION

The main objective of this study was evaluate and compare ability of different marine algae from Vona Bay to produce bioactive compounds of potential therapeutic interest. The antibacterial and antifungal activities of several of the marine algae reported in the literature [18,19]. In this study, *S. aureus* and *S. typhi* were the most sensitive than bacteria, *A. niger* was the most sensitive than fungus and *B. cereus* was more resistant against all the ethanol extracts of the marine algae tested. Besides, all marine alg extract in this study required an higher MIC value (>10 mg/ml) against *B. cereus*.

Several different organic solvents have been used to screening algae for antibacterial activity in previous studies. Tüney et al.[20] reported antibacterial activities in diethyl ether extracts from 11 macroalga species of Urla Coast from 6 species tested. Sastry et al.[21] related antibacterial activity in the benzene, chloroform and methanol extracts of some marine algae against gram positive and gram negative pathogenic strains [21]. Ballesteros et al.[11] found the methanol/toluene extract of *Padina pavonica* had no antibacterial and antifungal activity. In our studies, fractions of ethanol extract of *Padina pavonica* had shown good activity against *A. niger* and *C. albicans*.

Another significant result of the present study was showed that the ethanol extracts of *Enteromorpha linza* and *Padina pavonica* showed antibacteral activity against *E. coli* (13 mm and 15 mm/15 µl inhibition zone, respectively) and *S. aureus* (13 mm and 16 mm/15 µl inhibition zone, respectively). Similar observation were made by Ibtissam et al.[19] reported the same inhibition

Table 1. *Results of antimicrobial screening of marine algae extracts determined by the agar-well diffusion method (minimum inhibitory concentration, MIC, in mg/ml) and agar diffusion method (inhibition zone in mm)*

Tablo 1. *Deniz alglerinden elde edilen özütlerin antimikrobiyal etkilerinin agar-well difüsyon ve agar disk difüsyon metodu ile belirlenen sonuçları (Minimum İnhibisyon Konsantrasyonu-MIC değerleri (mg/ml) ve inhibisyon zonları-mm)*

TG	Taxa	Microorganisms															
		Inh. Zone (mm)								MIC (mg/ml)							
		Ec	Bc	Sa	St	Lm	Pa	Ca	An	Ec	Bc	Sa	St	Lm	Pa	Ca	An
Cholorophyta	*Cladophora glomerata*	16	9	17	16	16	17	16	16	>1.25	>10	>1.25	>1.25	>1.25	>1.25	>2.5	>5
	Enteromorpha linza	13	8	13	12	12	10	11	17	>5	>10	>2.5	>5	>2.5	>5	>10	>1.25
	Ulva rigida	11	10	15	15	10	14	12	12	>10	>10	>2.5	>1.25	>10	>2.5	>10	>10
Phaeophyta	*Cystoseira barbata*	12	9	16	12	14	9	12	11	>5	>10	>1.25	>2.5	>2.5	>10	>10	>10
	Padina pavonica	15	10	16	14	14	15	13	17	>1.25	>10	>1.25	>2.5	>2.5	>1.25	>5	>1.25
Rhodophyta	*Corallina officinalis*	15	8	13	13	12	11	12	14	>1.25	>10	>5	>2.5	>2.5	>5	>10	>5
	Ceramium ciliatum	13	8	7	15	14	11	14	14	>5	>10	>10	>2.5	>1.25	>2.5	>5	>2.5
Antibiotics	AM	15	26	10	22	24	27	NT	NT								
	CE	15	23	-	23	32	24	NT	NT								
	NYS	NT	NT	NT	NT	NT	NT	15	15								
	%70 Alkol	-	-	-	-	-	-	-	-								

Ec: *Escherichia coli,* ***Bc:*** *Bacillus cereus,* ***Sa:*** *Staphylococcus aureus,* ***St:*** *Salmonella typhimurium,* ***Lm:*** *Listeria monocytogenesis,* ***Pa:*** *P. aureginosa,* ***Ca:*** *C. albicans,* ***An:*** *Aspergillus niger;* ***Control : AM,*** *Ampicillin 10 µg;* ***CE,*** *Cefazolin 30 µg;* ***NYS,*** *Nystatin 100 Units;* ***TG:*** *Taxonomic group;* **** NT:*** *Not tested;* ***- :*** *No inhibition*

activity of ethanol extracts of this algae.

Tüney et al.[20] used fresh and dried materials of *Ulva rigida* for the extraction. They found that while dried samples had no activity against *S. aureus,* the extract prepared from fresh material has shown remarkably inhibitor activity to same strain. In contrast, our results showed that the crude extract of *Ulva rigida* inhibited all test organisms. Oral et al.[22] suggest that the use of some plant hydrosols as antimicrobial agents may be exploitable to prevent deterioration of stored foods by bacteria, as long as the taste impact is acceptable in targeted foods. Similarity, our study shown that some marine algae have antimicrobial effect. So, algae might be used for food protect. Each of the various types of antibiotics kill microorganisms in a unique way. Some disturb the structure of the bacterial cell wall; others interfere with the production of essential proteins; and still others interfere with the transformation (metabolism) of nucleic acid [23]. The algae samples used in the current study affect bacteria grow because they probably contain similar antibiotics substances.

In summary, our results indicate that these species of marine algae collected from coast of Vona Bay present a significant capacity to show a variety of antimicrobial and antifungal activity. Based on the results of this paper, we suggest that *Cladophora glomerata* and *Padina pavonica* may have potential as bioactive compounds and should be investigated for natural antibiotics. This study has shown that the production of antibacterial substances by macroalgae is a regular occurrence among those found on the coast of Vona Bay.

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