Antibacterial and Antifungal Effects of Some Marine Algae

Ömer ERTÜRK * Beyhan TAŞ *

* Department of Biology, Faculty of Arts and Sciences, Ordu University, Cumhuriyet Campus, TR-52000 Ordu - TURKEY

SUMMARY

Ethanolic crude extracts from seven marine algal species belong to Chlorophyceae (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

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. Marine algae are found to be important sources of useful bioactive substances since two decades. More than 150,000 macroalgae or seaweed species are found in the oceans of the globe but only a few of them were identified. Secondary or primary metabolites obtained from these organisms may be potential bioactive compounds of interest for the pharmacological industry. Many substances obtained from marine algae such as alginate, carrageenan and agar as phycocolloids have been used for decades in medicine and pharmacy. Marine secondary metabolites are organic compounds produced by microbes, sponges, seaweeds, and other marine organisms. 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.

Keywords: Antimicrobial effect, Marine algae

Bazı Deniz Algelerinin Antibakteriyal ve Antifungal Etkileri

Özet

Türkiye’de Vona kıyısından toplanan Chlorophyceae (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 ekstraktların alt bakteri ve iki fungusa karşı in vitro koşullarda antibakteriyal ve antifungal aktiviteleri kağıt disk difüzyon metodu ile değerlendirilmiştir. Deniz algelerinin analizi sonucunda Cladophora glomerata ve Padina pavonica türleri test edilen organizmaları karşısında 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 yanı sıra tüm alg türlerinin ekstraktları Staphylococcus aureus, Salmonella typhimurium ve Aspergillus niger türlerine karşı yüksek derecede antimikrobiyel aktiviteye göstermiştir. Enteromorpha linza ve Padina pavonica’dan elde edilen kuru ekstraktların minimal inhibisyon konsantrasyonu (MİK) sırasıyla 2.5-10 mg/ml ve 1.25-10 mg/ml değişen aralıktadır.

Anahtar sözcükler: Antimikrobiyel etki, Deniz algıları
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 monocytogenes*, *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 the study of Aysel et al.\(^\text{13}\)

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.\(^\text{14}\) and Holopainen et al.\(^\text{15}\), 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 Broth (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\(^\text{16}\). 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\(^\text{17}\). 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, nystatin 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.
**DISCUSSION**

The main objective of this study was to evaluate and compare the ability of different marine algae from Vona Bay to produce bioactive compounds of potential therapeutic interest. The antibacterial and antifungal activities of several marine algae reported in the literature [16,19]. 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 algae 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. reported antibacterial activities in diethyl ether extracts from 11 macroalga species of Urla Coast from 6 species tested. Sastry et al. related antibacterial activity in the benzene, chloroform and methanol extracts of some marine algae against gram positive and gram negative pathogenic strains. Ballesteros et al. 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 that the ethanol extracts of *Enteromorpha linza* and *Padina pavonica* showed antibacterial 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. reported the same inhibition against gram negative pathogenic strains.

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**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)**

<table>
<thead>
<tr>
<th>TG</th>
<th>Taxa</th>
<th>Microorganisms</th>
<th>Inh. Zone (mm)</th>
<th>MIC (mg/ml)</th>
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<td></td>
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<td></td>
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<td>Ec</td>
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<tr>
<td>Chorophyta</td>
<td><em>Cladophora glomerata</em></td>
<td></td>
<td>16</td>
<td>9</td>
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<tr>
<td></td>
<td><em>Enteromorpha linza</em></td>
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<td>13</td>
<td>8</td>
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<tr>
<td></td>
<td><em>Ulva rigida</em></td>
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<td>11</td>
<td>10</td>
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<tr>
<td>Phaeophyta</td>
<td><em>Corallina officinalis</em></td>
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<td>15</td>
<td>8</td>
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<tr>
<td></td>
<td><em>Padina pavonica</em></td>
<td></td>
<td>15</td>
<td>10</td>
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<tr>
<td>Rhodophyta</td>
<td><em>Ceramium ciliatum</em></td>
<td></td>
<td>13</td>
<td>8</td>
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<tr>
<td>Antibiotics</td>
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<td>NYS</td>
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</table>

Ec: Escherichia coli; Bc: Bacillus cereus; Sa: Staphylococcus aureus; St: Salmonella typhimurium; Lm: Listeria monocytogenes; Pa: P. aeruginosa; 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.\(^2\) 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.\(^2\) suggest that the use of some plant hydrosols as antimicrobial agents may be exploit- able 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.\(^2\) The algae samples used in the current study affect bacteria grow because they probably contain similar anti- biotics 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.

REFERENCES


