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Antimicrobial and antioxidant activities of *Cystoseira crinita* Duby and *Ulva intestinalis* Linnaeus from the coastal region of Sinop, Turkey

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ABSTRACT

Objective: To evaluate *in vitro* antimicrobial, antioxidant activities and total phenol contents of *Cystoseira crinita* and *Ulva intestinalis* species collected from the coastal region of Sinop.

Methods: The antimicrobial activity of each methanolic algae sample was screened by using disc diffusion method against to 15 bacteria and 3 yeasts. The antioxidant potential of the extracts on the stable radical 1,1-diphenyl-2-picrylhydrazyl was determined. The total phenolic content of the 3 methanolic extracts of the seaweed samples were determined by the Folin-Ciocalteu method.

Results: The results of antimicrobial assay showed that extracts were more effective against Gram-positive bacteria rather than Gram-negative. In addition, while the algal extracts had antifungal efficacy against *Candida krusei*, the other yeast strains were not affected at all. According to the findings of antioxidant activity, all methanolic extracts displayed good free radical scavenging activity ranging from $IC_{50} = (32.19 \pm 0.08)$ mg/mL to the $IC_{50} = (37.57 \pm 0.11)$ mg/mL. The total phenols content of the macroalgal extracts were found as between (5.10 ± 0.16) mg gallic acid equivalent/g and (87.70 ± 1.03) mg gallic acid equivalent/g. In this sense, our findings confirmed that there was a positive linear correlations ($r = 0.86$) between total phenol contents and the IC_{50} values.

Conclusions: The data gathered from this study suggested that the seaweeds can be used as a potential natural seafood sources owing to the antimicrobial efficiency and good antioxidant activity.

1. Introduction

The marine world, due to its phenomenal biodiversity, is an important natural resource of many bioactive substances such as polyunsaturated fatty acids, sterols, proteins, polysaccharides, antioxidants and pigments[1]. Seaweeds known as macroalgae produce many biologically active phytochemicals such as carotenoids, terpenoids, xanthophylls, chlorophylls, phycobilins, polyunsaturated fatty acids, polysaccharides, vitamins, sterols, tocopherols and phycocyanins[2]. Nowadays seaweeds are used as dietary supplements in daily life and metabolize human health[3]. Marine macroalgae are also important ecologically and commercially to many regions of the world, especially in Asian countries such as China, Japan and Korea. The Japanese and

Chinese use brown algae in the treatment of hyperthyroidism and other glandular disorders.

Lipids, proteins and nucleic acids involve in reactive oxygen species that cause oxidative damage. It may trigger various chronic diseases such as coronary heart disease, atherosclerosis, cancer, and aging[4]. Epidemiological studies have indicated that intake of certain vitamins, minerals and other nutrients help to protect the body against heart disease, cancer and aging process. Antioxidants may have a protective effect in preventing or reducing the severity of these diseases[5]. The synthetic antioxidants, such as butylated hydroxyanisole and butylated hydroxytoluene have been suspected to use their effects of carcinogenesis and liver damage[6]. Concerns about the reliability of synthetic antioxidants have increased the interest in plants and algae commonly present in natural antioxidants[7,8].

Cystoseira crinita (*C. crinita*) and *Ulva intestinalis* (*U. intestinalis*) are plentiful and extensive seaweed species along the northern of Black Sea seacoast of Turkey[9]. Two species of marine algae are highly valuable marine resources in Asian

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country and Europe due to using human-edible, food additives, fertilizers and animal feed[10]. They are also too sensitive against anthropogenic effect and pollution[11,12]. In recent years, many microorganisms are developing resistance against to synthetic and semi-synthetic drugs used for microbial infections treatment. However, the synthetic antimicrobial agents have the side effects. Thus, numerous scientists supposed that there is an urgent need to develop or discover new antimicrobial substances[13,14]. The results of numerous studies indicated that seaweed extracts had selective and effective antimicrobial activities against bacteria, fungi and virus in the parts of the world[15]. However, our knowledge obtained from literature indicated that data related to antioxidant and antimicrobial activity of seaweeds throughout Black Sea coastal line of Turkey are very limited. Hence, the aim of the present study was to investigate the activity of the antioxidant and antimicrobial of two common species of seaweeds in Sinop Peninsula coast of the Black Sea, Turkey.

2. Materials and methods

2.1. Samples collection

The samples of *C. crinita* and *U. intestinalis* were collected from Sinop Peninsula coast of the Black Sea, Turkey, in December 2013. After collection, the seaweeds were washed with fresh water to remove associated debris and epiphytes. The cleaned material was then air dried to dryness in the shade at 30 °C. The dried samples were finely powdered and stored at -20 °C until the next analysis were carried out.

2.2. Preparation of algae extracts

About 10 g of dried samples were extracted by 100 mL of methanol for 24 h in a shaking water bath. The combined extracts were filtered and concentrated under vacuum to obtain a crude extract.

2.3. Microorganisms

The test microorganisms, 15 Gram-positive bacteria [*Bacillus subtilis*, *Bacillus megaterium* ATCC 1842 (*B. megaterium*), *Bacillus thuringiensis* var. *israelensis*, *Bacillus cereus* ATCC 7064, *Bacillus sphaericus* (*B. sphaericus*), *B. sphaericus* ATCC 2362, *B. megaterium* MRS 400, *B. megaterium* DSM 32, *Bacillus licheniformis*, *Micrococcus luteus* ATCC 9345, *Staphylococcus aureus* (*S. aureus*) (clinical sp.), *S. aureus* ATCC 25923, *Staphylococcus epidermidis* (*S. epidermidis*), vancomycin resistant *Enterococcus*, *Enterococcus faecalis* ATCC 51299 (*E. faecalis*)], 3 Gram-negative bacteria [*Escherichia coli* ATCC 11293 (*E. coli*), *E. coli* extended spectrum beta-lactamase-positive (ESBL+), *Klebsiella pneumonia* (*K. pneumonia*)] and 3 yeast species [*Candida albicans* ATCC 14053 (*C. albicans*), *Candida krusei* ATCC 6258 (*C. krusei*) and *Candida parapsilosis* ATCC 22019 (*C. parapsilosis*)] supplied

from the Molecular Biology and Microbiology Laboratory, Department of Biology, Faculty of Arts and Science, Sinop University, Turkey.

2.4. Antimicrobial assay

The antimicrobial activity of each methanolic algae sample was screened by using disc diffusion method[16]. All the microorganisms were maintained at -20 °C in nutrient agar for *Bacillus* spp., Mueller-Hinton agar for *Staphylococcus* spp., Luria agar for *Enterococcus* spp. and *E. coli* and Sabouraud dextrose agar for yeast (Difco) containing 17% (v/v) glycerol. Before testing, the microorganisms were transferred in nutrient broth for *Bacillus* spp., Mueller-Hinton broth for *Staphylococcus* spp., Luria broth for *Enterococcus* spp. and *E. coli* and Sabouraud dextrose broth for fungus (Difco) and cultured overnight at 37 °C. Then, the turbidity was adjusted equivalent to 0.5 McFarland standards and 100 µL of microorganisms was spread over the surface of an agar plate. The filter paper discs (6 mm) were loaded with methanolic algae extracts (2 mg/disc) and was allowed to dry completely. Afterwards, it was placed on the surface of the freshly inoculated medium. The plates were incubated for 24 h at 37 °C. Penicillin G (10 mg/mL), chloramphenicol (10 mg/mL), erythromycin (10 mg/mL) and cycloheximide (10 mg/mL) were used as positive control and negative control was 12.5% dimethyl sulfoxide. The antimicrobial activity was evaluated by measuring the diameter of inhibition zone.

2.5. Determination of free radical scavenging activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method

The antioxidant potential (free radical scavenging activity) of the extracts on the stable radical DPPH was determined by Blois[17] and Kumar *et al.*[18] methods. The seaweed samples were dissolved as 1 000 µg/mL in ethanol. Then, the solutions at different concentrations such as 1 000, 500, 250, 125 and 62.5 µg/mL were prepared with serial dilution technique. About 1 mL of the ethanol solution of each concentration containing the seaweed extract was mixed with 4 mL of a DPPH-ethanol solution (0.1 mmol/L). The samples were shaken well and kept in dark for 30 min at room temperature. The absorbance was measured at 517 nm. The free radical scavenging activity was calculated by using the following equation:

$$\% \text{ Inhibition} = \frac{A_B - A_S}{A_B} \times 100$$

Where A_B is the absorbance of the control reaction and A_S is the absorbance of the test compound in this equation. Ascorbic acid was used as a standard or positive control. Not contained compound/standard was used as the negative control.

2.6. Determination of total phenolic contents (TPCs)

The TPCs of the 3 methanolic extracts (1 000 µg/mL) of the seaweed samples were determined by the Folin-Ciocalteu method[19].

About 100 µL of diluted sample was added to 2 mL of 2% Na₂CO₃ reagent. After 2 min room temperature incubation, 100 µL of 50% Folin-Ciocalteu reagent was added. After 30 min of incubation at room temperature in the dark, the absorbance was measured at 720 nm by spectrophotometer (Thermo Scientific, Helios Alpha). Gallic acid (0.05 to 1 mg/mL) was used for the standard calibration curve. The results were expressed as gallic acid equivalent (GAE)/g dry weight of extracts.

2.7. Statistical analysis

All the experiments were carried out in triplicates and values were expressed as mean ± SD. Graphics were made using Microsoft Office Excel 2007. In addition, extract IC₅₀ was calculated from the graph plotting inhibition percentage against extract concentration in Excel 2007. Pearson's correlation coefficient was calculated using Excel 2007.

3. Results

3.1. Antioxidant activity

The percentages of free radical scavenging activity of methanolic algae extracts are given in Figure 1. The IC₅₀ values of the extracts are shown in Figure 2. The IC₅₀ values of seaweed extracts were found ranging from (32.19 ± 0.08) mg/mL to (37.57 ± 0.11) mg/mL and this value for ascorbic acid was determined as (32.19 ± 0.08) mg/mL.

According to determined IC₅₀ values, the extract of *C. crinita* (thallus) exhibited strong antioxidant activity with value IC₅₀

(35.46 ± 0.09) mg/mL, compared to control and other seaweed extracts (Figure 2).

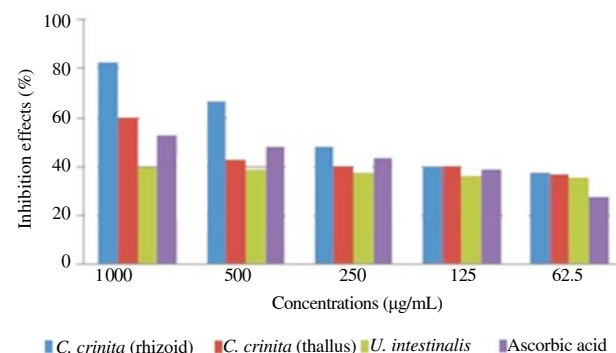


Figure 1. Free radical scavenging activity of methanolic seaweed extracts and ascorbic acid.

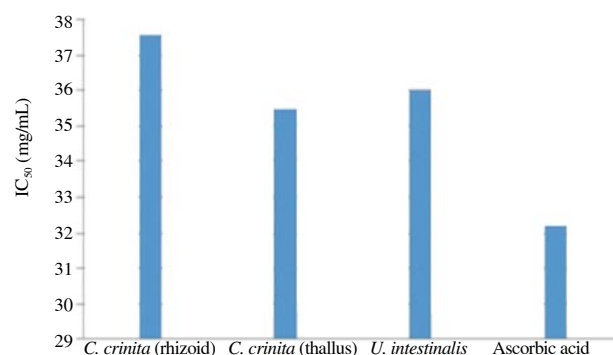


Figure 2. IC₅₀ values of methanolic extracts of seaweed and ascorbic acid.

3.2. Antimicrobial activity

The antimicrobial activities of the seaweed extracts against 18

Table 1

Antimicrobial activity of the seaweed extracts against tested microorganisms and some standard drugs.

Microorganisms	Inhibition zone (mm)							
	<i>C. crinita</i> (thallus)	<i>C. crinita</i> (rhizoid)	<i>U. intestinalis</i>	Penicillin G	Chloramphenicol	Erythromycin	Cycloheximide	Dimethyl sulfoxide (12.5%)
<i>Bacillus subtilis</i>	9	10	10	10	15	18	*	-
<i>B. megaterium</i> ATCC 1842	-	10	10	16	20	24	*	-
<i>Bacillus thuringiensis</i> var. <i>israelensis</i>	-	13	-	12	22	30	*	-
<i>Bacillus cereus</i> ATCC 7064	12	12	11	6	25	31	*	-
<i>B. sphaericus</i>	10	11	10	-	-	35	*	-
<i>B. sphaericus</i> ATCC 2362	-	10	11	18	24	36	*	-
<i>B. megaterium</i> MRS 400	-	12	-	20	28	35	*	-
<i>B. megaterium</i> DSM 32	-	11	11	16	25	28	*	-
<i>Bacillus licheniformis</i>	-	13	-	16	-	-	*	-
<i>K. pneumonia</i> ESBL+	-	10	10	11	26	22	*	-
<i>E. faecalis</i> ATCC 51299	-	9	-	10	12	9	*	-
<i>Micrococcus luteus</i> ATCC 9345	-	15	-	12	23	21	*	-
<i>S. aureus</i> ATCC 25923	-	17	-	12	28	40	*	-
<i>S. aureus</i> (clinical sp.)	-	20	11	11	12	37	*	-
<i>S. epidermidis</i>	20	17	15	11	11	24	*	-
Vancomycin resistant <i>Enterococcus</i>	11	12	13	-	22	-	*	-
<i>E. coli</i> ESBL+	-	-	-	11	24	11	*	-
<i>E. coli</i> ATCC 11293	-	-	-	11	14	18	*	-
<i>C. krusei</i> ATCC 6258	12	11	12	*	*	*	40	-
<i>C. albicans</i> ATCC 14053	-	-	-	*	*	*	41	-
<i>C. parapsilosis</i> ATCC 22019	-	-	-	*	*	*	42	-

-: No effect; *: Not tested.

bacterial strains and 3 *Candida* species are shown in Table 1. In the disc diffusion assays, the rhizoid extract of *C. crinita* displayed the antimicrobial activity at the different levels towards all tested microorganisms. The efficacy of *C. crinita* (rhizoid) was also found high against staphylococcal strains compared to other strains. On the other hands, there was no activity against to *E. coli* ESBL+ and *E. coli* ATCC 11293. All marine algal extracts were found more effective against Gram-positive bacteria than Gram-negative. In addition, it was determined that the tested extracts had weakly inhibitor effect on the growth of *C. krusei*, although there was no activity against *C. albicans* and *C. parapsilosis*. The antibacterial activity of extracts was found more effective than some of commercial antibiotics (Table 1).

3.3. TPCs

TPCs of the macroalgae extracts were calculated from the regression equation of calibration curve ($y = 0.0018x + 0.0159$; $R^2 = 0.999$) and expressed as mg GAE/g in dried weight. TPC of the methanol extracts are given in Table 2. The TPCs of algal methanolic extracts were found ranging from (5.10 ± 0.16) mg GAE/g to (87.70 ± 1.03) mg GAE/g. The highest amounts of the TPC were determined in the rhizoid extract of *C. crinita* with value (87.70 ± 1.03) mg GAE/g in dried sample.

Table 2

TPCs of 2 macro algae.

Algae species	TPCs (mg GAE/g)
<i>C. crinita</i> (rhizoid)	87.70 ± 1.03
<i>C. crinita</i> (thallus)	27.70 ± 0.83
<i>U. intestinalis</i>	5.10 ± 0.16

Our findings validated that there was a positive linear correlation ($r = 0.86$) between the TPCs and IC_{50} values.

4. Discussion

Many marine seaweed species include many phytochemicals such as flavonoids, terpenes and polyphenolic compounds[20]. Ethnopharmacologic studies proposed that such substances produced by plants have been used to treat adverse effects of several bacterial, fungal and viral infections due to high levels of antimicrobial activity against microorganisms[21]. Multi-drug resistant microorganisms pose a serious challenge to the medical community in many countries over the world and there is an urgent need to develop new agents[22].

Naturally growing seaweeds contain novel possible antioxidant compounds which scavenge side effects of the free radical generated by metabolic reactions. Several valuable studies suggested that brown algae have significantly high phenolic content[23]. Nakamura *et al.* reported that the members of Phaeophyta include a kind of polyphenol called phlorotannins[24]. This polyphenol had been reported as potential antioxidant, anticancer, antibacterial and antifungal compounds. A few researches were investigated

antioxidant properties of three brown algae *Sargassum wightii*, *C. crinita*, *Cystoseira myrica* and a green algae *Ulva lactuca*[25-27]. The results of the above mentioned studies offered that *Sargassum wightii*, *C. crinita* and *Cystoseira myrica* had higher antioxidant activity than *Ulva lactuca*. According to the results of our study, the thallus extract of *C. crinita* showed good antioxidant activity with IC_{50} value (35.46 ± 0.09) mg/mL compared to *U. intestinalis* extract and the rhizoid extract of *C. crinita*.

Some studies suggested that brown algae species had good antioxidant efficiency due to high phenolic compound contents[28]. The data gathered from this research demonstrated that the TPCs of algal extracts were ranging from (5.10 ± 0.16) mg GAE/g to (87.70 ± 1.03) mg GAE/g. The rhizoid extract of *C. crinita* had the highest phenolic content with value (87.70 ± 1.03) mg GAE/g, compared to other algal methanolic extracts. In this case, our results were coherent with the previous studies. Our findings also showed that there was a positive linear correlations ($r = 0.86$) between TPCs and the IC_{50} values.

A vast number of studies have been conducted about the antimicrobial effects of marine organisms, such as sponge, sea grass and seaweeds in recent years[14,26,28]. In a study done by Mhadhebi *et al.*, *C. crinita* extracts showed low activity against tested Gram-positive and Gram-negative bacteria but was no activity against yeast[26]. According to Tüney *et al.*, the methanol extracts of *Ulva rigida* and *Ulva linza* were no activity against *E. faecalis* and *E. coli*[29]. On the other hand, Taskin *et al.* determined that a pathogenic bacterium *E. coli* O157:H7 was sensitive against *Coronosphaera mediterranea*[30]. In the present study, it was determined that the rhizoid extract of a brown algae *C. crinita* showed high antimicrobial activity compared to other extracts and some standard antibiotics. Thallus extract of *C. crinita* was no activity against Gram-negative bacteria such as *E. coli* ESBL and *K. pneumonia* whereas showed high antibacterial efficiency against Gram-positive bacteria especially *S. epidermidis* and *S. aureus*. In this case, it can be said that the rhizoid extract of *C. crinita* had good antimicrobial efficacy rather than its thallus extract.

A green algae *U. intestinalis* extract showed considerable antimicrobial effect toward some tested microorganism. The methanolic extract of *U. intestinalis* indicated good activity against a Gram-positive bacterium *S. epidermidis*. Our study also showed that *U. intestinalis* was no activity against *E. faecalis* ATCC 51299 and *E. coli*. The results of this study confirmed that the seaweed extracts displayed higher antimicrobial activity against Gram-positive bacteria than Gram-negative bacteria such as *E. coli* ESBL, *E. coli* ATCC 11293 and *K. pneumonia*. This case may be ascribed to the existence of an extra outer membrane (lipopolysaccharide) in the cell walls structure of Gram-negative bacteria, possibly be said to inhibit cell entry of the active substance. In addition, all macroalgae extracts demonstrated weakly antifungal efficacy against *C. krusei*, but there was no activity against *C. albicans* and *C. parapsilosis*. Therefore, it can be said that the thallus extract of *C. crinita* had high antioxidant activity and low antimicrobial activity, whereas the rhizoid extract of *C. crinita* had low antioxidant activity and high antimicrobial

activity.

Our findings supposed that two marine algae called *C. crinita* and *U. intestinalis* species harvested from Sinop coastal line can be used as a potential natural seafood source owing to the antimicrobial efficiency and good free radical scavenging activity. We also put forward to an idea about the determination of these two seaweed species including important bioactive substance in terms of medicine and pharmaceuticals.

Conflict of interest statement

We declare that we have no conflict of interest.

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