



Antimicrobial Activities of Some Marine Macroalgae Species from Iskenderun Bay

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ABSTRACT

In the present study, the seaweeds belong to Phaeophyceae (*Halopteris scoparia* (Linnaeus) Sauvageau 1904, *Cystoseria mediterranea* Sauvageau 1912), Rhodophyceae (*Liagora viscida* (Forsskål) C. Agardh 1822, *Laurencia nidifica* J. Agardh 1852) and Chlorophyceae (*Enteromorpha multiramosa* Bliding, nom. inval. 1960) collected from nearby Iskenderun-Turkey of Mediterranean Sea were detected for their antimicrobial activities against seven bacterial (*Escherichia coli* ATCC 35218, *Bacillus cereus* NRRL B-371, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883, *Salmonella typhimurium* ATCC 14028, *Proteus vulgaris* RSKK 96029) and three fungal strains (*Candida albicans* ATCC 10231, *C. krusei* ATCC 6258, *C. tropicalis* Y-12968). The antimicrobial activities were expressed as minimum inhibitory concentrations (MICs), minimum bacterial concentrations (MBCs) and minimum fungicidal concentrations (MFCs) were determined. According to the results obtained from MIC values of the extracts on pathogenic microorganisms were between 50 and <0.39 mg / mL, while MBCs/MFCs values were ranged from > 50 and <0.39 mg / mL. These results suggest that brown, red, green algae secondary metabolites are important sources that could be used as broad spectrum of biological activities.

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Introduction

Seaweeds belong to primary producers in aquatic ecosystems a group of plants known as macroalgae and unique biological activities. Seaweeds are classified into three broad groups based on their nutrient and chemical composition; brown (Phaeophyta), red (Rhodophyta), or green (Chlorophyta) algae. Like other plants, seaweeds reveals that these natural biological active compounds in algae are an important resource for human health (Kuda et al., 2002). Seaweeds are defined as the main source of bioactive components that have the ability to produce a large variety of secondary metabolites distinguished by a wide range of natural biological activities.

Seaweeds are very rich in protein, carbohydrate, lipid, pigments, amino acids, fatty acids and glycerol, and also contain bioactive substances with antiviral, antifungal and antibacterial properties such as polyphenols and polysaccharides, vitamins and minerals. Secondary metabolites as essential oils, alginic acid, agar, carrageenan, organic acids, cellulose, alkaloid, sterol,

phenolic and many other substances extracted from structural materials in algae cells or cell walls (Mohammed et al., 2018; Mohammed et al., 2020). Because of these properties, seaweeds are used in many areas such as medical, pharmaceutical, microbiology, biotechnology and food, cosmetics, animal feed, fertilizer industries (Watson et al., 2003; Yuan et al., 2005; Bansemir et al., 2006; Chew et al., 2008). Various studies have been reported that seaweeds used in several implementations such as antioxidant (Devi et al., 2011), antimicrobial (Chiheb et al., 2011), antiviral (Bouhlal et al., 2011), antifungal (De Felicio et al., 2010), antibiotic (Chew et al., 2008), antiinflammatory (Moshfegh et al., 2019), antiallergic (Na et al., 2005), antitumoral and anticancer (Kim et al., 2011; Martins et al., 2018) and antifouling (Bhadury and Wright, 2004) anticoagulant activities (Dayong et al., 2008).

As a consequence of an increasing demand in screening for research on the use of algae as pharmacological and nutraceutical agents from natural products, there is more

interest in aquatic organisms in research on this issue. Many seaweed species with bioactive compounds have been shown to inhibit the growth of some gram-negative and gram-positive bacterial pathogens and identified as potential a source of natural antioxidants (Matanjun et al., 2008). Several researchers investigated the antimicrobial potential of seaweeds detected in different regions of our country (Battu et al., 2011, Tuney et al., 2006). Harder (1917) was pioneer of studies detecting the antimicrobial activities of seaweeds. Antibacterial compounds identified in seaweeds have been shown to have bactericidal agents as alkaloids, aminoacids, fatty acids, acrylic acid, terpenoids, phlorotannins, oil, lipophilic compound, phenolics, steroids, polyphenols, halogenates and isopyrenoid compounds (Glombitza, 1979; Watson and Cruz-Rivera, 2003; Paul and Puglisi, 2004). In the present study, we report that the effect of brown, red and green macroalgae collected from the Iskenderun Bay against pathogens.

Materials and Methods

Macroalgae Species

Macroalgae species of *Halopteris scoparia* (Linnaeus) Sauvageau 1904, *Cystoseria mediterranea* Sauvageau 1912, *Liagora viscida* (Forsskål) C.Agardh 1822, *Laurencia nidifica* J.Agardh 1852) and *Enteromorpha multiramosa* Bliding, nom. inval. 1960 were collected nearby Iskenderun-Turkey of Mediterranean Sea. Macroalgae were washed with distilled water and dried under room temperature.

Microorganisms and Growth Conditions

Seven bacterial and three fungal strains have been used to detect the antimicrobial activities of the extracts. Bacterial strains were as follows; *Escherichia coli* ATCC 35218, *Bacillus cereus* NRRL B-371, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883, *Salmonella typhimurium* ATCC 14028, *Proteus vulgaris* RSKK 96029; and fungal strains were *Candida albicans* ATCC 10231, *C. krusei* ATCC 6258, *C. tropicalis* Y-12968.

Preparation of Algal Extracts for Antimicrobial Activity

All of the extracts were prepared in DMSO at a concentration of 250 mg / mL and sterilized by a 0.45 mm pore sized syringe filter.

Broth Microdilution Assay

The minimum inhibitory concentrations (MIC) of the extracts were evaluated by the broth microdilution assay in 96-well microtitre plates according to CLSI reference methods for bacteria M7-A7 (CLSI, 2018) and yeasts M27-A3 (CLSI, 2008). 100 µL of the medium (Mueller Hinton broth for bacteria, Saboroud Dextrose broth for fungi) was added to each well for antimicrobial testing. At first well, 250 mg / mL of extract solution was added and then two-fold serial dilutions were made at a concentration range of 250–0.98 mg / mL on 96-well microtitre plate in Mueller – Hinton broth for bacteria or Saboroud Dextrose broth for fungi. Antibiotics used as positive controls include ampicillin, chloramphenicol and ketoconazole (Sigma). Microorganism inoculums were prepared from a 24-hour culture and suspensions in 0.9 % of NaCl were adjusted to 0.5 McFarland standard turbidity. Five microliters of microorganisms have been added to all wells. The microtitre plates were then incubated bacterial strains for 24 h at 37°C and fungal strains for 48 h at 30°C. After incubation, MICs of the extracts were obtained by lack of visual turbidity. The minimum bacterial concentration (MBC) and minimum fungicidal concentration (MFC) of the were detected by subculturing ten microliters volumes from non-turbid wells and spot inoculating onto an appropriate growth medium. After incubation, growth was reported and MBCs/MFCs were defined as the lowest concentration resulting in the death of 99.9% of the inoculum compared to the initial valid counts. The assay was recurrent at least three times and the characteristic values of MIC and MBC / MFC were selected.

Results and Discussion

Many natural resources in natural ecosystems have antimicrobial properties (Sevindik, et al., 2017; Sevindik, 2020). In this study we conducted, the antimicrobial potentials of macroalgae were determined. Extracts of macroalgae species (*H. scoparia*, *L. viscida*, *E. multiramosa*, *C. mediterranea*, *L. nidifica*) were tested against seven bacteria (*E. coli*, *B. cereus*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, *S. typhimurium*, *P. vulgaris*) and three fungal strains (*C. albicans*, *C. krusei*, *C. tropicalis*). MICs values of the the extracts of macroalgae species possessed antimicrobial activity were summarized in Table 1. The minimum bacterial (MBCs) and minimum fungicidal concentration (MFCs) values of the extracts of macroalgae species were presented in Table 2.

Table 1. Minimum inhibitory concentration (MICs) values of the macroalgae species extracts

Species	<i>E. coli</i> ATCC 35218	<i>B. cereus</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	<i>S. typhimurium</i>	<i>P. vulgaris</i>
<i>H. scoparia</i>	25	6.25	12.5	12.5	25	50	25
<i>L. viscida</i>	62.5	<0.98	3.91	31.25	31.25	31.25	31.25
<i>E. multiramosa</i>	25	<0.39	1.56	12.5	12.5	50	25
<i>C. mediterranea</i>	15.63	15.63	<0.98	15.63	7.81	15.63	3.91
<i>L. nidifica</i>	31.25	<0.98	3.91	31.25	31.25	31.25	62.5
Ampicillin	>125	31.25	62.5	>125	125	62.5	>125
Chloramphenicol	>125	125	125	>125	15.63	125	125

Table 2. Minimum bacterial concentration (MFC) values of the macroalgae species extracts

Species	<i>E. coli</i> ATCC 35218	<i>B. cereus</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	<i>S. typhimurium</i>	<i>P. vulgaris</i>
<i>H. scoparia</i>	50	>50	12.5	12.5	25	50	25
<i>L. viscida</i>	125	<0.98	15.63	62.5	125	125	62.5
<i>E. multiramosa</i>	25	<0.39	6.25	12.5	12.5	50	25
<i>C. mediterranea</i>	15.63	15.63	7.81	15.63	15.63	15.63	3.91
<i>L. nidifica</i>	125	<0.98	15.63	62.5	125	125	62.5
Ampicillin	>125	>125	62.5	>125	125	62.5	>125
Chloramphenicol	>125	125	>125	>125	15.63	125	>125

According to the results obtained from the present study, MICs values of the extracts on pathogenic microorganisms were between 50 and <0.39 mg / mL (Table 1), while MBCs / MFCs values were ranged from > 50 and <0.39 mg / mL. Also, the most effective extract on bacteria was *E. multiramosa* extract with the lowest MICs and MBCs (<0.39 mg / mL) on *B. cereus* (Table 2). Besides, this value was even lower than that of ampicillin and Chloramphenicol antibiotics used as positive controls.

In addition, all extracts used in the study had better antimicrobial effects on *E. coli* ATCC 35218, *B. cereus*, *S. aureus*, *P. aeruginosa*, *S. typhimurium* and *P. vulgaris* than ampicillin and chloramphenicol antibiotics. Other species except *C. mediterranea* showed the highest effect on *B. cereus*. On the contrary, the least effect was observed on *S. typhimurium* bacteria.

Dulger et al. (2009) have shown that the methanolic extracts of *H. scoparia* inhibits *S. aureus*, *E. coli*, *P. aeruginosa* and have antifungus activity on *Candida sp.* and *Kluyveromyces fragilis*. Febles et al (1995) have found that ethanol extracts of *H. scoparia* that collected in winter and autumn have antibacterial activity against *B. cereus* and *B. subtilis* and have no antimicrobial activity on *S. aureus*. Although, methanol extracts of *H. scoparia* inhibits *S. aureus* in winter and autumn.

Tuney et al. (2006) have demonstrated that the diethyl ether extracts of *C. mediterranea* have antimicrobial activity against *E. coli*, *E. faecalis* and *P. aeruginosa* and have antifungus activity against *Candida sp.*

Vairappan et al. (2001) have shown that halogenated metabolites and their antibacterial potential of the isolated of Okinawan *Laurencia sp.* were tested against eight species of marine bacteria the results have demonstrated that *Laurencia sp.* inhibits *Alcaligenes aquamarinus*, *Azomonas agilis*, *Azotobacter beijerinckii*, *Erwinia amylovora*, and *E. coli*. Salvador et al. (2007) tested *L. viscida* against *B. subtilis*, *B. cereus*, *S. aureus*, *E. coli* and *P. aeruginosa* and it has not shown antibacterial activity.

As Tables 1 and 2 presented, all extracts have antimicrobial effects against the tested macroalgae species. The extracts performed a higher antimicrobial activity than antifungal activity. Among the seaweed species used in this study, *C. mediterranea*, *L. viscida*, *L. nidifica* have been determined antimicrobial activity against both bacteria and fungi. In previous study, researchers the antimicrobial activity of the seaweeds in Turkey which were to evaluated. They determined that among the seaweed species used in this study, *C. compressa* have shown an excellent antimicrobial activity against both bacteria and fungi (Dulger et al., 2009). In another study, Salvador et al. (2007) reported that 82 different marine macroalgae

classified the Chlorophyceae, Phaeophyceae, and Rhodophyceae investigated for antimicrobial activity. Among the selected taxa of brown algae as known Phaeophyceae were determined that the most effective antimicrobial activity.

The high activity of the MICs and MBCs of macroalgae extracts against bacteria and fungi which were identified in the present study, are consistent with the results of MICs and MBCs / MFCs that were previously reported in other studies (Lambert et al., 2001; Stirck et al., 2007; El-Baky et al., 2009; Ertürk and Taş, 2011; Salem et al., 2011; Hernandez et al., 2013; Venugopa et al., 2014; Al-Judaibi et al., 2014; Zahrani et al., 2014; Veeramohan et al., 2017; Srikong et al., 2017; Martins et al. 2018; Erdogan Eliuz et al., 2019; El-Sheekh et al., 2020).

Pesando (1990) reported that all types of antimicrobial metabolites including (green, brown red algae) exist in temperate or tropical marine. In this study, significant levels of antimicrobial effects were observed in macroalgae extracts. Similar results were also obtained by Kandhasamy and Arunachalam (2008) and Karthikaidevi et al. (2009).

Previous studies reported that red, brown and green macroalgae extracts showed antimicrobial properties against various microorganisms and this antimicrobial activity was found in macroalgae extracts due to the presence of bioactive compounds also known as secondary metabolites. Secondary metabolites that differ regarding their species are defined as molecules those are responsible for antimicrobial activity (Bourgougnon and Stiger Pouvreau, 2012, Mohammed et al., 2018; Mohammed et al., 2020). Studies previously reported that chemical composition and antimicrobial activity of macroalgae vary depending on different species, the region where the thalli are located, physiological and environmental (climate, region, salinity, temperature) conditions, pollution, growth conditions, harvest time and epiphytic organisms (Nagayama et al., 2002; Demirel et al., 2009; Alghazeer et al., 2013; Parsaeimehr and Lutz, 2016; Sevindik et al., 2017; Gümüş et al., 2018; Güner et al., 2019; Moshfeigh et al., 2019; Sevindik 2020; Wang et al., 2020).

Conclusion

Macroalgae have been commonly used in food, medicine, pharmacy, cosmetics and other industrial fields for a long time because they contain bioactive components. On the other hand, the antimicrobial capacity of macroalgae extract depends on different variables such as macroalgae type, solvent, extraction method, extract concentration and the type of the microorganisms used. As

a result; the influences of the antimicrobial extract, which exists in macroalgae, may replace among natural protective antimicrobial agents in different areas of the industry with the aid of further detailed studies.

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