Evaluation of Antimicrobial Effects of Four Selected Marine Macroalgae from Iskenderun Bay

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A B S T R A C T

In the present study, the seaweeds belong to Phaeophyacea (Cystoseira elegans Sauvageau 1912, Cystoseira amentacea (C.Agardh) Bory 1832, Padina crassa Yamada 1931) and Florideophyceae (Corallina elongata J. Ellis & Solander 1786) collected from nearby Iskenderun-Turkey of Mediterranean Sea were detected for their antimicrobial activities against seven bacterial strains (Escherichia coli ATCC 35218, Bacillus cereus NRRL B-371, Staphylococcus aureus ATCC 29523, Pseudomonas aeruginosa ATCC 27853, Klebsiella pneumoniae ATCC 13883, Salmomella typhimurium ATCC 14028, Proteus vulgaris RSKK 96029). The antimicrobial activities were expressed as minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs). According to the results obtained from MICs and MBCs values of the extracts on pathogenic microorganisms were between 0.78 to 50 mg/mL. The lowest MICs and MBCs values were recorded for C. elegans extract against B. cereus with a MIC value of 0.78 mg/mL. These results suggest that secondary metabolites of brown and red algae are important sources that could be used as broad spectrum of biological and pharmaceutical activities.

Introduction

As photosynthetic organisms, algae are the primary producers in aquatic ecosystems with rich biodiversity, especially marine environments. Seaweed, also known as macroalgae, have unique biological activities (Blunt et al., 2007). Seaweeds are divided into broad groups depending on their nutritional and chemical composition, and brown, red or green algae are the main groups. Seaweeds are defined as the main source of bioactive components that can produce a large variety of secondary metabolites distinguished by a wide range of natural biological activities.

Seaweeds have been the subject of numerous studies and many important metabolites such as amino acids, vitamins, proteins, carbohydrates, pigments, terpenoids, lipids, fatty acids, minerals, glycerol, sterols, polysaccharides, tocopherol, phenolic compounds, and halogenated ketones (Mohamed et al., 2012). Secondary metabolites as essential oils, alginic acid, fucoxids, laminarine, fucans, agar, carrageenan, fluorotannines, phloroglucilin, terpenes, organic acids, cellulose, alkaloid, sterol, phenolic, and many other substances are extracted from structural materials in algae cells (Abouririche et al., 1999; Nagayama, 2002; Smith et al., 2002; Ghannadi et al., 2013; Yegdaneh et al., 2016). Various studies have been reported proving that the strong secondary components obtained from extracts of seaweeds species of these metabolites have antioxidant, antimicrobial (Chiheb et al., 2009; Cornish and Garbary 2010; Salem et al., 2011; Tambekar et al., 2011; Mayer et al., 2011; Güner et al., 2015; Silva et al., 2020), antiviral (Musale et al., 2020; Hans et al., 2021), antifungal (Oumaskour et al., 2012; Mickymaray et al., 2018; Biris-Dorhoi et al., 2020), antibiotics (Braña et al., 2015; Bhowmick et al., 2020), antiinflammatory (Kazlowska et al., 2010; Jaswir and Dorhoi, 2016), antiallergic, antihypertensive, antitumor (Güner et al., 2015; Seca and Pinto 2018; Güven et al., 2020; Wang et al., 2020), anticancer (Güner et al., 2019), antifouling (Dahms and Dobretsov, 2017), and...
Seaweeds have been described as an agent and have been widely used in many areas in many countries, especially China, Japan and India. In addition to its ecological importance, seaweeds are used as raw materials in many areas of the industry, such as food, agriculture, renewable energy, biodiesel, medical, pharmaceutical, nutraceutical, medicine, microbiology, biotechnology, cosmetics, animal feed and fertilizer (Watson et al., 2003; Yuan et al., 2005; Bansemir et al., 2006; Chew et al., 2008; Silva et al., 2020). In recent years, due to the increasing interest in natural product-based foods and medicines, there is more need for studies on this subject in seaweeds with rich content. Considering the side effects, immunization and toxic effects of conventional treatments, especially in cancer treatments, the opportunity to use algae with high antioxidant content, more effective and relatively fewer toxic properties has emerged as an alternative application in this field.

As a consequence of increasing demand in screening for research on the use of algae as antimicrobial agents that inhibit or kill the growth of microorganisms and are used to treat microbial infections. Antimicrobial drugs have been found in the form of antibacterial, antiviral, antifungal and plant-derived bioactive compounds, either synthetic or natural, depending on the microorganisms acting primarily in response to their activities (Pérez et al., 2016). 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 sources of natural antioxidants (Matanjun et al., 2008). The antimicrobial activity of macroalgae is due to chlorophyll derivatives, alkaloids, aminoacids, fatty acids, lipophilic compound, acrylic acid, terpenes, phenolic substances, cyclic polysulfides, steroids, fluorotannines, halogenates (ketones and alkanes), isopyrenoid and sulfur-containing heterocyclic components (Glombitza, 1979; Watson and Cruz-Rivera, 2003; Paul and Puglisi, 2004; Mohamed et al., 2012). Although the functions of secondary products differ, those with cytotoxic effects against microbial pathogens are used as antimicrobial agents in medicine. Considering the research in this field, natural products with biological and pharmacological effects are predominantly species belonging to marine brown, red and green macroalgae (Bhadury and Wright, 2004). Mostly red and brown algae of seaweed species have been used as human food resource and traditional medicine since ancient times (Smith, 2004).

The fact that seaweeds species with such intensive use in the world but, few studies on antimicrobial activities of investigation of pharmacological properties of these compounds have been published in our country. Therefore, in this study, the effect of the antimicrobial activities of brown algae Cystoseira elegans Sauvageau 1912 (Phaeophyceae), Cystoseira amentacea (C.Agardh) Bory 1832 (Phaeophyceae), Padina crassss Yamada 1931 (Phaeophyceae) and red algae Corallina elongata J.Ellis & Solander 1786 (Florideophyceae) marine macroalgae collected from the Iskenderun Bay, against pathogenic bacteria were investigated. At the same time, another purpose of the present study is to give direction to future pharmaceutical or nutraceutical uses by comparing algae groups with different secondary metabolites together with the antimicrobial effects of algae.

Materials and Methods

Macroalgae Species

Macroalgae species of Cystoseira elegans Sauvageau 1912, Cystoseira amentacea (C.Agardh) Bory 1832, Padina crassa Yamada 1931, Corallina elongata J.Ellis & Solander 1786 were collected nearby Iskenderun-Turkey of Mediterranean Sea. Macroalgae were washed with distilled water and dried under room temperature. The brown algae Cystoseira elegans and Cystoseira amentacea belongs to Sargassaceae family of Phaeophyceae class and Padina crassa belongs to Dictyotaceae family of Phaeophyceae class. The red algae Corallina elongata belongs to Corallinaceae family of Florideophyceae class. Macroalgae species were presented in Table 1.

Preparation of the Extracts

The dried algal samples were extracted by maceration in 1:4 (w/v) biomass/solvent ratio with methanol for 2 weeks at room temperature in a dark environment. The obtained methanolic extract was filtered through filter paper. After filtration, the solvent was evaporated at 50 °C under reduced pressure in a rotary evaporator (Heidolph, Germany), and deposited at +4 °C before further usage. For antimicrobial analysis, the extracts were dissolved in DMSO at a concentration of 250 mg/mL and sterilized by a 0.45 mm pore sized syringe filter.

Microorganisms and Growth Conditions

Seven bacterial strains have been used to evaluate the antibacterial activities of the extracts. Bacterial strains were as follows: Escherichia coli ATCC 35218, Bacillus cereus NRRL B-3711, Staphylococcus aureus ATCC 25923, Pseudomonas aeruginosa ATCC 27853, Klebsiella pneumoniae ATCC 13883, Salmonella typhimurium ATCC 14028, Proteus vulgaris RSKK 96029. All bacterial cultures were incubated in Tryptic soy agar at 37 °C for 24h.

Broth Microdilution Assay

The minimum inhibitory concentrations (MICs) 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). Antibiotics used as positive controls included Ampicillin and Chloramphenicol. Two-fold serial dilutions of the extracts and antibiotics in Mueller hinton broth were made at a concentration range of 50-0.39 for the extracts and 250-0.98 mg/mL for the antibiotics on 96-well microtitre plates. Microorganism inoculums were prepared from a 24-hour culture and suspensions in 0.9 % of NaCl were adjusted to 0.5 McFarland (1.5x10^8 cfu/mL) standard turbidity. Five microliters of microorganism suspension have been added to all wells. The microtitre plates were then incubated for 24 h at 37°C. After incubation, MIC values of the extracts were determined by lack of the visual turbidity. The minimum bactericidal concentrations (MBCs) values were determined by subculturing ten microletters volumes from non-turbid wells and spot inoculating onto an appropriate growth medium. After incubation, growth was recorded and MBCs were defined as the lowest concentration resulting in the death of 99.9% of the inoculum compared to the initial viable counts. The assay was repeated at least three times and the average values of MICs and MBCs were selected.
Table 1. Seaweed species which were used in this study

| Seaweed species                     | Class            |
|-------------------------------------|------------------|
| Cystoseira elegans Sauvageau 1912   | Phaeophyceae     |
| Cystoseira amentacea (C.Agardh) Bory 1832 | Phaeophyceae     |
| Padina crassa Yamada 1931           | Phaeophyceae     |
| Corallina elongata J.Ellis & Solander 1786 | Florideophyceae |

Table 2. Minimum inhibitory concentration (MIC) values of the macroalgae species extracts (mg/mL)

| Microorganisms | Macroalgae Species          | Positive controls |
|----------------|----------------------------|-------------------|
|                | Cystoseira elegans         |                   |
| E. coli        | 50                        | 50                |
| B. cereus      | 0.78                      | 3.13              |
| S. aureus      | 6.25                      | 12.5              |
| P. aeruginosa  | 12.5                      | 12.5              |
| K. pneumoniae  | 12.5                      | 12.5              |
| S. typhimurium | 50                        | 25                |
| P. vulgaris    | 25                        | 25                |

Table 3. Minimum bactericidal concentration (MBC) values of the macroalgae species extracts (mg/mL)

| Microorganisms | Macroalgae Species         | Positive controls |
|----------------|----------------------------|-------------------|
|                | Cystoseira elegans         |                   |
| E. coli        | 50                        | 50                |
| B. cereus      | 0.78                      | 3.13              |
| S. aureus      | 6.25                      | 25                |
| P. aeruginosa  | 12.5                      | 12.5              |
| K. pneumoniae  | 12.5                      | 12.5              |
| S. typhimurium | 50                        | 25                |
| P. vulgaris    | 25                        | 25                |

Results and Discussion

Extracts of macroalgae species (Cystoseira elegans, Cystoseira amentacea, Padina crassa, Corallina elongata) were tested against seven bacteria (Escherichia coli, Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhimurium, Proteus vulgaris). MICs and MBCs values of four seaweed extracts on tested pathogen bacteria are given in Table 2 and Table 3, respectively. Depending on the results obtained, the MICs and MBCs values vary from 0.78 to 50 mg/mL. All extracts have a significant bactericidal and bacteriostatic effect on the studied bacteria.

According to the results obtained from the present study, among the tested bacteria, extracts demonstrated the strongest antimicrobial effect on B. cereus. The lowest MIC and MBC values were recorded for C. elegans extract against B. cereus with a MIC value of 0.78 mg/mL (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, B. cereus, S. aureus, P. aeruginosa, K. pneumoniae, S. typhimurium and P. vulgaris than Ampicillin and Chloramphenicol antibiotics.

As Tables 2 and 3 presented, all extracts have antimicrobial effects against the tested macroalgae species. Among the seaweed species used in this study, Cystoseira elegans, Cystoseira amentacea, Padina crassa, Corallina elongata have been determined antimicrobial activity were tested against seven bacteria. According to previous studies, results similar to antimicrobial effects were obtained in this study (Smith 2004; Gouveia et al., 2013; El Shafay et al., 2016; Jegan et al., 2019).

Tuney et al. (2006) obtained that the diethyl ether extracts of Cystoseira mediterranea have antimicrobial activity against E. coli, E. faecalis and P. aeruginosa and have antifungal activity against Candida sp. In previous study, researchers evaluated the antimicrobial activity of the seaweeds in Turkey. They determined that among the seaweed species used in this study, Cystoseria compressa have shown an excellent antimicrobial activity against pathogen bacteria (Dulger et al., 2009). In another study, Salvador et al. (2007) reported that 82 different marine macroalgae classified the among the selected taxa of brown algae as known Phaeophycaceae have antifungal activity against 3 gram positive bacteria (E. faecalis) and
2 gram-negative (E. coli, Klebsiella sp.) bacterial strains (Rhimou et al., 2010). In another study conducted with 32 algae species collected from Pakistani Karachi, researcher reported that almost all algae show different levels of antimicrobial activity against common gram-positive and gram-negative strains in humans, animals and plants (Rizvi and Shammel, 2004). Divya et al. (2011) determined in their study that Saragassum cinerereum methanol extracts showed high activity against S. typhi, K. pneumoniae, P. aeruginosa and S. aureus strains.

The high activity of the MICs and MBCs of macroalgae extracts against bacteria which were identified in the present study, are consistent with the results of that were previously reported in other studies (Banaigs et al., 1983; Smith et al., 2002; Kamenarska et al. 2009; Osman et al., 2010; Er tü rök and Taş, 2011; Salem et al., 2011; Ghannadi et al., 2013; Oumaskour et al., 2013; Yegdanem et al., 2013; Al Zahrani et al., 2014; Parseaimehr and Lutzu 2016; Martins et al, 2018; Arguelles et al., 2019; Eliuz et al., 2019; El-Sheekh et al., 2020; Mirzadeh et al., 2020; Arguelles et al., 2021:). Data from previous studies reveals that these natural biologically active compounds in algae are an important resource for human health. Several researchers investigated the antimicrobial potential of seaweeds detected in different regions of our country (Demirel et al., 2009; Battu et al., 2011, Tuney et al., 2006; Gümüş et al., 2018; G ü n er et al., 2019). In this study, significant levels of antimicrobial effects were observed in macroalgae extracts. Similar results in this study were reported by Jing-Wen and Wei-ci 1984, Yegdanem et al. 2016, Zouaoui and Ghalem 2017 and Negara et al. 2020 have also been determined.

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 that are responsible for antimicrobial activity (Bourgougnon and Stiger 2012). Secondary metabolites demonstrated that these active compounds produced by macroalgae have antiviral, antimicrobial, antithrombic, anticoagulant and cell growth inhibitory effects on many biological activities beneficial for human health. Considering the research in this field, natural products with biological and pharmacological effect are predominantly species belonging to marine brown, red and green macroalgae (Bhadury and Wright, 2004). 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; Gümüş et al., 2018; G ü n er et al., 2019; Moshfegh et al., 2019; Wang et al., 2020; Silva et al., 2020; Kuda et al., 2021).

Macroalgae have been commonly used in food, medicine, pharmacy, cosmetics, feed, fertilizer and other industrial fields for a long time because they contain bioactive components. On the other hand, the antimicrobial capacity of macroalgae extracts 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. Hence, it is necessary to conduct the more comprehensive studies on screening of the antioxidant, antibacterial and antimicrobial activities of macroalgae.

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