International Journal of Secondary Metabolite
2021, Vol. 8, No. 2, 117–123
https://dx.doi.org/10.21448/ijsm.894564
Published at https://dergipark.org.tr/en/pub/ijsm
Research Article

Antibacterial Activities of Methanolic Extracts of Different Seaweeds from Iskenderun Bay, Turkey

Betul Aydin[1,*]

[1]Department of Biology, Faculty of Science, Gazi University, Ankara, TURKEY

Abstract: Antibacterial activities of the methanolic extracts of *Stypopodium schimperi* (Kützing) Verlaque and Boudouresque, *Halopteris filicina* (Grateloup) Kützing, *Dictyota dichotoma* (Hudson) J.V.Lamouroux, *Gracilaria bursa-pastoris* (S.G.Gmelin) P.C.Silva, *Ulva intestinalis* Linnaeus species from the Iskenderun Bay, Turkey against *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhimurium and Proteus vulgaris* were evaluated by using the microdilution method. Results of the study showed that all of the extracts had an antimicrobial effect on the tested bacteria. A minimum inhibitor concentration and minimum bactericidal concentrations of the extracts ranged from >125 to <0.39 mg mL^{-1}. Furthermore, this is the first report on antibacterial effects of the methanolic extracts of *S. schimperi* (Kützing) Verlaque and Boudouresque and *G. bursa-pastoris* (S.G.Gmelin) P.C.Silva species. The present findings revealed that all studied seaweed species could act as a natural source of bioactive compounds for the treatment of infectious diseases.

ARTICLE HISTORY
Received: Mar. 10, 2021
Revised: Apr. 26, 2021
Accepted: May 05, 2021

KEYWORDS
Antimicrobial activity,
Brown macroalgae,
Red macroalgae,
Green macroalgae.

1. INTRODUCTION

For centuries, human beings have suffered from infectious diseases caused by various pathogenic microorganisms. The discovery of antibiotics used to control infectious diseases has been a beacon of hope to reduce the deaths caused by these diseases. Today, however, people are in danger of losing this powerful weapon due to resistant strains caused by the improper use of antibiotics. For this reason, researchers have been examining many natural resources for the discovery of new antibiotics (Mohr, 2016; Zaman *et al*., 2017). One of these natural resources is seaweed, which has many important medical properties due to the valuable components it contains (Shelar *et al*., 2012). Seaweeds also known as macroalgae are photosynthetic eukaryotic organisms that are essential components of the living resources of the sea. Seaweeds consist of three main classes; namely, Rhodophtya (red algae), Chlorophyta (green algae) and Phaeophyta (brown algae) (Wang *et al*., 2017). In some studies to date, it has been observed

CONTACT, Betül AYDIN barslan@gazi.edu.tr Department of Biology, Faculty of Science, Gazi University, Ankara, Turkey

e-ISSN: 2148-6905 / © IJSM 2021

that many species of algae can synthesize antimicrobial metabolites such as bromophenols, indolocarbazoles, cycloeudesmol, laurinterol, and elatol to kill or inactivate the pathogen microorganisms (Parsaeimehr and Lutzu, 2016).

There is a very rich variety of seaweed in Turkey, which has coasts in the Mediterranean, Black Sea and Aegean Sea, as well as an inland sea called the Marmara Sea (Aysel and Erdugan, 1996; Ribera *et al*., 1992; Taskin, 2014). There are some studies on the biochemical composition (total protein, carbohydrate, phenolic, chlorophyll-a and carotene contents) of some brown, green and red seaweeds from Iskenderun Bay in the Mediterranean Sea in the south of Turkey (Ozgun *et al*., 2015; Turan *et al*., 2015). On the other hand, there are no studies in the literature showing the antimicrobial effects of the methanolic extracts of *Stypopodium schimperi* (Kützing) Verlaque and Boudouresque and *Gracilaria bursa-pastoris* (S.G.Gmelin) P.C.Silva species.

The aim of this study is to determine the antimicrobial effect of methanol extract of seaweeds; namely, *Stypopodium schimperi* (Kützing) Verlaque and Boudouresque (Phaeophyta), *Halopteris filicina* (Grateloup) Kützing (Phaeophyta), *Dictyota dichotoma* (Hudson) J.V.Lamouroux (Phaeophyta), *Gracilaria bursa-pastoris* (S.G.Gmelin) P.C.Silva (Rhodophyta), and *Ulva intestinalis* Linnaeus (Chlorophyta) collected from the Iskenderun Bay, on various bacteria.

2. MATERIAL and METHODS

2.1. Sample Collection

Antimicrobial activity analysis was performed on *Stypopodium schimperi*, *Halopteris filicina*, *Dictyota dichotoma*, *Gracilaria bursa-pastoris,* and *Ulva intestinalis* species (Table 1). Sampling studies were carried out in June 2018 at 0-20 m depth from free dives on the Iskenderun Gulf coast, Hatay, Turkey. The seaweeds were collected underwater in gathered mesh bags. The collected seaweeds were washed with water to remove the epiphytes, rocks, sand and mud that could be present. In the laboratory, the washed materials were dried in a shaded environment without sun exposure for further study. Some of the collected seaweeds were stored in jars with a 4-6 % neutralized formaldehyde solution prepared with seawater for identification. The identification of the seaweeds was carried out using the Olympus brand Ckx41sf model stereo inverted light microscope.

Table 1. Macroalgae species used in antimicrobial activity test.

Seaweed species	Class
Stypopodium schimperi (Kützing) Verlaque and Boudouresque 1991	
Halopteris filicina (Grateloup) Kützing 1843	Brown algae
Dictyota dichotoma (Hudson) J.V.Lamouroux 1809	
Gracilaria bursa-pastoris (S.G.Gmelin) P.C.Silva 1952	Red algae
Ulva intestinalis Linnaeus 1753	Green algae

2.2. 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 the 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^{-1} and sterilized by a 0.45 mm pore sized syringe filter.

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2.3. Microorganisms and Growth Conditions

Seven bacterial strains have been used to detect the antimicrobial activities of the extracts. Bacterial strains were as follows: Gram positive bacteria, *Bacillus cereus* NRRL B-371, *Staphylococcus aureus* ATCC 25923, gram negative bacteria, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883, *Salmonella typhimurium* ATCC 14028, *Proteus vulgaris* RSKK 96029. All the bacterial cultures were incubated in Tryptic soy agar at 37 °C for 24h.

2.4. Broth Microdilution Assay

The minimum inhibitory concentrations (MIC) of the extracts were evaluated by the broth microdilution assay in 96-well microtiter plates according to CLSI reference methods for bacteria M07 (CLSI, 2018). Serial dilutions of the samples were made in Mueller Hinton broth at a concentration range of 125–0.39 mg mL^{-1} on 96-well microtiter plates. The antibiotics ampicillin and chloramphenicol (Sigma) were used as positive control. Microorganism inoculums were prepared from a 24-hour culture, suspensions in 0.9 % of NaCl were adjusted to 0.5 McFarland standard turbidity ($1.5x10^8$ cfu mL^{-1}), and this microbial suspension was diluted 10^{-1} in 0.9 % of NaCl solution. Five microliters of the diluted microbial suspension had been added to all wells. The plates were then incubated for 24 h at 37°C. After the incubation, the minimum inhibitory concentration (MIC) values of the extracts were determined by absence of visual turbidity. The minimum bactericidal concentration (MBC) values were determined by subculturing ten microliters from non-turbid wells and spot inoculated onto an appropriate growth medium. After the incubation, the 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 mean values of MIC and MBC were selected.

3. RESULTS and DISCUSSION

Antimicrobial activity of the seaweed extracts was evaluated by using the microdilution method. MIC and MBC values of five methanolic seaweed extracts against tested bacteria are given in Table 2 and Table 3, respectively. According to the obtained results MIC and MBC values ranged from <0.39 to >125 mg mL^{-1}. All extracts were found to have a strong bactericidal and bacteriostatic effect on the studied bacteria.

Table 2. Minimum inhibitory concentration (MIC) values of the extracts (mg mL^{-1}).

	Gram positive			Gram negative			
	S. aureus	*B. cereus*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*S. typhimurium*	*P. vulgaris*
Stypopodium schimperi	3.13	3.13	12.5	3.13	1.56	6.25	12.5
Halopteris filicina	15.63	15.63	125	31.25	31.25	31.25	31.25
Dictyota dichotoma	50	1.56	50	<0.39	12.5	50	12.5
Gracilaria bursa-pastoris	50	6.25	50	1.56	12.5	50	25
Ulva intestinalis	12.5	1.56	25	12.5	12.5	50	25
Ampicillin	62.5	31.25	>125	>125	125	62.5	>125
Chloramphenicol	125	125	>125	>125	15.63	125	125

Table 3. Minimum bactericidal concentration (MBC) values of the extracts (mg mL^{-1}).

	Gram positive			Gram negative			
	S. aureus	*B. cereus*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*S. typhimurium*	*P. vulgaris*
Stypopodium schimperi	6.25	3.13	12.5	3.13	6.25	6.25	12.5
Halopteris filicina	62.5	>125	>125	62.5	>125	125	62.5
Dictyota dichotoma	50	1.56	50	<0.39	50	50	12.5
Gracilaria bursa-pastoris	50	25	50	1.56	50	50	25
Ulva intestinalis	25	1.56	50	12.5	50	50	25
Ampicillin	62.5	>125	>125	>125	125	62.5	>125
Chloramphenicol	>125	125	>125	>125	15.63	125	>125

Among the bacteria tested, extracts were found to demonstrate the highest antimicrobial effect on *B. cereus*. The lowest MIC and MBC values were recorded for the *D. dichotoma* extract against *P. aeruginosa* (<0.39 mg mL^{-1}). Methanolic extract of *S. schimperi* showed a higher antimicrobial effect against the tested bacteria than the other algal species.

Ballesteros *et al*. screened the antibacterial, antifungal, antiviral, cytotoxic, and antimytotic properties of seventy-one different macroalgae species collected from the Central Mediterranean (Ballesteros *et al*., 1992). They said that antifungal activity (against *Candida albicans* and *Aspergillus niger*) was more common than antibacterial activity (against *E. coli* and *B. subtilis*) among these species and *H. filicina* did not have antibacterial effects. Val *et al*. studied the antibacterial and antifungal activities of the methanol extracts from 44 species harvested from Gran Canaria (Canary Islands, Spain) against *B. subtilis* MB964, *Enterococcus faecium* MB5571, *S. aureus* MB5393, *P. aeruginosa* MB979, *Serratia marcescens* MB252, *Mycobacterium smegmatis* MB2233, *Candida albicans* MY1055, *Saccharomyces cerevisiae* W303, *Aspergillus fumigatus* MF5668 (Val *et al*., 2001). They found that *H. filicina* extract did not have any antimicrobial effects against these microorganisms. However, Alghazeer *et al*. evaluated the antibacterial activity of crude methanolic and water extracts of 19 seaweed species collected from the western coast of Libya against *S. aureus*, *B. subtilis*, *Bacillus* spp., *Staphylococcus epidermidis*, *E. coli*, *Salmonella typhi*, *Klebsiella* spp., and *P. aeruginosa* (Alghazeer *et al*., 2013). They found that methanol extract of *H. filicina* was less effective than the positive control antibiotic, ciprofloxacin. Taskin *et al*. studied the antibacterial activity of methanol extracts of six marine algae from the North Aegean Sea (Turkey) against *S. aureus*, *Micrococcus luteus*, *Enterococcus faecalis*, *E. coli*, *Enterobacter aerogenes* and *E. coli* O157,H7 (Taskin *et al*., 2007). They determined that *H. filicina* methanol extract had a moderate antibacterial effect only on *S. aureus* among the bacteria studied. In our study, it was observed that *H. filicina* extract was less effective on the tested bacteria than the other extracts. This difference between the results could have been due to the difference in preparation methods such as concentration of extract, type of the solvent and extraction technique or some ecological factors such as the location and season of sampling.

D. dichotoma extract showed an antibacterial effect in variable values against studied bacteria. This was in consistence with the finding of Ibraheem *et al*. and Demirel *et al*. who

found that the extracts of *D. dichotoma* obtained from Hurghada on the Red-Sea coast of Egypt and Aegean Sea had antimicrobial properties on both gram positive and gram negative bacteria (Ibraheem *et al*., 2017; Demirel *et al*., 2009).

Like other species, *U. intestinalis* extract has been found to have a fairly high antimicrobial effect, similar to previous studies (Abdel-Khaliq *et al*., 2014; Berber *et al*., 2015; Sahnouni *et al*., 2016; Srikong *et al*., 2017; Srikong *et al*., 2015). Berber *et al*. tested the antimicrobial activities of the methanolic extracts of *Cystoseira crinita* Duby and *U. intestinalis* from the coastal region of Sinop, Turkey against 18 bacteria and 3 yeasts. According to their results, *U. intestinalis* extract had a moderate antibacterial effect on 10 bacteria out of 18 species (Berber *et al*., 2015). Srikong *et al*. evaluated the antibacterial activities of methanol, ethanol, dichloromethane, and hexane extracts of *U. intestinalis* harvested from Thailand on *Bacillus cereus* TISTR 687*, Enterococcus faecalis* ATCC 29212*, Listeria monocytogenes* DMST 4553*, S. aureus* ATCC 29213 *and* methicillin-resistant *S. aureus* 001 R (MRSA 001 R), *E. coli* ATCC 25922*, K. pneumonia* PSU 1*, Proteus mirabilis* PSU 1*, P. aeruginosa* ATCC 27853*, Salmonella typhi* PSU 1*, Vibrio alginolyticus* PSU VA 1*, V. harveyi* PSU 4109 *and V. parahaemolyticus* PSU 5124. They found that only the hexane extract had a significant antibacterial activity against Gram-positive bacteria but not against Gram-negative bacteria (Srikong *et al*., 2017). Moreover, Sahnouni *et al.* studied *Ulva rigida* C. Agardh and *U. intestinalis* methanolic extracts for antimicrobial activities on *E. coli*, *Salmonella* sp., *Shigella dysentriae*, *P. aeruginosa*, multi-drug resistant *Proteus mirabilis,* sensitive *Streptococcus pyogenes,* methicillin-resistant *S.aureus* (MRSA), *Staphylococcus epidermidis*, *K. pneumoniae*, vancomycin resistant *Enterococcus faecalis*, *C. albicans*, *A. niger* and *Cryptococcus neoformans*. They showed that *E. coli*, *S. pyogenes* and *S. epidermidis* bacteria were more susceptible to *U. intestinalis* extract than the other bacteria studied (Sahnouni *et al*., 2016).

There are no studies on the antimicrobial effects of the *S. schimperi* and *G. bursa-pastoris* species. Both of the species have been found to inhibit the growth of all bacteria studied. Therefore, this initial data is very important as it reveals the antimicrobial potential of these species.

4. CONCLUSION

Findings obtained from this study suggest that methanolic extracts of *S. schimperi*, *H. filicina*, *D. dichotoma*, *G. bursa-pastoris* and *U. intestinalis* species from Iskenderun Bay are potential natural source of antibacterial agents. In particular, the lack of any study showing the antimicrobial effectiveness of the *S. schimperi* and *G. bursa-pastoris* species has made this study the first research to reveal the antimicrobial properties of these species. Further studies will pave the way for the use of active substances contained in these algae in the field of pharmacy and medicine. Therefore, detailed research should be carried out for the detection of their active components.

Declaration of Conflicting Interests and Ethics

The author declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the author.

Orcid

All authors must write ORCID
Betül Aydın https//orcid.org/0000-0002-9092-1350

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