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Determination of antimicrobial activity of different extracts of *Enteromorpha intestinalis* (Linnaeus Nees.1820) against pathogenic microorganisms

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Abstract: It is known that components such as polyphenols, flavonoids and polysaccharides have antimicrobial activity in macroalgae in aquatic environments. In this study, it was aimed to determine the antimicrobial activities of *Enteromorpha intestinalis* (L.) Nees methanol, 70% ethanol and water extracts against different pathogenic microorganisms, which are economically important marine macroalgae. According to Broth Microdilution Method; Antimicrobial activities of different extracts of the studied species against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Salmonella enteritidis*, *Sarcina lutea* and *Bacillus cereus* standard bacterial strains and *Candida albicans* fungus strain were evaluated by determining the Minimum Inhibition Concentration (MIC). According to the findings, it was observed that the water extract of the studied species showed no antimicrobial activity against any test organism. The methanol extract of *E.intestinalis* was found to have antimicrobial activity against *P. aeruginosa*, *S. aureus*, *S. lutea*, *B. cereus* and *C. albicans* strain, while ethanol extract was observed against *P. aeruginosa*, *S. lutea* and *C. albicans* strains. The highest antibacterial effect was observed in *E.intestinalis* (L.) Nees methanol extract and the most effective strain was *Bacillus cereus* (0.390 mg/mL). Nowadays, algae are of great importance in terms of being very useful drug raw material, containing the basic components, being more effective and less toxic, as well as being models for drugs with original drug-like physiological activity. Since the data obtained with this study are intended for prospective application, it is thought that alternative drug applications will be provided as a basis for the studies aimed at reducing the use of commonly used antibiotic and antifungal agents against pathogenic microorganisms.

Keywords: *Enteromorpha intestinalis*; macroalgae; antimicrobial activity; pathogen microorganism.

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1 Introduction

In recent years, algae are among the natural resources that people started to use for their own benefit (Yoldaş et al. 2003; Sevindik, 2020). Algae are important bioactive molecule sources used in human and animal nutrition. There are many types of antimicrobial compounds that play an important role in the natural defense of all living species in nature (Rauha et al. 2000; Mohammed et al., 2019).

Antibiotic, antiviral, anticancer, antifungal, antibacterial, anti-inflammatory effects, as well as hypocholesterolemic, enzyme inhibition and some other pharmacological effects of these molecules from microalgae and macroalgae have been observed in the last two decades. These natural products are not only used as pharmaceutical raw materials, but also as structural models in the production of synthetic molecules (Sevindik, 2018). Macroalgae have low calorie,

high vitamin, mineral and fiber content, and because of that, they become an attractive material for researchers. *E. intestinalis* (L.) Nees. is macroalgae and can grow in all natural waters (Akköz et al. 2011) (Fig 1.)

The antimicrobial activity of macroalgae is due to chlorophyll derivatives, acrylic acid, terpenes, phenolic substances, halogenated aliphatic components and sulfur-containing heterocyclic components. Besides these components, antimicrobial activity is stated to be caused by some amino acids, fluorotannins, steroids, halogenated ketones and alkanes, cyclic polysulfides and fatty acids (Gupta et al. 2012).

The aim of the present study was to determine antimicrobial activity of different extracts of *E. intestinalis* against pathogenic microorganisms. Systematic of *E.intestinalis* that we use in our study is as given in Table 1.



Fig. 1 *Enteromorpha intestinalis* (Linnaeus) Nees 1820

Table 1 Systematic of *Enteromorpha intestinalis* (Linnaeus) Nees 1820 (AlgaeBase)

Species	Genus	Family	Ordo	Classis	Subphylum	Phylum	Subkingdom	Kingdom
<i>E. intestinalis</i>	Enteromorpha	Ulveaceae	Ulvales	Ulvophyceae	Chlorophytina	Chlorophyta	Viridiplantae	Plantae

2 Materials and Method

2.1 Algae Samples Collection

Samples of *E. intestinalis* seaweed was collected from a sampling as deep as 0-1bm, station in Demre, far 150 km from Antalya, The position of Demre is centered on, 36°13'42.30"N -29°56'23.31"E. *E. intestinalis*, whose geographical information has been given above, was washed with the ambient waters in order to remove foreign substances first and brought to the laboratory environment in sterile polyethylene bags. Washed with distilled water in the hydrobiology laboratory to remove epiphytic creatures and necrotic particles on the samples.

In order to accelerate the drying process of the macroalgae which drained the water and after this process, the algae were placed in an oven set at 40°C to prevent the phytochemical compounds from being damaged, and pre-drying was carried out by keeping it for 17 hours. Algae that were properly dried and ground with the help of hand homogenizer were kept airtight at room temperature until extraction.

2.2 Preparation of Algae Extracts

Algae collected from the field were cleaned and dried in the shade. Soxhlet extraction method (Thermal) was applied after grinding with the help of a mechanical grinder to obtain extracts of shade-dried algae samples.

Powdered samples (20 g) were extracted for 6 hours at 65 °C in a soxhlet extraction method using 180 mL of methanol solvent. After the extraction phase was obtained, after drying the samples, extraction was carried out with other solvents (70% ethanol (at 75 °C) and water). The extracts obtained were filtered through Whatman No: 1 filter paper and then

evaporated (until 1-2 mL thick) at 40 °C under reduced pressure and stored in vials at -20 °C until analysis. (Kaufman et al., 1995). The amount of extracts, organic solvent type, drug amount and extraction efficiency are as shown in Table 2. The graph showing the yields of algae extracts is as shown in Fig 2.

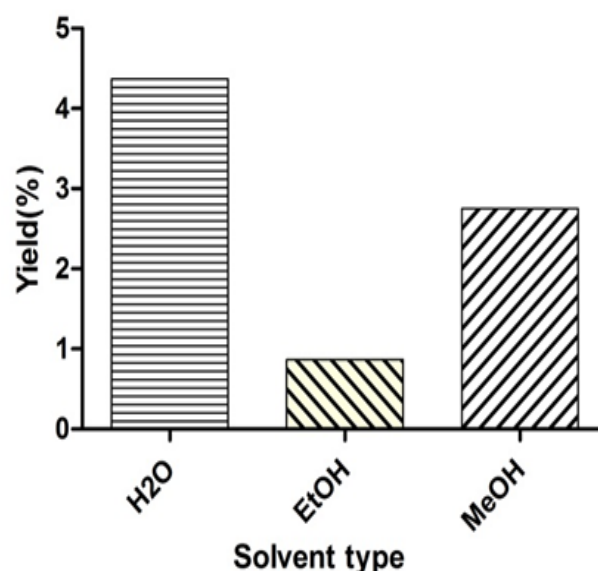


Fig. 2 The yields (%) of algae extracts.

When the extracts were to be tested for their antimicrobial activities, they were dissolved in DMSO at a concentration of 25 mg / mL for use in the broth microdilution method, and after being passed through 0.45 µm milipore filters, they were divided into small volumes in tubes with an extract number and stored at +4 °C. (Buruk 2002).

Table 2 Amount of algae extracts, organic solvent type, drug amount and extraction yields.

Algae name	Collection area	Coordinate	Solvent type	Drog amount (gr)	Balloon joje tare(gr)	Last weighing (gr)	Extract amount	Yield %
<i>E. intestinalis</i>	Antalya Demre creek	36°13'42.30"K 29°56'23.31"D	H ₂ O	20	69.91	70.78	0.87	4.37
			EtOH	20	112.28	112.45	0.17	0.87
			MeOH	20	113.89	114.44	0.55	2.75

2.3 Preparation of Broth

To determine the antimicrobial activities of the extracts, some modifications were applied and the broth microdilution method reported by Abbasoğlu et al.(1995) was used (Abbasoğlu et al. 1995). Mueller-Hinton broth was used in this method. In addition, Brain Heart Infusion Broth broth was prepared to prepare fresh cultures of the test microorganisms overnight. After the Brain Heart Infusion Broth (BHIB) medium was homogeneously dissolved in distilled water with 37 g/L, it was distributed to 10-mL tubes with screw cap and sterilized in an autoclave at 121 ° C for 15 minutes. After the Mueller Hinton Broth medium (21 g / L) was dissolved in distilled water, it was dispensed into test tubes at 10 mL and sterilized in an autoclave at 121 ° C for 15 minutes.

2.4 Test microorganisms

The strains of three gram positive bacteria (*Staphylococcus aureus* ATCC 43300, *Bacillus cereus* ATTC 11778, *Sarcina lutea* ATTC 9341) and four gram negative bacteria (*Escherichia coli* ATTC 25922, *Pseudomonas aeruginosa* ATTC 27853, *Klebsiella pneumoniae* ATTC 70603, *Salmonella enteritidis* ATTC 13076) and also one strain of fungus (*Candida albicans*) were obtained from the culture collection of the Department of Biology Microbiology Laboratory of Selcuk University, Konya, Turkey, and were maintained on Brain Heart Infusion (BHI) agar medium at 4 °C until testing.

2.5 Antimicrobial testing

Minimum inhibitor concentration (MIC) tests were performed in accordance with the M27-A8 CLSI (Clinical Laboratory Standards Institute) criteria for bacteria (CLSI). Antimicrobial activity was evaluated using Broth Microdilution method. Briefly, the stock solution of the extract was obtained using solvent (Grierson and Afolayan,

1999) or DMSO (Salie et al. 1996; Nostro et al. 2000; Baris et al. 2006; Mendoza 1998). The inoculum volume for this procedure is usually 5 x 10⁵ cfu / mL (Lourens et al. 2004; Basri and Fan 2005).

Microbial culture with 0.4 optical density at 620 nm or 12 hour broth culture set with 0.5 McFarland turbidity standard was used (Baris et al. 2006). 100 µL of the plates were added to the first wells of the extracts diluted with microbial culture and DMSO at concentrations of 25 mg/mL and incubated at 37 ° C for 24 hours (Lourens et al. 2004).

After incubation, plates were examined for changes in turbidity as an indicator of growth. The first well that appeared clear was considered the MIC of the extract. Dilutions of the extracts according to Log₂ base (12.5 mg/ mL - 12.2 µg/mL) were prepared. No extract or culture was placed in the last well of the plate for media control. Algae extracts were diluted between concentrations of 6.25 mg / mL and 6.1 µg/mL. In parallel with this study, negative control (DMSO) and control antibiotic gentamicin were prepared in different plates. Reconstitution of gentamicin from 0.1 mg/ mL concentration was made with serial dilution at concentrations of 0.025 mg/mL-0.02 µg / mL. After 18 hours incubation at 37 ° C, 20 µL of aqueous (aqueous) TTC (0.5%) was added to the wells and incubated for another 30 minutes at 37 ° C. At the end of the incubation period, the growth in the plates was checked, and the lowest extract concentration in which there was no visible growth (uncolored areas) and hence inhibition of growth was evaluated as MIC.

3 Results

The antimicrobial activity results obtained in the broth microdilution method of *E. intestinalis* extracts are given in Table 2 and MIC of gentamisin(0.1mg/mL) and DMSO(% 100) are demonstrated in Table 3.

Table 2. Antimicrobial activity of *E. intestinalis* water, methanol and 70% ethanol extract

Test Microorganism	MIC (mg/mL)		
	<i>E.intestinalis</i> H ₂ O	<i>E.intestinalis</i> Methanol	<i>E.intestinalis</i> Ethanol(%70)
A2	—	—	—
A8	—	1.562 mg/mL (3.well)	1.562 mg/mL (3.well)
A10	—	—	—
A11	—	3.125 mg/mL (2.well)	3.125 mg/mL (2.well)
A12	—	—	—
A14	—	0.781 mg/mL (4. well)	1.562 mg/mL (3. well)
A55	—	0.390 mg/mL (5.well)	1.562 mg/mL (3. well)
A17	—	< 0.006 mg/mL	0.012 mg/mL (10. well)

A2: *E. coli* ATCC 25922
A8: *P. aeruginosa* ATCC 27853
A10: *K. pneumoniae* ATCC 70603
A11: *S. aureus* ATCC 43300
A12: *S. enteritidis* ATCC 13076
A14: *S. lutea* ATCC 9341

A55: *B. cereus* ATCC 11778
A17: *C. albicans*
— : There is bacterial growth

Table 3. MIC of gentamisin(0.1mg/mL) and DMSO(% 100)

Test Microorganism	Gentamisin (Positive control) (0.1 mg/mL)	DMSO (Negative control) (%100)
A2	< 0.02 mg/mL	%12.5
A8	<0.02 mg/mL	%12.5
A10	0.78 mg/mL	%12.5
A11	<0.02 mg/mL	%25
A12	0.04 mg/mL	%12.5
A14	<0.02 mg/mL	%12.5
A55	<0.02 mg/mL	%12.5
A17	<0.02 mg/mL	%12.5

4 Discussion

According to the findings, it was observed that the water extract of the studied species showed no antimicrobial activity against any test organism. The methanol extract of *E.intestinalis* was found to be effective in terms of antimicrobial activity against *P. aeruginosa*, *S. aureus*, *S. lutea* and *B. cereus* strains of bacteria and *Candida albicans* fungus strain. It was observed that *E.intestinalis* methanol and ethanol extract were effective only against *P. aeruginosa* strain from gram negative bacteria, while methanol extract did not show antimicrobial effects only against *S. enteritidis*. In other words, it can be said that the methanol extract of the species is more effective against gram positive bacterial strains.

Mansuya et al. (2010) studied antibacterial activity of *Cladophora glomerata* Grunow, *Ulva lactuca* L., *U. reticulata* Forsskål, *Gracilaria corticata* (J.Agardh) J.Agardh, *Kappaphycus alvarezii* (Doty) Doty ex P.C.Silva and *Sargassum wightii* Greville ex J.Agardh extracts by well diffusion method. Maximum activity (45 mm) was recorded against *U. reticulata* 200 mg of *Salmonella typhi* and minimum activity (9 mm) was recorded against *Streptococcus pyogenes*, while 50 mg of *U. lactuca* water extract was recorded and methanol extracts have been reported to have higher antimicrobial activity than water extracts (Manyusa et al. 2010)

Zbakh et al. (2012) tested the antibacterial activity of 20 macroalgae species (9 green algae, 3 brown algae and 8 red algae) collected from the Morocco coasts of the Mediterranean against *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis*. Among algae tested, 17 were found to show antibacterial activity. Extracts of *U. lactuca*, *Gracilaria bursa-pastoris* (S.G.Gmelin) P.C.Silva and *Chaetomorpha linum* (O.F.Müller) Kützinger were determined to have the highest antibacterial activity. Methanol extract of *Ulva rigida* C.Agardh has been reported to have an inhibitory effect against all strains tested (Zbakh et al. 2012).

Varier et al. (2013) determined the antimicrobial activity of red algae (*Gelidiella acerosa* (Forsskål) Feldmann & Hamel, *Gracilaria verrucosa* (Hudson) Papenfuss and *Hypnea musciformis* (Wulfen) J.V.Lamouroux) against gram positive and negative bacteria by disc diffusion method. Methanol, ethanol, chloroform and water were used as the solvent. While chloroform extracts of *G. verrucosa* were reported to constitute the highest inhibition zone (21 mm) against *Salmonella paratyphi*, none of the water extracts showed antibacterial activity (Varier et al. 2013).

5 Conclusion

As a result, it has been determined that extracts obtained using different solvents belonging to algae type used in our study have different degrees of antibacterial effects against the tested bacteria, and it will be appropriate to optimize the production conditions of the active substance, characterize it and to clarify the mechanism of action. It has been determined that the species used in the study has high antimicrobial activity, and it is suggested that different studies can be conducted in the future for the use of new

drugs produced for the treatment of infectious diseases as antimicrobial agents. In the light of new studies, it will be possible to contribute to the development of herbal natural products that can be used against various diseases by pharmaceutical companies and relevant scientific authorities.

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