

Full Length Research Paper

Antibacterial activities of some marine algae from the Aegean Sea (Turkey)

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In this study, methanolic extracts of six marine algae belong to Rhodophyceae (*Corallina officinalis*), Phaeophyceae (*Cystoseira barbata*, *Dictyota dichotoma*, *Halopteris filicina*, *Cladostephus spongiosus* f. *verticillatus*) and Chlorophyceae (*Ulva rigida*) from the North Aegean Sea (Turkey) were studied for their antibacterial activity against pathogenic microbes, 3 gram positive (*Staphylococcus aureus*, *Micrococcus luteus* and *Enterococcus faecalis*) and 3 Gram negative (*Escherichia coli*, *Enterobacter aerogenes* and *E. coli* O157:H7) *in vitro*. Extracts of all the test marine algae except *C. officinalis* showed inhibition against *S. aureus*. On the other hand, highest inhibiton activity among all the extratcs was shown to *E. aerogenes* by *C. officinalis*. The extract from *C. barbata* has shown broader activity spectrum against all the test organisms.

Key words: Aegean sea, antibacterial activity, *Corallina officinalis*, marine algae.

INTRODUCTION

Many substances obtained from marine algae such as alginate, carragenean and agar as phycocollids have been used for decades in medicine and pharmacy (Taskin et al., 2001). Since algae have been used in traditional medicine for a long time (Fitton, 2006) and also some algal substances have bacteriostatic and bactericidal activity, they have been extensively studied by several researchers (Burkholder et al., 1960; Ehresmann et al., 1977; Moreau et al., 1984; Reichelt and Borowitzka, 1984; Hornsey and Hide, 1985; Vlachos et al., 1999; Gonzalez del Val et al., 2001; Nora et al., 2003; Ghosh et al., 2004; Freile-Pelegrin and Morales, 2004; Salvador et al., 2007). Among the algal substances which have this kind of activity amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones and alkanes, cyclic polysulphides, fatty acids and acrylic acid can be counted (Mtolera and Semesi, 1996) as well as antimicrobial activity, antitumor activity of seaweeds studied by Yamamoto et al. (1984), Noda et

al. (1990), Harada et al. (1997, 2002), Abourriche et al. (1999) and of marine organisms by Ely et al. (2004).

There are some studies on antimicrobial activity of marine algae from Turkey (Haliki et al., 2005; Tuney et al., 2006, 2007; Ozdemir et al., 2006; Karabay-Yavasoglu et al., 2007). In this paper, some marine algae belonging to Phaeophyceae (*Dictyopteris membranaceae*, *Cystoseira barbata*, *Cystoseira compressa*, *Cystoseira mediterranea*, *Halopteris scoparia*, *Dictyota dichotoma*, *Colpomenia sinuosa*, *Ectocarpus siliculosus*, *Padina pavonica*, *Dictyota linearis*), Rhodophyceae (*Jania rubens*, *Acanthophora najadiformis*, *Laurencia papillosa*, *Hypnea musciformis*, *Gracilaria gracilis*, *Ceramium rubrum*) and Chlorophyceae (*Enteromorpha linza*, *Ulva rigida*) were studied.

In this investigation, antibacterial activity of six marine algae belonging to Rhodophyceae (*Corallina officinalis*), Phaeophyceae (*Cystoseira barbata*, *D. dichotoma*, *Halopteris filicina*, *Cladostephus spongiosus* f. *Verticillatus*) and Chlorophyceae (*U.rigida*) were studied against pathogenic microbes (*Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*, *Enterobacter aerogenes*, *Enterococcus faecalis* and *E. coli* O157:H7) *in vitro*. *C. officinalis* and *C. spongiosus* f. *verticillatus* were studied

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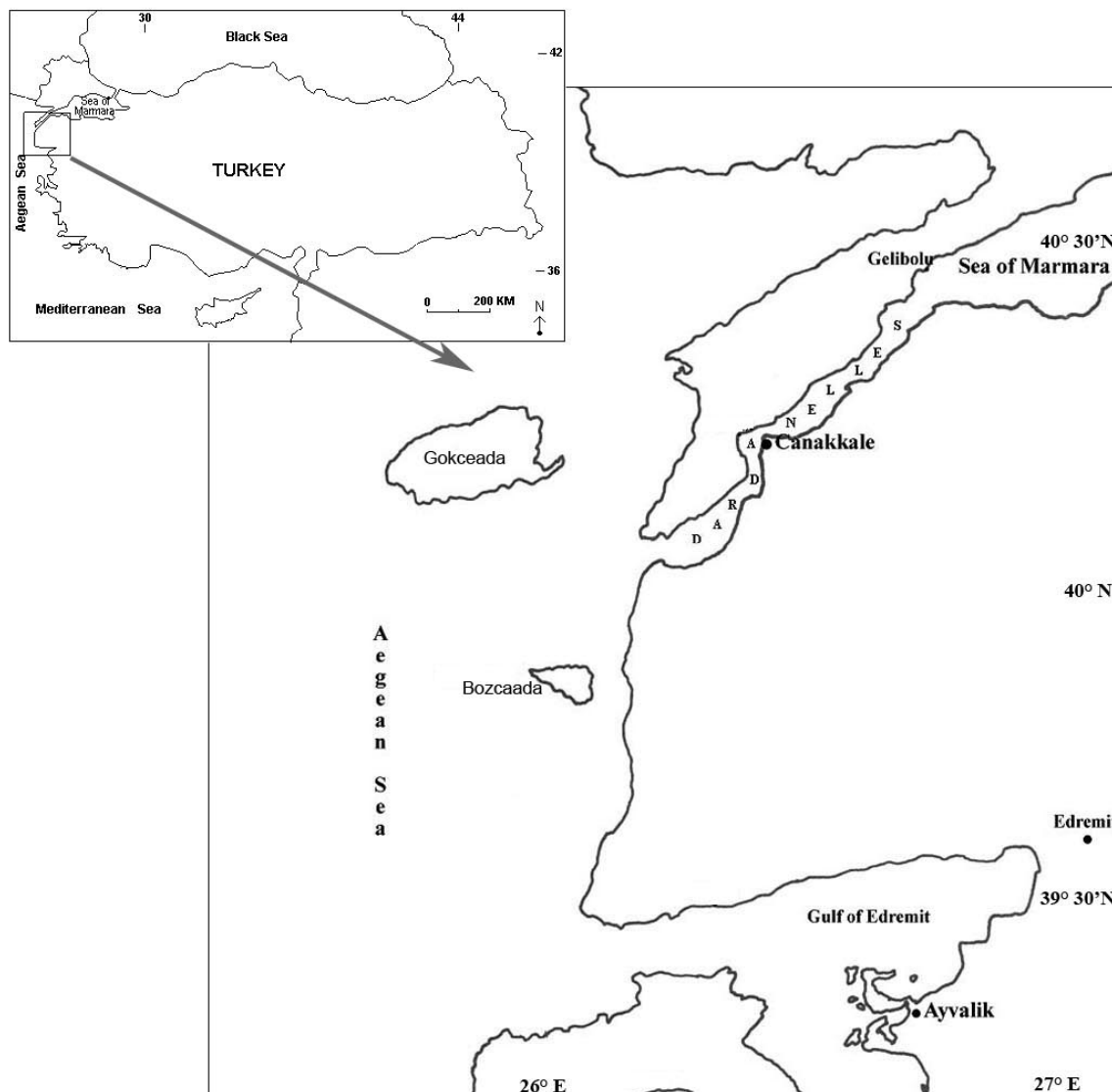


Figure 1. Location of study area.

for the first time for antibacterial activity from the Aegean Sea (Turkey).

MATERIALS AND METHODS

Sampling

Sampling was made in the midlittoral zone from Ayvalik (*C. officinalis*, *D. dichotoma*, *H. filicina*, *C. spongiosus* f. *verticillatus*) and Canakkale (*C. barbata* and *U. rigida*) by snorkeling (Figure 1).

Extract preparation and antibacterial assay

Collected samples were washed with tap water to remove epiphytes and other marine organisms and then washed with distilled water. Samples were dried at 45°C and powdered. This material mixed

with methanol (1:50, w/v) and placed into the soxhlet apparatus. Extraction solvent was evaporated under vacuum and used for antibacterial assay by paper disc diffusion method (El-Masry et al., 2000).

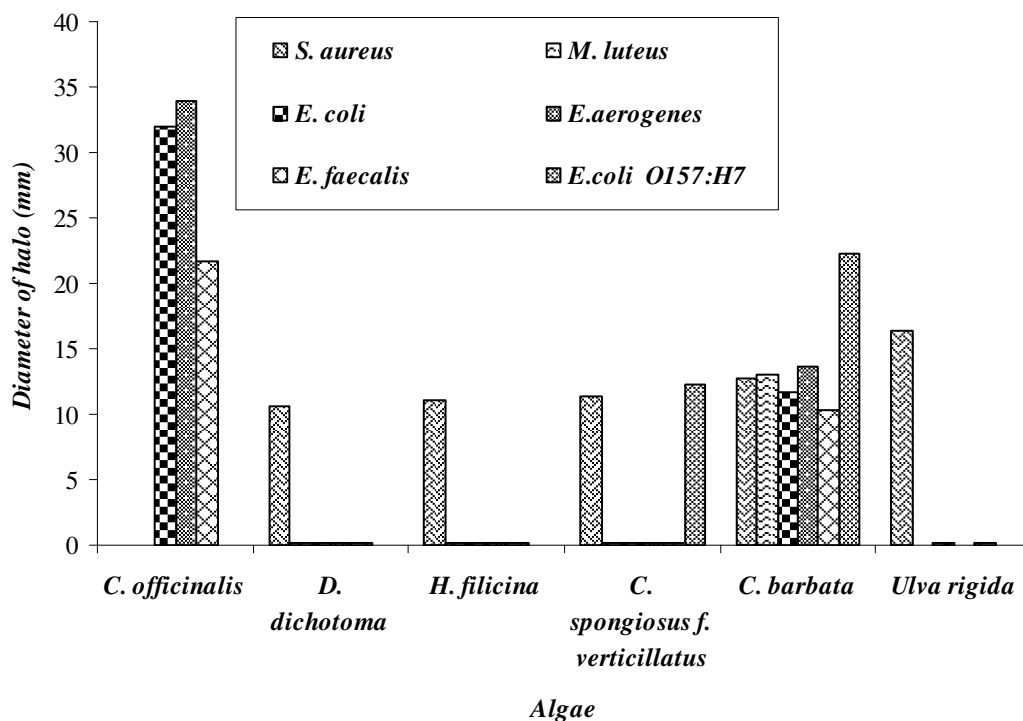
Test microorganisms were cultivated on Mueller Hinton Broth at 37°C for 18 h before inoculation for assay. 100 µl of broth culture which contains 10^7 - 10^8 number of bacteria/mL was added to Tryptic Soy Agar (Merck) medium and poured to sterile petri dishes. After medium solidified, the discs impregnated with extracts were placed onto the surface. Dishes were incubated at the same conditions mentioned above. Assays were run in triplicate. After incubation the clearance zones around the discs were measured and expressed in millimeter.

The test organisms used in this study included 3 gram positive (*S. aureus* ATCC 6538P, *M. luteus* ATCC 9341, *E. faecalis* ATCC 8043) and 3 Gram negative (*E. coli* ATCC 29998, *E. aerogenes* ATCC 13048, *E. coli* O157:H7) bacteria.

Table 1. Antibacterial activity of methanolic extracts of some marine algae from the Aegean Sea (Turkey) against to test organisms.

Algae	Microorganisms					
	Gram +			Gram -		
	<i>Staphylococcus aureus</i>	<i>Micrococcus luteus</i>	<i>Enterococcus faecalis</i>	<i>Enterobacter aerogenes</i>	<i>Escherichia coli</i>	<i>E. coli</i> O157:H7
<i>Corallina officinalis</i>	nt	nt	21,66±0,57	34,00±1,00	32,00±1,73	nt
<i>Dictyota dichotoma</i>	10.66±1.52	--	--	nt	--	--
<i>Halopteris filicina</i>	11.00±1.00	--	--	nt	--	--
<i>Cladostephus spongiosus</i> f. <i>verticillatus</i>	11.33±0.57	--	--	--	--	12.33±0.57
<i>Cystoseira barbata</i>	12.66±0.57	13.00±1.00	10.33±1.15	13.66±1.52	11.66±1.15	22.33±0.57
<i>Ulva rigida</i>	16.33±1.15	nt	--	nt	--	nt

--: inactive, nt: not tested, diameter of halo in mm.

**Figure 2.** Diameter of inhibition halo of marine algae against each test microorganisms.

RESULTS AND DISCUSSION

Antibacterial activities of crude extracts of six marine algae from the North Aegean Sea (Turkey) were determined by paper disc diffusion method and the results are summarised in Table 1 and Figure 2. Crude

extracts of all tested algae except *C. officinalis* showed inhibition against *S. aureus* and the extract of *U. rigida* was the most effective. On the other hand, highest inhibition activity was shown in *E. aerogenes* (34.00 ± 1.00 mm) by *C. officinalis* and it was followed with *E. coli* and *E. faecalis* (Figures 2 and 3). *D. dichotoma* has the

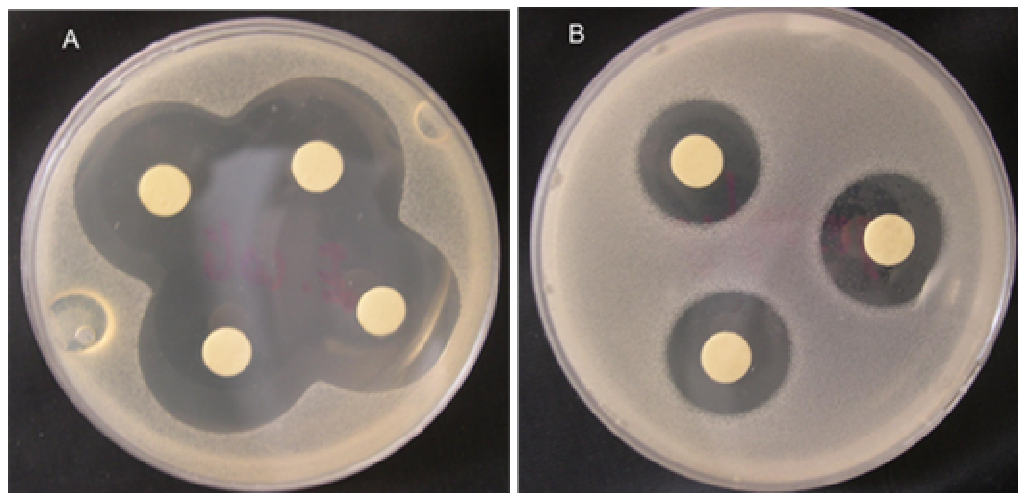


Figure 3. Inhibition zones obtained by the methanolic extract of *Corallina officinalis* against A) *E. coli* and B) *E. faecalis* on Tryptic Soy Agar (TSA) media.

lowest inhibitor activity (10.66 ± 1.52 mm). While the extract of *C. barbata* had the broadest activity spectrum, *D. dichotoma* and *H. filicina* had the lowest against test microorganisms.

The growth of food-borne pathogen *E. coli* O157:H7 was inhibited by only the extracts of *C. spongiosus* f. *verticillatus* with moderate and of *C. barbata* with the strong inhibition level (22.33 ± 0.57).

Studies that were carried on antimicrobial activity of some algae from the different parts of the world are summarized in Table 2. Many marine algae were screened for their antimicrobial activity by Reichelt and Borowitzka (1984) and Salvador et al. (2007). Salvador et al. (2007) studied antimicrobial activities of 82 marine algae as fresh and lyophilized forms. It was reported that the members of the red algal order, Bonnemaisoniales were the most active. In this study, they also studied with *C. spongiosus* f. *verticillatus*, *C. barbata*, *D. dichotoma* and *U. rigida*, as the same taxa that we used and inhibitor activities of these algae species against *S. aureus* ATCC 29213 were 14.1, 18 and 12.3 mm in diameter, respectively. They have also reported any inhibitor activity was obtained by *U. rigida*.

Tuney et al. (2007) used fresh and dried materials of *U. rigida* for the extraction. They found that while dried samples had no activity against *S. Aureus*, the extract prepared from fresh material has shown remarkably inhibitor activity to same strain. They have also obtained inhibitor activity against *E. faecalis* and *E. coli*.

Karabay-Yavasoglu et al. (2007) reported that the only methanolic and chloroform-prepared extracts of *Jania rubens* (Corallinales) have significant antimicrobial activity, however volatile oil did not inhibit significantly test

microorganisms. Ozdemir et al. (2006) indicated that the volatile oils of *D. membranacea* and *C. barbata* did not remarkably inhibit test organisms and the methanolic extracts of both algae have shown lower inhibitor activity than the hexane extracts.

Bansemir et al. (2006) have investigated the antibacterial activities of the extracts from 26 algae species prepared by dichlorometane, methanol and water against five fish-pathogenic bacteria. The highest activities were obtained by the dichloromethane prepared extracts. They have reported that the most active algal species was *Asparagopsis armata* against all tested bacteria.

Ely et al. (2004) have shown the methanolic extract of *Cladophora prolifera* had moderate bactericidal activity against *S. aureus* and *Vibrio cholerae*. Freile-Pelegrin and Morales (2004) studied ethanolic extracts from different thallus regions (apical, basal and stolon) of *Caulerpa* spp. They indicated that the stolon was the region having the highest antibacterial activity.

Conclusion

C. officinalis and *C. spongiosus* f. *verticillatus* were studied for the first time for antibacterial activity from the Aegean Sea (Turkey). Methanolic extract of *C. officinalis* showed good activity against *E. aerogenes*, *E. coli* and *E. faecalis*. The growth of food-borne pathogen *E. coli* O157:H7 was inhibited by the extract of *C. barbata* with the strong inhibition level (22.33 ± 0.57 mm) and as far as we know this strain was not used before as test organisms. It was indicated before that methanolic extract of *C. barbata* had no antibacterial activity against *E. coli* by

Table 2. Antimicrobial activity of the some marine algae from different parts of the world.

Algae	Microorganisms							References
	MI	Sa	Bs	Pa	Ec	Ef	Ca	
<i>Cladophora prolifera</i>	-	7-10	-	In	In	-	In	Ely et al., 2004 (India)
<i>Caulerpa prolifera</i>	7.7	-	10.0	-	-	11.3	-	Freile-Pelegri'n and Morales, 2004 (Mexico)
<i>Ceramium nitens</i>	23.0	-	22.3	-	-	-	-	
<i>Asparagopsis armata</i>	-	35.1	38.9	27.3	39.9	-	53.2	Salvador et al., 2007 (Spain)
<i>Bonnemaisonia asparagoides</i>	-	70.5	69.9	-	22.7	-	42.7	
<i>Hapalospongidion macrocarpum</i> (Fresh)	-	17.2	18.7	-	13.0	-	12.3	
	-	-	-	10-15	10-15	10-15	10-15	
<i>Ulva rigida</i>	-	In	-	In	In	-	In	Tuney et al., 2006 (Turkey) Reichelt and Borowitzka, 1984 (Australia)
<i>Caulerpa racemosa</i>	-	2.5-10	2.5-10	-	11-20	-	In	Mtolera and Semesi, 1996 (Tanzania)
<i>Laminaria pallida</i>	-	8.0	10.0	-	8.0	-	-	Vlachos et al., 1999 (South Africa)
<i>Enteromorpha intestinalis</i>	-	In	19-25	-	15-19	-	-	Glombitza, 1969 (Helgoland)
<i>Cystoseira barbata</i>	-	16.0	In	8.0	In	In	In	Ozdemir et al., 2006 (Turkey)
	-	12.5	12.1	-	In	-	In	Salvador et al., 2007 (Spain)

MI: *Micrococcus luteus*, Sa: *Staphylococcus aureus*, Bs: *Bacillus subtilis*, Pa: *Pseudomonas aeruginosa*, Ec: *Escherichia coli*, Ef: *Enterococcus faecalis*, Ca: *Candida albicans*.

--: inactive, nt: not tested, diameter of halo in mm.

other authors (Ozdemir et al., 2006; Salvador et al., 2007) but in this study our extract inhibited in diameter of 11.66 ± 1.15 mm.

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