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# Inhibitor Activities of some Seaweeds from the Aegean Coast of Turkey

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**Abstract**

In this study methanolic extracts of four seaweeds belong to Phaeophyceae (*Petalonia fascia, Stypocaulon scoparium*) and Chlorophyceae (*Cladophora prolifera, Codium fragile*) that were collected from the Aegean coast of Turkey have been studied for their inhibitor activity against pathogenic microbes (*Staphylococcus aureus, Staphylococcus epidermidis, Salmonella typhimurium, Enterobacter aerogenes, Escherichia coli, Enterococcus faecalis* and *Escherichia coli* O157:H7), *in vitro*. Against the extracts of all the tested marine algae, S. aureus was the most sensitive bacteria since it was inhibited by most of the extracts. On the other hand, the highest inhibitor activity was shown to *Enterobacter faecalis* by the extract of *Codium fragile*. Whereas, the growth of *Salmonella typhimurium* and *Enterobacter aerogenes* were not inhibited by any of the extracts.

**Key Words:** Aegean Sea, inhibitor activity, pathogenic microbes, seaweeds.

## INTRODUCTION

Many substances obtained from seaweeds have been used in traditional medicine and pharmacy for a long time [1,2]. Mtolera and Semesi [3] counted many kind of activity amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones and alkanes, cyclic polysulphides, fatty acids and acrylic acid among the algal substances. Some algal substances have antimicrobial activity and antitumoral activity they have been extensively studied in worldwide by several researchers [4-21].

There are numerous studies on antimicrobial activities of seaweeds (*Dictyopteris membranaceae, Cystoseira barbata, Cystoseira compressa, Cystoseira mediterranea, Halopteris scoparia, Halopteris filicina, Cladostephus spongiosus f. verticillatus Dictyota dichotoma, Colpomenia sinuosa, Ectocarpus siliculosus, Padina pavonica, Dictyota linearis, Corallina officinalis, Jania rubens, Acanthophora najadiformis, Laurencia papillosa, Hypnea musciformis, Gracilaria gracilis, Ceramium rubrum, Enteromorpha linza, Ulva rigida*) from Turkey which were carried by Haliki et al. [22], Tuney et al. [23,24], Ozdemir et al. [25], Karabay-Yavasoglu et al. [26], Taskin et al. [21] and Dulger et al. [27].

In this paper, the antimicrobial activity of four seaweeds belong to Phaeophyceae (*Petalonia fascia, Stypocaulon scoparium*) and Chlorophyceae (*Cladophora prolifera, Codium fragile*) were studied against pathogenic microbes (*Staphylococcus aureus, Staphylococcus epidermidis, Salmonella typhimurium, Enterobacter aerogenes, Escherichia coli, Enterococcus faecalis* and *Escherichia coli* O157:H7), *in vitro*.

## MATERIALS AND METHODS

**Sampling**

Sampling was made in the midlittoral zone from Ayvalik [*Stypocaulon scoparium* (L.) Kützing] and Canakkale [*Petalonia fascia* (O.F. Müller) Kuntze, *Cladophora prolifera* (Roth) Kützing and *Codium fragile* (Suringar) Hariot] by snorkerling in Summer 2006 and 2007 (Fig. 1).

**Extract preparation and antimicrobial assay**

Collected samples were washed under tap water to remove epiphytes and other marine organisms and then washed with distilled water. Samples were dried at 45 ºC and powdered. Seaweed powder was then mixed with methanol (1:15, w/v) and placed into the soxhlet apparatus at 50 °C for 8 hours. After extraction was completed, solvent was then evaporated under vacuum and reduced pressure then the residue was redissolved in dimethylsulfoxide (DMSO) and used for antibacterial assay by well - diffusion method [28].

**Cultivation of microorganisms and antibacterial assay**

Test microorganisms (*Staphylococcus aureus, Staphylococcus epidermidis, Salmonella typhimurium, Enterococcus faecalis, Escherichia coli, Enterobacter*

*aerogenes, E. coli* O157:H7) were cultivated on Mueller Hinton Broth at 37 ºC for 18 hours before inoculation for the assay. One hundred µl of broth culture which contains 107- 108 number of bacteria/mL was spread onto Tryptic Soy Agar (CASO Agar, Merck, 1.07324, Darmstadt, Germany) medium that were poured to sterile petri dishes. Then, 4 mm holes were punched onto the medium using a cork borer. Petri dishes left for 15 minutes until bacteria were absorbed to medium, then extracts (50 µl) were poured into wells. Dishes were incubated at the same incubation conditions mentioned above. Assays were run in triplicate. After incubation the inhibition zones around the wells as an evidence of antibacterial activity were measured underside and expressed in milimeter. The solvent and DMSO were used as a negative control while Streptomycin (30 µg) as positive control.

## RESULTS AND DISCUSSION

Inhibitor activities of crude extracts of four seaweeds that were collected from the Aegean coast of Turkey were determined by well-diffusion method and the results were summarised in Table 1 and Fig. 2.

The inhibition zones (IZ) that were observed in petri dishes were grouped as following: in the ranges between 9-12 mm as moderate, 13-15 mm as good, 16-18 mm as better, >18 mm as high.

Most of the extracts except that were obtained from *Petalonia fascia* have shown inhibitor activities against *S. aureus*. It was the most sensitive bacteria among the test bacteria. The broadest inhibitor activities were shown by the extracts of *Codium fragile* and *Cladophora prolifera* against test bacteria. The highest inhibitor activity was shown by the extract of *Codium fragile* against *E. faecalis* (20 mm= IZ) and it was followed by the bacteria *E. coli* and *E. coli* O157:H7 (18.5 and 18 mm IZ, respectively). On the other hand, the extract of *Petalonia fascia* which was applied against only two of test microorganisms, did not inhibit the growth of any of test microorganisms. The growth of *Salmonella typhimurium* and *Enterobacter aerogenes* were not inhibited by any of the extracts applied.

The growth of food-borne pathogen E. coli O157:H7 was only inhibited by the extracts of *Cladophora prolifera* and *Codium fragile* with the strong inhibition level (19 and 18 mm IZs, respectively).

Taşkın et al. [21] have showed that the growth of E. aerogenes has been strongly inhibited by the methanolic extract of red alga *Corallina officinalis* (34 mm inhibition zone). They also reported that brown algae members *Dictyota dichotoma* and *Halopteris filicina* had the lowest against test microorganisms while *Cystoseira barbata* had the broadest activity spectrum.

Ely et al. [19] reported that the methanolic extract of *Cladophora prolifera* had moderate bactericidal activity against *S. aureus* (7-10 mm inhibition zone) while the inhibitory activity of the extract of Turkish *Cladophora prolifera* was better (16 mm inhibition zone). Tuney et al. [24] investigated the antimicrobial effects of different organic extracts of seaweeds collected from the coast of Izmir. Different organic extracts of *Cladophora* sp. which was collected from two different sites were tried against test microorganisms, however, methanol and diethlyether extracts did not show any inhibitor activity. Only methanol extract showed good inhibitory activity against *E. faecalis* (IZ as 10-15 mm). Another *Chlorophyceae* member *Ulva rigida* was also studied and the only the diethyl ether extract of this species showed high and broad inhibitor activity against three test microorganisms (*E. coli, Pseudomonas aeruginosa* and *Candida sp*.) with

**Table 1.** Inhibition zones* of test microorganisms against methanolic extracts of seaweeds collected from Aegean Sea (*: diameter of inhibiton zones in mm, --: inactive, nt: not tested.).

| Seaweeds | Test Microorganisms | | | | | | |
|---|---|---|---|---|---|---|---|
| | Gram positive bacteria | | | | Gram negative bacteria | | |
| | *S. aureus* | *S. typhimurium* | *S. epidermidis* | *E. faecalis* | *E. aerogenes* | *E. coli* | *E. coli* O157:H7 |
| *Petalonia fascia* | nt | nt | nt | nt | -- | -- | nt |
| *Stypocaulon scoparium* | 11 | -- | Nt | nt | nt | -- | -- |
| *Cladophora prolifera* | 16 | -- | 13 | 19 | -- | -- | 19 |
| *Codium fragile* | 9 | -- | nt | 20 | -- | 18.5 | 18 |


**Figure 1.** Location of study area.

the inhibition zones range between 10-15 mm.

Many seaweeds have been investigated for their antimicrobial activities by Glombitza [29], Reichelt and Borowitzka [8], Ballesteros et al. [30], Caccamese et al. [31-33], Pesando and Caram [34] and Salvador et al. [20]. Salvador et al. [20] have investigated antimicrobial activity of 82 seaweeds and they have reported that the highest inhibitor activity was shown by the extract of red alga *Bonnemaisonia asparagoides* against *S. aureus* (70.5 mm inhibition zone). They have also reported that in general, the antimicrobial activities of Phaeophyceae and Rhodophyceae members were much higher than the Chlorophyceae members. However, in relation to taxonomic groups the saeson with the highest percentage of active taxa was autumn for Phaeophyceae and Rhodophyceae, and summer for Chlorophyceae. The extracts prepared from lyophilized specimens of Chlorophyceae members showed inhibitor activity against Gram positive bacteria in the range between 10.6-12.6 mm as IZs.

Choudhury et al. [35] and Bansemir et al. [36] have investigated the extracts of some algae against to fish pathogens. Bansemir et al. [36] have reported that the most active algal species was red alga *Asparagopsis armata* to against all tested bacteria. Freile-Pelegrin and Morales [17] studied ethanolic extracts from different thallus regions (apical, basal and stolon) of *Caulerpa* spp. and indicated that the stolon was the region having the

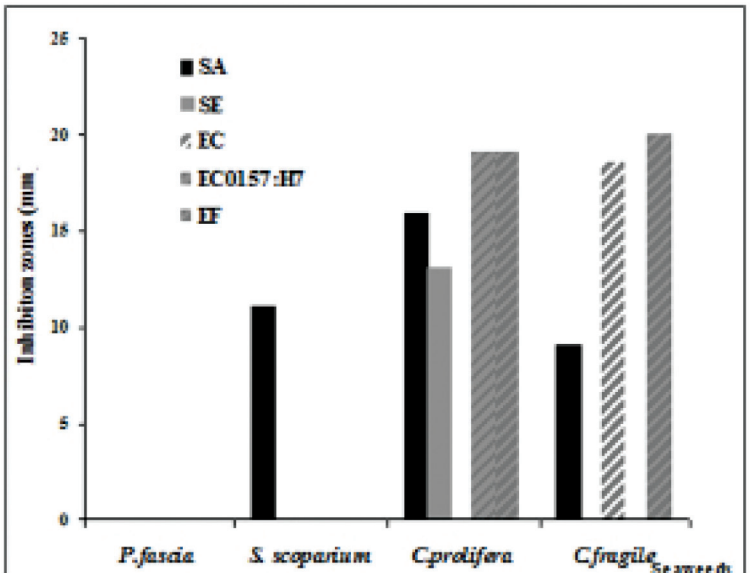


**Figure 2.** Diameter of inhibition zones of inhibited microorganisms against the crude extracts of seaweeds. SA: *Staphylococcus aureus,* SE: *Staphylococcus epidermidis*, EC: *Escherichia coli*, ECO157:H7: *Escherichia coli* O157:H7, EF: *Enterococcus faecalis.*

highest antibacterial activity.

Because of the intra specific variability in the production of seconder metabolites and related to seasonal and locational variation there are differences in the inhibitor activities of seaweeds. In this study, Chlorophyceae members showed broader and higher inhibitor activity than the two members of Phaeophyceae that were studied from Turkey.

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