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Antibacterial Activities of Some Seaweeds from Northern Cyprus Against Some Food-related Pathogens

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ABSTRACT

In this study, antibacterial activities of some seaweeds from Northern Cyprus against some food-related pathogens were examined. The methanolic extracts of eight seaweeds belonging to Rhodophyceae (*Haliptilon attenuatum, Dasya rigidula, Spyridia filamentosa*), Phaeophyceae (*Cystoseira foeniculacea, Dictyota dichotoma* var. *intricata*) and Chlorophyceae (*Caulerpa racemosa, Caulerpa prolifera, Ulva linza*) classes collected from North Cyprus (Eastern Mediterranean Sea) during late summer in 2006 and 2007 were studied for their antibacterial activities against some common food-borne pathogens species: *Staphylococcus aureus*, *Salmonella* Typhimurium (*S. enterica* subsp. *enterica*), *Escherichia coli* O157:H7, *Aeromonas hydrophila* and *Yersinia enterocolitica, in vitro*. *S. aureus* was the most sensitive bacterium since it was inhibited by most of the extracts. On the other hand, *A. hydrophila* was not inhibited by any of the extracts. The greatest inhibitory activity against *Salmonella* Typhimurium was shown by *C. foeniculacea* extract. Most of the extracts inhibited the growth of *E. coli* O157:H7. The *C. foeniculacea* extract also showed the broadest inhibitor activity against food-related pathogens. Six species (*D. rigidula, S. filamentosa, C. foeniculacea, D. dichotoma* var. *intricata, C. racemosa* and *C. prolifera*) were shown inhibitor activity against food-related pathogens.

Key words: Seaweeds, bacterial inhibition, crude extract, eastern Mediterranean, Soxhlet extraction

INTRODUCTION

Because of the emerging resistance of pathogenic microorganisms to existing antibiotics, and the fast spread of resistant microorganisms, there is an increasing need for new antibiotics. In marine environments, competition for space and nutrients led to the evolution of antimicrobial defence strategies such as production of chemically active metabolites in their surroundings potentially as an aid to protect themselves against other settling organisms. Marine algae are one of the largest producers of biomass in the marine environments. Therefore, algae offer a rich source of potential new drugs (Bhadury and Wright, 2004; Bansemir *et al*., 2006).

Phycocolloid substances from marine algae such as alginate, carrageenan and agar have been used globally for decades in medicine and pharmacy. Since the traditional medicinal properties of algae are long known, their antimicrobial activities have been investigated by many researchers

and extensive results have been published. In those papers the organic crude extracts of some marine algae belonging to the Phaeophyceae, Rhodophyta and Chlorophyta were studied for their potential inhibitory activities against bacteria and fungi (Burkholder *et al*., 1960; Moreau *et al*., 1984; Reichelt and Borowitzka, 1984; Hornsey and Hide, 1985; Vlachos *et al*., 1999; Gonzalez-del-Val *et al*., 2001; Freile-Pelegrin and Morales, 2004; Salvador *et al*., 2007; Taskin *et al*., 2007; Tuney *et al*., 2007; Shanmughapriya *et al*., 2008; Manilal *et al*., 2009; Koz *et al*., 2009; Lategan *et al*., 2009; Taskin *et al*., 2010).

It is now collectively acknowledged that the use of antimicrobials for both animals and humans can select for resistant bacterial populations. Emergence of antimicrobial resistance has become a serious worldwide problem. In fact, emerging antimicrobial resistance phenotypes have been recognized among multiple zoonotic pathogens including *Salmonella enterica* serovar Typhimurium, *Escherichia coli*, *Campylobacter jejuni*, *Listeria monocytogenes* and *Yersinia enterocolitica* (White *et al*., 2002). Despite advances in hygiene, consumer knowledge, food treatment and processing, food-borne diseases mediated by pathogenic microorganisms or microbial toxins still represent a significant threat to public health worldwide. The global incidence of food-borne diseases is difficult to estimate but it has been reported that in 2005 alone 1.8 million people died from diarrhoeal diseases (WHO, 2007) and a significant proportion of these results from consumption of mainly food of animal origin with microbial pathogens and toxins. *E. coli* is a large and diverse group of bacteria. Although most strains are harmless, some kinds of *E. coli* cause urinary tract diseases, respiratory illness and pneumoniae. *E. coli* O157:H7 was first described in 1982. Subsequently, it has emerged rapidly as a major cause of bloody diarrhoea and acute renal failure and producer of deadly Shiga-toxin. *Salmonella* is the most common bacterial cause of diarrhea and the most common cause of food-borne deaths. Staphylococcal food intoxication is one of the most common types of food-related diseases around the world. Staphylococcal food poisoning result from the ingestion of food containing preformed staphylococcal enterotoxins produced by the enterotoxigenic staphylococci mainly *Staphylococcus aureus* (Erol and Iseri, 2004).

However, the investigation of the bioactivities of crude or pure extracts that were obtained from marine algae against food-related pathogens are scarce. Bansemir *et al*. (2006) studied theeinhibitory activities of various organic extracts of algae against some fish pathogenic bacteria and their results confirmed the possible use of algae as a source of antimicrobial compounds or as a health-promoting food.

The marine algae flora of Northern cyprus was studied by Ozturk *et al*. (2008) and about 300 species were reported.

The main objective of this study was to evaluate the inhibitory effects of the crude extracts that were obtained from marine algae which collected from Northern cyprus against some food-related pathogens.

MATERIALS AND METHODS

Sampling: Algal species (*Haliptilon attenuatum, Spyridia filamentosa, Cystoseira foeniculacea, Dictyota dichotoma* var. *intricata, Caulerpa racemosa* and *Caulerpa prolifera, Dasya rigidula* and *Ulva linza*) were sampled from the mid-littoral zone by snorkeling. Samples were identified by Assoc. Prof. Ergun Taskin (Celal Bayar University, Turkey) according to the accounts in Fletcher (1987), Maggs and Hommersand (1993) and Siguan (2001). Voucher specimens are deposited at his personal herbarium.

Extract preparation and antibacterial assay: Collected samples were brought immediately to the laboratory on ice. Samples were washed with tap water to remove epiphytes and other marine organisms and then washed with distilled water. They were dried at 45°C and powdered by milling.

This algae powder was mixed with methanol (1:15, w/v) and placed into a Soxhlet apparatus at 50°C for 8 h. After extraction was complete, the solvent was then evaporated under vacuum and reduced pressure then the residue was used for antibacterial assay by the well-diffusion method (Perez *et al*., 1990). At first, 4 mm holes were punched in tryptic soy agar medium (CASO Agar, Merck, 1.07324, Darmstadt, Germany) using a cork borer. Test microorganisms were cultivated on Mueller Hinton Broth (MHB, Merck, 1.10293, Darmstadt, Germany) at 37°C for 18 h before inoculation for the assay and broth culture, which contained 10^7-10^8 bacteria mL^{-1}, was added to medium that was prepared a priori.

Petri dishes were left for 15 min until bacteria absorbed to the medium, then extracts (50 μL) were poured into wells. Dishes were incubated under the same incubation conditions mentioned above. Assays were run in triplicate. After incubation the inhibition zones around the wells were measured on the underside of petri dishes and expressed in mm. The solvent was used as a negative control and streptomycin (30 μg) antibiotic discs as positive control.

The test microorganisms used in this study included one Gram-positive (*Staphylococcus aureus* (SA) and four Gram-negative (*Escherichia coli* (EC), *E. coli* O157:H7 (ECO157:H7), *Aeromonas hydrophila* (AH) and *Yersinia enterocolitica* (YE) bacteria which are known as food-related pathogens isolated from different food products. All of the seaweed crude extracts were tested against *S. aureus*, *E. coli* and *E. coli* O157:H7, while five of them were tested against *A. hydrophila* and *Y. enterocolitica*.

RESULTS

The inhibitory activities of the extracts of seaweeds were classified from moderate to high according to the diameters of obtained Inhibition Zones (IZs) surrounding the wells in mm on petri dishes as follows:

- 9-12 mm = moderate
- 13-15 mm = good
- 16-18 mm = very good
- >18 mm = high

Inhibition zones using methanol as the negative control were also observed. Moderate inhibition zones (9-12 mm IZs) were observed by the streptomycin discs as positive control.

Antibacterial activities of the crude extracts of 8 seaweeds that were collected from Northern Cyprus against five food-related pathogen bacteria were determined by the well-diffusion method and the results are summarised in Fig. 1.

Most of the extracts except for a few showed inhibitory activity against tested bacteria as the IZs fell between 12 and 15 mm. The broadest inhibitory activities were shown by the extracts of both *S. filamentosa* and *C. foeniculacea* and only those two extracts showed inhibitory activities against *Salmonella* Typhimurium (11 and 21 mm IZs, respectively) (Fig. 1).

Most of the extracts (except for two) showed inhibitory activity against *S. aureus*, which was the most sensitive bacteria among the test bacteria. The highest inhibitory activity was shown by

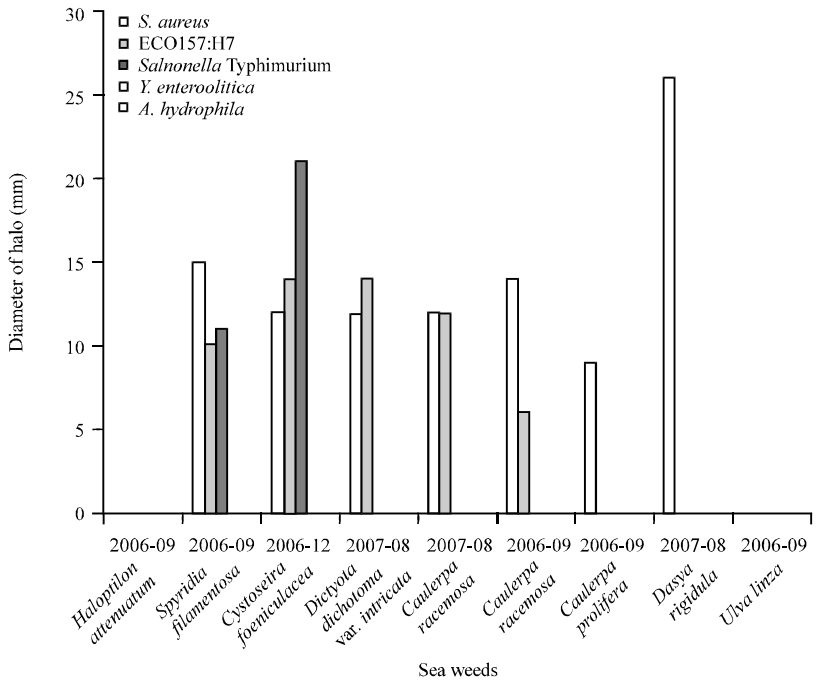

Fig. 1: Measurements of diameter of inhibition zones of test microorganisms obtained by the crude extracts of eight seaweeds on petri dishes

the *D. rigidula* extract (IZ = 26 mm) (Fig. 1). *Y. enterocolitica* and *A. hydrophila* were the most resistant bacteria against the 5 seaweed extracts. A moderate inhibition zone (IZ = 12 mm) against *Y. enterocolitica* was shown only by the *D. dichotoma* var. *intricata* extract (collection time 2006-09) (Fig. 1). On the other hand, the *U. linza* and *H. attenuatum* extracts did not inhibit the growth of any test bacteria *in vitro* (Fig. 1). Growth of *A. hydrophila* was not inhibited by any of the extracts. Choudhury *et al.* (2005) investigated the inhibitory activities of various extracts of some marine algae and mangroves against some fish pathogens. They reported that the algal extracts showed weak inhibitor activities against pathogens, with IZs ranging between <2 and = 2 mm. The growth of *A. hydrophila* was only inhibited by various extracts of a mangrove, *Cynometra iripa* with the measured IZ = 2 mm.

DISCUSSION

According to the results of this study, the extracts prepared from the samples of Phaeophyceae and Rhodophyceae classes showed broader and higher inhibitory activities against the growth of test bacteria than the extracts of the Chlorophyceae members. These results are in agree with Salvador *et al.* (2007). They have investigated the antibacterial and antifungal activities of 82 marine macroalgae and they have found that the highest percentage of active taxa are in Phaeophyceae (84%). Much of the *in vitro* activity observed in the brown algae could be attributed to various phenols which occur commonly in these plants (Reichelt and Borowitzka, 1984).

While the antibacterial effects of plant extracts against food-related pathogens were studied, the algae extracts were rarely tried against those pathogens. Bansemir *et al.* (2006) have studied the inhibitory activities of the dichloromethane extracts of 26 cultured seaweeds against five fish pathogenic bacteria which were *Vibrio anguillarum* (VA), *Pseudomonas anguilliseptica* (PA),

Aeromonas salmonicida (AS), *A. hydrophila* (AH) and *Yersinia ruckeri* (YR). Extract of the *Asparagopsis armata* exhibited strong inhibitory activities against all the test bacteria. Obtained inhibition zones were 26.9, 19.3, 17, 14.9, 15.3 mm against VA, PA, AS, AH and YR, respectively. AH and YR were weakly inhibited by only the four of extracts of seaweeds among the 26 extracts of algae. The extracts of *Ulva* spp. were only and weakly inhibited the growth of VA and PA, the IZs were obtained as 3 and 3.1, respectively. *D. dichotoma* extract did not inhibit the growth of *Aeromonas* spp. and YE. Tuney *et al.* (2007) have found the extracts of *U. rigida* prepared from dried material (Coast of Izmir, Turkey) as inactive against *S. aureus* and weakly active against *E. coli* (8.5 mm=IZ) while the extract prepared from fresh material was highly active against *S. aureus* (38 m=IZ) and *E. coli* (22.5 mm=IZ).

In another study that was carried by the algae samples collected from Aegean Sea (Turkey), the growth of ECO157:H7 was inhibited by the extracts of *Cladostephus spongiosus* f. *verticillatus* in moderate level (12.33 mm= IZ) and of *Cystoseira barbata* in the strong (22.33 mm= IZ) inhibition level. The growth of *E. coli* ATCC29998 has been strongly inhibited by the extract of *Corallina officinalis* (32 mm= IZ) (Tuney *et al.*, 2007). Vairappan *et al.* (2001) investigated the antibacterial effects of halogenated compounds isolated from *Laurencia* spp. Two halogenated compounds named as elatol and iso-obtusol were tried against the strain of *E. coli* and it was inhibited in the range between 7-12 mm IZ. Based on their findings they have firstly offered the possible role of halogenated metabolites in antibacterial defense in algal habitats. Besides halogenated compounds (sesquiterpenes, diterpenes and acetylenes), fatty acids, sterols, bromophenol compounds have been identified as antimicrobial substances in algae.

The antibacterial activities of six seeweeds from Southeast coast of India were investigated against test bacteria *E. coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae and Enterococcus faecalis*, recently (Kolanjinathan and Stella, 2009). They have found that acetone extracts of fresh material gave better results than extracts that were prepared by other solvents e.g., ethanol and methanol and than the dried material. All the extracts were active against test bacteria. According to the results, the growth of test bacteria were inhibited as the lowest by the extract of *Ulva lactuca* (the IZs between 8-10 mm) while highly inhibited by the extract of *Sargassum myriocystum* (IZs between 19-32 mm).

The antibacterial activities of five brown algae that were collected from the Izmir coast of Turkey were assessed against nine of test microorganisms one of which was a yeast strain (Demirel *et al.*, 2009). They have found that dichloromethane extracts of the samples exhibited a higher degree of activity as compared to the methanol and hexane extracts. They have reported the inhibition zones between 6.5-11 mm (filter paper disc included) at 1.5 mg disc^{-1} concentration of the extract of *D. dichotoma* against test microorganisms. *Proteus vulgaris* was the mostly inhibited test bacterium while *S. aureus* was not inhibited.

By this current study, the inhibitory activities of extracts that were prepared from marine algae collected from NC were firstly evaluated against some common food-related pathogens. As stated in literature, the presence of the inhibitory activities of extracts of seaweeds or the degrees of this activities depend on the extraction procedure parameters such as quantity of sample, type and solvent of extraction, sampling time and even the localities of seaweeds. Therefore, the results of this study are in preinformative nature and in order to obtain wide and stable viewpoint about the antimicrobial activities of these seaweeds, they should be monitored and investigated in the next studies.

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