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Antimicrobial Activity of Some Brown Algae from Turkey

BASARAN DULGER*, NURCIHAN HACIOGLU, HUSEYIN ERDUGAN and VEYSEL AYSEL
Department of Biology, Faculty of Science and Arts
Canakkale Onsekiz Mart University, 17100 Canakkale, Turkey
Fax: (90)(286)2180533; E-mail: basarandulger@yahoo.com

In vitro antimicrobial screening of methanolic extracts obtained from 6 brown algae (*Calpomenia peregrine* (Sauvageau) Hamel, *Halopteris scoparia* (Linnaeus) Sauvageau, *Padina pavonica* (Linnaeus) Thivy, *Cystoseria compressa* (Esper) Gerloff et. Nizamuddin, *Cladostephus spongiosus* (Hudson) C.Agardh and *Scytosiphon simplicissimus* (Clemente) Cremades) (Heterokontophyta) collected from coast of Canakkale, Turkey, against *Escherichia coli* ATCC 11230, *Staphylococcus aureus* ATCC 6538P, *Klebsiella pneumoniae* UC57, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 8427, *Bacillus subtilis* ATCC 6633, *Mycobacterium smegmatis* CCM 2067, *Listeria monocytogenes* ATCC 15313, *Micrococcus luteus* CCM 169, *Candida albicans* ATCC 10231, *Rhodotorula rubra* DSM 70403, *Kluyveromyces fragilis* ATCC 8608 and *Cryptococcus neoformans* ATCC 90112 was conducted in this study by disc diffusion method. *Cystoseria compressa* was the most active exhibiting a broad spectrum antimicrobial activity against each of the microorganims tested. Contrary to this, the lowest effect was observed for *Calpomenia peregrine*. The other algae species have moderate effects. The extracts showing good antimicrobial activity are undergoing further analysis to identify the active constituents.

Key Words: Brown algae, Antimicrobial activity.

INTRODUCTION

Seaweeds provide a rich source of structurally diverse secondary metabolites. There are numerous of compounds derived from seaweeds with a broad range of biological activities, such as antibiotics, antivirals, antitumorals and antiinflammatories[1], as well as neurotoxins[2]. In Western countries, seaweeds are mainly use as sources of alginate, carrageenan and agar in addition to ingredients in the content of many beauty products. The greatest use of seaweeds in the worldwide is for food, most probably by reason of rich in non-digestible fibers, mineral salts, vitamins and protein, but low in fat content[3-5].

To date, research on antimicrobial activity of seaweeds in Turkey is scarce. The aim of this works was to evaluate the antimicrobial activity of 6 brown algae (Heterokontophyta) species *i.e.*, *Calpomenia peregrine* (Sauvageau) Hamel, *Halopteris scoparia* (Linnaeus) Sauvageau, *Padina pavonica* (Linnaeus) Thivy, *Cystoseria compressa* (Esper) Gerloff et. Nizamuddin, *Cladostephus spongiosus* (Hudson) C.Agardh and *Scytosiphon simplicissimus* (Clemente) Cremades as wild-growing in Turkey.

EXPERIMENTAL

Seaweeds were collected at a depth of 1-2 m from the coast of Canakkale, Turkey in May 2008 and were identified by Dr. Huseyin Erdugan. Algae samples were cleaned of epiphytes and necrotic parts were removed. Then the samples were rinsed with sterile water to remove any associated debris as described by Gonzales del Val *et al.*[6].

Preparation of extracts: 25 g of each air-dried seeweed samples were extracted in 150 mL of 80 % methanol (Merck, Darmstadt, Germany) for 24 h by using Soxhlet equipment[7]. The extract was filtered using Whatmann no.1 and the filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55 °C. Dried extract were stored in labeled sterile screw-capped bottles at -20 °C.

Microorganisms: *Escherichia coli* ATCC 11230, *Staphylococcus aureus* ATCC 6538P, *Klebsiella pneumoniae* UC57, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 8427, *Bacillus subtilis* ATCC 6633, *Mycobacterium smegmatis* CCM 2067, *Listeria monocytogenes* ATCC 15313, *Micrococcus luteus* CCM 169, *Candida albicans* ATCC 10231, *Rhodotorula rubra* DSM 70403, *Kluyveromyces fragilis* ATCC 8608 and *Cryptococcus neoformans* ATCC 90112 were used as test microorganisms.

Screening for antimicrobial activities: The seaweed extracts were tested for antimicrobial activity through the disc diffusion method according to the National Committee for Clinical Laboratory Standards[8]. The dried seaweed extracts were dissolved in 10 % aqueous dimethyl sulfoxide to a final concentration of 200 mg/mL and sterilized by filtration through an 0.45 µm membrane filter. Empty sterilized antibiotic discs having a diameter of 6 mm (Schleicher & Schull No. 2668, Dassel, Germany) were each impregnated with 50 µL extract (10 mg/disc) at concentration of 200 mg/mL. All the bacteria mentioned above were incubated 35 ± 0.1 ºC for 24 h inoculation into Nutrient Broth (Difco Laboratories, MI, USA) and the yeast cultures studied were incubated in Malt Extract Broth (Difco Laboratories, MI, USA) at 25 ± 0.1 ºC for 48 h. An inoculum containing 10^6 bacterial cells or 10^8 yeast cells/mL was spread on Mueller Hinton Agar (Oxoid Ltd., Hampshire, UK) plates (1 mL inoculum/plate), the discs injected with extracts were placed at 4 ºC for 2 h, plaques injected with the yeast cultures were incubated at 25 ± 0.1 ºC for 72 h and bacteria were incubated at 35 ± 0.1 ºC for 24 h. At the end of the period, inhibition zones formed on the medium were evaluated appropriate reference antibiotic disc was applied, depending on the test microorganisms for comparison.

RESULTS AND DISCUSSION

The antimicrobial activity of the methanol extracts obtained from six species of the brown algae is shown in Table-1. Besides, the inhibition zones formed by standard antibiotic discs are indicated in Table-2.

TABLE-1
ANTIMICROBIAL ACTIVITY OF THE SEAWEEDS

Microorganisms	Zone of inhibition (mm)*				
	Methanolic extracts				
	1	2	3	4	5
Bacteria					
Escherichia coli	10.5	11.4	11.4	14.6	11.8
Staphylococcus aureus	12.1	14.4	12.8	17.8	10.2
Klebsiella pneumoniae	9.6	13.0	13.2	15.2	14.2
Pseudomonas aeruginosa	-	12.8	9.6	14.4	13.2
Proteus vulgaris	9.2	11.0	9.2	12.6	11.8
Bacillus subtilis	-	9.6	10.2	12.2	12.0
Listeria monocytogenes	-	10.2	10.4	12.0	10.6
Micrococcus luteus	10.2	12.2	9.8	12.4	9.8
Mycobacterium smegmatis	-	14.2	10.2	14.0	9.2
Fungi					
Candida albicans	9.6	9.2	9.2	18.6	10.2
Cryptococcus neoformans	-	11.2	11.2	19.0	10.0
Kluyveromyces fragilis	10.2	10.2	9.6	19.6	10.8
Rhodotorula rubra	-	10.0	10.2	16.6	11.4

1 = *C. peregrine*, 2 = *H. scoparia*, 3 = *P. pavonica*, 4 = *C. compressa*, 5 = *C. spongiosus*, 6 = *S. simplicissimus*; *Values, including diameter of the filter paper disc (6.0 mm), are means of three replicates.

TABLE-2
ANTIMICROBIAL ACTIVITY OF SOME STANDARD ANTIBITOTICS

Microorganisms	Zone of Inhibition (mm)*						
	Standard antibiotics						
	P10	SAM20	CTX30	VA30	OFX5	TE30	NY100
Bacteria							
Escherichia coli	18.2	12.2	10.4	22.0	30.8	28.2	-
Staphylococcus aureus	13.4	16.8	12.6	13.4	24.4	26.4	-
Klebsiella pneumonia	18.2	14.4	13.4	22.4	28.2	30.6	-
Pseudomonas aeruginosa	8.6	10.8	54.2	10.8	44.0	34.8	-
Proteus vulgaris	10.2	16.2	18.4	20.0	28.6	26.2	-
Bacillus subtilis	14.4	12.4	14.6	18.6	30.2	25.4	-
Mycobacterium smegmatis	15.8	21.0	11.8	20.0	32.2	24.6	-
Listeria monocytogenes	10.6	12.4	16.6	26.4	30.2	28.2	-
Micrococcus luteus	36.2	32.0	32.2	34.2	28.8	22.4	-
Fungi							
Candia albicans	-	-	-	-	-	-	18.6
Cryptococcus neoformans	-	-	-	-	-	-	18.0
Kluveromyces fragilis	-	-	-	-	-	-	18.2
Rhodotorula rubra	-	-	-	-	-	-	17.2

*Includes diameter of disk (6 mm); P10 = Penicillin G (10 Units), SAM20 = Ampicillin 10 µg, CTX30 = Cefotaxime 30 µg, V30 = Vancomycin 30 µg, OFX 5 = Oflaxacin 5 µg, TE30 = Tetracyclin 30 µg, NY100 = Nystatin 100 µg.

As can clearly be seen from Tables 1 and 2, all extracts have antimicrobial effects against the tested microorganism cultures in various inhibition zones. Notably, the extracts showed a stronger antifungal activity than antibacterial activity. Among the seaweed species used in this study, *Cystoseria compressa* have shown an excellent antimicrobial activity against both bacteria and fungi. However, the lowest effect was observed for *Calpomenia peregrina* forming with the inhibition zones against only 5 bacteria and 2 yeast cultures. *Staphylococcus aureus* is more susceptible the extract of *C. compressa* as compared to all standard antibacterial antibiotics, except for OFX5 and TE30. A moderate effect against the other bacteria is seen. In addition, the extract of *C. compressa* has a strong antiyeast effect against all yeast cultures. Antifungal effect against *Candida albicans* is equal to that of the extract in comparison to the standard antifungal antibiotic Nystatin. The extracts have higher antifungal activity than those of standard Nystatin against *Cryptococcus neoformans* and *Kluyveromyces fragilis*. A moderate activity is seen against *Rhodotorula rubra*.

In previous study, it is determined that ethanol extracts of *Padina pavonica* show antibacterial activity only against *Bacillus subtilis*[6]. In another study, acetone, methanol and diethyl ether extracts of *P. pavonica* had no antibacterial or antifungal activities, but the ethanol extract of *P. pavonica* showed weak activity against *Candida* sp., *Enterococcus faecalis, Pseudomonas aeruginosa* and *Escherichia coli*[9]. In contrast, present results showed that the methanol extract of *P. pavonica* inhibited all the test microorganisms. The differences between present results and the others may be due to several factors, for example the infra-specific variability in the production of secondary metabolites[10,11]. Also, as reported that the efficacy of macro algae extracts against microorganisms is mostly influenced by factors such as location and seasonality[10] and another study that macro algae showed a high percentage of species with antimicrobial activity, 73 % in the case of Chlorophyta (green algae), 69 % in Rhodophyta (red algae) and 53 % in Phaeophyta (brown algae)[12].

The extract from *C. compressa* showed a strong antifungal activity against the yeast cultures. The antimicrobial effect of several types of extracts (methanol, ethanol, diethyl ether, hexane, chloroform and water) of *Cystoseria tamariscifolia* (Hudson) Papenfuss was carried out on yeast and only ethanol extracts showed antimicrobial activity against yeasts[13]. Also, the ethereal fraction of *C. tamariscifolia* showed more potent antibacterial activity than hexane and dichloromethane fractions[14]. In another study, none of the extracts (methanol, dichloromethane, hexane and chloroform) from *Cystoseria barbata* (Good et Woodw.) J.Agardh showed activity against *C. albicans*[15]. This difference may have been due to species variation. Also, there may be differences in the extraction protocols to recover the active metabolites and differences in the assay methods.

Taskin *et al.*[16] reported that the growth of food-borne pathogen *E. coli* 0157:H7 was inhibited by only the extracts of *Cladostephus spongiosus* f. *verticillatus* with moderate of *Cystoseria barbata* with the strong inhibition level. In the same study, it is determined that *Halopteris filicina* (Grateloup) Kutzing has a low antibacterial

effect against tested bacteria. In present study, the extracts from *Cladostephus spongiosus* and *Halopteris scoparia* have a moderate activity against all tested microorganisms.

Some studies concerning the effectiveness of extraction methods highlight that methanol extraction yields higher antimicrobial activity than the other solvents[10]. According to present results, methanol extract has stronger and broader spectrum of antimicrobial activity. This information confirmed that the methanol has higher effective solvent for extraction of antimicrobial substances in the seaweed species. Among the algal substances, amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones and alkanes, cyclic polysulphides, fatty acids and acrylic acid can be counted[17]. Terpenoids, polyphenols and C_{11} metabolites are broadly distributed among brown seaweeds[18]. Several authors[19-22] had found antibacterial activities of microalgae due to fatty acids. The mentioned substances may be responsible for the antimicrobial activity in seaweeds used in this study.

In conclusion, this preliminary evaluation indicated that the seaweeds used in this study have significant activity against tested microorganisms. Further analyses are necessary to identify the main active constituents.

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