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Antimicrobial Activities of Some Marine Algae and Some Cyanobacteria from Çanakkale (Turkey)

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Abstract

Methanol, chloroform, diethylether, dichloromethane, ethanol extracts of *Rivularia bullata* (Poir) Berkeley ex Bornet & Flahault, *Nostoc* spongiaeforme C. Agardh ex Bornet & Flahault, *Codium fragile* (Suringar) Hariot, *Colpomenia peregrina* Sauvageau, *Cystoseira barbata* (Stackhouse) C. Agardh, *Zanardinia typus* (Nardo) P. C. Silva were tested *in vitro* for their antimicrobial activities against some Gram negative and Gram positive bacteria, some yeast. Antimicrobial activities were evaluated by using the disc diffusion method in petri plates according to the National Committee for Clinical Laboratory Standards. The antifungal activities of all species are more than their antibacterial activities. *Z. typus* have shown a broad antimicrobial activity against bacteria and yeast strains. The lowest effect was observed for *C. fragile* forming with the inhibition zones against only two bacteria and all yeast strains with its ethanol extracts. Chloroform is the best solvent for algal bioactive compounds and the algae belongs to Phaeophyceae have more antimicrobial activity than the other groups.

Keywords: algae, antimicrobial activity, bacteria, yeast.

Introduction

The seaweeds and Cyanobacteria have many different metabolites on their body. Biological and pharmaceutical characteristics of these compounds are researched and they are used as antimicrobial substances. Microorganisms especially bacteria have the genetic resistance against to antibiotics. So; pharmaceutical industries have fabricated many new antibiotics for the microorganisms that sick the humans but firstly bioactive compounds should be obtained. The antimicrobial studies were focused on the higher plants (Saify *et al.*, 2000; Mufti *et al.*, 2012; Mahmood *et al.*, 2012). But recent years; alternatively algae are the most important living groups for the sources of useful bioactive compounds especially secondary or primary metabolites, since two decades (Ertürk and Taş, 2011). In late years, there have been many studies of algae reproduced compounds that have a broad range of biological activities, such as antibacterial, antiviral, antifungal, antioxidant, anti-inflammatory, cytotoxic activities (Demirel *et al.*, 2009). Some algal compounds have bacteriostatic and bactericidal activities; they have been researched by several scientists (Taşkın *et al.*, 2007). Many researchers studied about antimicrobial activity of algae in Turkey, too (Haliki *et al.*, 2005; Tüney *et al.*, 2006; Tüney *et al.*, 2007; Özdemir *et al.*, 2006; Sukatar *et al.*, 2006; Karabay-Yavaşoğlu *et al.*, 2007; Taşkın *et al.*, 2007; Demirel *et al.*, 2009; Ertürk and Taş, 2011).

In this investigation, antibacterial and antifungal activities of methanol, chloroform, diethyl ether, dichloromethane (DCM) and ethanol extracts of six algae biomasses obtained from the coast of Dardanelles and Ayazma Stream (Çanakkale, Turkey) were studied against some pathogenic microorganisms.

Materials and Methods

Algal Material and Preparation of Algal Extract

The biomasses of *Rivularia bullata* (Poir) Berkeley ex Bornet & Flahault (Cyanobacteria, Rivulariaceae), *Colpomenia peregrina* Sauvageau (Phaeophyceae, Scytosiphonaceae), *Cystoseira barbata* (Stackhouse) C. Agardh (Phaeophyceae, Sargassaceae), *Zanardinia typus* (Nardo) P. C. Silva (Phaeophyceae, Cutleriaceae), *Codium fragile* (Suringar) Hariot (Chlorophyta, Codiaceae) were collected from the coasts of Dardanelles, the biomass of *Nostoc spongiaeforme* C. Agardh ex Bornet & Flahault (Cyanobacteria, Nostocaceae), was collected from the Ayazma Stream, in December 2011 and were identified by Dr. Rıza AKGÜL and Dr. Hüseyin ERDUĞAN. Epiphytes on the algae samples were cleaned and injured parts were removed. Samples were washed with sterile water to remove any associated sandy particles. They were then pressed

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between filter papers and subsequently dried at room temperature. After drying the sample, it was ground thoroughly to powder form. Air-dried biomass samples were extracted in 150 ml of methanol, chloroform, diethyl ether, DCM and ethanol for 8 hour by using soxhlet extraction apparatus. 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. The dried extract was then re-dissolved in 10% dimethyl sulfoxide (DMSO) (v/v) to yield solution containing 40 mg/ml.

Test Microorganisms

Two Gram-positive bacterial strains (*Staphylococcus hominis, Staphylococcus aureus*), six Gram-negative bacterial strains (*Enterobacter cloaceae, Escherichia coli, Acinetobacter boumannii, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella oxytora*) and ten yeast strains (*Candida tropicalis, C. albicans, C. parapsilosis, C. pelliculosa, Kluyveromyces marxianus, Rhodotorula glutinis, Debaryomyces occidentalis, Pichia anomala, Cryptococcus neoformans* and *Metschnikowia pulcherrima*) were used as test microorganisms. They were obtained from stocks of BIM (Basic and Industrial Microbiology) and Yeast Genetics Research Laboratories of Çanakkale Onsekiz Mart University, Turkey.

Antimicrobial processing

Antimicrobial activities of biomass extracts were evaluated by using the disc diffusion method in petri plates according to the National Committee for Clinical Laboratory Standards (NCCLS, 2002). 50 μ L of each extract was loaded on sterile filter paper discs 6 mm in diameter and air-dried (2mg/disc). Bacterial strains were incubated on Nutrient Broth (Scharlau Chemie S.A Barcelona, Spain) growth medium at 37°C ± 0.1°C for 24 h. The yeast strains were incubated in enrichment medium (Yeast Extract 10 g/L, Bacto Peptone 20 g/L, Dextrose 20 g/L) for 24 h at 120 rpm and 27°C ± 0.1°C. From overnight cultures, 100 μ L test microorganisms (approximately 10⁶ bacterial cells/mL and 10⁸ yeast cells/mL) were transferred to Müeller Hinton Agar (Scharlau Chemie S.A Barcelona, Spain) plates and spreading. All plates were allowed to air dry for nearly 30 minutes and extract absorbed discs were located on plates. Then all bacterial plates were incubated for 24 hour at 37°C ± 0.1°C and yeast plates were incubated for 2-3 days at 28°C ± 0.1°C. After incubation, all plates were observed for a clear zone of growth inhibition around each disc. Diameters of these zones of inhibition were measured with inhibition zone ruler (Bioanalyse) in millimeters. All tests were done duplicate and three times. DMSO was used as negative control. Gentamicin (CN10), Ampicillin/Sulbactam (SAM30), Penicillin G (P10) and Chloramphenicol (C30) were used as reference antibacterial antibiotics and Nystatin (NS100) and Ketoconazole (KTC50) were used as reference antifungal antibiotics.

Results

Extracts of algae and Cyanobacteria species were tested against bacteria and yeast strains. The antimicrobial activities of algae and Cyanobacteria are summarized in Table 1 that shows diameter of the inhibition zones according to type of the algal species, type of solvent used and tested bacteria and yeasts.

As is apparent from Table 1; extracts of all biomass have antimicrobial effects against the tested microorganism strains in various inhibition zones. It can be said that antifungal activities of the extracts are more than their antibacterial activities. Among the algae species used in this study, *Z. typus* have shown a broad antimicrobial activity against both bacteria and yeast strains. However, the lowest effect was observed for *C. fragile* forming with the inhibition zones against only two bacteria and all yeast strains with its chloroform extracts and against five bacteria strains with its ethanol extracts.

Chloroform extracts of all biomass have high antimicrobial activities, especially antifungal. Otherwise, methanol extracts of all biomass except *R. bullata;* have not any antibacterial and antifungal activity or weakly, as in *Z. typus* and *C. barbata*. Ethanol extracts have stronger antimicrobial activity than diethyl ether and DCM extracts. Especially, ethanol is very effective about solving the antibacterial substances in algae. Diethyl ether is inadequate for solving the antimicrobial activity against *Cryptococcus neoformans* and *Metschnikowia pulcherrima*. When DCM can solve the antibacterial substances in *R. bullata*, *Z. typus*, C. *barbata*; can solve both of antibacterial and antifungal substances in *N. spongiaeforme*. Particularly; DCM extract of *N. spongiaeforme* has strong antifungal activity against to *Debaryomyces occidentalis*; as much as NS100 antibiotic. Antimicrobial activities of some standard antibiotics have showed in Table 2.

In Cyanobacteria; *N. spongiaeforme* has more antimicrobial activity than *R. bullata*. In Phaeophyceae; respectively *Z. typus, C. peregrina*, C. *barbata* have the highest activity. In Chlorophyta; only one sample is *C. fragile* has very low effect against the microorganisms.

Discussion

When considering on studies at this field, similar results with our study were found. Yılmaz-Koz *et al.* (2009) researched antimicrobial activity of methanol, dichloromethane and hexane extracts of *C. fragile* and indicated that all the extracts of *C. fragile* have low antimicrobial activity. In contrast; some researchers detected that same alga has an important inhibitor

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activity against the Gram positive bacteria and *Codium bursa*, *C. coralloides*, *C. vermilara* have no activity (Salvador *et al.*, 2007). In some green microalgae; Uma et al. (2011) detected that the highest inhibition zone was observed in acetone extract of *Chlorococcum sp* against gram positive and gram negative bacteria.

In another study similar to our study; Karabay-Yavaşoğlu *et al.* (2007) used methanol, DCM, hexane, chloroform and volatile oil extracts of red alga *Jania rubens* against Gram-positive and Gram negative bacteria and *Candida albicans* and they reported that the methanol and chloroform extracts showed more potent antimicrobial activity than the other solvents.

According to our results; methanol extracts of all samples except *R. bullata*; have not any antibacterial and antifungal activity or weakly, as in *Z. typus* and *C. barbata*. In contrast to our study; Sasidharan *et al.* (2010) studied methanol extract of the *Gracilaria changii* and reported that extracts show high antimicrobial activity against *P. aeruginosa*. Vijayabaskar and Shiyamala (2011) indicated that the methanol extract of brown marine algae have a strong antimicrobial activity against *Gram-negative* bacteria as well as ampicillin. Taşkın *et al.* (2007) found that methanol extracts of some marine algae have moderate inhibitor activity against the Gram-positive and Gram-negative bacteria and a broad activity spectrum for *C. barbata*, like to our study. Some results are different from ours; these differences can be associated with collection of algae at different times in different places and use of different test microorganisms.

We found that diethyl ether is inadequate for solving the antimicrobial materials in all samples, expect *C. barbata*. As similar to our study; Tüney *et al.* (2006) detected that diethyl ether extraction of *Cystoseria mediterranea* inhibited the growth of yeasts.

In our results; *N. spongiaeforme* has more antimicrobial activity than *R. bullata*. Zarmouh (2010) detected that diethyl ether extracts of *Rivularia* species have moderate antibacterial activity and ethylacetate extract of that species showed low activity and ethanol extract of *Rivularia* species have no activity to that selected bacteria. This result is similar to ours that ethanol extract of *R. bullata* showed weak antibacterial activity against to only one bacterium. And also Tiwari and Sharma (2013) found that ethanol:acetic acid extract of *Anabaena variabilis* had an inhibition effect against *E. coli* and methanol extract of *Synechococcus elongates* had the highest antimicrobial activity against *E. coli* and *Enterococcus sp.*.

In Phaeophyceae; respectively Z. typus, C. peregrina, C. barbata have the highest activity. In Chlorophyta; C. fragile has very low impact against the microorganisms; Yılmaz-Koz et al. (2009) found same result. But in contrast to our study; Govindasamy et al. (2011) said that methanolic extracts of Halimeda macroloba (Chlorophyta) observed maximum activity against the some pathogens. A study detected that Phaeopycae has more antimicrobial activity than Rhodophyceae (Caccamese et al., 1985). Vlachos et al. (2001) specified that extracts of the Phaeophyta showed the highest antibacterial activity, respectively Rhodophyta and Chlorophyta. Yi et al. (2001) reported that Rhodophyta has the highest antibacterial activity. Salem et al. (2011) reported that Chlorophyta have more antimicrobial activity than the Rhodophyta and Phaeophyceae. Salvador et al. (2007) specified that red algae have higher antimicrobial activity compared to other groups.

Conclusions

Due to the different results of previous studies, it is hard to generalize the antimicrobial activities of studied groups. Some researchs are compatible to our study but some of them are not. This unconformity may be due to diversity of algae and Cyanobacteria samples, location and time of collection, solvents and test microorganisms. But we found that algae in Phaeophyceae have more antimicrobial activity than the other groups studied.

According to this study; the algae from region of Çanakkale are potential sources of bioactive compounds and should be investigated for natural antibiotics. Further works may be determining the compounds causing the activity, evaluating specific antimicrobial activity against pathogenic microorganisms especially those threatening the humanity.

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Table 1: Antimicrobial activity of algae extracts against to test microorganisms; -: no inhibition zone; *: Values, including diameter of the filter paper disc (6.0 mm); N.T.: Not Tested; 1: Enterobacter cloaceae, 2: Escherichia coli, 3: Acinetobacter boumannii, 4: Pseudomonas aeruginosa,, 5: Staphylococcus hominis, 6: Klebsiella oxytora, 7: Proteus mirabilis, 8: Staphylococcus aureus, 9: Candida tropicalis, 10: C. albicans, 11: C. parapsilosis, 12: C. pelliculosa, 13: Kluyveromyces marxianus, 14: Rhodotorula glutinis, 15: Debaryomyces occidentalis, 16: Pichia anomala, 17: Cryptococcus neoformans, 18: Metschnikowia pulcherrima, DMSO: dimethyl sulfoxide

	Diameter of İnhibition zone (mm)																		
		Bact	erial	Strai	ns					Ŋ	east	Strai	ns						
Algal Species	Organic Solvents	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	Methanol	-	-	-	-	-	-	-	-	-	8	7	-	-	-	7	8	-	-
	Chloroform	10*	10	8	8	10	10	8	8	10	10	12	10	10	10	10	10	8	8
Rivularia bullata	Diethyl ether	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Dichloromethane	-	7	-	8	8	10	10	8	-	-	-	-	-	-	10	-	-	-
	Ethanol	8	-	-	-	-	-	-	-	8	-	-	-	8	-	-	-	-	-
	Methanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Chloroform	10	10	9	8	8	10	11	8	12	8	12	8	12	8	8	8	8	10
Nostoc spongiaeforme	Diethyl ether	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Dichloromethane	10	8	8	-	7	7	7	8	7	7	10	-	-	-	20	12	13	8
	Ethanol	10	8	11	-	11	11	11	11	-	7	-	7	-	-	8	-	8	-
	Methanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Chloroform	9	9	8	9	8	8	-	7	12	12	8	10	8	8	10	10	8	10
Colpomenia peregrina	Diethyl ether	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Dichloromethane	-	9	9	-	-	-	-	-	-	7	-	-	-	-	-	7	-	8
	Ethanol	11	10	11	10	8	9	10	12	-	7	-	10	-	-	10	10	8	8
	Methanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Chloroform	-	8	-	-	-	-	7	-	8	12	10	8	10	8	-	8	8	8
Codium fragile	Diethyl ether	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Dichloromethane	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Ethanol	8	10	7	-	-	-	8	10	-	-	-	-	-	-	-	-	-	-
	Methanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-
	Chloroform	10	10	9	10	8	9	9	8	12	10	10	12	12	12	10	10	10	8
Zanardinia typus	Diethyl ether	-	-	-	8	-	-	-	9	-	-	-	8	8	-	-	7	-	-
	Dichloromethane	7	9	-	9	-	-	-	-	-	-	-	-	-	-	7	8	-	-
	Ethanol	12	12	12	-	12	13	11	13	10	10	10	10	10	10	12	10	12	10

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Cystoseira barbata	Methanol	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-
	Chloroform	-	-	-	-	8	8	8	9	8	8	8	10	-	10	10	10	8	8
	Diethyl ether	-	-	-	-	-	10	-	-	-	-	-	-	-	-	-	-	8	10
	Dichloromethane	7	-	7	7	7	7	-	-	-	-	-	-	-	-	-	-	-	-
	Ethanol	-	-	8	-	-	7	7	7	7	7	-	8	10	8	-	8	8	-
DMSO	DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 2: Antimicrobial activity of some standard antibiotics; CN10: Gentamicin (10 µg), SAM30: Ampicillin/Sulbactam (30µg), P10: Penicillin G (10 Units), C30: Chloramphenicol (30 µg), KTC50: Ketoconazole (50 µg), NS100: Nystatin (100 units)

Mikroorganisms/Antibiotics	CN10	SAM30	P10	C30	KTC50	NS100
Enterobacter cloaceae	-	8	-	8	N.T	N.T
Escherichia coli	14	18	-	14	N.T	N.T
Acinetobacter boumannii	8	-	-	-	N.T	N.T
Pseudomonas aeruginosa	20	12	-	24	N.T	N.T
Staphylococcus hominis	16	16	-	10	N.T	N.T
Klebsiella oxytora	12	8	-	22	N.T	N.T
Proteus mirabilis	18	20	16	14	N.T	N.T
Staphylococcus aureus	22	26	24	22	N.T	N.T
Candida tropicalis	N.T	N.T	N.T	N.T	38	22
C. albicans	N.T	N.T	N.T	N.T	32	22
C. parapsilosis	N.T	N.T	N.T	N.T	46	24
C. pelliculosa	N.T	N.T	N.T	N.T	42	24
Kluyveromyces marxianus Rhodotorula	N.T	N.T	N.T	N.T	24	18
glutinis	N.T	N.T	N.T	N.T	34	22
Debaryomyces occidentalis Pichia	N.T	N.T	N.T	N.T	58	30
anomala	N.T	N.T	N.T	N.T	36	22
Cryptococcus neoformans	N.T	N.T	N.T	N.T	-	-
Metschnikowia ulcherrima	N.T	N.T	N.T	N.T	38	22